2nd Joint Meeting

of the

Bone Research Society

and the

British Society for Matrix Biology

14-16 June 2009

LONDON, UK

BONE RESEARCH SOCIETY
www.brsoc.org.uk

British Society for Matrix Biology
www.bsmb.ac.uk

FINAL PROGRAMME & ABSTRACTS

2nd Joint Meeting

of the

Bone Research Society

and the

British Society for Matrix Biology

14-16 June 2009

LONDON, UK

FINAL PROGRAMME AND ABSTRACTS

Contents

Local Organisers

Tim Arnett/Andy Pitsillides

with Sanjeev Patel/Tonia Vincent

web addresses

www.brsoc.org.uk

www.bsmb.ac.uk

BONE RESEARCH SOCIETY

www.brsoc.org.uk

The Society (formerly known as the Bone and Tooth Society) is the oldest and largest scientific society in Europe that is dedicated to further research into clinical and basic science problems related to mineralised tissues. The meeting attracts a wide audience from throughout the UK and, increasingly, from continental Europe and further afield. The presentations are traditionally a balance between clinical and laboratory-based studies. The participation of young scientists and clinicians is actively encouraged.

Committee 2009

President: Cyrus Cooper (Oxford and Southampton)

President Elect: Jonathan Tobias (Bristol)

Secretary: Colin Farquharson (Edinburgh)

Treasurer: Nigel Loveridge (Cambridge)

Jacqueline Berry (Manchester)

Kay Colston (London)

David Marsh (London)

Eugene McCloskey (Sheffield)

Andy Pitsillides (London)

Ken Poole (Cambridge)

Student representatives:

Claire Clarkin (London)

Nick Harvey (Southampton)

Next Year's BRS Meeting

Will be held in association with the European Calcified Tissue Society

Glasgow, 26-30 June 2010

British Society for Matrix Biology

www.bsmb.ac.uk

Formerly known as the British Connective Tissue Society (BCTS), the BSMB is a learned society of professional scientists with an interest in all aspects of the extracellular matrix. The society is a member of the UK Biosciences Federation and the Federation of European Connective Tissue Societies (FECTS). BSMB members come from diverse scientific backgrounds, working in universities, hospitals and industry. As you might expect, most of the membership live and work in the UK, although we do have members from the USA, Europe and Australia.

Committee 2009

Chair: Bruce Caterson (Cardiff)

Secretary: John Couchman (Copenhagen)

Treasurer: Graham Riley (Norwich)

Ann Canfield (Manchester)

Jelena Gavrilovic (Norwich)

Wa'el Kafienah (Bristol)

Nikki Kuiper (Keele)

Andy Pitsillides (London)

Mandy Plumb (Aberdeen)

Tonia Vincent (London)

David Young (Newcastle upon Tyne)

Student representatives:

Emma Blain (Cardiff)

Chris Watson (Manchester)

Next Year's BSMB Meeting

Manchester, 29-30 March 2010

SPONSORS AND OTHER SUPPORTERS

GOLD SPONSORS

Amgen

Novartis Pharmaceuticals

Procter and Gamble Pharmaceuticals

BRONZE SPONSORS

Company of Biologists

Eli Lilly

Roche

Servier

OTHER SUPPORTERS

Advanced Molecular Vision

e2v Scientific Instruments/Skyscan

IDS

PeproTech

Promocell

Scanco

Wyeth

Sunday 14 June

REGISTRATION 13:30-18:00

PARALLEL SESSIONS

13.30 Registration & Coffee

14.00 Joint Symposium: New imaging technologies for cartilage and bone
CRUCIFORM LECTURE THEATRE 1

15.30 Coffee
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

16.00 Joint Symposium: Life in an extreme environment
CRUCIFORM LECTURE THEATRE 1

17.00 New Investigator/ Trainee Session
CRUCIFORM LECTURE THEATRE 1

19.00 Meet-the-Professor-in-the-Pub!

Monday 15 June

BASIC SCIENCE PROGRAMME

REGISTRATION 08:30-18:00

PARALLEL SESSIONS

08.30 Registration & Coffee

09.30 Basic Science Symposium: New frontiers
CRUCIFORM LECTURE THEATRE 1

10.30 Coffee and commercial exhibition
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

11.30 Joint Symposium: The control of angiogenesis in bone and cartilage
CRUCIFORM LECTURE THEATRE 1

12.30 Lunch, commercial exhibition, posters
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

13.15-14.00 Attended Posters – odd numbers

14.00 *The control of angiogenesis in bone and cartilage (continued)*
CRUCIFORM LECTURE THEATRE 1

15.00 Joint Oral Communications (basic science)
CRUCIFORM LECTURE THEATRE 1

16.00 Tea & commercial exhibition
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

17.00 BRS Oral Communications (basic)
CRUCIFORM LECTURE THEATRE 1

17.00 BSMB Oral Communications
CRUCIFORM LECTURE THEATRE 2

18.00 Joint Plenary Lectures
CRUCIFORM LECTURE THEATRE 1

19.00 Extended Reception and poster viewing
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

18:45-19:15 Attended Posters – even numbers

19:15-19:45 Attended Posters – odd numbers

Monday 15 June

BRS CLINICAL DAY

REGISTRATION 08:30-18:00

08.30 **Registration & Coffee**

09.00 **IS12**
PEARSON LECTURE THEATRE

09.30 **Bone Consults**
PEARSON LECTURE THEATRE

10.00

10.30 **Coffee and commercial exhibition**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

11.00

11.30 **Joint Symposium: The control of angiogenesis in bone and cartilage**
CRUCIFORM LECTURE THEATRE 1

12.00

12.30 **Lunch and commercial exhibition**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

13.00 13.15-14.00 Attended Posters – odd numbers

13.30

14.00 ***The control of angiogenesis in bone and cartilage (continued)***
CRUCIFORM LECTURE THEATRE 1

14.30 **IS13**
PEARSON LECTURE THEATRE

15.00 **Clinical Cases**
PEARSON LECTURE THEATRE

15.30

16.00 **Tea & commercial exhibition**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

16.30 **Satellite Symposium**
PEARSON LECTURE THEATRE

17.00

17.30

18.00 **Joint Plenary Lectures**
CRUCIFORM LECTURE THEATRE 1

18.30 **Extended Reception and poster viewing**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

19.00

19.30

20.00

20.30

PARALLEL SESSIONS

Tuesday 16 June

REGISTRATION 08:00-17:00

08.30 **Joint Symposium: Skeletal development**
CRUCIFORM LECTURE THEATRE 1

09.00

09.30

10.00 **Coffee and commercial exhibition**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

10.30 **BRS Oral Communications**
CRUCIFORM LECTURE THEATRE 1

11.00

11.30

12.00 **BRS - Cancer and bone**
CRUCIFORM LECTURE THEATRE 1

12.30

13.00 **Lunch and commercial exhibition**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

13.30 13.15-14.00 Attended Posters – even numbers

14.00 **BRS AGM**
CRUCIFORM LECTURE THEATRE 1

14.30 **Joint Oral Posters**
CRUCIFORM LECTURE THEATRE 1

15.00

15.30 **Tea**
WILKINS SOUTH CLOISTERS AND JEREMY BENTHAM ROOM

16.00 **Joint Symposium: Dynamic relationships with the extracellular matrix (ECM): lessons from dysplasias**
CRUCIFORM LECTURE THEATRE 1

16.30

17.00 **Awards**
CRUCIFORM LECTURE THEATRE 1

17.30 **Close**

18.00

18.30

19.00

19.30

20.00

20.30

PARALLEL SESSIONS

BSMB Open Session
CRUCIFORM LECTURE THEATRE 2

BSMB - Matricellular proteins in health and disease
CRUCIFORM LECTURE THEATRE 2

BSMB AGM
CRUCIFORM LECTURE THEATRE 2

SUNDAY 14 JUNE

13:30-14:00 **Wilkins South Cloisters**

REGISTRATION AND COFFEE

14:00-15:15 **Cruciform Lecture Theatre 1**

Joint Symposium: New imaging technologies for cartilage and bone

Chairs: Miep Helfrich (Aberdeen, UK) & Michael Sherratt (Manchester, UK)

14:00 IS01 USING μCT TO STUDY BONE ARCHITECTURE
Rob van't Hof *(Edinburgh, UK)*

14:25 IS02 IMAGING OF OSTEOCHONDRAL TISSUES
Alan Boyde *(London, UK)*

14:50 IS03 TOWARDS UNDERSTANDING OF OSTEOARTHRITIS AND JOINT HEALTH BY CONTRAST-ENHANCED MRI OF CARTILAGE
Leif Dahlberg *(Malmö, Sweden)*

15:15-15:50 **Wilkins South Cloisters and Jeremy Bentham Room**

COFFEE

15:50-16:40 **Cruciform Lecture Theatre 1**

Joint Symposium: Life in an extreme environment

Chairs: Alan Boyde (London UK) & Tim Hardingham (Manchester, UK)

15:50 IS04 EARTH (WATER), WIND (OXYGEN) AND FIRE (ENERGY): A RECIPE FOR CELL SURVIVAL IN A HOSTILE ENVIRONMENT
Irving Shapiro *(Philadelphia, USA)*

16:15 IS05 OSTEOCYTES: BIRTH, LIFE AND DEATH IN A MINERALIZED TISSUE
Nigel Loveridge *(Cambridge, UK)*

16:40 **BREAK**

16:50-17:50 **Cruciform Lecture Theatre 1**

New Investigator/ Trainee Session

Chairs: Emma Blain (Cardiff, UK) & Nick Harvey (Southampton, UK)

John Williams *(London, UK): Wellcome Trust Clinical/ Basic Fellowship Schemes*

Karl Kadler *(Manchester, UK): MRC training fellowships / Fellowship versus lecturer routes in academia*

Keith Thompson *(Aberdeen, UK): ARC Fellow*

Vicky MacRae *(Edinburgh, UK): BBSRC Fellow and 2008 Barbara Mawer Visiting Fellowship recipient*

19:00-20:15 See board next to registration desk

'Meet-the-Professor-in-the-Pub!'

This novel concept will enable you to discuss your research with key experts in an informal, convivial setting. Afterwards, you will be able to explore the amazingly diverse café/bar/restaurant/gastropub/club scene in Central London using our specially-compiled guide (see page 108) with recommendations that will suit all pockets and interests.

See information board next to registration desk for full details and locations. Please sign in for the session you would like to attend. Places are limited and will be allocated on a first-come first-served basis.

Charlie Archer & Bruce Caterson:
Cartilage zonation

Jill Cornish & Chantal Chenu:
Bone, brain and fat

Jim Gallagher & Alison Gartland:
Nucleotides and bone

Tim Hardingham & Jerry Saklatvala:
Can we fix articular cartilage?

Ken Poole & Nigel Loveridge:
Sclerostin

Graham Riley & Roger Smith:
Trouble with tendons

Tim Skerry & Brendon Noble:
Bone responses to mechanical loading

David Abraham & Tim Cawston:
Matrix repair

BASIC SCIENCE PROGRAMME

(IN PARALLEL WITH BRS CLINICAL DAY – SEE PAGES 10-11)

MONDAY 15 JUNE - BASIC SCIENCE PROGRAMME

08:30-09:15 Wilkins South Cloisters and Jeremy Bentham Room

REGISTRATION AND COFFEE

09:15-10:30 Cruciform Lecture Theatre 1

Basic Science Symposium: New frontiers

Chairs: Bruce Caterson (Cardiff, UK) Peter Croucher (Sheffield, UK)

09:15 IS06 MICRO RNAS AND THE SKELETON
Ian Clark (Norwich, UK)

09:40 IS07 ZEBRAFISH AS A TOOL FOR SKELETAL BIOLOGY
Henry Roehl (Sheffield, UK)

10:05 IS08 EX-VIVO AND IN-VIVO GENE DELIVERY STRATEGIES USING MATRIX PATHOLOGY AS A TARGET
Yuti Chernajovsky (London, UK)

10:30-11:20 Wilkins South Cloisters and Jeremy Bentham Room

COFFEE & COMMERCIAL EXHIBITION

11:20-12:10 Cruciform Lecture Theatre 1

Joint Symposium: The control of angiogenesis in bone and cartilage

Chairs: Jerry Saklatvala (London, UK) & Claire Clarkin (London, UK)

11:20 IS09 VASCULARISATION AND BONE REMODELLING
Marie-Hélène Lafage-Proust (St Etienne, France)

11:45 IS10 TARGETING THE HIF-1 PATHWAY FOR BONE REPAIR
Tom Clemens (Birmingham, USA)

12:10-12:15 Cruciform Lecture Theatre 1

Developmental biology at UCL

Lewis Wolpert (London, UK)

12:15-14:00 Wilkins South Cloisters and Jeremy Bentham Room

LUNCH, COMMERCIAL EXHIBITION, POSTERS

13:15-14:00 Wilkins South Cloisters and Jeremy Bentham Room

ATTENDED POSTERS – ODD NUMBERS

14:00-14:25 Cruciform Lecture Theatre 1

Joint Symposium: The control of angiogenesis in bone and cartilage

(continued)

14:00 IS11 MOLECULAR REGULATION OF VASCULAR CALCIFICATION
Ann Canfield (Manchester, UK)

14:25 BREAK

14:30-16:00 Cruciform Lecture Theatre 1

Joint Oral Communications (basic science)

Chairs: Colin Farquharson (Edinburgh, UK) & John Couchman (Copenhagen, Denmark)

14:30 OC1 MATRIX MINERALISATION: A DRIVER FOR OSTEOCYTOGENESIS?
***M Prideaux**, AA Pitsillides, C Farquharson (Edinburgh, UK)*

14:40 P031 THE WISKOTT-ALDRICH SYNDROME PROTEIN VERPROLIN HOMOLOGOUS (WAVE) 1 IS ESSENTIAL TO LOCALIZE MT1-MMP AT THE LEADING EDGE OF MIGRATING CELLS
***BK Wolters**, K Sato-Kusubata, N Ito, S Suetsugu, T Takenawa, Y Itoh (London, UK)*

14:50 OC2 TWO POPULATIONS OF ENDOCHONDRAL OSTEOBLASTS WITH DIFFERENTIAL SENSITIVITY TO HEDGEHOG SIGNALLING
***CL Hammond**, S Schulte-Merker (Utrecht, The Netherlands)*

15:00 P019 CHONDROITIN SULPHATION MOTIFS IDENTIFY PUTATIVE CHONDROPROGENITOR CELLS IN HUMAN OSTEOARTHRITIC CARTILAGE FOR USE IN ACT TECHNOLOGIES
***S Mukhopadhyay**, S Li, AJ Hayes, CE Hughes, MA Nowell, B Caterson (Cardiff, UK)*

15:10 OC3 ENHANCED OSTEOGENEISIS OF FOETAL AND ADULT HUMAN BONE MARROW STROMAL CELLS COCULTURED WITH HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS
***JM Kanczler,** ROC Oreffo (Southampton, UK)*

15:20 P020 FRACTURE HEALING IN GDF-5 DEFICIENT MICE
***CM Coleman**, BH Scheremeta, A Boyce, RL Mauck, RS Tuan (Galway City, Eire)*

15:30 OC4 PHENOTYPIC EFFECTS OF HYPOXIA AND HYPOXIA-INDUCIBLE FACTOR IN EWING'S SARCOMA
***HJ Knowles**, L Schaefer, NA Athanasou (Oxford, UK)*

15:40 P033 CTGF PLAYS A PIVOTAL ROLE IN LUNG FIBROSIS THROUGH THE MAPK DEPENDENT ACTIVATION OF COLLAGEN TYPE I
***M Ponticos**, X Shi-wen, K Khan, P Leoni, GE Lindah, DJ Abraham (London, UK)*

15:50-16:30 Wilkins South Cloisters and Jeremy Bentham Room

TEA AND COMMERCIAL EXHIBITION

PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS

16:30-17:30 Cruciform Lecture Theatre 1

BRS Oral Communications (basic)

Chairs: Jackie Berry (Manchester, UK) & Kay Colston (London, UK)

16:30 OC5 LOCALLY GENERATED GLUCOCORTICOIDS, RATHER THAN PRO-INFLAMMATORY CYTOKINES, DIRECTLY REGULATE SYNOVIAL DKK-1 EXPRESSION IN INFLAMMATORY ARTHRITIS
***RS Hardy**, M Ahasan, P Patel, A Ahmed, A Filer, K Raza, CD Buckley, PM Stewart, MS Cooper (Birmingham, UK)*

16:40 OC6 BLOCKADE OF THE ALK1 RECEPTOR REDUCES TUMOUR MICROVESSEL DENSITY AND PREVENTS THE DEVELOPMENT OF OSTEOLYTIC BONE DISEASE IN THE 5T2MM MURINE MODEL OF MYELOMA
***AD Chantry**, L Coulton, O Gallagher, H Evans, J Seehra, S Pearsall, H De Raeve, K Vanderkerken, PI Croucher (Sheffield, UK)*

16:50 OC7 OSTEOCYTES AS YOU'VE NEVER SEEN THEM BEFORE
***GM Warnock**, JS Gregory, KS Mackenzie, JG Greenhorn, AD McKinnon, MH Helfrich (Aberdeen, UK)*

17:00 OC8 APRIL, BAFF AND NGF: NOVEL RANKL SUBSTITUTES
***F Jones**, R Taylor, HJ Knowles, NA Athanasou (Oxford, UK)*

17:10 OC9 NOVEL USE OF DIGITAL IMAGE CORRELATION TO MEASURE SURFACE STRAINS IN LOADED MOUSE TIBIAE DISCLOSES DIRECT EVIDENCE OF FUNCTIONAL ADAPTATION TO LIMIT PEAK STRAINS
***SJ Shefelbine**, P Sztefek, M Vanleene, R Olsson, RC Collinson, AA Pitsillides (London, UK)*

17:20 OC10 OPTIMAL BONE MINERALIZATION AND STRENGTH REQUIRES THE TYPE 2 IODOTHYRONINE DEIODINASE IN OSTEOBLASTS
***JHD Bassett**, A Boyde, PGT Howell, RH Bassett, TM Galliford, M Archanco, DL St. Germain, VA Galton, GR Williams (London, UK)*

16:30-17:30 Cruciform Lecture Theatre 2

BSMB Oral Communications

Chairs: Graham Riley (Norwich, UK) & Chris Watson (Manchester, UK)

16:30 P074 MOLECULAR INTERACTIONS OF ADAMTS-4 IN THE VEGFR2 SIGNALLING COMPLEX
***YP Hsu**, CA Staton, DJ Buttle (Sheffield, UK)*

16:40 P086 HISTONE DEACETYLASE INHIBITORS AS CHONDROPROTECTIVE AGENTS
***KL Culley**, S Fenwick, IM Clark (Norwich, UK)*

16:50 P091 MIR-675 REGULATES COLLAGEN II LEVELS IN HUMAN ARTICULAR CHONDROCYTES
***K Dudek**, JE Lafont, CL Murphy (London, UK)*

17:00 P082 KNOCKOUT OF P58IPK, A KNOWN INHIBITOR OF PKR, IN MICE RESULTS IN A DEGENERATIVE PHENOTYPE IN THE KNEE JOINT
***SJ Gilbert**, M Patel, H Toumi, VC Duance, DJ Mason (Cardiff, UK)*

17:10 P081 APOPTOSIS IN ANTEROMEDIAL GONARTHROSIS - EVIDENCE FOR THE ROLE OF REACTIVE OXYGEN SPECIES
***R Rout**, S Mcdonnell, S Snelling, X Dao, AJ Price, PA Hulley (Oxford, UK)*

17:20 P080 IMAGING EARLY MOLECULAR ALTERATIONS IN ARTICULAR CARTILAGE DEGENERATION BY RAMAN SPECTROSCOPY:DIAGNOSTIC APPLICATIONS
***J Dudhia**, S Firth, PF McMillan, ERC Draper (North Mymms, UK)*

17:30 BREAK

17:40-18:30 Cruciform Lecture Theatre 1

Joint Plenary Lectures

Chairs: Tim Arnett (London, UK) & Andy Pitsillides (London, UK)

17:40 BRS DENT LECTURE:
DENTING THE IMAGE OF BONE
***Alan Boyde** (London, UK)*

18:05 BSMB FELL-MUIR LECTURE:
ARTICULAR CARTILAGE: THE KNOWN AND THE UNKNOWN UNKNOWNS
***Tim Hardingham** (Manchester, UK)*

18:30-20:30 Wilkins South Cloisters and Jeremy Bentham Room

Extended Reception and poster viewing

18:45-19:15 ATTENDED POSTERS – EVEN NUMBERS

19:15-19:45 ATTENDED POSTERS – ODD NUMBERS

BRS CLINICAL DAY

(IN PARALLEL WITH BASIC SCIENCE PROGRAMME – SEE PAGES 8-9)

MONDAY 15 JUNE - BRS CLINICAL DAY

08:30-09:00

REGISTRATION AND COFFEE

09:00-09:25 Pearson Lecture Theatre

IS12
Advances in bone biology relevant to clinicians

Jon Tobias (Bristol, UK)

Chairs: Cyrus Cooper (Oxford and Southampton, UK) & Ken Poole (Cambridge, UK)

09:25-10:40 Pearson Lecture Theatre

Bone Consults

Chairs: Juliet Compston (Cambridge, UK) & Sanjeev Patel (London, UK)

AN OPPORTUNITY TO SEE HOW YOUR CLINICAL PRACTICE COMPARES TO ACADEMIC CLINICAL EXPERTS IN THE FIELD

How should I use the FRAX tool in an osteoporosis clinic?

What do I do when a patient with OP treated with a bisphosphonate has a further fracture?

Should you ask for serum PTH when measuring serum 25OHD levels?

How do you use bone markers when treating patients with PTH?

When and how to treat an asymptomatic patient with Paget's disease of bone?

When do I refer a patient with an acute vertebral fracture for vertebroplasty?

SPEAKERS:
Bill Fraser (Liverpool, UK)
Eugene McCloskey (Sheffield, UK)
Peter Selby (Manchester, UK)

10:40-11:20 Wilkins South Cloisters and Jeremy Bentham Room

COFFEE & COMMERCIAL EXHIBITION

11:20-12:10 Cruciform Lecture Theatre 1

Joint Symposium: The control of angiogenesis in bone and cartilage

11:20 IS09 VASCULARISATION AND BONE REMODELLING
Marie-Hélène Lafage-Proust (St Etienne, France)

11:45 IS10 TARGETING THE HIF-1 PATHWAY FOR BONE REPAIR
Tom Clemens (Birmingham, USA)

12:10-12:15 Cruciform Lecture Theatre 1

Developmental biology at UCL

Lewis Wolpert (London, UK)

12:15-14:00 Wilkins South Cloisters and Jeremy Bentham Room

LUNCH, COMMERCIAL EXHIBITION, POSTERS

13:15-14:00 Wilkins South Cloisters and Jeremy Bentham Room

ATTENDED POSTERS – ODD NUMBERS

14:00-14:25 Cruciform Lecture Theatre 1

Joint Symposium: The control of angiogenesis in bone and cartilage

(continued)

14:00 IS11 MOLECULAR REGULATION OF VASCULAR CALCIFICATION
Ann Canfield (Manchester, UK)

14:25 BREAK

14:30 Pearson Lecture Theatre

IS13
Developmental mechanisms in the pathogenesis of osteoporosis

Cyrus Cooper (Oxford and Southampton, UK)

Chairs: Eugene McCloskey (Sheffield, UK) & Caje Moniz (London, UK)

14:50-15:50 Pearson Lecture Theatre

Clinical cases

14:50 CC1 CLINICAL AND RADIOLOGICAL FEATURES OF MELORHEOSTOSIS
M Chan, Y Zhang, P Wordsworth (Oxford, UK)

15:05 CC2 CHRONIC HYPOMAGNESAEMIA – A NOVEL CAUSE OF TERTIARY HYPERPARATHYROIDISM?
B Lopez, JH Tobias, Chris Probert, A Day (Bristol, UK)

15:20 CC3 BILATERAL ATYPICAL FEMORAL STRESS FRACTURES IN PATIENTS RECEIVING BISPHOSPHONATES
Thinn Hlaing, Juliet Compston (Cambridge UK)

15:35 CC4 A CASE OF MALE OSTEOPOROSIS AND INDOLENT SYSTEMIC MASTOCYTOSIS
P Manghat, D Radia, M Moonim, C Grattan, G Hampson (London, UK)

15:50-16:30 Wilkins South Cloisters and Jeremy Bentham Room

TEA AND COMMERCIAL EXHIBITION

16:30-17:30 **Pearson Lecture Theatre**

Satellite Symposium
The role of RANK ligand inhibition in osteoporosis and rheumatoid arthritis: Clinical trial update

Organised by Amgen

Chairs: Sanjeev Patel (London, UK) & Jon Tobias (Bristol, UK)

TREATMENT OF POSTMENOPAUSAL BONE LOSS WITH RANKL INHIBITION: THE STAND AND DECIDE STUDY
Juliet Compston *(Cambridge, UK)*

RANK LIGAND INHIBITION:
PHASE 2 DATA IN RHEUMATOID ARTHRITIS
David Reid *(Aberdeen, UK)*

17:30 **BREAK**

17:40-18:30 **Cruciform Lecture Theatre 1**

Joint Plenary Lectures

Chairs: Tim Arnett (London, UK) & Andy Pitsillides (London, UK)

17:40 DENT LECTURE:
DENTING THE IMAGE OF BONE
Alan Boyde *(London, UK)*

18:05 BSMB FELL-MUIR LECTURE:
ARTICULAR CARTILAGE: THE KNOWN AND THE UNKNOWN UNKNOWNS
Tim Hardingham *(Manchester, UK)*

18:30-20:30 **Wilkins South Cloisters and Jeremy Bentham Room**

EXTENDED RECEPTION AND POSTER VIEWING

18:45-19:15 ATTENDED POSTERS – ODD NUMBERS

19:15-19:45 ATTENDED POSTERS – EVEN NUMBERS

TUESDAY 16 JUNE

08:00-08:30

COFFEE / CROISSANTS / FRUIT

08:30-09:45 Cruciform Lecture Theatre 1

Joint Symposium: Skeletal development

Chairs: Irving Shapiro (Philadelphia, USA) & Charlie Archer (Cardiff, UK)

08:30 IS14 MOLECULAR MECHANISMS OF SKELETAL MORPHOGENESIS
Bjorn Olsen *(Boston, USA)*

08:55 IS15 BONE DEVELOPMENT AND DYSPLASIAS
Stefan Mundlos *(Berlin, Germany)*

09:20 IS16 OSTEOBLAST DIFFERENTIATION IS REGULATED BY MULTIPLE SIGNALLING PATHWAYS IN THE ZEBRAFISH EMBRYO
Henry Roehl *(Sheffield, UK)*

09:45-10:30 Wilkins South Cloisters and Jeremy Bentham Room

Coffee and commercial exhibition

PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS

10:30-11:50 Cruciform Lecture Theatre 1

BRS Oral Communications

Chairs: Marie-Hélène Lafage-Proust (St. Etienne, France) & Nick Harvey (Southampton, UK)

10:30 OC11 GENDER DIFFERENCES IN HIP STRUCTURE IN PERIPUBERTAL CHILDREN
A Sayers*, JH Tobias (Bristol, UK)*

10:40 OC12 A PILOT TRIAL OF THE EFFECTS OF VITAMIN D_2 SUPPLEMENTATION UPON BONE IN POST-PUBERTAL GIRLS
KA Ward*, G Das, SA Roberts, JL Berry, JE Adams, MZ Mughal (Cambridge, UK)*

10:50 OC13 ABNORMALITIES IN THE DISTRIBUTION OF SCLEROSTIN POSITIVE AND NEGATIVE OSTEOCYTES IN THE CORTICAL OSTEON IN A HIP FRACTURE MODEL OF OSTEOPOROSIS
J Reeve*, J Power, M Doube, R van Bezooijen, KES Poole, N Loveridge (Cambridge, UK)*

11:00 OC14 RELATIONSHIP BETWEEN DIFFERENT DOMAINS OF PHYSICAL ACTIVITY AND FRACTURES: RESULTS FROM THE EPIC-NORFOLK STUDY
A Moayyeri*, R Luben, S Bingham, N Wareham, KT Khaw (Cambridge, UK)*

11:10 OC15 EFFECT OF INTERMITTENT HPTH (1-34) ON 1,25 (OH)2 VITAMIN D, BONE FORMATION AND FIBROBLAST GROWTH FACTOR-23 (FGF-23) IN POST-MENOPAUSAL OSTEOPOROSIS
M Sridharan*, P Manghat, I Fogelman, WD Fraser, G Hampson (London, UK)*

10:30-11:50 Cruciform Lecture Theatre 2

BSMB Open Session

Chairs: Tonia Vincent (London, UK) & Bjorn Olsen (Boston, USA)

10:30 P064 FGF RECEPTOR-DEPENDENT, INTEGRIN-INDEPENDENT PHOSPHORYLATION OF P38, JNK AND ERK MAPKS FOLLOWING MECHANICAL STIMULATION OF HUMAN ARTICULAR CHONDROCYTES IN PRIMARY MONOLAYER CELL CULTURE
Y Zhou*, C Beadle, DM Salter, G Nuki (Edinburgh, UK)*

10:40 P034 STRUCTURAL AND REGULATORY COMPONENTS OF THE PERICELLULAR MATRIX OF YOUNG AND MATURE ARTICULAR CARTILAGE
C McLean, J Saklatvala, ***T Vincent*** *(London, UK)*

10:50 P021 EXPANSION ON A HYALURONAN COATED SURFACE ENHANCES THE CHONDROGENIC POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS
C Ryan*, C Coleman, M Murphy, F Barry (Galway, Eire)*

11:00 P066 MOLECULAR RESPONSE OF ARTICULAR CARTILAGE TO INJURY: WNT SIGNALING IN AN IN VIVO MODEL OF JOINT SURFACE INJURY
N Eltawil*, C Pitzalis, F Dell'Accio (London, UK)*

11:10 P088 INVOLVEMENT OF NMDAR IN CHONDROCYTE CELL DEATH AND MATRIX DEGENERATION
L Ramage*, GE Hardingham, DM Salter (Edinburgh, UK)*

PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS

11:20 OC16 INACTIVATING MUTATIONS IN RANK RESULT IN DISEASES WITH OPPOSITE PHENOTYPES
***DJ Mellis**, A Duthie, M Guerrini, A Villa, MH Helfrich, MJ Rogers, JC Crockett (Aberdeen, UK)*

11:30 OC17 INHIBITING GLYCOGEN SYNTHASE KINASE-3 (GSK-3) PREVENTS THE DEVELOPMENT OF MYELOMA BONE DISEASE
***NG Abdul**, W Stoop, W Koopman, M Djerbi, AD Chantry, H Evans, K Vanderkerken, PI Croucher (Sheffield, UK)*

11:40 OC18 METABOLOMIC STUDIES IDENTIFY TRUE RESPONDERS FROM NON-RESPONDERS TO VITAMIN D SUPPLEMENTATION IN POSTMENOPAUSAL WOMEN
***C Moniz**, R Chandra, TJ Ong, C Meredith, C Mugglestone (London, UK)*

11:50 **Break**

12:00-12:50 **Cruciform Lecture Theatre 1**

BRS - Cancer and bone

Chairs: Tim Chambers (London, UK) & Tom Clemens (Alabama, USA)

12.00 IS17 CANCERS IN BONE: AN OVERVIEW
***Nick Athanasou** (Oxford, UK)*

12.25 IS18 INTERACTIONS BETWEEN CANCER CELLS AND BONE CELLS: UNRESOLVED ISSUES
***Peter Croucher** (Sheffield, UK)*

11:20 P022 CLONAL MESENCHYMAL STEM CELL POPULATIONS DERIVED FROM THE SYNOVIAL FAT PAD EXHIBIT A SIMILAR CELL SURFACE CHARACTERISATION PROFILE BUT VARIABLE OSTEOGENIC AND CHONDROGENIC DIFFERENTIATION POTENTIAL
***WS Khan**, SR Tew, A Adesida, JG Andrew, TE Hardingham (Manchester, UK)*

11:30 P060 MECHANICAL STRAIN MODULATION OF MATRIX METALLOPROTEINASES IN HUMAN TENOCYTES
***ER Jones**, GC Jones, GP Riley (Norwich, UK)*

11:40 P065 MECHANICAL LOADING OF MURINE KNEE JOINTS: DETERMINING THE INTERPLAY BETWEEN GENETICS AND MECHANICAL LOADING IN THE DEVELOPMENT OF OA
***B Poulet**, AA Pitsillides (London, UK)*

11:50 **Break**

12:00-13:10 **Cruciform Lecture Theatre 2**

BSMB - Matricellular proteins in health and disease

Chairs: David Abraham (London, UK) & Hideaki Nagase (London, UK)

12.00 IS19 THE ROLES OF CONNECTIVE TISSUE GROWTH FACTOR AND CYR61 IN SKELETAL DEVELOPMENT
***Karen Lyons** (Los Angeles, USA)*

12.25 IS20 ROLES OF MATRILINS AND COMP IN CARTILAGE MATRIX ASSEMBLY AND IN THE PATHOGENESIS OF CHONDRODYSPLASIAS
***Mats Paulsson** (Cologne, Germany)*

12:50 BSMB YOUNG INVESTIGATOR AWARD

12:50-14:20 **Wilkins South Cloisters and Jeremy Bentham Room**

LUNCH & COMMERCIAL EXHIBITION

13:15-14:00 **Wilkins South Cloisters and Jeremy Bentham Room**

ATTENDED POSTERS – EVEN NUMBERS

PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS PARALLEL SESSIONS

13:45 **Cruciform Lecture Theatre 1**

BRS AGM

13:45 **Cruciform Lecture Theatre 2**

BSMB AGM

14 :10 **Cruciform Lecture Theatre 1**

Musculoskeletal research opportunities

Mark Pitman (MRC)

14:20-15:20 **Cruciform Lecture Theatre 1**

Joint Oral Posters

Chairs: Ray Boot-Handford (Manchester, UK) & Nigel Loveridge (Cambridge, UK)

14:20 P098 THE ELASTIC NETWORK IN HUMAN ARTICULAR CARTILAGE: AN IMMUNOHISTOCHEMICAL STUDY OF ELASTIN FIBRES AND MICROFIBRILS
J Yu, S Roberts, EH Evans, JP Urban (Oxford, UK)

14:25 P071 ENOS NULL OSTEOBLASTS PRODUCE NITRIC OXIDE IN RESPONSE TO FLUID FLOW BUT DO NOT TRANSLOCATE β-CATENIN
C Huesa, RM Aspden, MH Helfrich (Aberdeen, UK)

14:30 P076 ANTI-ANGIOGENIC PROPERTIES OF PROTEOGLYCAN IN CARTILAGE EXPLANT CULTURES
JJ Bara, S Roberts, WEB Johnson (Oswestry, UK)

14:35 P130 INCREASED FAT MASS AND REDUCED SERUM OSTEOCALCIN IN INDIVIDUALS WITH HIGH BONE MASS: POSSIBLE CROSS-TALK BETWEEN FAT AND BONE METABOLISM
CL Gregson, S Steel, WD Fraser, JH Tobias (Bristol, UK)

14:40 P023 EVALUATION OF BONE MARROW MESENCHYMAL STEM CELL THERAPY FOR TENDON REGENERATION IN A LARGE ANIMAL MODEL
NJ Young, J Dudhia, AE Goodship, RK Smith (North Mymms, UK)

14:45 P053 CALCIUM-INDUCED SECRETION OF DKK-1 IN OSTEOBLASTIC CELLS
HS McCarthy, JH Williams, MWJ Davie, MJ Marshall (Gobowen, UK)

14:50 P029 THE PPARα AGONIST ROSIGLITAZONE REVERSES THE PERSISTENT FIBROTIC PHENOTYPE OF SCLERODERMA FIBROBLASTS
X Shi-wen, M Eastwood, CP Denton, A Leask, DJ Abraham (London, UK)

14:55 P008 THE INHIBITORY ROLE OF SUPPRESSOR OF CYTOKINE SIGNALLING-2 ON STAT SIGNALLING IN THE GROWTH PLATE
C Pass, VE MacRae, SF Ahmed, C Farquharson (Edinburgh, UK)

15:00 P061 LOVASTATIN UPREGULATES CHONDROCYTE MARKER EXPRESSION IN TENOCYTES
HR Cornell, AJ Carr, PA Hulley (Oxford, UK)

15:05 P055 MILD HYPOTHERMIA PROMOTES OSTEOCLASTOGENESIS WHILST RETARDING OSTEOBLAST DIFFERENTIATION AND BONE FORMATION
JJ Patel, IR Orriss, ML Key, SEB Taylor, K Karnik, TR Arnett (London, UK)

15:10 P079 IN SITU ULTRASTRUCTURAL IMAGING OF NATIVE EXTRACELLULAR MATRIX MACRO-MOLECULES
HK Graham, CEM Griffiths, NW Hodson, JA Hoyland, J Millward-Sadler, AW Trafford, REB Watson, MJ Sherratt (Manchester, UK)

15:15 P041 A ROLE FOR TRPV1 AND K+ CHANNELS IN THE REGULATION OF OSTEOBLAST TRANSDIFFERENTIATION TO ADIPOCYTES
NC Henney, A Thomas, BAJ Evans, KT Wann (Cardiff, UK)

15:20-15:45 **Wilkins South Cloisters and Jeremy Bentham Room**

TEA

15:45-17:00 **Cruciform Lecture Theatre 1**

Joint Symposium: Dynamic relationships with the extracellular matrix (ECM): lessons from dysplasias

Chairs: Stefan Mundlos (Berlin, Germany) & Bjorn Olsen (Boston, USA)

15:45 IS21 INTRINSIC MATRIX PROPERTIES DIRECT STEM CELL FATE: INSIGHTS INTO ANOMALOUS BONE FORMATION
Adam Engler (San Diego, USA)

16:10 IS22 FIBROUS DYSPLASIA - MODELS OF DISEASE, MODELS OF THERAPY
Paolo Bianco (Rome, Italy)

16:35 IS23 ER (ENDOPLASMIC RETICULUM) STRESS AS A PATHOGENIC FACTOR IN CHONDRODYSPLASIA
Ray Boot-Handford (Manchester, UK)

17:00-17:15 **Cruciform Lecture Theatre 1**

Awards

Chairs: Bruce Caterson (Cardiff, UK) & Jon Tobias (Bristol, UK)

17:15

Close

ABSTRACTS

The following abstracts are published exactly as received from the submitting authors. The opinions and views expressed are those of the authors and have not been verified by the Meeting Organisers, who accept no responsibility for the statements made or the accuracy of the data presented.

IS01

Using µCT to study bone architecture

R van't Hof

Rheumatic Diseases Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, UK

The relatively strong absorption of X-rays by mineralised tissue has been used for bone research for a long time, and DEXA is the standard for bone density assessment in clinical practice. However, standard X-ray and DEXA do not provide three-dimensional (3D) information on the architecture of bone samples, and do not provide sufficient resolution to image individual trabeculae. Micro-CT was developed specifically for high resolution 3D imaging. The technique is based on taking a series of X-ray images of a sample at different rotations, and then uses a computer algorithm to reconstruct a 3D image. The current generation of µCT scanners has easily sufficient resolution to image the trabecular and cortical structure of bone, even of small mouse bones. Compared to histomorphometry, µCT is a very rapid technique (minutes vs weeks), and in our laboratory µCT is the technique of choice for the initial phenotyping of transgenic mice. In contrast to histomorphometry, µCT is a non-destructive technique, and specialised instruments for µCT imaging of live animals are now available, allowing us to follow bone development in experimental mice and rats. In this presentation I will explain the principles of µCT imaging and show examples of its use in analysing bone architecture in several different mouse models. This will include the ovariectomised mouse model of osteoporosis and the collagen induced arthritis model of rheumatoid arthritis. Furthermore, there will be examples of bone phenotyping of transgenic mouse strains, including a model for Paget's disease of bone, using both in vivo and ex vivo µCT imaging.

IS02

Abstract not received

IS03

Towards understanding of joint health and disease by contrast-enhanced MRI of cartilage

L Dahlberg

Department of Orthopaedics, Joint and Soft Tissue Unit, Department of Clinical Sciences, Malmö University Hospital, Lund University, Sweden

Joint cartilage, organized as a composite tissue, dissipates load and enables almost frictionless movements. Its reinforced fibril-network consists primarily of type II collagen filled with aggrecan. Aggrecan consists of a core protein to which negatively charged glycosaminoglycans (GAG) are attached. Due to the high fixed charged density of the GAG, water is drawn into the tissue generating a swelling pressure counteracted by the tensile properties of the collagen network. The chondrocyte maintains matrix integrity by replacing non-functional molecules. Morphologically intact cartilage may have different molecular content (quality) and constitute different mechanical properties.

dGEMRIC, (delayed Gadolinium Enhanced MRI of cartilage) is a specific magnetic resonance imaging method based on the principle that a negatively charged paramagnetic contrast agent (Gd-DTPA2-) distributes in the cartilage inversely to the negatively charged cartilage GAG. In conditions of low GAG content a higher concentration Gd-DTPA2- will be distributed within the cartilage changing the cartilage T1 relaxation time (T1Gd). T1Gd quantification (dGEMRIC), as an estimate of the cartilage aggrecan content, monitors cartilage quality and mechanical properties.

Recent dGEMRIC cross-sectional examination of healthy volunteers and an RCT in middle-aged meniscectomized subjects suggest a relationship between exercise level and cartilage T1Gd (GAG content). These studies provide evidence that articular cartilage adapts by increasing knee cartilage GAG to improve mechanical properties.

To understand joint health and pathogenesis in joint disease, molecular correlates to extrinsic factors should be identified. Age seems unrelated to T1Gd signal. dGEMRIC suggests a relationship between cartilage GAG content and weight (low T1Gd with increased weight) and muscle strength (low T1Gd with weak muscle). Two years after an anterior cruciate ligament (ACL) injury, dGEMRIC shows that the cartilage in the medial compartment, where most ACL injured subjects are at risk to develop OA, has still not recovered the GAG loss that occurred at the injury. However, even more intriguing is the fact that the cartilage GAG content was lower in meniscectomized compartments suggesting similar molecular changes in OA risk factors meniscus injury and overweight.

IS04

Earth (Water), Wind (Oxygen) and Fire (Energy): a recipe for cell survival in a hostile environment

IM Shapiro, V Srinivas, M Risbud

Thomas Jefferson University, Philadelphia USA

In most connective tissues, cells survive in microenvironments in which there are limitations in oxygen and nutrient supply and an elevation in osmotic pressure. We advance the concept that these cells sense the physical environment and generate signals that auto-regulate their own function. The tissue oxemic state is regulated by the transcription factor HIF-1α. We now report that HIF-1 regulates a new maturation stage, autophagy. Cells in the growth plate, cartilage and bone exhibit particulate expression of the autophagic proteins Beclin-1 and LC3. Aside from reliance on HIF-1, we found that induction of autophagy was dependent on the expression of mTOR, a nutrient sensor and AMPK a regulator of energy status. We found that an increase in AMPK, caused inhibition of mTOR; when TOR activity is blocked, autophagy is induced. Noteworthy, when autophagy is suppressed, there was a marked decrease in apoptogen sensitivity. Hence, a low energy status activates AMPK which by promoting autophagy, enhances survival in the hostile cartilage microenvironment. To explore the impact of osmolarity on cell function, we focusing on the aggrecan-rich tissue, the nucleus pulposus. Here, aggrecan-bound water permits the disc to resist compression. We found that the osmo-regulatory protein, TonEBP and its downstream target genes are robustly expressed in the nucleus pulposus. Above 450 mosmol/kg, nucleus pulposus cells up-regulate target genes TauT, BGT-1, and SMIT and HSP-70. In hypertonic media there is activation of TauT and HSP-70 reporter activity and increased binding of TonEBP to the TonE motif. Gene analysis indicates that the aggrecan promoter contains two conserved TonE motifs. To evaluate their importance, we overexpressed DN-TonEBP and partially silenced TonEBP. Both approaches resulted in suppression of aggrecan

promoter activity. Based on these findings, it is concluded that TonEBP permits the disc cells to adapt to the hyperosmotic milieu while autoregulating the expression of molecules that generate the unique extracellular environment. These studies lend strength to the notion that in tissue-specific niches, cells express genes that regulate their energy state (fire), ionic milieu (water) and accommodate available levels of gases (wind).

IS05

Osteocytes: birth, life and death in a mineralised tissue

N Loveridge

Bone Research Group, University of Cambridge, UK

Osteocytes are the most abundant cell type in bone and it is generally well accepted that they are the predominant cell type which transduces the response to load[1,2]. However, little is known about how they are formed from mature osteoblasts (osteocytogenesis:[3,4]) or how they survive within a hostile environment[5,6]. While their apoptotic death is considered to be an important cue for the targeted bone resoprtion[7] associated with oestrogen deficiency[8] and alterations in mechanical loading[9], it has become increasingly apparent that through the expression of sclerostin[10] and its effect on the Wnt pathway[11] osteocytes may control bone formation as well[12].

Given their apparent importance in the regulation of bone turnover it is not surprising that the possible role of osteocytes in the aetiology of musculoskeletal disease has received much recent attention[13]. Increased rates of osteocytogenesis through modulating of the TGFβ signalling pathway results on an osteoporotic phenotype[14]. In femoral neck fracture (FNF) osteocytic expression of eNOS and nNOS is reduced[15,16], while the location of NOS positive osteocytes suggests a major role in limiting bone resorption within existing BMUs, possibly through the local production of other factors. Hip OA is associated with increased bone formation[17] and a reduced expression of sclerostin, although this may not be causal.

REFERENCES

[1]Skerry et al 1989, J Bone Miner Res 4:783-8; [2]Pitsillides et al 1995, Faseb J 9: 1614-22; [3]Bonewald 2007, Ann NY Acad Sci 1116:281-90; [4]Prideaux et al 2009, BRS/BSMB meeting OC1; [5]Gross et al 2001, J Appl Physiol 90:2514-9; [6]Gross et al 2005, J Bone Miner Res 20:250-6; [7]Noble & Reeve 2000, Mol Cell Endocrinol 159:7-13; [8]Tomkinson et al 1998, J Bone Miner Res, 3: 1243-50; [9]Verborgt et al 2000, J Bone Miner Res 15:60-7; [10]Poole et al 2005, Faseb J 19:1842-4; [11]ten Dijke et al 2008, J Bone Joint Surg Am, 90 Suppl 1:31-5; [12]Martin RB 2000, Bone, 26:1-6; [13]Power et al 2002, Bone, 30:859-65; [14]Borton et al 2001, J Bone Miner Res, 16:1754-64; [15]Caballero-Alias et al 2004, Calcif Tissue Int, 75:78-84; [16]Caballero-Alias et al, 2005. J Bone Miner Res, 20:268-73; [17]Jordan et al 2003, Osteoporos Int, 14: 160-5.

IS06

Micro RNAs and the skeleton

TE Swingler[1], F Nicolas[1], S Soond[1], A Chantry[1], T Dalmay[1], DA Young[2], IM Clark[1]

[1]School of Biological Sciences, University of East Anglia, Norwich, UK; [2]Musculoskeletal Research Group, Newcastle University, UK

Small non-coding RNAs (19-24 nt long) known as microRNAs (miRNAs) have recently been recognised as important regulators of gene expression in mammalian cells. miRNAs are first transcribed as primary transcripts (pri-miRNA) with a cap and poly-A tail and processed to short, 70-nucleotide stem-loop structures(pre-miRNA) in the nucleus. The pre-miRNA is then processed by the ribonuclease, Dicer, forming two complementary short RNA molecules one of which is integrated into the RNA-induced silencing complex (RISC). After integration into the active RISC complex, miRNAs base pair with their complementary mRNA molecules, usually in the 3'UTR. Depending on the level of sequence homology between miRNA and target mRNA, RISC either cleaves the target mRNA (if homology is high) or suppresses translation of the mRNA (if homology is lower).

The expression of miRNAs can be measured using conventional techniques such as northern blot, qRT-PCR or microarray, but with specific modifications or short-comings when applied to miRNAs. Similarly, miRNAs can be detected in tissue sections by in situ hybridization. A key challenge is the identification of miRNA targets. Computer algorithms exist to predict which miRNAs target a specific gene or which genes are targeted by a specific miRNA. Our incomplete understanding of the targeting process means that these algorithms are poor. A variety of experimental techniques must therefore be drawn upon either to identify the genes targeted by a miRNA or to validate predictions. These include reporter assays, western blots, microarrays, proteomics, pull down assays and transgenic mice.

Using both hybridization arrays and Taqman low density arrays, we have profiled the expression of microRNAs in human articular cartilage and in a cell model of chondrogenesis. Using these data we have identified key microRNAs involved in chondrocyte differentiation and potentially in osteoarthritis and localized these in the developing skeleton. We are identifying the molecular targets for these microRNAs in order to build testable hypotheses of their function.

IS07

Zebrafish as a tool for skeletal biology

HH Roehl

MRC Centre for Developmental and Biomedical Genetics, University of Sheffield, Sheffield, United Kingdom

In recent years, zebrafish has become a desirable model for many aspects of vertebrate development and human disease. Zebrafish are easy to maintain, generate thousands of offspring, develop ex utero and are optically clear. These attributes make zebrafish suitable for high through-put forward genetic and pharmaceutical approaches. In addition, genes can be knocked out and transgenic lines that label cells fluorescently or manipulate molecular pathways are easy to establish and maintain. These technologies allow for rapid analysis of gene function in vivo.

In the area of zebrafish skeletal research, embryonic differentiation of chondrocytes and osteoblasts has been well characterized, however analysis of osteoclast development has been hampered due to their late appearance. Although much of the research has focused on the early development of the skull, several studies have looked the formation of the vertebrae and fin skeleton. The morphology the adult skeleton has been examined for genetic disease models of multiple osteochondromas and osteogenesis imperfecta. Analysis of bone repair is ongoing in several laboratories.

In this workshop, I will present a survey of current research in fish skeletal biology, focusing on zebrafish but also including work done with medaka. I will also outline several strengths and weaknesses of the fish model in comparison to other vertebrate models.

IS08

Ex-vivo and in-vivo gene delivery strategies using matrix pathology as a target

Y Chernajovsky, A Nissim, C Hughes, S Vessillier, G Adams, DJ Gould

Bone and Joint Research Unit, William Harvey Research Institute, Barts and The London School of Medicine, Charterhouse Square, London EC1M 6BQ

The extracellular matrix changes during pathological processes. In particular, we are in interested in inflammatory processes that occur in rheumatoid arthritis and osteoarthritis. In both conditions, there is production of free radicals either by incoming inflammatory cells or by the resident chondrocytes. These free radicals modify proteins, lipids (and DNA of cells) and these new 'chemical tags' can be used for targeting therapeutic molecules specifically to disease tissue.

Using phage display we have isolated human antibody fragments that recognise free radical-induced 'chemical tags' on collagen type II and have used this antibody binding specificity to target therapeutic molecules to arthritic joints.

Another hallmark in these diseases is the high level of matrix metalloproteinases (MMPs) that degrade the extracellular matrix. We have developed a latent cytokine technology (1) that enables the delivery of therapeutics to disease sites with high levels of MMP activity seen in autoimmune diseases, inflammation and cancer. Our latent cytokine technology confers longer half-life, improved pharmacokinetics and specific delivery to sites of disease.

As these diseases are chronic, long term administration of biologicals is necessary and genetic delivery has been shown to be a feasible and safe method that can also provide for local expression and gene expression that can be activated both through drug-mediated regulation (2) or disease-specific promoters and can be used systemically or locally. These genetic delivery can be achieved directly in vivo or indirectly ex vivo by growing autologous cells and engineering them to express the therapeutic proteins using viruses or plasmids and transplanting them back to the patient.

The combination of transcriptional control and targeted delivery provides for two layers of safety needed to treat long term non fatal inflammatory diseases and allows for withdrawal of treatment during remission phases.

REFERENCES

1. Adams, G., Vessillier, S., Dreja, H., and Chernajovsky Y. Targeting cytokines to sites of inflammation. Nature Biotechnology 21: 1314- 1320, 2003

2. Gould DJ, Yousaf N, Fatah R, Subang MC and Chernajovsky Y. Gene therapy with an improved doxycycline regulated plasmid encoding a TNF inhibitor in experimental arthritis. Arthritis Res Ther. 2007 Jan 25;9(1):R7 [Epub ahead of print]

IS09

Vascularisation and bone remodeling

MH Lafage-Proust

INSERM 890, Université de Lyon, Saint-Etienne, France

While the role of bone vascularisation is rather well known in bone development, growth or fracture repair, its role in physiological bone remodelling and metabolic bone diseases has been less investigated. Yet, relationships between bone marrow perfusion and bone mass were reported in both humans and animals. Bone is a composite tissue that supports various functions including locomotion, calcium/phosphate metabolism and haematopoiesis, all depending on bone remodelling, and bone vessels appear to be the common link between these functions. Indeed, bone marrow sinusoids together with osteoblasts and osteoclasts play a major role in the hematopoïetic stem cell niche. Further, osteoblasts and osteoclasts belong to specific microanatomy entities tightly associated with capillaries that bring, in addition to oxygen and nutrients, bone precursor cells to the bone remodelling sites. In turn, bone cells secrete many factors which target vessel cells and alter capillary number or functions such as VEGF. In this context, we hypothesised that challenging bone remodelling would alter bone blood vessel network and that manipulating bone vascularisation could consecutively modulate bone remodelling and bone mass. We developed, in the rat, reproductive quantitative measurements of vessels in bone using intravascular infusion of barium as contrast product. We showed that the bone gain and rise in osteoblastic formation induced by treadmill running was associated with bone angiogenesis which were both prevented by the administration of anti VEGF antibodies. Conversely, hind limb unloading decreased bone formation together with bone vessel number while VEGF receptor agonists partially prevented these changes. Thus, when bone was mechanically challenged bone formation and vessel number were tightly coupled. However, this was not true for other conditions. Actually, we found that three weeks of hypoxia dramatically impaired osteoblast function whereas bone angiogenesis was significantly stimulated. In addition, intermittent 1-84 PTH enhanced bone mass and formation while, unexpectedly, it reduced the number of vessels but spatially redistributed them closer to sites of new bone formation. In conclusion, the bone vessel network varies as bone metabolic demands change, however the functional relationships between bone cell activities and vessels are complex, presumably involving blood flow as an additional factor to take into account. Measuring both kinetics of bone blood perfusion adaptation and vascular micro-architectural modifications during bone remodelling should help to better understand how vessels influence bone metabolism and vice versa.

IS10

Targeting the HIF-1 pathway for bone repair

TL Clemens

Department of Pathology, University of Alabama, Birmingham, USA

Angiogenesis and osteogenesis are tightly coupled during bone development and regeneration. The vasculature supplies oxygen to developing and regenerating bone and also delivers critical signals to the stroma that stimulate mesenchymal cell specification to promote bone formation. Recent studies suggest that the hypoxia inducible factors are required for initiating the angiogenic-osteogenic cascade. Genetic manipulation of individual components of the HIF/VEGF pathway in mice has provided clues to how coupling is achieved. Here we review the current understanding of cellular and molecular mechanisms responsible for angiogenic-osteogenic coupling. We also describe the development of small molecules that mimic hypoxia and activate HIF. Such discoveries suggest a promising approach for the development of novel therapies to improve bone accretion and repair.

IS11

Molecular regulation of vascular calcification

A Canfield

Wellcome Trust Centre for Cell-Matrix Research, Faculty of Medical & Human Sciences, University of Manchester, UK

Vascular calcification is a common complication of many diseases including atherosclerosis, diabetes, and end-stage kidney disease. It appears as dispersed crystals of hydroxyapatite, large calcified deposits and mineralised tissue comprising both bone and cartilage. Within atheromatous lesions, calcification is irregularly distributed along the thickened intima and underlying media, leading to an increased risk of plaque rupture, thrombosis and subsequent myocardial infarction. Calcification of the media is a highly characteristic feature of arteries from patients with diabetes and end-stage kidney disease and leads to increased mortality and amputation risk.

It is now well established that vascular calcification is a complex process which involves the osteo/chondrogenic differentiation of vascular smooth muscle cells (VSMC) and pericytes present in the vessel wall, the release of apoptotic bodies and matrix vesicles which provide the nucleation sites for mineralisation, the loss of natural inhibitory molecules (e.g. MGP) and the expression of stimulatory molecules (e.g. BMP-2, cbfa1, sox-9) for osteo/chondrogenesis.

We have recently identified several key regulators of pericyte and VSMC differentiation and vascular calcification, including members of the Wnt, TGFβ and receptor tyrosine kinase (RTK) signalling pathways. We have also discovered that the RTK Axl and its ligand Gas6, and HtrA1 (a serine protease which degrades specific matrix proteins and which can also regulate TGFβ signalling) are novel inhibitors of this process. We also have new data showing that farnesyl transferase inhibitors prevent vascular calcification both in vitro and in vivo. We are currently using molecular, cellular and biochemical approaches to elucidate the mechanisms by which these regulatory factors regulates cell differentiation and vascular calcification and some of these studies will be presented at this meeting.

IS12

Advances in bone biology relevant to clinicians

J Tobias

Academic Rheumatology, Clinical Science at North Bristol, University of Bristol

Bone provides an endoskeleton, a mineral store for calcium homeostasis, and a site for haematopoiesis. Recent insights into how these functions are regulated have improved our understanding of the pathogenesis of conditions where these are compromised, and identified new opportunities for diagnosis and treatment. For example, in the case of calcium homeostasis, cloning of the calcium sensing receptor gene (CaSR) responsible for regulating PTH secretion led to the identification of calcimimetic agents such as cinacalcet, which ameliorate hyperparathyroidism through CaSR activation. Similarly, recent identification of the role of FGF23 and related proteins in regulating phosphate excretion has led to the use of FGF23 assays in the diagnosis of oncogenic osteomalacia, and PHEX gene mutational analysis in prenatal screening for sex-linked hypophosphataemic rickets. In terms of the regulation bone structure, the latter reflects the coordinated activity of osteoblasts and osteoclasts responsible for bone formation and resorption respectively. Several genetic mutations have been identified in essential pathways involved in regulating osteoblast and osteoclast function, which are responsible for rare familial disorders caused by excessive bone formation or resorption. For example, LRP5 and SOST gene mutations have been implicated in skeletal dysplasias associated with high bone mass and overgrowth of the skull base, which are thought to affect essential osteoblast regulatory pathways. Conversely, activating mutations of the TNFRSF11A gene, which encodes the receptor for RANKL (the main stimulator of osteoclast differentiation) have been implicated in rare familial forms of Paget's disease. These regulatory pathways have also provided targets for new therapies. For example, denosumab is a RANKL antibody which increases bone mass through its ability to suppress osteoclast activity, and is likely to be available as a new treatment for osteoporosis in the near future. Likewise, several new anabolic treatments for osteoporosis are in development which stimulate bone formation by targeting LRP5, SOST and related pathways.

IS13

Developmental mechanisms in the pathogenesis of osteoporosis

C Cooper[1,2], N Harvey[1], Z Cole[1], E Dennison[1].

[1]MRC Epidemiology Resource Centre, University of Southampton; [2]INstitute of Musculoskeletal Sciences, University of Oxford, UK.

Osteoporosis is a skeletal disease characterised by low bone mass and susceptibility to fracture. Preventive strategies against osteoporotic fracture can be targeted throughout the life course. Although there is evidence to suggest that peak bone mass is inherited, current genetic markers are able to explain only a small proportion of the variation in individual bone mass or fracture risk. Evidence has begun to accrue that fracture risk might be modified by environmental influences during intrauterine or early postnatal life: (1) Epidemiological studies which confirm that subjects who are born light and whose growth falters in the first year of postnatal life, have significantly lower bone size and mineral content, at age 60 to 75 years; (2) Cohort studies demonstrating that subsequent lower trajectories of childhood growth are associated with an increased risk of hip fracture among such men and women; (3) Detailed physiological studies of candidate endocrine systems which might be programmed have shown that birthweight and growth in infancy alter the functional settings of the GH/IGF-1, and vitamin d/PTH axes; (4) Studies characterising the nutrition, body build and lifestyle of pregnant women which relate these to the bone mass of their newborn offspring, have identified a number of important determinants of reduced fetal mineral accrual (maternal smoking, low maternal fat stores and maternal vitamin D deficiency, intense levels of weight-bearing physical activity in late pregnancy). Follow-up studies of randomised controlled trials of vitamin D supplementation in infancy suggest persisting benefits in adolescence and young adulthood. These data suggest that undernutrition and other adverse influences arising in fetal life or immediately after birth have a permanent effect on body structure, physiology and metabolism, which might independently influence the later risk of osteoporotic fracture.

IS14

Molecular mechanisms of skeletal morphogenesis

BR Olsen

Professor of Developmental Biology/Harvard School of Dental Medicine

Hersey Professor of Cell Biology/Harvard Medical School, USA

Insights into mechanisms that regulate skeletal development have come from different types of studies. In many cases, genetic studies of human single gene disorders have led to discoveries that paved the way for more detailed and mechanistic work in animals or cell culture. Experimental studies in mice have been extremely important as well. However, it is clear that elucidation of signaling pathways and transcription factors is not sufficient for understanding how the architecture of each and every bone in the skeleton is specified and how osteoblasts build bone along trajectories of mechanical stress. Such understanding requires investigations of morphogenetic mechanisms associated with "architectural" properties of cells and tissues; namely, studies of cytoskeletal functions, mechanisms of cellular polarity, and mechanical stress responses of cells and pericellular matrices. With the discovery that hedgehog signaling is coupled to processes regulating protein trafficking into and out of primary cilia, a major signaling pathway associated with skeletal development has been coupled to cellular polarity mechanisms. Also, recent studies indicate that the primary cilia-associated cation channel polycystin 1/2 is important for the proliferative response of osteochondroprogenitor cells to postnatal mechanical stress in craniofacial sutures and synchondroses at the skull base. This work represents the beginning of what may lead to deeper understanding of skeletal morphogenesis.

IS15

Bone development and dysplasias

S Mundlos

Max Planck Institute for Molecular Genetics and Institute for Medical Genetics, Charité, Berlin, Germany

Genetic disorders affecting the skeleton comprise a large group of clinically distinct and genetically heterogenous conditions. The clinical manifestation ranges from neonatal lethality to only mild growth retardation. Although they are individually rare, disorders of the skeleton are of clinical relevance because of their overall frequency. Traditionally, skeletal disorders have been subdivided into dysostoses, defined as malformations of individual bones or groups of bones and osteochondrodysplasias, defined as developmental disorders of chondro-osseos tissue. The genetic analysis of these conditions has greatly increased our knowledge about the molecular mechanisms of skeletal development, growth and homeostasis. Hox genes have long been regarded as master regulators of early patterning processes, as demonstrated by the homeotic transformation phenotypes in Drosophila such as antennapedia and bithorax. In mammals they play important roles in the development of the limb and the spine and mutations in several HOX genes are associated with human limb malformations. For example, poly-alanine expansions in HOXD13 result in digit duplications and fusions (synpolydactyly, SPD). We used the mouse mutant synpolydactly homologe (spdh) to investigate the molecular pathogenesis of SPD. Spdh/spdh mice show multiple extra digits with fusions. Chondrocytes within the anlagen do not differentiate and bne formation does not take place until P7. We identified retinaldehyde dehydrogenase-2 (Raldh2), the rate limiting enzyme for retinoic acid (RA) synthesis in the limb, as down regulated in spdh/spdh limbs. Raldh2 is co-expressed with and directly regulated by Hoxd13. RA levels are lower in the mutant and intrauterine treatment with RA restores pentadactyly in spdh/spdh mice. We show that RA and Hoxd13 suppress chondrogenesis in mesenchymal progenitor cells. In contrast, Hoxd13 with an expanded Ala repeat promotes cartilage formation which is associated with an upregulation of Sox6/9. Increased Sox9 expression and ectopic cartilage formation in the interdigital mesenchyme of spdh/spdh limbs suggest uncontrolled differentiation of these cells into the chondrocytic lineage. We propose that polydactyly in SPD is caused by increased interdigital chondrogenesis due to reduced RA levels and the prochondrogenic effect of mutated Hoxd13. Thus, Hox genes pattern the limb by controling cell differentiation and proliferation.

IS16

Osteoblast differentiation is regulated by multiple signalling pathways in the zebrafish embryo

Nan Li[1], Katharina Felber[1], Phil Elks[1,2], Peter Croucher[2], Henry H Roehl[1]

[1]MRC Centre for Developmental and Biomedical Genetics, University of Sheffield, Sheffield, United Kingdom; [2]Academic Unit of Bone Biology, Section of Musculoskeletal Science, University of Sheffield Medical School, Beech Hill Road, Sheffield, United Kingdom

Most of our understanding of bone formation is derived from analysis based upon the mesodermally derived endochondral bones found in the limbs of tetrapods. Considerably less is known about the development of the skull which originates from both the mesoderm and neural crest and forms via both dermal and endochondral ossification routes. Our aim is to determine the factors that specify osteoblast differentiation from neural crest precursors in the context of zebrafish cranial bones.

Molecular and genetic analysis performed in mice has identified two transcription factors Runx2 and Osterix that act sequentially to direct mammalian osteoblast differentiation. Runx2 is required during the early stages of commitment and acts in part to activate Osx transcription. Osterix and Runx2 then act to direct transcription of bone matrix proteins. We have investigated the expression of these genes during zebrafish cranial osteoblastogenesis by using whole-mount in situ hybridization. We find that during the formation of a given bone, the mammalian expression sequence is conserved.

To investigate the role that signaling pathways play during osteoblastogenesis we have used a heat shock (hs) promoter to drive misexpression of ligands or inhibitors for a given pathway. In addition we have used chemical intervention to precisely define the timing of activity for each pathway. We find that BMP and RA signalling act at the earliest stages of commitment by influencing runx2 expression, FGF, BMP and WNT signalling act on osterix expression and BMP, RA and FGF pathways all act on mature osteoblasts to regulate bone deposition. Our data provide an in vivo model to address how osteoblast recruitment and maturation is coordinated with other processes such as skeletal morphogenesis as well as the development of other neural crest derived structures.

IS17

Cancers in bone: an overview

NA Athanasou

Department of Pathology, Nuffield Department of Orthopaedic Surgery, University of Oxford OX37LD

Cancers in bone may be either primary or secondary in origin. Bone is the third most common site of metastatic cancer and metastatic carcinoma is by far the most common malignant tumour of bone. Bone is a well-vascularised tissue and tumour cells reach the bone via the bloodstream; there are no lymphatics in bone. Increased bone isotope uptake suggestive of skeletal metastasis has been noted in up to 85% of cancer patients. Although primary malignant bone tumours are rare, many different types have been described; the histogenesis of many primary bone tumours is not known and a number of bone tumours are by convention characterised by their distinct clinico-pathological features or by karyotypic and molecular genetic abnormalities. Bone sarcomas spread locally, causing extensive bone destruction, and produce distant metastases, most commonly via the bloodstream. The approach to the diagnosis of malignant bone tumours needs to take into account the clinical and radiological features of the lesion, the results of laboratory investigation and histology of the tumour. This protocol demands close cooperation between the clinician, radiologist and pathologist, all of whom should be experienced in the assessment of these uncommon tumours.

Both primary and metastatic tumours cause considerable bone destruction. The extensive lacunar resorption required for the establishment of tumour metastasis in bone or growth of a primary sarcoma is carried out by osteoclasts; tumour cells may facilitate this process by contributing to bone matrix degradation. Tumour-associated macrophages within carcinomas and sarcomas are known to be capable of osteoclast differentiation. Carcinoma and sarcoma cells secrete a number of humoral factors which stimulate macrophage-osteoclast differentiation and osteoclast resorptive activity, including prostaglandins, growth factors (eg M-CSF, GM-CSF) and cytokines (eg IL-6, TNFα and TGFβ). Some tumour cells also secrete factors that promote osteoblastic activity/bone formation and angiogenesis (eg HIF, VEGF). The treatment of primary and metastatic tumours of bone involve surgical removal and/or adjuvant chemotherapy/radiotherapy to destroy tumour cells, but inhibition of tumour osteolysis by targeting osteoclast resorption/formation is also useful in controlling tumour growth and preventing complications such as pathological fracture.

IS18

Interactions between cancer cells and bone cells: unresolved issues

PI Croucher

Department of Human Metabolism, University of Sheffield, Sheffield, United Kingdom

Tumour cells either develop directly in the skeleton, such as in multiple myeloma, or metastasise to bone, for example in breast and prostate cancer. Once in the skeleton, tumour cells hijack normal bone remodelling and cause the development of osteolytic and/or osteosclerotic disease. There is also evidence for an interdependence between tumour cells and bone, with cancer cells producing molecules that stimulate changes in bone and the changes in bone providing a supportive microenvironment for the growth and survival of cancer cells. This is the so-called 'vicious cycle'. Despite significant progress in identify some of the molecular mechanisms responsible for these interactions many issues remain unresolved. For example, the spacial and temporal development of tumour/bone interactions is poorly understood. Tumours cells are likely localise to a specific microenvironment in bone, a 'bone metastasis niche' yet the nature of this 'niche' is unclear. The tumour cells are able to interact with osteoclasts to promote bone resorption and bone disease, yet the role of these cells in supporting tumour cell survival remains to be defined. It is also understood that increased osteoblastic activity is important in the development of osteosclerotic disease and suppression of osteoblastic bone formation has been shown to be a key feature of the development of osteolytic bone disease. However, the molecular mechanisms responsible are only now being defined. Wnt signalling antagonists, such as dickkopf-1 (dkk1) may be critical mediators. Certainly targeting dkk1 and blocking downstream signalling prevents the development of osteolytic disease. In some cases this is independent of effects on bone resorption. This may suggest that osteoblasts are critical in determining the nature of tumour-induced bone disease. Studies in experimental models have also demonstrated that inhibiting bone resorption and/or stimulating bone formation is associated with a reduction in tumour burden in the skeleton. In some cases this has been attributed to direct anti-tumour effects although it is likely that this is mediated indirectly by changes in the bone microenvironment. Whether this reflects effects on tumour cell colonisation of bone, their survival in bone or their growth is unclear.

Understanding some of these issues, particularly the nature of the 'bone metastasis niche', the temporal development of tumours in the skeleton and the role of osteoblasts, is likely to be critical if we are to prevent tumour growth in the skeleton and treat the tumour-induced bone disease effectively.

IS19

The roles of connective tissue growth factor and Cyr61 in skeletal development

F Hall-Glenn[1,2], B-L Huang[1,3], K Lyons[1,3]

[1]UCLA/Orthopaedic Hospital Department of Orthopaedic Surgery, Los Angeles, USA; [2]UCLA Department of Molecular, Cell, and Developmental Biology, Los Angeles, USA; [3]Department of Oral Biology, UCLA School of Dentistry, Los Angeles, USA

Connective Tissue Growth Factor (CTGF) and Cyr61 are matricellular proteins that exert their functions by binding to and activating integrins. They also bind to many growth factors, including Bone Morphogenetic Proteins (BMPs), Transforming Growth Factor b (TGFb), and Platelet-derived growth factor (PDGF). CTGF has also been shown to interact with and modify the activity of matrix metalloproteinases (MMPs). We have shown previously that CTGF is required for multiple aspects of chondrogenesis. These studies revealed that CTGF regulates the production of cartilage ECM via integrin-mediated signaling. We also showed that CTGF is required for growth plate angiogenesis, via upregulation of VEGF expression in hypertrophic chondrocytes.

We now show that the expression of CTGF in hypertrophic chondrocytes is activated by Wnt signaling., and that CTGF expression in proliferating chondrocytes is repressed by Sox9.

Using transgenic mice and chromatin immunoprecipitation studies, we have identified a region in the CTGF promoter that regulates stage-specific expression in hypertrophic chondrocytes. These studies have identified a single element that is responsive to Sox9, b-catenin, and Runx2 that is sufficient for stage-specific gene expression. Moreover, these studies have revealed a previously unrecognized role for Runx2 as a repressor of chondrocyte-specific gene expression in proliferating chondrocytes.

Whether or not CTGF and Cyr61 have overlapping functions is unknown. Using BAC transgenic mice, we have compared the patterns of CTGF and Cyr61 expression in developing cartilage. These studies reveal largely overlapping expression. Using conditional mutants, we now show that CTGF and Cyr61 have both unique and overlapping functions in chondrogenesis and osteogenesis. As shown previously CTGF is required for proliferation and clearance of hypertrophic chondrocytes. Cyr61 is essential for proper joint formation. However, CTGF/Cyr61 double mutants exhibit profound chondrodyslasia, due to overlapping effects on chondrocyte proliferation.

Finally, we are investigating the role of CTGF in assembly of basement membranes and the pericellular matrix of blood vessels and chondorcytes, respectively. These studies reveal that CTGF plays a role intracellularly in the export of ECM components, and their final assembly into an organized ECM.

IS20

Roles of matrilins and COMP in cartilage matrix assembly and in the pathogenesis of chondrodysplasias

M Paulsson[1,2,3], K Blumbach[1], G Groma[1], C Otten[1], A-K Becker[1], C Neascu[1], R Wagener[1], F Zaucke[1,2]

[1]Center for Biochemistry, Medical Faculty; [2]Center for Molecular Medicine; [3]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Germany

Matrilins and COMP are non-collagenous proteins in the cartilage extracellular matrix and have been implicated as mediators of interactions between cartilage collagen fibrils and other matrix components. While the matrilins form a unique four-member protein family, COMP is a thrombospondin and is also referred to as TSP5. In biochemical studies strong interactions have been demonstrated between matrilins and COMP and between these proteins and collagen IX, which is present at the surface of the cartilage collagen fibril. It was therefore surprising that mice in which COMP or each of the matrilins have been ablated do not show any pronounced skeletal phenotype, a fact that is probably due to a functional redundancy between the proteins found at the collagen fibril surface. To analyse the function of these peri-fibrillar proteins as a group, we have created mice that lack expression of more than one of the genes and also turned to genetic analysis in the zebrafish system. In both kinds of experiments distinct skeletal changes are seen that we now analyse to determine the biological importance of this interacting set of proteins at the collagen fibril surface.

Patient mutations in matrilin-3, COMP and collagen IX have been shown to cause several forms of chondrodysplasia and, in case of matrilin-3, also to predispose for osteoarthritis. The common view has been that such mutations cause a protein misfolding, leading to intracellular protein accumulation, an ER stress response and chondrocyte death. We have analysed a set of COMP and matrilin-3 mutations by biochemical, cell biological and genetic means and found a considerable heterogeneity in the molecular and cellular mechanisms by which they cause disease. While some mutations indeed induce intracellular retention and ER stress, proteins carrying other mutations are readily secreted, but still cause disease. Mechanistic investigation of the mutations that have extracellular effects show a dominant negative influence on collagen fibril formation, a process that is not strongly affected by the lack of the same protein.

IS21

Intrinsic matrix properties direct stem cell fate: insights into anomalous bone formation

A Engler[1], D Discher[2]

[1]Univ of California, San Diego, La Jolla, CA USA; [2]Univ of Pennsylvania; Philadelphia, PA USA

Microenvironments appear important in stem cell lineage specification but can be difficult to adequately characterize or control with soft tissues.

Naive mesenchymal stem cells (MSCs) are shown here to specify lineage and commit to phenotypes with extreme sensitivity to tissue-level elasticity.

Soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic. Abnormally stiff environments block or alter normal differentiation: naïve MSCs injected into an ischemic muscle scar tissue downstream of a blocked artery in a heart attack do not become muscle but rather express bone-like properties. Additionally, extracellular matrix (ECM) composition, e.g. collagen vs. fibronectin, as well as its structure, e.g. ligand coating vs. fibrillar matrix, appears to govern lineage specification in embryonic stem cells into endoderm-like cells, arguing for the broad application of intrinsic matrix properties as differentiation regulators for stem cells.

IS22

Fibrous dysplasia - models of disease, models of therapy

P Bianco

Sapienza Universita'di Roma, Italy

Almost two decades after the identification of the causative mutations, Fibrous Dysplasia (OMIM#174800) remains a major challenge. There is no cure for the severe, at times even lethal, forms of the disease, and there is actually no rational treatment beyond palliation. In addition, unexpected levels of complexity in the relationship between genotype and phenotype in FD underlie the partial-at-best understanding of the pathogenesis of the disease and its skeletal expressions. Clinical and pathological studies have been significantly advancing our understanding of the disease over the past 10 years. This applies to mechanisms of disease (e.g. the role of FGF-23 in FD-associated phosphate wasting syndromes), and in a more limited, somewhat negative way, to treatment (the limited efficacy of widely used drugs in reverting bone lesions, but also the apparent ability of certain lesions to self-sterilize over time). In order to gain further insight into disease mechanisms, and to provide the sorely needed animal models of the disease, we set out to generate transgenic models of the disease in which the

causative mutation is either targeted to osteoblasts or ubiquitously expressed. Analysis of these models, complemented by studies on human stem cells in which the same transgene is expressed from the same vectors as in the transgenic mice, is now revealing novel and potentially important aspects of the disease. These include for example the demonstration of specific effects of the targeted vs ubiquitous transgenes, reflected in specific tissue changes of direct clinical bearing, or b) the elucidation of a remarkable adaptive response whereby cells cope with the effects of the mutation. This response and its failure over time, may contribute to explain the de novo appearance of focal FD lesions in the postnatal life, both in humans, and in transgenic models (in which somatic mosaicism is excluded), and perhaps provide a clue as to mechanisms whereby nature can keep the effects of mutation at bay. Meanwhile, experimental approaches to gene therapy in human stem cells have been developed, showing the feasibility of specific silencing of the mutated allele. Further developments of these studies will hopefully tell which of the effects of the mutation beyond excess production of cAMP can be reverted, and availability of animal models will allow to determine, in preclinical models, the potential relevance of this approach for therapy.

IS23

ER stress as a pathogenic factor in chondrodysplasia

R Boot-Handford[1], H Rajpar[1], B McDermott[1], L Kung, J Bateman[2], R Wilson[2], P Arvan[3], K Kadler[1], D Thornton[1], M Briggs[1]

[1]Wellcome Trust Centre for Cell-Matrix Research, Fac. Life Sciences, University of Manchester, UK; [2]Murdoch Childrens Research Inst., Royal Children's Hospital, Melbourne, Aus; [3]Div. Metabolism, Endocrinology & Diabetes, University of Michigan Medical Center, Ann Arbor, USA

Eukaryotic cells have a sophisticated machinery located within the endoplasmic reticulum (ER) to promote protein folding and deal with the consequences of protein misfolding. Protein folding homeostasis is maintained within the ER by a series of ER stress sensors which, if activated due to rises in the concentrations of misfolded protein, can down-regulate general protein synthesis, stimulate the synthesis of chaperones to enhance the folding capacity of the ER, and activate proteasomal and autophagic routes for disposing of unfolded protein. The set of responses triggered by accumulating misfolded protein is known as the unfolded protein response (or UPR). If protein folding homeostasis cannot be restored, affected cells can apoptose. 'Professional secretory cells' such as hepatocytes and insulin-producing cells in the pancreas are particularly susceptible to increases in ER stress due to the large quantities of protein passing through their ER. Studies by others that I will briefly review and studies from my own lab which I shall describe in greater detail indicate that connective tissue cells such as chondrocytes and fibroblasts are susceptible to increases in ER stress and often exhibit a UPR when expressing mutant forms of ECM genes[1]. I shall present evidence that the essential pathological features of metaphyseal chondrodysplasia type Schmid (MCDS), a dwarfism caused by mutations in type X collagen, can be phenocopied by targeting ER stress to the hypertrophic chondrocyte. These studies demonstrate the central role played by ER stress in the pathological mechanism linking genotype and phenotype in MCDS. Furthermore, these studies indicate that for connective tissue diseases caused by mutations in extracellular matrix genes, the consequences of ER stress in addition to a deficient or defective extracellular matrix should be considered when examining disease mechanisms and potential therapies.

REFERENCES

1. Bateman JF, Boot-Handford RP, Lamande S (2009) Genetic diseases of connective tissues: cellular and extracellular effects of ECM mutations. Nat Rev Genet. 10(3):173-83.

OC01

Matrix mineralisation: A driver for osteocytogenesis?

M Prideaux[1], *AA Pitsillides*[2], *C Farquharson*[1]

[1]The Roslin Institute, R(D)SVS University of Edinburgh, Edinburgh, EH25 9PS, United Kingdom; [2]Royal Veterinary College, London, NW1 0TU, United Kingdom

Introduction

The regulation of osteocytogenesis is poorly understood and the genetic and environmental factors responsible remain undefined. Previous studies have suggested that mineralisation of the extracellular matrix (ECM) is important in osteocyte maturation (Irie et al. 2008) but whether it coordinates osteocytogenesis has not been confirmed.

Materials and Methods

We have utilised the MLO-A5 cell line and western blotting and quantitative RT-PCR to examine whether the expression of osteocyte-selective markers is elevated concurrently with the onset of ECM mineralisation. Secondly, if mineralisation of the ECM is indeed a driver of osteocytogenesis, we reasoned that impairment of matrix mineralisation would result in a reversible inhibition of osteocytogenesis.

Results

Supplementation of MLO-A5 cell cultures with ascorbic acid and phosphate promoted progressive ECM mineralisation as well as temporally associated increases in expression of the osteocyte-selective markers, E11 and sclerostin. Consistent with a primary role for mineralisation in osteocytogenesis, we also found that inhibition of mineralisation, by omitting phosphate or adding sodium pyrophosphate (PPi), resulted in a 15-fold ($P<0.001$) decrease in mineral deposition that was closely accompanied by lower E11 protein expression throughout the culture period. Indeed, the most marked restriction of ECM mineralisation in response to treatment with PPi or omission of phosphate also produced a 6-fold ($P<0.001$) and 3-fold ($P<0.001$) reduction in E11 protein expression, respectively. Expression of the osteocyte markers DMP1, CD44 and SOST were also reduced in the mineralisation-inhibited cultures, whilst the deposition of collagen, an indicator of ECM synthesis, was unaffected.

A restriction upon matrix mineralisation may irreversibly modify osteoblast behaviour to limit osteocytogenesis. We therefore measured the capacity of MLO-A5 cells to re-enter the osteocyte differentiation programme, by first restricting ECM mineralisation, for 6 days, followed by promoting mineralisation for a further 9 days. We found that re-initiation of the mineralisation process rescued the blockade on mineralisation evident at 6 days ($P<0.001$), and this recovery in ECM mineralisation was closely allied with increased E11 protein expression, indicating a mineralisation-induced restoration in osteocytogenesis.

Discussion

These results emphasise the importance of cell-matrix interactions in regulating osteoblast behaviour and importantly suggest that ECM mineralisation exerts pivotal control during terminal osteoblast differentiation and acquisition of the osteocyte phenotype.

OC02

Two populations of endochondral osteoblasts with differential sensitivity to Hedgehog signalling

CL Hammond[1], *S Schulte-Merker*[1]

[1]Hubrecht Institute of Developmental Biology and Stem Cell Research and the University Medical Center Utrecht, Uppsalalaan 8, Utrecht, 3584CT, The Netherlands

Introduction

Hedgehog signalling, regulated by the membrane receptor Patched, has been implicated in the development of both osteoblasts (bone matrix-secreting cells) and osteoclasts (bone resorbing cells), the balanced activities of which being critical for proper bone formation. Yet, due to early embryonic lethality of many mouse null mutants in the Hedgehog pathway, a number of questions on the exact effects of Hedgehog signalling on osteogenesis remain.

Materials and Methods

Using the zebrafish as a model for bone development; using genetic approaches and studying transgenic fish with fluorescent proteins under the control of osteoblast promoters, we show that within the endochondral bone there are two populations of osteoblasts with differential sensitivity to Hedgehog signalling.

Results

One population of osteoblasts, which forms just outside the cartilage structure requires a low level of Hedgehog signalling and fails to differentiate in the Indian hedgehog mutant. The second population of osteoblasts comes from transdifferentiation of chondrocytes and requires a higher level of Hedgehog signalling for their formation. This population of osteoblasts forms significantly earlier in mutants with increased levels of Hedgehog signaling such as the Patched1 and Patched2 receptor mutants than in wild type larvae, leading to premature endochondral ossification, while they fail to differentiate in the Indian Hedgehog mutant leading to an absence of endochondral ossification. Additionally, we demonstrate that the timing of first osteoclast activity positively correlates to hedgehog signalling levels in both endochondral and dermal bone. Finally, we show that Hedgehog signalling is important for proper vertebral patterning and the formation of the neural and haemal arches, the zebrafish equivalent of amniote ribs.

Discussion

In summary not only do we demonstrate that zebrafish are an ideal model for the in vivo study of bone development which allow us to use the genetic advantages of the zebrafish to answer questions that are difficult to approach in mammalian models, but we also show that there are two populations of endochondral osteoblasts with different sensitivities to Hedgehog signalling.

OC03

Enhanced osteogeneisis of foetal and adult human bone marrow stromal cells cocultured with human umbilical vein endothelial cells

JM Kanczler[1], *ROC Oreffo*[1]

[1]Bone & Joint Research Group, Centre for Human Development Stem Cells and Regeneration DOHaD, Institute of Developmental Sciences, University of Southampton, SO16 6YD, United Kingdom

Introduction

The microvascular endothelium plays a fundamental role in the development and growth of the skeleton and in bone fracture repair. Integral to these processes are endothelial cells and the role they play in osteoprogenitor cell differentiation. Understanding the intercellular signalling and interactive processes of vascular cells such as endothelial cells with osteoprogenitor stem cells could lead to future therapies particularly in the field of bone regenerative medicine.

Materials and Methods

Human umbilical vein endothelial cells (HUVEC) were cocultured with either foetal femur derived human bone marrow stromal cells (HBMSC), adult HBMSC or Stro-1+ selected HBMSC for 7 days either in a cell-contact culture system or in a transwell non-contact culture system. Using Real-Time PCR assays cocultured HUVEC and osteoprogenitor cells were analysed for Alkaline Phosphatase (AP) and Type-1 collagen expression, while the osteogenic differentiation marker, alkaline phosphatase was analysed using histochemical and biochemical protocols.

Results

A significant increase in alkaline phosphatase (nmols PNPP hr-1 mg DNA-1) was detected after 7 days in the coculture system groups of osteoprogenitor cells with HUVECS (Foetal HBMSC-HUVEC = 303 ± 21; Adult HBMSC-HUVEC = 320 ± 41; Stro-1+ HBMSC-HUVEC = 263.4 ± 34) (***p, 0.001). In contrast, after 7 days in culture negligible alkaline phosphatase activity was observed in the transwell cultures (non contact of the two cell types) of all three osteoprogenitor cell types and also negligible activity of alkaline phosphatase was observed in the osteoprogenitor cells or HUVECS grown individually. Alkaline phosphatase mRNA expression was elevated in the adult Stro-1+ HBMSC-HUVEC cultures compared to Stro-1+ HBMSC or HUVEC cultured individually, whilst foetal HBMSC–HUVEC cocultures showed a decrease in alkaline phosphatase mRNA expression compared to the foetal HBMSC or HUVEC cultured individually. Type 1 collagen mRNA expression was significantly elevated in the both coculture groups compared to the individual osteoprogenitor HBMSC or HUVEC cultures.

Discussion

We have demonstrated an increase in osteogenic differentiation of foetal and adult HBMSC-HUVEC cocultures when in physical contact with each other. Understanding the synergistic actions of the osteoprogenitor-endothelial interaction in inducing an osteogenic response offers a unique approach for the vascularisation and regeneration of bone tissue for solving problems such as delayed and non-union bone fracture pathologies.

OC04

Phenotypic effects of hypoxia and Hypoxia-Inducible Factor in Ewing's sarcoma

HJ Knowles[1], *L Schaefer*[2], *NA Athanasou*[3]

[1]University of Oxford, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Science, Botnar Research Centre, Nuffield Orthopaedic Centre, Oxford, OX3 7LD, United Kingdom; [2]Heinrich-Heine-University Düsseldorf, Dusseldorf, Germany; [3]Department of Pathology, Nuffield Orthopaedic Centre, University of Oxford, Oxford, OX3 7LD, United Kingdom

Introduction

Hypoxia, a feature of solid tumours, regulates gene expression via stabilisation of the Hypoxia-Inducible Factor (HIF) transcription factor. HIF-induced genes modulate pathways central to cell survival and expansion within an oxygen-deficient environment. In carcinomas, hypoxia and / or HIF promote tumour progression and resistance to therapy and are predictive of adverse patient outcome. However, limited information is available regarding HIF expression and function in primary bone sarcomas. We describe HIF expression in Ewing's sarcoma and effects of hypoxia and HIF on Ewing's cell lines.

Materials and Methods

HIF-1alpha and HIF-2alpha were detected in Ewing's sarcomas by immunohistochemistry and in Ewing's cell lines (A673, RD-ES, SKES-1, SK-N-MC, TC-71) by Western blot. Transcriptional activation was assessed by promoter-linked luciferase assay and expression of downstream genes by Western blot (Glut-1) and ELISA (VEGF). Phenotypic analysis of proliferation (CellBlue assay, mitotic index), apoptosis (caspase 3/7 activation), migration (scratch assay) and angiogenic capacity (tube formation) was assessed in the presence and absence of siRNA against HIF-1alpha and / or HIF-2alpha.

Results

Of 53 Ewing's tumours analysed, 28% were positive for HIF-1alpha alone, 26% for HIF-2alpha alone and 19% for both HIF-1alpha and HIF-2alpha. HIF-1alpha immunoreactivity corresponded with areas of necrosis and expression of cleaved caspase 3. In culture, hypoxic induction and transcriptional activation of HIF peaked at 0.1% O2. siRNA indicated that gene expression downstream of HIF is predominantly regulated by HIF-1alpha. Glucose-deprivation induced expression of HIF-2alpha, but not HIF-1alpha or downstream genes, supporting results with siRNA. Hypoxia (24 h, 0.1% O2) caused a 17 ± 3% reduction in Ewing's cell number associated with a 1.5- to 4-fold increase in apoptosis. Hypoxia also reduced Ewing's cell migration by 33 ± 9% and caused a modest increase in tube formation.

Discussion

The presence of HIF adjacent to areas of necrosis in Ewing's sarcoma suggests a role for hypoxia in in vivo induction of the transcription factor. Co-localisation with cleaved caspase 3 additionally implies a role for the transcription factor in the regulation of apoptosis. Initial results with Ewing's cell lines in culture support these hypotheses and indicate a role for HIF in the pathobiology of Ewing's sarcoma.

OC05

Locally generated glucocorticoids, rather than pro-inflammatory cytokines, directly regulate synovial DKK-1 expression in inflammatory arthritis

RS Hardy[1], *M Ahasan*[1], *P Patel*[1], *A Ahmed*[2], *A Filer*[2], *K Raza*[2], *CD Buckley*[1], *PM Stewart*[1], *MS Cooper*[1]

[1]School of Clinical and experimental Medicine, 2nd floor IBR, University of Birmingham, Birmingham, B15 2TT, United Kingdom; [2]School Immunity and Infection, 2nd floor IBR, University of Birmingham, Birmingham, B15 2TT, United Kingdom

Introduction

We have previously proposed a central role for locally generated glucocorticoids in the periarticular and systemic osteoporosis seen in rheumatoid arthritis (RA). Synovial fibroblasts (SFs) express the 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) enzyme and this expression increases dramatically with inflammation. This expression is significantly increased in response to pro-inflammatory cytokines. Recently, production of DKK-1 (a Wnt signalling inhibitor known to inhibit bone formation and support bone resorption) by SF in response to inflammation has been proposed to be a master regulator of inflammatory bone loss. We tested the hypothesis that DKK-1 production in inflamed synovium was primarily mediated by glucocorticoids and indirectly by inflammatory cytokines.

Materials and Methods

Primary SF were isolated from synovial biopsies from 4 patients with RA undergoing orthopaedic surgery. Glucocorticoid metabolism by 11beta-HSD1 was measured in assays using tritiated tracers and TLC analysis. DKK-1 expression was measured by real-time RT-PCR and ELISA on cell culture supernatant.

Results

High basal DKK-1 mRNA and protein expression were found in SF. In the RA group TNFalpha treatment resulted in a small increase in expression (2.3 fold in mRNA; 1.4 fold in protein) whereas IL-1 had no effect. The active glucocorticoids cortisol and dexamethasone caused a substantially greater increase in mRNA and protein expression (3.1 and 3.2 fold increase in mRNA, $p<0.05$; 2.3 and 2.8 fold in protein respectively, $p<0.05$). Importantly, the inactive glucocorticoid cortisone also increased DKK-1 expression in SF (2.7 fold in mRNA, $p<0.05$; and 1.7 fold in protein, $p<0.05$) to a much greater degree than TNFalpha/IL1, an effect blocked by an 11beta-HSD1 inhibitor. Similar changes were seen in DKK-1 promoter reporter assays. When glucocorticoids and TNFalpha/IL1 were combined the effect on DKK-1 expression was similar to that of glucocorticoids alone. Even though the direct effects of TNFalpha/IL-1 on DKK1 were modest/absent both cytokines were able to substantially increase 11beta-HSD1 expression and glucocorticoid production in these cells.

Discussion

These results show that the effect of TNFalpha/IL-1 on SF DKK-1 expression, and any consequences on bone remodelling, are unlikely to be direct but instead are mediated indirectly through increased local glucocorticoid generation.

OC06

Blockade of the ALK1 receptor reduces tumour microvessel density and prevents the development of osteolytic bone disease in the 5T2MM murine model of myeloma

AD Chantry[1], *L Coulton*[1], *O Gallagher*[1], *H Evans*[1], *J Seehra*[2], *S Pearsall*[2], *H De Raeve*[3], *K Vanderkerken*[3], *PI Croucher*[1]

[1]Academic Unit of Bone Biology Dept of Human Metabolism, University of Sheffield School Of Medicine and Biomedical Sciences, Beech Hill Road, Sheffield, S10 2DF, United Kingdom; [2]Acceleron Pharma, 149 Sydney Street, Cambridge, MA 02139, USA; [3]Department Hematology and Immunology Vrije Universiteit Brussel (VUB), Brussels, BI 090, Belgium

Introduction

Myeloma is a malignancy of differentiated B-lymphocytes. Myeloma growth in bone is associated with increased angiogenesis and the development of osteolytic lesions. Loss of function mutations in activin receptor-like kinase 1 (ALK1), a type 1 TGF-beta superfamily receptor, result in vascular defects. Recently, blockade of ALK1 has been shown to inhibit angiogenesis.

Materials and Methods

In the present study we sought to investigate the effect of blocking signalling through ALK1 using a soluble receptor comprised of the extra-cellular domain of ALK1 fused to the Fc portion of a mouse IgG immunoglobulin (RAP-041). C57/BLK/KalWrj mice were injected with 5T2MM murine myeloma cells and treated with RAP-041 (10mg/kg/ twice weekly / intra-peritoneal) or vehicle from the time of tumour cell injection (n=10/group). Additionally, naïve non tumour bearing mice were analysed as controls (n=10/group). Mice were sacrificed at week 12. Microvessel density was measured on histological sections. Bone volume and osteolytic lesions were analysed using microCT. Tumour burden, osteoblast and osteoclast number were measured using histomorphometry.

Results

Microvessel density was increased in mice bearing 5T2MM cells compared with naïve controls ($p<0.001$). Treatment with RAP-041 reduced microvessel density ($p<0.001$). Trabecular bone volume was reduced in mice bearing 5T2MM cells ($p<0.05$). This reduction was completely reversed in 5T2MM bearing mice treated with RAP-041 ($p<0.05$). Analysis of activated osteoclast number revealed significantly increased numbers of activated osteoclasts in 5T2MM bearing mice ($p<0.05$). Treatment with RAP-041 reduced the number of activated osteoclasts ($p<0.05$). Furthermore, treatment with RAP-041 reduced the number of osteolytic bone lesions seen in 5T2MM bearing mice compared with vehicle treated mice. No significant differences in osteoblast number or tumour load were observed in RAP-041 treated mice bearing 5T2MM cells compared with vehicle treated mice.

Discussion

These data suggest that blockade of ALK1 signaling in a tumor model reduces angiogenesis, osteoclast activation and prevents the development of osteolytic bone lesions.

OC07

Osteocytes as you've never seen them before

G. M. Warnock[1], *J. S. Gregory*[1], *K. S. Mackenzie*[2], *J. G. Greenhorn*[1], *A. D. McKinnon*[2], *M. H. Helfrich*[1]

[1]Bone Research Programme Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom; [2]Histology-EM Facility, Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom

Introduction

Osteocytes are notoriously difficult to visualise due to their position deep within bone. However, the perceived geometry of osteocytes and their environment has a major affect on calculated forces supposed to be imparted on the cells following loading (Anderson, Knothe Tate 2008). Previous data has been derived from chemically fixed specimens, wherein shrinkage of cells is inevitable. High pressure freezing (HPF) provides a novel method for preparing bone specimens for transmission electron microscopy (TEM) which limits fixation artefacts. In addition, a range of fluorescent probes allows staining of osteocytes in unfixed bone specimens, which when visualised by confocal scanning microscopy and multiphoton microscopy, allow in-depth resolution of their connective network in situ. Micro and nano computer tomography offer complementary imaging options for osteocytes and their network in bone, as does the well-established method of etching resin-embedded tissue followed by examination in the scanning EM. Our current work is applying this range of methodologies to obtain novel information on the shape and connectivity of osteocytes and visualise their canalicular network. Here we show examples of the full range of imaging options available and specifically report on the measurements obtained on osteocyte lacunae in HPF material.

Materials and Methods

For HPF, calvaria of 4-day old mouse pups were frozen using an EMPACT2 and tissue processed to Epon resin using freeze substitution in a Leica AFS2 system. Micrographs of osteocytes were taken at a magnification of x3400 in a Philips CM10 microscope with Gatan Multiscan 600W camera and measurements made off-line using ImageJ software (v1.41o) and a graphics tablet.

Results

The mean area between osteocyte cell body and bone matrix was 42.15μm (SD 31.59μm). Mean circularity and Feret's diameter were 0.48 (SD 0.23) and 15.93μm (SD 4.35μm) respectively. The mean distance between the osteocyte cell body and the bone matrix was 1.02μm (SD 0.59μm) which is less than half the previously published estimate (2.7μm) (Wasserman, Yaeger 1965).

Discussion

Better imaging of osteocytes will have important implications for the validation of models used to calculate forces experienced by bone cells after loading and will help to identify the exact mechanisms by which the network of osteocytes exerts its mechanosensory function.

REFERENCES

Anderson, E.J. and Knothe Tate, M.L., 2008. Idealization of pericellular fluid space geometry and dimension results in a profound underprediction of nano-microscale stresses imparted by fluid drag on osteocytes. Journal of Biomechanics, 41(8), pp. 1736-1746.

Wasserman, F. and Yaeger, J.A., 1965. Fine structure of the osteocyte capsule and of the wall of the lacunae in bone. Zeitschrift fur Zellforschung, 67, pp. 636-652.

OC08

APRIL, BAFF and NGF: Novel RANKL substitutes

F Jones[1], *R Taylor*[1], *HJ Knowles*[1], *NA Athanasou*[1]

[1]Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences University of Oxford, Oxford, OX7 3LD, United Kingdom

Introduction

Osteoclasts are differentiated and activated by two key growth factors; M-CSF, a macrophage survival and proliferation factor, and RANKL, a key growth factor required for osteoclast formation. A number of other cytokines and growth factors are able to substitute, to some extent, for either M-CSF or RANKL. In this study we identified three novel RANKL substitutes, APRIL and BAFF, both members of the TNF superfamily, and NGF, a member of the neurotrophin family. We studied the effect these had on osteoclast differentiation and formation plus their effect on the resorptive activity of giant cells extracted from giant cell tumour of bone (GCTB).

Materials and Methods

Peripheral blood mononuclear cells extracted from Buffy coat blood were seeded onto coverslips and dentine slices. These were cultured with M-CSF (25ng/ml) and either RANKL (50ng/ml) or 5-200ng/ml of APRIL, BAFF, or NGF. Osteoprotegrin (50ng/ml) and 25ng/ml of APRIL, BAFF or NGF was added to additional cultures. At day 14 the coverslips were stained for TRAP and VNR. At day 21 the dentine slices were stained for resorption pits.

Giant cells were extracted by curetting GCTB samples, seeded onto dentine slices and cultured for 24 hours with media, RANKL, APRIL, BAFF, or NGF.

GCTB paraffin-embedded sections and a tissue micro-array were stained for APRIL.

Results

At 25-200ng/ml APRIL, BAFF, and NGF induced the formation of a significant number of TRAP+ and VNR+ multinucleated cells that were able to resorb dentine. The addition of osteoprotegrin, the endogenous decoy receptor of RANKL, revealed that this resorption was independent of RANKL. When giant cells extracted from seven GCTB samples were cultured with these growth factors, the resorptive activity of three samples was increased 2-4 fold above the media control. The other four samples showed little or no increase in resorptive activity. Initial IHC staining showed that APRIL is expressed by all three GCTB cell populations.

Discussion

In this study we identified several new RANKL substitutes which may help to further our understanding of the normal osteoclastogenic process and the pathogenesis of various bone tumours, including GCTB.

OC10

Optimal bone mineralization and strength requires the type 2 iodothyronine deiodinase in osteoblasts

JHD Bassett[1], A Boyde[2], PGT Howell[3], RH Bassett[4], TM Galliford[1], M Archanco[1], DL St. Germain[5], VA Galton[5], GR Williams[1]

[1]Molecular Endocrinology Group, Division of Medicine and MRC Clinical Sciences Centre, Imperial College London Hammersmith Hospital, London, W12 0NN, United Kingdom; [2]Queen Mary University of London Oral Growth and Development, Institute of Dentistry Bart's and The London School of Medicine, London, E1 1BB, United Kingdom; [3]Division of Restorative Dental Sciences University College London Eastman Dental Institute, London, WC1X 8LD, United Kingdom; [4]Department of Civil and Environmental Engineering University College London, London, WC1E 6BT, United Kingdom; [5] Departments of Physiology and Medicine Dartmouth Medical School, Lebanon, NH 03756, USA

Introduction

Thyroid hormones are essential for growth and acquisition of peak bone mass and both hypothyroidism and thyrotoxicosis are associated with an increased risk of fracture. The pro-hormone T4 is the predominant circulating thyroid hormone, but thyroid hormone action in target cells is determined by local availability of the active hormone T3. The D1 and D2 iodothyronine deiodinases convert T4 to T3 in peripheral tissues but in the skeleton T4 to T3 conversion is restricted to osteoblasts (Williams et al, 2008). To investigate the role of locally produced T3 in bone, we characterized mice harbouring a deletion of Dio2 (D2KO) and mutants with deletions of both Dio1 and Dio2 (D1/D2KO), both of which had normal circulating T3 concentrations (Schneider et al, 2001; Galton et al, 2009)

Materials and Methods

Mice were analyzed at postnatal days P1, P14, P21, P28, P56 and P112. Bone development was characterized in skeletal whole mounts stained with alizarin red and alcian blue and histological sections of long bones stained with van Gieson and alcian blue. Bone micro-architecture was analyzed by back scattered electron scanning electron microscopy (BSE-SEM) and skeletal mineralization was determined by Faxitron analysis and quantitative BSE-SEM. Biomechanical characteristics and strength properties were examined in destructive three point bend tests. Osteoclast parameters were determined in sections stained for tartrate resistant acid phosphatase activity (TRAP) and areas of osteoclastic resorption on endosteal and trabecular bone surfaces were quantified by BSE-SEM. Bone formation was investigated by confocal autofluorescence scanning light microscopy (CSLM) and histomophometry using samples form calcein double-labelled mice

Results

D2KO mice had increased trabecular and cortical bone mineralization density and brittle bones accompanied by reduced parameters of osteoblastic bone formation. Osteoclast numbers and resorption surfaces were similar in D2KO and wild-type mice, and D2KO mutants displayed normal endochondral and intramembranous bone formation and linear growth.

Discussion

These data demonstrate that D2KO mice have brittle bones due to a discrete osteoblast defect. Additional deletion of D1 in D1/D2KO double mutants did not modify the D2KO phenotype, identifying an essential role for D2 in osteoblasts that is required for optimal bone mineralization and strength.

REFERENCES

Galton V.A., Schneider M.J., Clark A.S. & St. Germain D.L. (2009) Life without T4 to T3 conversion: Studies in mice devoid of the 5'-deiodinases. Endocrinol. as doi:10.1210/en.2008-1572.

Schneider M.J., Fiering S.N., Pallud S.E., Parlow A.F., St Germain D.L. & Galton V.A. (2001) Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. Mol. Endocrinol. 15,2137-2148.

Williams A.J., Robson H., Kester M.H., van Leeuwen J.P, Shalet S.M., Visser T.J. & Williams GR (2008) Iodothyronine deiodinase enzyme activities in bone. Bone 43,126-134.

OC11

Gender differences in hip structure in peripubertal children

A Sayers[1], JH Tobias[1]

[1]Academic Rheumatology Clinical Science at North Bristol University of Bristol, Bristol, BS10 5NB, United Kingdom

Introduction

Gender differences in skeletal development are well recognised, but their relevance to fracture risk is unknown.

Materials and Methods

We investigated whether bone strength differs between boys and girls at the hip, the impact of puberty on any differences, and whether the differences explain the higher fracture risk of boys. Hip DXA scans were performed in 5884 children mean 13.9 years of age, and hip structural parameters were derived for the femoral neck.

Results

Femoral neck (FN) BMD was slightly higher in the girls (P=0.01), more marked gender differences were observed for indices of hip structure: minimum neck width and cortical thickness were higher in boys, as was resistance to bending, reflected by greater cross sectional moment of inertia (CSMI) (P=0.001). In contrast, cortical stability was greater in girls as shown by lower buckling ratio (BR) (P=0.001). These gender differences were generally evident in early pubertal children, but typically magnified as puberty advanced (P<0.0001 for puberty-gender interaction). Similar associations were observed after adjusting for height, fat mass and lean mass, with the exception that cortical thickness was now greater in girls (P<0.001). A higher proportion of 16 year old boys reported ever having had a fracture compared to girls (OR 1.55 [95% CI: 1.36, 1.77]). To determine whether gender differences in bone geometry contribute to those in fracture risk, we examined the relationship between hip parameters and fracture risk. In fully adjusted models, structural indices such as CSMI and BR were only weakly associated with fracture risk (OR 0.90 [95% CI: 0.77, 1.04] and 1.11 [95% CI: 1.01, 1.22] respectively). The relationship between fracture risk and total hip and FN BMD was somewhat stronger (OR 0.85 [95% CI: 0.77, 0.95] and 0.86 [95% CI: 0.77, 0.96] respectively).

Discussion

We conclude that in peripubertal children, gender differences in hip geometry more marked than those in FN BMD, characterised by greater bending strength in boys but greater cortical stability in girls. However, fracture risk in children is related to FN BMD more strongly than hip structural parameters, possibly reflecting the important contribution of deficits in cortical BMD to fracture risk in the growing skeleton.

OC12

A pilot trial of the effects of vitamin D2 supplementation upon bone in post-pubertal girls

KA Ward[1], *G Das*[2], *SA Roberts*[3], *JL Berry*[3], *JE Adams*[3], *MZ Mughal*[4]

[1]MRC-Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, CB[1] 9NL, United Kingdom; [2]Central Manchester Primary Care NHS Trust, Manchester, United Kingdom; [3]University of Manchester, Manchester, M13 9PT, United Kingdom; [4]Central Manchester University Hospitals Foundation Trust, Manchester, M13 OJH, United Kingdom

Introduction

In a previous study 73% of girls from an Inner City school were found to have serum 25(OH)D levels <37.5 nmol/l. We therefore undertook an exploratory double blind, placebo controlled RCT to determine whether vitamin D supplementation would increase bone mineral density (BMD) and content (BMC) in the treated girls compared to controls. The secondary aims were to study the effects of supplementation on bone geometry and strength (stress strain index (SSI))

Materials and Methods

Ninety-nine post-menarchal girls, aged 12-14 years, were screened for inclusion in the trial, 73 had 25(OH)D levels < 37.5nmol/l and were recruited (n= 37 intervention (I), 36 control (C)); 4 were lost to follow-up. Peripheral QCT measurements of radius and tibia were made. 4 doses of 150000 IU of vitamin D2 were given over 12 months; placebo tablets were given to C at same time. A treatment effect was tested after adjustment for baseline height and bone measurement.

Results

Mean baseline 25(OH)D and PTH levels were: 25(OH)D I=17.9±8.0 nmol/l C=17.8±7.3 nmol/l; PTH I=4.8±3.9 pmol/l, C=6.5±5.7 pmol/l. At follow up they were I=57.0±8.9 nmol/l, C=15.7±6.6 nmol/l; PTH I=2.0±1.0 pmol/l, C=2.6±1.6 pmol/l. The treatment effect sizes (range), for the radius and tibia respectively were: Total BMD (mg/cm3) 11.3 (-5.6 to 28.3), 1.97 (-3.67 to 7.60); Trabecular BMD (mg/cm3) 0.10 (-4.8 to –5.1), 2.32 (-2.59 to 7.22); Cortical BMC (mg/mm) 0.08 (-1.10 to 1.27), 0.56 (-5.06 to 6.18); Cortical BMD (mg/cm3) -4.1 (-15.0 to 6.7), -3.92 (-12.4 to 4.51); Total area (cm2) 1.27 (-4.04 to 6.56), 8.34 (-8.65 to 25.3); Medullary area (cm2) 0.63 (-4.22 to 5.48), 13.4 (-6.38 to 33.2); SSI (mm3) -1.62 (-6.67 to 3.43), 38.8 (-16.8 to 94.4).

Discussion

Treatment with vitamin D2 did not significantly alter BMD, BMC, geometry or strength. The majority of skeletal growth is complete after menarche and mineralization has passed its peak rate. These data suggest that targeting vitamin D supplementation during the post pubertal period is not appropriate to improve bone status.

OC13

Abnormalities in the distribution of sclerostin positive and negative osteocytes in the cortical osteon in a hip fracture model of osteoporosis

J Reeve[1], *J Power*[1], *M Doube*[2], *R van Bezooijen*[3], *KES Poole*[1], *N Loveridge*[1]

[1]box 157 University Dept Medicine, Addenbrooke\\\'s Hospital, Cambridge, CB[2] 2QQ, United Kingdom; [2]Imperial College, London, United Kingdom; [3]University of Leiden, Leiden, The Netherlands

Introduction

The activation of wnt signaling via the canonical pathway promotes bone formation and inhibits osteoblast and osteocyte apoptosis. Osteocytic sclerostin inhibits wnt signalling and might play a role in osteoporosis. If premature death of osteoblasts contributes to osteoporosis, the formation of osteocytes (osteocytogenesis) may be compromised, leading to lowered numbers of osteocytes close to the canal in mature osteons. We therefore studied the distribution of osteocytes within cortical osteons in 10 cases of hip fracture(FNF) and 11 controls (C).

Materials and Methods

8 micrometer frozen sections of mid-femoral neck bone were cut and one stained for sclerostin by reacting with a mouse monoclonal antibody and counterstaining with toluidine blue. The adjacent section was reacted for alkaline phosphatase to identify osteons that were forming mineralized bone. Cortical fields (approximately 0.5mm dia) were identified containing at least one unremodelled osteon that could be matched in each section-pair. Sclerostin positive and negative osteocytes were identified and their coordinates registered using Image J (NIH). Canal surfaces and cement lines defining osteonal boundaries were captured. Using special purpose software, minimum distances from the canal boundary were recorded for each osteocyte. Non parametric statistics were used to characterize the distributions of osteocyte distances from the canal surface.

Results

In all 421 BMUs studied, the distance from the canal within which 25% of osteocytes were located (25th quantile of osteocyte distance) was significantly larger in FNF than C: 35 vs 27 micrometers (p=0.002, Wilcoxon). In ALP+ osteons the 25th quantiles of distance for scl- (19 vs 11)& scl+ osteocytes (39 vs 28 micrometers) were also significantly larger in FNF. Distributions of osteocyte distance were significantly more negatively skewed in FNF than C there being substantial osteocytes close to the cement line, but a relative deficit close to the canal.

Discussion

Power et al showed that at the tissue level, osteocyte densities were unremarkable in FNF; but these new data show that the distribution of osteocytes within the maturing osteon is abnormal in FNF. This might reflect osteoblast or osteocyte death late in BMU maturation or alternatively abnormal osteocytogenesis and requires further investigation.

OC14

Relationship between different domains of physical activity and fractures: Results from the EPIC-Norfolk study

A Moayyeri[1], R Luben[1], S Bingham[1], N Wareham[1], KT Khaw[1]

[1]Department of Public Health and Primary Care Institute of Public Health University of Cambridge, Cambridge, CB[1]8RN, United Kingdom

Introduction

A large body of epidemiological evidence suggests an inverse relationship between physical activity and risk of fractures. However, it is unclear how this association varies according to the domain of life in which the activity is undertaken. The purpose of this study was to describe the association between the overall and domain-specific physical activity and risk of prospective fractures.

Materials and Methods

In the context of the European Prospective Investigation of Cancer- Norfolk study, total and domain-specific physical activity was assessed in 14,903 participants (6,514 men, mean age 62 yr) using a validated questionnaire (EPAQ2). After a median follow-up of 8 years, there were 504 fractures of which 164 were hip fractures. Metabolic equivalent measures were calculated according to frequency and duration of different activities for all participants.

Results

The hazard ratios (95%CI) for any fracture due to physical activity undertaken at home, during exercise, at work, for transport, and in total were 1.14 (0.87–1.49), 0.91 (0.69–1.19), 1.31 (0.76–2.23), 1.33 (0.80–2.24), and 1.31 (0.99–1.74), respectively, after adjustment for baseline age, sex, history of fracture, body mass index, smoking status, alcohol intake, and heel broadband ultrasound attenuation in Cox proportional-hazards models. Hip fracture was inversely associated with moderate physical activity at home (HR=0.55, 95%CI 0.30-0.98) and for exercise (HR=0.52, 95%CI 0.29-0.93) among women. Sport/recreational activities were associated with highly reduced risk of hip fracture among men (HR=0.18, 95%CI 0.05-0.62; P for trend=0.004). Walking for leisure or transport for >90 min/week was associated with reduced risk of any fracture (HR=0.74, 95%CI 0.58-0.95) and hip fracture (HR=0.57, 95%CI 0.37-0.87) in both men and women. The associations between different domains of physical activity and fractures were more evident in younger participants (age <65 yr) and those without previous history of fracture.

Discussion

This study suggests that physical activities at home and during exercise are associated with lower risk of hip fracture, whereas occupational and transportation-related activities are not. Further attention to the interactions between different domains of physical activity and known fracture risk factors for prediction of fractures is recommended.

OC15

Effect of intermittent hPTH (1-34) on 1,25 (OH)2 vitamin D, bone formation and Fibroblast Growth Factor-23 (FGF-23) in post-menopausal osteoporosis

M Sridharan[1], P Manghat[1], I Fogelman[2], WD Fraser[3], G Hampson[1]

[1]Department of Clinical Chemistry/Blood Sciences, 5th Floor North Wing, St Thomas\' Hospital, London, SE[1]7EH, United Kingdom; [2]Department of Nuclear Medicine, Guy's Hospital, London, SE[1]3RT, United Kingdom; [3]Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool, United Kingdom

Introduction

The role of FGF-23 in the regulation of phosphate and 1,25 (OH)2vitamin D is well established. However, the relationship between FGF-23 and PTH remains unclear. FGF-23 is produced by osteoblasts and osteocytes and may also be implicated in bone formation. Intermittent PTH administration has anabolic effects on bone. We investigated the effect of intermittent PTH on FGF-23.

Materials and Methods

Twenty seven women aged (75.8 [5.4] years) were treated with the PTH analogue 1-34 (Teriparatide) for established postmenopausal osteoporosis. We investigated their response following treatment with Teriparatide. Bone mineral density (BMD) was measured at the lumbar spine (LS) and Total Hip (TH) at baseline, at 6-9 months and 18 months. Serum calcium, phosphate, PTH, 25(OH)vitamin D, 1,25 (OH)2 vitamin D and the bone formation marker ; PINP were measured at baseline, 1-3 months, 6-9, 12-15 and 15-18 months. Circulating FGF-23 was determined at the same time-points. Other biochemical markers of bone turnover; Bone alkaline phosphatase (BALP), osteocalcin (OC) and urine CTX were measured at baseline, 1-3 months and 6-9 months only.

Results

BMD at the LS increased significantly (6-9 months: 6.5% [4.3], 18 months : 12.9% [3.9] p <0.0001). Serum calcium increased significantly and peaked at 6-9 months (baseline 2.37 [0.02], 6-9 months 2.47 [0.02]). This was accompanied by a significant reduction in PTH ng/L (baseline 40.7 [4.2], 6-9 months 33.1 [5.3] p<0.01). No significant change in serum phosphate was seen. 1, 25 (OH)2vitamin D increased steadily from 114.5 [10.7] pmol/L to reach a peak 157 [16.6] pmol/L p= 0.037) at 12-15 months. FGF-23 increased from baseline (128 [43] RU/L) with maximal concentrations at 6-9 months (210 [73] RU/L p = 0.017) and declined after (18 months : 134[85] RU/L). The same pattern was observed with PINP (baseline : 39.8[4.7] ug/L, 6-9 months: 149[16.5] p<0.001 , 18 months 75.3[13.2] p = 0.035).

Discussion

In conclusion, intermittent PTH increases bone formation, 1,25 (OH) 2 vitamin D and circulating FGF-23 concentrations. Although, the increases in 1,25 (OH) 2vitamin D following PTH may stimulate FGF-23 production, our study suggests that the stimulatory effect of PTH on osteoblast numbers may also contribute, at least partly, to the up-regulation of FGF-23.

OC16

Inactivating mutations in RANK result in diseases with opposite phenotypes

DJ Mellis[1], A Duthie[1], M Guerrini[2], A Villa[2], MH Helfrich[1], MJ Rogers[1], JC Crockett[1]

[1]Bone and Musculoskeletal Research Programme, Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB252ZD, United Kingdom; [2]CNR-ITBA, Via Fratelli Cervi 93, 20090 Segrate (MI), Milan, Italy

Introduction

RANK is critical for osteoclastogenesis and, following activation by RANKL, causes NFkB translocation to the nucleus. Causative mutations have been identified in RANK (TNFRSF11a) in patients with osteoclast-poor osteopetrosis and early onset forms of Paget's disease, including familial expansile osteolysis (FEO). We previously described a substitution mutation in RANK (WX434), associated with osteoclast-poor osteopetrosis, resulting in premature termination of translation and lack of intracellular domains required for downstream signalling. Heterozygous insertion mutations in the RANK signal peptide cause the increased osteoclastogenesis observed in FEO. Here, we investigated the cellular mechanisms leading to these opposite disease phenotypes.

Materials and Methods

Cells were transfected or transduced with wildtype (WT) or mutant forms of RANK, stimulated with RANKL and analysed by immunostaining (followed by confocal and TEM) or by western blot analysis.

Results

Whilst over-expression of WT-RANK in COS7 cells resulted in RANKL dependent NFkB translocation to the nucleus (detected by immunostaining for p65), over-expression of WX434-RANK prevented RANKL-dependent NFkB translocation.

We found that adenoviral transduction of mouse osteoclast precursors with FEO-RANK resulted in a significant increase in osteoclast formation compared to osteoclast formation from precursors transduced with WT-RANK. Immunostaining followed by confocal microscopic and TEM analysis demonstrated that FEO-RANK accumulated within membranous ER-like structures in the cytosol and was undetectable at the plasma membrane. In FEO-RANK transfected 293 cells, RANKL-dependent activation of NFkB and phosphorylation of ERK1/2 and p38 was prevented. By contrast, co-expression of FEO-RANK with WT-RANK (to mimic the heterozygous expression of the protein in FEO patients), resulted in detection of both WT-RANK and FEO-RANK in the golgi and on the plasma membrane. In addition, FEO- and WT-RANK co-expression rescued RANKL-induced signalling downstream of RANK.

Discussion

These data illustrate that, although the different mutations in RANK cause diseases with contrasting phenotypes, all mutations studied inactivate RANKL-dependent signalling. The WX434 mutation results in absence of osteoclasts in patients, probably as a direct consequence of impaired NFkB signalling. By contrast, the inactivating FEO mutation appears to increase osteoclast formation but only when co-expressed with WT-RANK. The exact nature of this interaction and the mechanism leading to its dominant effect on osteoclast formation is under investigation.

OC17

Inhibiting glycogen synthase kinase-3 (GSK-3) prevents the development of myeloma bone disease

NG Abdul[1], W Stoop[2], W Koopman[3], M Djerbi[3], AD Chantry[1], H Evans[1], K Vanderkerken[2], PI Croucher[1]

[1]Academic Unit of Bone Biology, Department of Human Metabolism, University of Sheffield Medical School, Sheffield, S10 2RX, United Kingdom; [2]Vrije Universiteit Brussel (VUB), Dept Hematology & Immunology, Laarbeeklaan 103, Brussels, B-1090, Belgium; [3]AstraZeneca R&D, Södertälje, S-15185, Sweden

Introduction

Multiple myeloma is a B-cell malignancy characterised by the growth of tumour cells in the bone marrow and the development of osteolytic bone disease. This is mediated by increased osteoclastic bone resorption and a suppression of bone formation. Regulators of the bone resorption have been identified; however, understanding of the mechanism responsible for suppressing bone formation is poor. Recently, the Wnt pathway has been implicated in regulating bone formation and myeloma cells produce soluble antagonists of Wnt signalling, which suppress bone formation. GSK-3 phosphorylates beta-catenin and blocks Wnt signalling. Thus, inhibiting this enzyme may remove myeloma-induced suppression of osteoblastic bone formation.

Materials and Methods

In this study we investigated whether blocking GSK-3, with AR28, a potent GSK-3 inhibitor, prevents myeloma-induced suppression of bone formation and the development of osteolytic disease in the 5T2MM model of myeloma.

Results

Injection of C57Bl/KaLwRij mice with 5T2MM myeloma cells suppressed osteoblast numbers ($p<0.05$) and promoted formation of osteolytic lesions ($p<0.05$). Treatment of C57B1/KaLwRij mice with AR28 (15 µmol/kg, twice daily, 4 weeks) increased cancellous bone volume. Treatment of C57B1/KaLwRij mice bearing 5T2MM cells with AR28 (15 µmol/kg or 45 µmol/kg) increased osteoblast numbers ($p<0.05$) and osteoblast perimeter ($p<0.05$), and prevented the development of osteolytic lesions ($p<0.05$). Treatment had no effect on osteoclast numbers. AR28 also had no effect on serum paraprotein, a marker of whole animal tumour burden, or spleen weight, a site of extramedullary proliferation. However, myeloma burden in bone was decreased by 40%.

Discussion

These data suggest that blocking GSK-3 with AR28 prevents myeloma suppression of bone formation and blocks the development of osteolytic bone disease, independent of effects on osteoclast numbers. This suggests that GSK-3 inhibitors may have therapeutic potential in patients with multiple myeloma.

OC18

Metabolomic studies identify true responders from non-responders to vitamin D supplementation in postmenopausal women

C Moniz[1], *R Chandra*[1], *TJ Ong*[1], *C Meredith*[1], *C Mugglestone*[1]

[1]Clinical Biochemistry, Kings College Hospital, Bessemer Road, London, SE5 9RS, United Kingdom

Introduction

Inconclusive studies showing fracture prevention from calcium and vitamin D supplementation in osteoporotic subjects and several reports showing high prevalence of hypovitaminosis D in the elderly population, suggest there may be genetic inter-individual variability in handling micronutrients. We therefore undertook a metabolomics study in morning fasting urine samples, in normal postmenopausal women before and after supplementation with calcium and vitamin D. We used serum parameters of calcium homeostasis to determine response and matched these with urinary patterns of unique metabolites determined by NMR spectroscopy.

Materials and Methods

112 postmenopausal women (69 ± 10 yrs) gave informed consent to giving a blood and morning fasting urine sample before and after 3 months. At entry they were age matched to receive either oral 800 units of vitamin D and 1200mgs calcium or no supplementation. Blood was taken for a biochemical profile to include calcium and albumin, vitamin D and PTH. Urinary calcium and creatinine were measured and putative NMR biomarkers were examined after filtration (molecular cut-off 10kDa, centrifuged, freeze-dried and reconstituted in phosphate buffer made up with TMSP as internal standard. NMR spectra were recorded in triplicate in a fully automated manner on a Varian UNITY 400 MHz spectrometer, processed using the standard Varian software and converted to a data file suitable for multivariate analysis applications (MVDA). Complete data were obtained in 47 subjects. Data was normalized in an appropriate way, centred and scaled.

Results

Significant changes in calcium, PTH and Vit D, differentiated responders (22) from non-responders (14) and controls (11) at 3 months, despite good compliance. The NMR data show distinct patterns in distinguishing controls, responders & non-responders and segregation was recognisable even before treatment commenced.The preliminary analyses of the 'NMR fingerprint' suggest that folate and vitamin B metabolism are implicated in Ca/VitD status and response to supplementation.

Discussion

Regarding deficiency and how micronutrients interact in this population would be premature without larger studies to include genetic polymorphisms. Nevertheless, there is tremendous potential to develop tests for patients who do not need (nor respond) to Ca/VitD supplementation and target those that do.

OC9

Novel use of digital image correlation to measure surface strains in loaded mouse tibiae discloses direct evidence of functional adaptation to limit peak strains

SJ Shefelbine[1], *P Sztefek*[1], *M Vanleene*[1], *R Olsson*[1], *RC Collinson*[2], *AA Pitsillides*[2]

[1]Imperial College London UK, London, SW7 2AZ, United Kingdom; [2]Department of Veterinary Basic Sciences, The Royal Veterinary College, London, NW[1]0TU, United Kingdom

Introduction

Animal models of bone adaptation to loading are currently calibrated by strain gauges or finite element modeling. Gauges measure strain on a limited area of bone surface and finite element models likely oversimplify material properties, geometry and loading conditions. Neither is likely, therefore, to accurately measure strain distribution over the bone surface. In contrast, digital image correlation (DIC) is an optical full-field method for non-contact, 3-D measurement of deformation. We have used DIC to measure strains across the entire cortical surface of loaded murine tibiae and examined whether these are modified, 'adapted', following a period of in vivo loading.

Materials and Methods

Right tibiae of 8-week C57Bl/6J mice were loaded at 12N (three days/week for two weeks (1). For DIC, left (non-loaded, control) and right ('adapted') limbs were coated with white paint and then speckled with black paint, using high precision airbrushes. Limbs were placed in the loading device and speckle movement tracked on medial and lateral sides during load application. Image post-processing produced ~900 measurements on each surface.

Results

DIC revealed lower transverse/shear strains than axial strain and non-uniform surface strain fields with predominant load-induced tension/compression on medial/lateral sides, respectively. Unexpectedly, localized regions of high strain (0.5%) and significant variation in magnitude were evident on the medial aspect. Two weeks of loading was sufficient to significantly decrease strains in 'adapted' tibiae. Mean strain across the entire medial aspect decreased from 0.144±0.042% on non-loaded (left) tibiae to 0.117±0.041% following adaptation. In contrast, mean lateral compressive strains did not change. DIC was validated by small experimental errors in paired images and by near identical repeat load-deformation curves in the same limb.

Discussion

DIC accurately measures surface strain, discloses reproducible gradients and localised areas of higher strain. It confirms decreases and greater uniformity of strain in load-adapted tibiae. Strains engendered by 12N in adapted tibiae were similar to those generated by 8N in control limbs. As loads of 8N do not induce osteogenic adaptive responses, this suggests the existence of a 'preferred' strain field, above which bone will adapt. Accurate strain measurement will allow us to link specific mechanical stimuli to local changes in osteogenic/anti-resorptive cellular behaviour.

REFERENCES

1. De Souza RL, Matsuura M, Eckstein F, Rawlinson SC, Lanyon LE, Pitsillides AA (2005) Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element. Bone, 37: 810

P001

Abstract withdrawn

P002

Abstract withdrawn

P003

A functional role of PHOSPHO1 in mineralisation during chick limb development

VE MacRae[1], MG Davey[1], L McTeir[1], S Narisawa[2], MC Yadav[2], JL Millan[2], C Farquharson[1]

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, Division of Developmental Biology, University of Edinburgh, Roslin, EH25 9PS, United Kingdom; [2]Sanford Children's Health Research Center Burnham Institute for Medical Research, La Jolla, CA 92037, USA

Introduction

PHOSPHO1 is a bone specific phosphatase implicated in the initiation of inorganic phosphate generation for matrix mineralisation. This study investigated the temporal expression and functional role of PHOSPHO1 within the developing chick limb.

Materials and Methods

PHOSPHO1 and tissue-nonspecific alkaline phosphatase (TNAP) expression was determined in chick limb bud mesenchymal micromass cultures (mRNA and protein) and also in wild-type and talpid3 chick mutants by whole mount in-situ hybridisation. In vivo studies also examined the effect of lansoprazole (a PHOSPHO1 inhibitor) on mineralisation in developing chick embryos.

Results

The ability of micromass cultures to differentiate into chondrocytes and mineralise their matrix was temporally associated with increased expression of both PHOSPHO1 and TNAP. Compared to day 0 cultures PHOSPHO1 expression was higher after 7 (6.1 fold; $P<0.05$) and 10-days (17 fold; $P<0.001$) of culture whereas higher expression of TNAP was not observed until culture day 10 (3.6 fold; $P<0.001$). Comparable changes in PHOSPHO1 and TNAP protein expression were observed.

FGF2, a known inhibitor of chondrocyte differentiation, caused a reduction in both PHOSPHO1 (0.56 fold; $P<0.001$) and TNAP (0.31 fold; $P<0.001$) gene expression and this was accompanied by decreased Alizarin red staining (17%; $P<0.05$). Lanzoprazole (100muM) exposure reduced Alizarin red staining after 3 (26%; $P<0.05$) and 7-days (34%; $P<0.05$) in culture. However, culture in control medium during 0-3 days with subsequent lanzoprazole exposure during 4-7 days had no effect on mineralisation confirming our hypothesis that PHOSPHO1 is pivotal to the first phase of matrix vesicle-mediated mineralisation.

In-situ hybridisation demonstrated that PHOSPHO1 and TNAP had similar developmental expression patterns in long bones. Treatment of developing embryos for 5 days with 100muM lansoprazole completely inhibited mineralisation of all leg and wing long bones. Furthermore, long bones of the talpid3 mutant were not mineralised and did not express PHOSPHO1 or TNAP. This contrasted with the normal expression of PHOSPHO1 and TNAP in the mineralising clavicle.

Discussion

We have established the temporal expression and functional role of PHOSPHO1 during mesenchymal differentiation, chondrogenesis and matrix mineralisation within the developing chick limb. These studies indicate that PHOSPHO1 plays a pivotal role in the mineralisation process in the developing chick limb.

P004

Bearing capacity of subchondral bone/subchondral bone replacement co-regulates the quality of cartilage matrix

M Petrtyl[1], L Senolt[2], P Cerny[1], J Lisal[1], H Hulejova[2], J Danesova[1], M Liskova[2]

[1]Czech Technical University FCE Department of Biomaterial Engineering Thakurova 7, Prague 6, 160 00, Czech Republic; [2]Institute of Rheumatology Na Slupi 4, Prague 2, 120 00, Czech Republic

Introduction

The repair of osteochondral defects in the big joints remains a clinical challenge. The insertion of crushed autologous grafts, thought to stimulate the regenerative processes, has been reported as possible therapy. Nevertheless the regenerative mechanical quality (after experimental verifications) was 60% - 70% of healthy cartilage for treatment and control, respectively. Our project has been focused on the design, development and clinical verification of two-component polymer replacements, which have a distal part in the subchondral bone and a proximal part partially in the space of the removed arthritic cartilage, creating the bearing basis for chondrogenesis.

Materials and Methods

This low component replacement has been created from a cyclic olefin copolymer blend, and the head component from hydrogel. The surface functionalization of the COC-blend and EXACT polymers were accomplished by several different surface treatments (low pressure MW plasma, UV+ozone, ion bombardment, atmospheric discharge, laser radiation). The upper components of the replacements were made from poly-hydroxyethylmethacrylat without plasma surface treating. Osteochondral defects (depth: 12 mm, diameter: 8 mm) were created in each lateral and medial tibial condyle of the right and left knee in 6 mature pigs. The histological analyses of the cartilage matrix were accomplished after 4 months.

Results

Developed and plasmatically modified/unmodified replacements in vivo conditions have been proven as bioactive, bioconductive and biotolerant materials. The developed subchondral bone around the COC-blend had the same quality as a natural healthy one. The new subchondral bone mineralized perfectly. The replacement installed into the artificially executed osteochondral defects of porcine tibial condyles, both modified as well as non-modified implants demonstrated perfect tolerability and appeared to heal into existing subchondral bone without displacement or evidence of necrosis. Histological findings and morphological changes of osteochondral samples did not demonstrate any pathological features. Moreover, the top of the implant was overgrowth with viable new articular cartilage or new fibrocartilage.

Discussion

The bearing capacities of subchondral bone replacements considerably contribute to the genesis of a new extracellular cartilage matrix. COC-blend copolymers and hydrogels (HEMA)

can be suggested as a reliable reconstructive alternative for local osteochondral defects and effective support as well as the creation of new articular cartilage having a surface without irregularities.

REFERENCES

Kleemann R. et al. (2006) Mechanical Influences on Cartilage Regeneration. In: The Interaction of Mechanics and Biology, Center for Musculoskeletal Surgery, Charité, Berlin, 31

P005

An analysis of orbital cartilage development

H Thompson[1], G Jeffery[2], I McGonnell[1]

[1]Dept VBS, Royal Veterinary College, Royal College Street, London, NW[1]0TU, United Kingdom; [2]Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, United Kingdom

Introduction

Orbital cartilage encircles the eye giving strength and support to the neural retina. It is derived from cranial neural crest cells (NCC), cells that migrate from the dorsal neural tube in vertebrate embryos. NCCs are pluripotent generating a number of cell types including neurons, glia, and melanocytes. Uniquely in the developing head, NCCs also make skeletal derivatives that form the majority of the craniofacial skeleton. Differentiation of NCCs into cartilage in the head requires inductive interactions between NCCs and the local environment. The nature of these interactions is largely unknown. We hypothesise that formation of the eye socket requires interactions between the eye and the NC during early development. This is supported by evidence in animals and humans where lack of eyes (anophthalmia) or formation of small eyes (microphthalmia) result in craniofacial abnormalities, in particular lack of a socket and misalignment of jaws.

Materials and Methods

Wholemount in situ hybridisations (ISH) were performed according to Neito et al, 1996, using chick probes against Sox9, Cart1 and Aggrecan. Definitive cartilage was visualised by staining with 1% Alcian blue/ 3% acetic acid/ ethanol for 5 days. The eyes were removed (enucleated) from Embryonic day (E) 2 chicks using a tungsten needle before re-incubating for <8 days, fixing in 4% PFA and analysing by ISH or alcian staining. RPE was isolated from the enucleated eyes of E3 chicks in HBSS media and grafted into E3 head mesenchyme before re-incubating for <7 days, fixing and analysing with ISH or alcian staining.

Results

We have examined gene expression patterns around the developing eye, including the early cartilage markers Cart1 and Sox9 and later cartilage marker Aggrecan. Expression is seen encircling the eye in the region of presumptive orbital cartilage. We also demonstrate that expression of these genes is altered following eye removal in early development. Additionally, the neural ectoderm derived- retinal pigment epithelium can induce ectopic cartilage to form in head NCCs.

Discussion

We have identified the spatial and temporal development of orbital cartilage in the chicken embryo and have highlighted the important role of the eye in orbital cartilage induction.

P006

The role of Gas6/Axl signalling in chondrocyte differentiation during endochondral ossification

PT Newton[1], C Farquharson[1], AE Canfield[2]

[1]Bone Biology Group, Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, EH25 9PS, United Kingdom; [2]Wellcome Trust Centre for Cell-Matrix Research, Cardiovascular Research Group, Michael Smith Building, Oxford Road, Manchester, M13 9PT, United Kingdom

Introduction

During endochondral ossification, mesenchymal cells undergo differentiation into proliferating chondrocytes and further differentiate to become hypertrophic. The chondrocytes secrete specific collagens and proteoglycans to form an extracellular matrix which becomes mineralised. Mineralisation of the extracellular matrix is associated with the apoptosis of hypertrophic chondrocytes.

A role for the tyrosine kinase Axl and its ligand, growth arrest-specific 6 (Gas6), has been determined in chondrocytes of the articular cartilage: to regulate growth and survival. Previous studies indicate that Gas6 transcription decreases upon chondrogenic differentiation and indeed that addition of recombinant Gas6 inhibits chondrogenesis. Gas6 signalling via Axl also plays a role in the prevention of ectopic mineralisation in the vasculature via anti-apoptotic mechanisms. This present study tests the hypothesis that Gas6 and Axl are pivotal in chondrocyte differentiation and matrix mineralisation.

Materials and Methods

ATDC5 cells have previously been used to study chondrocyte differentiation during endochondral ossification. The ATDC5 monolayer model was refined by the addition of beta-glycerophosphate to induce mineralisation at an earlier time-point and was characterised by histological and histochemical analysis. The temporal protein and mRNA expression of collagen types II and X, Axl, and Gas6 were assessed by Western blotting and qPCR over a 34 day time-course with ten time-points.

Results

ATDC5 cells undergo chondrogenic differentiation (as shown by staining with alcian blue) and express chondrocyte markers (collagen types II and X) at expected stages of development. Mineralisation occurred in these cultures from day 14, much earlier than in previous reports. Axl protein and mRNA expression both peaked at day 8 and decreased thereafter. Gas6 transcription was minimal at day 6 and increased to reach a maximum at day 13, after which transcription markedly decreased.

Discussion

Differential expression of Axl and Gas6 occurred during early stages of chondrocyte differentiation in this model and the expression of both Axl and Gas6 decreased prior to the deposition of a mineralised matrix by these cells. These results indicate that Axl and Gas6 may function in chondrogenesis, as previously suggested. Future studies will elucidate the role(s) played by Gas6/Axl signalling in regulating chondrocyte differentiation and cartilage matrix mineralisation.

P007

The expression of FGF23 signalling cascade components in growth plate chondrocytes

KA Staines[1], *VE MacRae*[1], *C Farquharson*[1]

[1]Roslin Institute and R(D)SVS, Roslin, EH25 9PS, United Kingdom

Introduction

Dysregulation of inorganic phosphate homeostasis manifests a wide spectrum of diseases. Recent advances in the molecular basis of hypophosphatemic disorders have identified a novel group of molecules that have also been implicated in bone mineralisation directly. Such molecules include FGF23, PHEX, MEPE and DMP1. Their role, along with that of tissue non – specific alkaline phosphatase (TNAP), in chondrocyte matrix mineralisation of the growth plate remains largely undefined and current concepts are speculative.

Materials and Methods

To establish the temporal expression pattern of FGF23, PHEX, MEPE, TNAP and DMP1 by growth plate chondrocytes, we have utilised the chondrogenic ATDC5 cell line, which represents the multistep chondrogenic differentiation observed during endochondral bone formation (Shukunami et al. 1997). Semi-quantitative PCR and RT-qPCR were used to establish their expression over a 20 day culture period along with Alizarin red staining to quantify matrix mineralisation.

Results

The cells underwent the expected developmental stages of differentiation and maturation as shown by collagen types II and X expression. Matrix mineralisation of ATDC5 cells was minimal at day-8 and increased significantly ($P<0.001$) at day-15 of culture. Semi-quantitative PCR confirmed the presence of FGF23, PHEX, DMP1, MEPE and TNAP in ATDC5 cells; RT-qPCR indicated quantitative changes in their expression throughout the culture period. Consistent with the onset of mineralisation, expression of TNAP and PHEX increased at day-15 where they levelled off for the remainder of the time course. In contrast, MEPE expression markedly decreased at day-15 (compared to day-8, $P<0.005$) before increasing at day-20. The opposite trend to MEPE was observed in DMP1 expression with the most significant fold-changes (up to 23 fold from days 8 and 20 levels, $P<0.005$) being observed at day-15. FGF23 expression has yet to be quantified in ATDC5 cells, potentially due to low transcript levels.

Discussion

Our findings indicate that FGF23, PHEX, MEPE, DMP1 and TNAP are all expressed by chondrocytes and their expression patterns are consistent with a role in chondrocyte matrix mineralisation. Further analysis by Western blotting and in situ hybridisation will further determine their temporal and spatial expression patterns and will warrant investigation into their functional roles within the growth plate

REFERENCES

Shukunami C., Ishizeki K., Atsumi T., Ohta Y., Suzuki F. & Hiraki Y. (1997) Cellular Hypertrophy and Calcification of Embryonal Carcinoma-Derived Chondrogenic Cell Line ATDC5 In Vitro. JBMR. 12, 1174-1188.

P008

The inhibitory role of suppressor of cytokine signalling-2 on STAT signalling in the growth plate

C. Pass[1], *V.E. MacRae*[1], *S.F. Ahmed*[2], *C. Farquharson*[1]

[1]The Roslin Institute and R(D)SVS The University of Edinburgh, Roslin, Midlothian, EH25 9PS, United Kingdom; [2]Bone & Endocrine Research Group, Royal Hospital for Sick Children, Glasgow, United Kingdom

Introduction

Suppressor of Cytokine Signalling-2 (SOCS2) tightly controls postnatal growth, possibly through the regulation of GH and IGF-1 signalling. SOCS2 null mice are characterised by enhanced body size and bone length. SOCS2 protein may also mediate the effects of IL-1beta and TNFalpha in chronic inflammatory disorders. The precise mechanisms by which SOCS2 inhibits the GH/IGF-1 axis and pro-inflammatory cytokine signalling are however unclear and was the focus of this study.

Materials and Methods

We compared STAT signalling by costochondral chondrocytes from wild-type and SOCS2 knockout mice in response to GH (500ng/ml), IGF-1 (50ng/ml), and the pro-inflammatory cytokines IL-1beta and TNFalpha (10ng/ml). Phosphorylation of STATs1, 3 and 5 was determined by Western Blotting.

Results

Phosphorylation of STATs1, 3 and 5 was increased in both wild-type and SOCS2 null chondrocytes in response to GH exposure for 15 minutes. In all cases total STATs1, 3 and 5 expression was unchanged. In comparison to wild-type chondrocytes, SOCS2 null cells showed increased STAT3 phosphorylation in response to exposure with GH (15mins), IL-1beta (24hrs) and TNFalpha (24hrs). STATs1 and 5 showed similar levels of activation in both wild-type and null cells. The noted lack of STAT phosphorylation in response to IGF-1 was not unexpected as the IGF-1 receptor lacks specific tyrosine-based motifs recognised by STATs. GH stimulation of wild-type chondrocytes for between 15 and 60 minutes resulted in the phosphorylation of STATs1, 3 and 5 that peaked at approximately 30 minutes and declined thereafter. In contrast, STATs1, 3 and 5 activation in SOCS2 null cells was moderately prolonged showing no decline by the end of the study period.

Discussion

We have confirmed that in growth plate chondrocytes, SOCS2 inhibits GH, IL-1beta and TNFalpha signalling via STAT phosphorylation. It is therefore likely that the increased length of long bones of SOCS2 knockout mice (MacRae et al. 2008) is due to increased and prolonged chondrocyte STAT signalling. In addition, SOCS2 may contribute to short stature observed during chronic pediatric inflammatory conditions such as inflammatory bowel disease. Hence, it is possible that SOCS2 may be a potential therapeutic target for enhancing GH signalling in children with short stature.

References

Macrae V.E., Horvat S., Pells S.C., Dale H., Collinson R.S., Pitsillides A.A., Ahmed S.F. & Farquharon C. (2009) Increased bone mass, altered trabecular architecture and modified growth plate organization in the growing skeleton of SOCS2 deficient mice. J. Cell. Physiol. 218, 276-284.

P009

Abstract withdrawn

P010

Biochemical characterization of Indian Hedgehog mutations associated with Brachydactyly Type A1 and Acrocapitofemoral Dysplasia

KA Patterson[1], *GRM Cunningham*[1], *MA Akimenko*[2], *DE Bulman*[1]

[1]Ottawa Health Research Institute 50[1]Smyth Road, Ottawa, ON, K1H 8L6, Canada; [2]Ottawa Health Research Institute 725 Parkdale Avenue, Ottawa, ON, K1Y 4E9, Canada

Introduction

Indian hedgehog (IHH) is a master regulator of bone development, playing a particularly important role in endochondral ossification. Mutations in the human IHH gene have been implicated in two bone developmental disorders, brachydactyly type A1 (BDA1) and acrocapitofemoral dysplasia (ACFD). We are interested in characterizing the mutations in IHH by elucidating the resulting defect in protein function.

Materials and Methods

Site-directed mutagenesis was used to induce point mutations into the full-length human IHH cDNA in pcDNA3 to reproduce the mutations detected in affected individuals. These mutant constructs were then stably transfected into HEK293 cells, media containing secreted IHH was collected, and the activity of each mutant construct was assessed using a variety of cell-based assays. In vivo characterization of mutant IHH function utilized morpholino knock-down of ihha in zebrafish and assessed the ability of injected mutant IHH mRNA to rescue the knock-down phenotype.

Results

Preliminary results suggest that processing of the IHH precursor into the active form is reduced in the mutants. Results from the activity assays suggest that several of the mutant constructs display decreased signaling ability, and may also act in a dominant negative manner. Morpholino knock-down of ihha in zebrafish resulted in a variety of phenotypes including decreased overall size of the larvae, as well as incomplete branching of the developing vasculature. Injection of human wild-type IHH mRNA was able to partially rescue the knock-down phenotype, with the mutants rescuing the phenotype to a lesser degree.

Discussion

The dysregulation of endochondral ossification seen in BDA1 and ACFD may be the result of the decreased signaling ability of the mutant IHH proteins at the growth plate. In the zebrafish model, morpholino knock-down of ihha illustrated its importance in early zebrafish angiogenesis. The in vitro and in vivo characterization of mutations in IHH has provided insights into the functionality of the IHH protein, as well as the pathogenesis of BDA1 and ACFD.

P011

Intra-uterine growth velocities of fetal abdominal circumference and femur length differentially predict bone size and volumetric density at 4 years

NC Harvey[1], *PA Mahon*[1], *SM Robinson*[1], *CE Nisbet*[1], *MK Javaid*[2], *SR Crozier*[1], *HM Inskip*[1], *KM Godfrey*[1], *EM Dennison*[1], *C Cooper*[1]

[1]MRC Epidemiology Resource Centre, Southampton General Hospital, Southampton, SO16 6YD, United Kingdom; [2]Botnar Research Centre, University of Oxford, Oxford, United Kingdom

Introduction

We used ultrasound to measure the velocities of fetal femur and abdominal growth between 19 and 34 weeks gestation and related these to bone size and density measured by DXA at 4 years old.

Materials and Methods

Participants were recruited from the Southampton Women's Survey, a unique prospective cohort of 12,583 initially non-pregnant women aged 20-34 years, resident in Southampton UK. These women were studied in detail before and during pregnancy. Measurement of fetal femur length and abdominal circumference were performed at 19 and 34 weeks gestation using ultrasound, and a subset of the offspring underwent bone mineral assessment (Hologic Discovery DXA instrument) at 4 years old [whole body minus head bone area (BA), bone mineral content (BMC), areal bone mineral density (aBMD) and estimated volumetric BMD (vBMD)]. Royston models were fitted to fetal measurements to create Z-scores for size and conditional growth velocity. Volumetric bone mineral density was estimated using BMC adjusted for BA, height and weight.

Results

There were 380 children (197 boys) with 19-34 week fetal ultrasound and 4 year DXA measurements. There were strongly statistically significant correlations between 19 to 34 week femur growth velocity and indices of skeletal size but not volumetric density (BA: $r=0.30$, $p<0.0001$; vBMD: $r=0.03$, $p=0.51$). Conversely, growth velocity of abdominal circumference was positively associated with indices of volumetric bone density more strongly than bone size (BA: $r=0.06$, $p=0.21$; vBMD: $r=0.15$, $p=0.004$). Both fetal measurements were positively associated with BMC and aBMD, indices influenced by both size and density ($r=0.15$-0.29, $p<0.005$). These associations persisted after controlling for maternal and childhood dietary, lifestyle and anthropometric factors.

Discussion

In our study femur length growth velocity from 19 to 34 weeks gestation predicted childhood skeletal size at 4 years whereas growth velocity of fetal abdominal circumference (a measure of liver volume and adiposity) predicted volumetric density. These results suggest a possible discordance between influences on volumetric density and skeletal size, and that tracking of skeletal growth has its origins in utero.

P012

Maternal dietary patterns during pregnancy and childhood bone mass: a longitudinal study

Z Cole[1], *C Gale*[1], *MK Javaid*[1], *SM Robinson*[1], *C Law*[2], *B Boucher*[3], *SR Crozier*[1], *KM Godfrey*[1], *EM Dennison*[1], *C Cooper*[1]

[1]MRC Epidemiology Resource Centre, Tremona Road, Southampton, SO16 6YD, United Kingdom; [2]Insitute of Child Health, University College London, London, United Kingdom; [3]Centre for Diabetes and Metabolic medicine, Barts and the London MDS, Queen Mary, London, United Kingdom

Introduction

Background: Epidemiological studies have suggested that early environment influences the skeletal growth trajectory and Peak bone mass accrual. Traditionally maternal diet has been considered in terms of intake of specific nutrients and mother offspring cohort studies have pointed to a potential role for insufficient maternal intake of calcium and poor vitamin D status. however these nutrients comprise parts of broader dietary patterns, which have not been explored in relation to skeletal health in the offspring. We therefore investigated the effects of maternal dietary patterns during pregnancy on the bone mass of her offspring at aged 9 years.

Materials and Methods

Methods: In a longitudinal study, we studied 198 children who were measured at birth and during infancy and for whom maternal anthropometric and lifestyle variables were ascertained during early and late pregnancy in a cohort from Southampton, UK. The nutritional status of their mothers was recorded in early and late pregnancy using a validated food frequency questionnaire (FFQ) and dietary scores were derived using principal component analysis. A higher, more prudent, diet score was associated with high intakes of fruit and vegetables, wholemeal bread, rice and pasta, yoghurt and breakfast cereals. The children were followed up at aged 9 years when detailed anthopometric data was collected and assessment of whole body bone mineral performed using a Hologic Discovery DXA instrument

Results

Results: Mothers with a a higher prudent dietary score during late pregnancy had children with significantly increased whole body BMC (r=0.17, p=0.02) aBMD (r = 0.14, p=0.05) and bone area (r =0.18, p=0.02)at aged 9 years. This relationship remained significant after adjustment for the child's height, weight, arm circumference maternal smoking, vitamin D status of the mother at 36 weeks gestation, social class and birthweight. Associations with prudent diet score in early pregnancy were much weaker.

Discussion

Conclusions: This is the first study to show that a well balanced healthy diet in pregnancy, as measured by principle component analysis is associated with increased bone size, BMC and BMD aged 9 years. Further work to replicate these findings is now indicated

P013

Early growth only partially predicts fetal skeletal size in late pregnancy

SC Earl[1], *PA Mahon*[1], *SM Robinson*[1], *MK Javaid*[2], *SR Crozier*[1], *HM Inskip*[1], *KM Godfrey*[1], *EM Dennison*[1], *C Cooper*[1], *N Harvey*[1]

[1]MRC Epidemiology Resource Centre, Southampton General Hospital, Southampton, SO16 6YD, United Kingdom; [2]Botnar Research Centre, University of Oxford, Oxford, United Kingdom

Introduction

Birth weight is mainly dependent on nutrient supply and maternal constraint in the second half of pregnancy. However, there are few data relating growth in this latter period to growth in the first half of pregnancy. We measured the correlation between fetal femur length, assessed by ultrasound, at 19 and 34 weeks gestation.

Materials and Methods

Participants were recruited from the Southampton Women's Survey, a unique prospective cohort of 12,583 initially non-pregnant women aged 20-34 years, resident in Southampton UK. These women were studied in detail before and during pregnancy. Measurement of fetal femur length was performed at 19 and 34 weeks gestation using ultrasound. Royston models were fitted to fetal measurements to create Z-scores for size. Pearson correlation and regression methods were used to investigate the relationship between size at 19 and 34 weeks.

Results

There were 482 children (242 boys) with 19-34 week fetal ultrasound measurements. There was a positive statistically significant relationship between femur length measured at 19 and 34 weeks (r=0.41, p<0.001). Thus 19-week femur length accounted for 17% of the variance in the 34-week measure.

Discussion

In our study 19-week femur length (at the end of the first half of pregnancy) accounted for only 17% of the variance in femur length measured at 34 weeks. This suggests that growth in the second half of pregnancy is only partly determined by that in the first half, consistent with the known effect of maternal constraint and environmental factors. Further work is underway to relate these fetal growth patterns to post-natal skeletal development.

P014

Musculoskeletal injury and disease – potential answers from development?

SJ Ainsworth[1], *R Stanley*[1], *V Perugini*[1], *DJR Evans*[1]

[1]Brighton & Sussex Medical School, Biology Road, Falmer, East Sussex, BN[1]9PX, United Kingdom

Introduction

The functional integrity of the musculoskeletal system relies on the coordinated interaction of constituent tissues. Damage to one or more of these tissues as a result of either an accumulation of age/exercise-related microdamage or a progressive disease process, has become an increasing clinical problem. This is exacerbated by the inability of some musculoskeletal tissues, namely cartilage and tendon to repair themselves or provide renewed structural integrity. Our group has focussed on these tissues during development and our previous studies suggest the cells forming both these tissues are lineage-related, with both tenocytes and chondrocytes able to contribute to developing

tendon and cartilage (1,2). The current study focuses on the capacity and tendency of specific cell populations to form cartilage or tendon when challenged by new environments.

Materials and Methods

Cranial neural crest cells, which readily give rise to a number of musculoskeletal tissues including cartilage and tendon in the head were isolated as explants, tagged with a LacZ-encoded replication-deficient retroviral vector and grafted into developing chick limb buds at different stages. Subsequent activity of the gene product was monitored histochemically in wholemount and tissue sections in all musculoskeletal tissues.

Results

Labelled chondrocytes were clearly identified in developing cartilage, classified by morphology and phenotypic assays, however labelling within tendons was rarely seen, suggesting cranial neural crest derived cells (CNCDC) are able to contribute to developing cartilage, but are less likely to contribute to tendon.

Discussion

The local environment may therefore have played a role in determining the potential plasticity of implanted cells. Our recent studies examining the appearance of ossified tendons during development suggest that it is the surrounding matrix that is likely to regulate the fate of tenocytes and drive them down a chondrocytic route. Therefore by altering the environment appropriately we may provide clues as how to manipulate cells in diseased or injured tissues to improve the potential for repair.

REFERENCES

1. Oldfield SF, Evans DJR. 2003. Tendon morphogenesis in the developing avian limb: plasticity of fetal tendon fibroblasts. J. Anat. 202:153-164.

2.Dowthwaite GP et al. 2004. The surface of articular cartilage contains a progenitor cell population. J. Cell Sci. 117:889-897.

P015

Pseudoachondroplasia resulting from a C-terminal COMP mutation is associated with a mild myopathy and abnormal changes in tendon and ligament

KA Pirog[1], Y Katakura[2], O Jaka[1], RS Meadows[1], MD Briggs[1]

[1]Wellcome Trust Centre for Cell Matrix Research (WTCCM) Faculty of Life Sciences University of Manchester UK, Manchester, M13 9PT, United Kingdom; [2]School of Material Sciences Faculty of Engineering University of Manchester UK, Manchester, United Kingdom

Introduction

Pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) are relatively common skeletal dysplasias belonging to a single skeletal dysplasia family and comprise a disease spectrum with an overlap between mild PSACH and severe MED. PSACH is an autosomal dominant skeletal dysplasia characterized by generalised epimetaphyseal dysplasia, joint laxity, short-limbed dwarfism and early onset osteoarthritis. PSACH and some forms of MED result from mutations in cartilage oligomeric matrix protein (COMP), a large pentameric glycoprotein found in cartilage, tendon, ligament and skeletal muscle. Some PSACH-MED patients suffer from a mild myopathy characterized by muscle weakness, difficulty standing up, mildly increased creatine kinase (CK) levels or small atrophic fibers in the muscle biopsy. Indeed, mild PSACH-MED patients are often referred to the neuromuscular clinic prior to the diagnosis of skeletal dysplasia. Unfortunately, a detailed study of the PSACH-MED musculoskeletal phenotype has never been undertaken, primarily due to the lack of suitable pathological samples.

Materials and Methods

T585M COMP-CTD knock-in mice (a model of mild PSACH) were used in this study. Muscle weakness was assessed at various ages using a digital force gauge. COMP expression was analysed by qRT-PCR and Western blotting. Skeletal muscle and tendon were analysed using various histological techniques. Collagen fibers in tendons/ligaments were visualised using transmission electron microscopy and proteoglycan content was analysed by DMMB assay. Tendons were submitted to tensile and cyclic testing using Instron 1122.

Results

Mutant mice exhibited a progressive mild muscle weakness and a mild myopathy localized to myotendinous junction. The expression and localisation of important skeletal muscle and tendon proteins was not dramatically altered. Interestingly, both the diameters and biomechanical properties of collagen fibers in the mutant tendons and ligaments were altered when compared to the wild type controls.

Discussion

The differences in the biomechanical and ultrastructural properties between the wild type and mutant tendons and ligaments provide a rationale for the myopathy localised to the myotendinous junction and for the ligamentous laxity seen in the PSACH-MED patients. This is the first comprehensive characterisation of the musculoskeletal phenotype of the PSACH-MED dysplasias and it may have a profound effect on the clinical management of the PSACH-MED patients in the future.

REFERENCES

Piróg-Garcia KA et al. "Reduced cell proliferation and increased apoptosis are significant pathological mechanisms in a murine model of mild pseudoachondroplasia resulting from a mutation in the C-terminal domain of COMP" Hum Mol Gen 2007; 16(17):2072-88

P016

Novel chondroitin sulphation motif expression delineates specific regions of musculoskeletal tissue differentiation in early human and chick limb development

S Li[1], A Bartlett[1], AJ Hayes[1], J Melrose[2], S Smith[2], CB Little[2], CE Hughes[1], B Caterson[1]

[1]Connective Tissue Biology Lab., Cardiff University, Cardiff, CF10 3US, United Kingdom; [2]Purves Bone & Joint Institute, Royal North Shore Hospital, University of Sydney, St Leonards, NSW, 2065, Australia

Introduction

Previous studies in our laboratory have used monoclonal antibodies (mAbs) that recognise CS sulphation motifs in the developing Bursae of Fabricus in the embryonic chicken (1) to identify putative stem/progenitor cell niches in the superficial/surface zone of bovine hyaline articular cartilage (2). Here we use these mAbs to study the differential expression of CS sulphation motifs in musculoskeletal connective tissues from the developing embryonic human and chick limbs.

Materials and Methods

Knee joint rudiments from 12-14 week human foetuses and embryonic chick limbs from foetal days 4 – 18 were fixed, processed into paraffin, sectioned and immunoperoxidase-stained with mAbs 3B3(-), 7D4, 4C3 or 6C3.

Results

Human Limbs - All three CS sulphation motif epitopes localised prominently at sites of incipient articular cartilage formation at a stage before there was any histological evidence of secondary ossification at the epiphysis. Their staining was detectable in very defined regions within the perichondrium; growth plate; fibrocartilage of both meniscus and enthesis; vasculature; and at sites of capillary invasion, with subtle differences in their distribution.

Chick Limbs - Differential patterns of 3-B-3(-) and 6-C-3 staining were observed at all developmental stages (E4 – E18) in locations where articular cartilage, meniscus, growth plate, ligament, bone and skin (feathers) were proceeding through different stages of development in to mature functional tissues.

Discussion

These results from two evolutionary distinct species show similarities in labelling patterns in a range of different developing/differentiating musculoskeletal tissues. The CS-labelling pattern in the human limb at the developing articular surface was intriguing as this closely matched similar findings in bovine articular cartilage (2). We believe that these CS sulphation motif patterns are part of a complex extracellular matrix milieu that contributes to the production of gradients of signalling molecules needed for proliferation/differentition events during development.

REFERENCES

Sorrell, J.M. et al (1988) J. Immunology 140:4263-4270

Hayes AJ et al (2008). J. Histochem. Cytochem 56: 125 – 128

P017

Temporal and spatial expression of MMP-14 during endochondral ossification in mice

KBS Paiva[1], JM Granjeiro[2], FD Nunes[1]

[1]Molecular Pathology Laboratory, Department of Oral Pathology, University of São Paulo Avenida Professor Lineu Prestes 2227, São Paulo, 05508-000 - São Paulo - SP, Brazil; [2] Depto Molecular and Cellular Biology Institute of, Biology Federal Fluminense University Niterói, Rio de Janeiro, Brazil

Introduction

MMPs are zinc-dependent endopeptidases that degrade all components of the extracellular matrix (ECM). The membrane-anchored MMPs (MT-MMP), containing an anchoring motif that facilitates to reach key membrane and peripheral proteins as well as closely associated ECM components thereby playing a pivotal role during alterations of the pericellular environment in both physiological and pathological conditions.

Membrane insertion confers MT-MMPs with a unique set of regulatory mechanisms that serve to control the pool of active protease at the cell surface including endocytosis, recycling, autocatalytic processing, and ectodomain shedding. MMPs and their inhibitors are responsable for bone matrix remodeling and, probably, determinate the level of its turnover. Thus, our aim was to evaluate the temporal-spatial expression of MMP-14 in mice embryos during endochondral ossification.

Materials and Methods

Femurs (n=5/period) were collected from foetuses (E13-E20) and newborns (PN1) and performed by avidin-biotin-immunoperoxidase technique in formalin-fixed paraffinembedded and by Real-Time PCR (SYBR Green Dye). We used GAPDH as housekeeping gene, E13 as reference sample and comparative Ct method for analysis.

Results

During cellular and vascular invasion (E15), MMP-14 immunostaining was observed in primary medullar cavity (osteoblast-like and/or osteclast/chondroclast-like cells) and

perichondrium. At later stages, this enzyme was restrict to osteoblasts at growth plate.

No difference in mRNA levels was verified in early stages (E13-E15).

Discussion

Taken together, our results show same evidences that MMP-14 may be regulated at enzymatic activity instead transcriptional level in early stages of endochondral ossification.

P018

Beukes Hip Dysplasia segregates with a mutation identified in the UFM1-specific peptidase 2 gene, UFSP2

CM Watson[1], P Beighton[2], R Ramesar[2], JBJ van Meurs[3], R Donn[4], G Wallis[1]

[1]Wellcome Trust Centre for Cell-Matrix Research, The School of Translational Medicine, The University of Manchester, Oxford Road, Manchester, M13 9PT, United Kingdom; [2]Division of Human Genetics, University of Cape Town, Faculty of Health Sciences, South Africa; [3]Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; [4]The School of Translational Medicine, The University of Manchester, Oxford Road, Manchester, M13 9PT, United Kingdom

Introduction

The autosomal dominant disorder, Beukes Hip Dysplasia (OMIM: 142669), results in premature onset osteoarthritis as described previously by Cilliers and Beighton (1990). Originally identified in an Afrikaner family in South Africa the disorder has been linked to chromosome 4q35, Roby et al. (1999). The reported locus spans a 2.79 Mb region encompassing 28 genes and is defined by markers D4S1535 and D4S3051.

Materials and Methods

Single nucleotide polymorphisms identified within the family were genotyped by direct sequencing and restriction fragment length polymorphism analysis. Following fine mapping candidate genes were screened for mutations. This included the UFM1-specific peptidase 2 (UFSP2) gene. Wild type and mutant recombinant UFSP2 and its substrate, ubiquitin-fold modifier 1 (UFM1), were expressed in bacterial expression systems and affinity purified. In vitro protease cleavage assays were performed and analysed by coomassie blue staining. Expression of UFSP2 mRNA was assayed in differentiated ATDC5 cells.

Results

Three SNPs indicated points of recombination thus decreasing the extent of the linked allele. The refined linkage peak spanned 1.3 Mb of sequence which encompassed 18 genes. Sequencing of UFSP2 revealed a heterozygous thymine to cytosine transition within exon 8 and the single point LOD score for the T > C sequence change was 10.4. The mutation predicted a tyrosine to histidine amino acid substitution and sequence alignments between multicellular organisms indicated the tyrosine residue was highly conserved. To ensure the mutation was not present in an unaffected population 360 Dutch individuals were screened. Using an in vitro protease cleavage assay it was determined mutant UFSP2 was unable to cleave it substrate, UFM1. Preliminary studies in the chondrogenic ATDC5 cell line suggest

a correlation between UFSP2 mRNA expression and chondrogenesis.

Discussion

To determine the role of the UFSP2 Y290H mutation in the aetiology of BHD further functional studies are being pursued.

REFERENCES

Cilliers H. & Beighton P. (1990) Beukes familial hip dysplasia: an autosomal dominant entity. Am. J. Med. Genet. 36, 386-390.

Roby P., Eyre S., Worthington J., Ramesar R., Cilliers H., Beighton P., Grant M. & Wallis G. (1999) Autosomal dominant (Beukes) premature degenerative osteoarthropathy of the hip joint maps to an 11-cM region on chromosome 4q35. Am. J. Hum. Genet. 64, 904-908.

P019

Chondroitin sulphation motifs identify putative chondroprogenitor cells in human osteoarthritic cartilage for use in ACT technologies

S Mukhopadhyay[2], S Li[1], AJ Hayes[1], CE Hughes[1], MA Nowell[2], B Caterson[1]

[1]School of Biosciences, Cardiff University, Cardiff, CF10 3AX, United Kingdom; [2]School of Medicine, Cardiff University, Cardiff, CF10 3AX, United Kingdom

Introduction

Previous studies have reported "chondrocyte clusters" present in osteoarthritic cartilage can be identified with monoclonal antibodies (mAbs) that recognise chondroitin sulphate (CS) sulphation motif epitopes (Visco et al, 1988; Slater et al 1990). Recent studies have also indicated that these same mAbs can identify putative stem/progenitor cells in immature bovine articular cartilage (Hayes et al, 2008). The objective of this study was to use these mAbs to isolate these chondrocyte clusters from pathological articular cartilage obtained from patients with osteoarthritis (OA).

Materials and Methods

Histologically graded severely degenerative cartilage samples were harvested from patients undergoing total knee replacement with ethical approval. Chondrocytes were isolated using rapid sequential digestion using pronase and collagenase with close monitoring in order to maximize the optimal harvesting. These digestion times ranged from 2 to 3 hours. Harvested chondrocytes were fluorescently labelled using mAbs 3B3, 7D4, 4C3 (1: 100) and analysed by flow cytometry.

Results

By carefully monitoring digestion, very good yields of viable chondrocytes were obtained for FACS. Analyses showed that each of the mAbs displayed a specific and overlapping sorting pattern suggestive of the presence of multiple CS sulphation motifs on some of the chondrocyte subpopulations isolated from these "clusters". We speculate that these subpopulations represent different/intermediate stages of chondroprogenitor cell differentiation; e.g. stem and progenitor cells preceding mature chondrocytes.

Discussion

These mAbs have been shown to identify cell surface markers on putative stem/progenitor cells. The significance of identifying a subpopulation of cells in degenerative human cartilage suggests the possibility of the presence of stem/progenitor cell niches in OA human cartilage that are possibly attempting to repair the cartilage in the adverse physiological conditions present in the pathogenesis of OA. In future studies, these mAbs will be used to sort these stem/progenitor cells, culture expand them ex vivo and use these to produce cartilage grafts in vitro for use in new generation ACT technologies.

REFERENCES

Visco et al (1993) Arthritis & Rheumatism 36, 1718; Slater et al (1995) Arthritis & Rheumatism 38, 655; Hayes et al (2008) J. Histochem. Cytochem 56: 125

P020

Fracture healing in GDF-5 deficient mice

CM Coleman[1], BH Scheremeta[2], A Boyce[3], RL Mauck[4], RS Tuan[5]

[1]Regenerative Medicine Institute, National University of Ireland Galway, Galway City, 00000, Irish Republic; [2]Department of Pediatrics, University of Maryland, Baltimore, 21201, USA; [3]National Institutes of Health, Division of Musculoskeletal Diseases, Bethesda, 20891, USA; [4]McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, 19104, USA; [5] National Institutes of Health, National Institute of Arthritis Musculoskeletal and Skin Diseases, Bethesda, 20892, USA

Introduction

Growth differentiation factor-5 (GDF-5) is expressed during the chondrogenic phase of embryogenesis, adult endochondral fracture repair and in healthy adult articular cartilage. In this study, we investigated tibial fracture healing in the GDF-5-deficient brachypod (bp) mouse to determine the role of GDF-5 in fracture healing by assessing the effect of impaired chondrogenesis on skeletal repair. These results may be extrapolated to enhance repair in clinically challenging situations such as non-union long bone fractures.

Materials and Methods

To address the shortened length characteristic of the bp tibiae, a novel surgical procedure was devised employing bone dissection scissors to create an osteotomy and two pins, intramedullary and extramedullary, to realign the bone. Tibae were assayed at 1, 5, 10, 14, 21, 28 and 56 days post fracture via radiographs, histologically using safranin-o/fast green staining, and mechanically by evaluating yield stress and Young's Modulus. Additionally the callus-cross sectional area and the composition of the callus (including cartilage, organized bone and woven bone) were evaluated.

Results

These data indicate that a deficiency in GDF-5 bioavailability impaired the chondrogenic phase of fracture repair as demonstrated by reduced deposition of cartilaginous matrix in the callus, increased population of granular tissue in the fracture site and a reduced callus cross sectional area. However, with time the repaired bp fracture was as mechanically sound as controls as demonstrated by yield stress and Young's modulus.

Discussion

Post-inflammatory fracture healing recapitulates embryonic endochondral ossification. Defects in this process reappear as defects in fracture repair. Here we demonstrate that GDF-5 deficiency alone does not impair fracture healing over time. However, a delay in callus formation, a reduction in chondrogenesis in the soft callus and altered callus remodeling demonstrate a role for GDF-5 in the chondrogenic phase of healing. No differences in mechanical integrity were observed between control and bp mice. An increase in granular tissue in the bp mice may impart the mechanical stability normally provided by cartilage. GDF-5 deficiency therefore impairs chondrogenesis during fracture healing, though ultimately the bones heal similarly to controls. GDF-5 is therefore a potent activator of chondrogenesis and subsequent bone formation, making it an interesting direction for future investigations.

P021

Expansion on a hyaluronan coated surface enhances the chondrogenic potential of human mesenchymal stem cells

C Ryan[1], C Coleman[1], M Murphy[1], F Barry[1]

[1]REMEDI, NCBES, NUI Galway, Galway, 0000, Irish Republic

Introduction

Degenerated cartilage as seen in osteoarthritis (OA) does not characteristically undergo repair, but progressively deteriorates. Hyaluronan (HA) is a major component of synovial fluid and cartilage matrix and serves to lubricate the joint. Although HA therapy results in pain relief for OA patients, its mode of action and capacity as a regenerative agent remains unclear. This study investigates the in vitro effect of HA on the chondrogenic differentiation of human mesenchymal stem cells (hMSCs), and the hypothesis that expansion on a HA coated surface increases the chondrogenic potential of hMSCs.

Materials and Methods

hMSCs isolated from the bone marrow of the iliac crest were seeded onto cell culture surfaces coated with 1mg/ml of either HA-A, HA-B or Chondroitin Sulphate. Alternatively, hMSCs were cultured with 1mg/ml soluble HA-A or HA-B. On day 7, the expression of chondrocyte marker genes Sox9, Agg and ColII was determined by real-time RT-PCR.

hMSCs exposed to HA for 7 days were induced towards chondrogenesis by pellet culture and 10ng/ml TGF-beta3. Glycosaminoglycan (GAG) content was visualised by staining with Safranin O and quantified by DMMB assay on day 21.

Results

Sox9 gene expression was up-regulated 2.5-fold in hMSCs expanded on HA-A and 5.8-fold on HA-B. While the expression levels of Agg and ColII were unchanged in hMSCs expanded on HA-A for 7 days, there was a 2.4-fold increase in Agg and a 4.8-fold increase in ColII expression in hMSCs expanded on HA-B. In contrast, there was no significant change in the expression of these chondrogenic marker genes in hMSCs cultured with soluble HA. Sox9 was upregulated in hMSCs expanded on chondroitin sulphate, but expression of both Agg and ColII were downregulated. Similarly, the GAG/DNA ratio was increased in chondrogenic pellet cultures of hMSCs expanded on HA, and unchanged in other treatment groups.

Discussion

Expansion on a HA-coated surface increased the chondrogenic potential of hMSCs. The increased expression of chondrocyte marker genes and GAG/DNA ratio observed was specific to un-sulphated GAGs and surface bound HA. This potentially represents a valuable tool for increasing the chondrogenic potential of hMSCs, and an option for clinical cartilage regeneration.

P022

Clonal mesenchymal stem cell populations derived from the synovial fat pad exhibit a similar cell surface characterisation profile but variable osteogenic and chondrogenic differentiation potential

WS Khan[1], SR Tew[1], A Adesida[1], JG Andrew[1], TE Hardingham[1]

[1]UK Centre for Tissue Engineering Faculty of Life Sciences University of Manchester Oxford Road, Manchester, M13 9PT, United Kingdom

Introduction

Mesenchymal stem cells are a potential source of cells for the repair of bone and articular cartilage defects. We have previously demonstrated that the infrapatellar synovial fat pad is a rich source of mesenchymal stem cells and these cells are able to undergo osteogenic and chondrogenic differentiation (Khan et al, 2008; Khan et al, in press). Although synovial fat pad derived mesenchymal stem cells may represent a heterogenous population, clonal populations derived from the synovial fat pad have not previously been studied.

Materials and Methods

Mesenchymal stem cells were isolated from the infrapatellar synovial fat pad of a patient undergoing total knee arthroplasty and expanded in culture. Six clonal populations were also isolated before initial plating using limiting dilution and expanded. The cells from the mixed parent population and the derived clonal populations were characterised for stem cell surface epitopes, and then cultured in osteogenic medium for 21 days and as cell aggregates in chondrogenic medium for 14 days. Gene expression analyses; alizarin red staining; alkaline phosphatase, glycosoaminoglycan and DNA assays; and immunohistochemical staining were determined to assess osteogenic and chondrogenic responses.

Results

Cells from the mixed parent population and the derived clonal populations stained strongly for markers of adult mesenchymal stem cells including CD44, CD90 and CD105, and they were negative for the haematopoietic marker CD34 and for the neural and myogenic marker CD56. Interestingly, a variable number of cells were also positive for the pericyte marker 3G5 both in the mixed parent and clonal populations. The clonal populations exhibited a variable osteogenic and chondrogenic response; two clonal cell populations exhibited a significantly greater osteogenic response, and one clonal cell population exhibited a significantly greater chondrogenic response when compared with the mixed parent population.

Discussion

Pericytes are a candidate stem cell in many tissue and our results show that all six clonal populations derived from the heterogenous synovial fat pad population express the pericyte marker 3G5. The variable osteogenic and chondrogenic responses suggest inherent differences between these populations. The osteogenic and chondrogenic potential of the synovial fat pad could be optimised by the identification of clonal populations with a propensity to differentiate down particular differentiation pathways.

REFERENCES

Khan W.S., Tew S.R., Adesida A.B. & Hardingham T.E. (2007) Human infrapatellar fat pad-derived stem cells express the pericyte marker 3G5 and show enhanced chondrogenesis after expansion in fibroblast growth factor-2. Arthritis. Res. Ther. 10, R74.

Khan W.S., Adesida A.B., Tew S.R. & Hardingham T.E. The epitope characterisation and the osteogenic differentiation potential of human fat pad-derived stem cells is maintained with ageing in later life. Injury. (in press).

P023

Evaluation of bone marrow mesenchymal stem cell therapy for tendon regeneration in a large animal model

NJ Young[1], J Dudhia[1], AE Goodship[2], RK Smith[1]

[1]Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA, United Kingdom; [2]Institute of Orthopaedics and Musculoskeletal Science, University College London, Brockley Hill, Stanmore, Middlesex, HA7 4LP, United Kingdom

Introduction

Exercise-induced tendinopathy is extremely common in both human and equine athletes yet no good treatment options are available. Healing results in a poorly functional and disorganised scar tissue that reduces performance and leads to high re-injury rates. Mesenchymal stem cells (MSC's) offer the potential for tendon regeneration and autologous implantation of MSCs into injured equine superficial digital flexor tendons (SDFT) has been used clinically with significant improvement of re-injury rate. However, a systematic evaluation of efficacy of repair has not been reported. This study aimed to establish whether MSC implantation could induce regeneration of normal tendon matrix.

Materials and Methods

SDFT's were removed from horses that were treated 6 months previously with either autologous MSC implantation or saline injection into the site of maximal injury. Tissue samples were taken from the site of implantation and a site proximal to the injury from the treated and contralateral tendon. Matrix organisation was assessed histologically and scored for cellularity, vascularity and organisation between grade 0 (normal) to 3 (markedly abnormal) and compositional analyses by tissue-linked fluorescence, DNA, glycosaminoglycan, total collagen and water content.

Results

MSC-treated horses had lower scores for cellularity and organisation at the injured site compared to the saline-treated group, and was comparable to the uninjured site of the treated tendon. Tissue-linked fluorescence, DNA and water content of the MSC-treated injured site were similar to uninjured sites and contralateral limbs. The MSC-treated injured site had higher total collagen and lower total glycosaminoglycan content compared to uninjured sites and the saline-treated group.

Discussion

The MSC-treated regions of SDFT were morphologically similar to both the uninjured site of the same tendon and the contralateral limb with evidence of lower cellularity and good linear organisation. The low glycosaminoglycan and high collagen content are also indicative of normal tendon matrix. The increased collagen content of the MSC-treated group may be new matrix synthesis although further assessment will determine the collagen type I/III ratio to indicate a normal or fibrotic matrix. These preliminary findings suggest MSC implantation results in a tissue more closely resembling that of normal tendon matrix rather than fibrous scar tissue formed subsequent to natural repair.

P024

The role of Ceramide in programmed cell death and autophagy in osteoblasts

R Al-Dabbagh[1], D Weekes[1], F McDonald[1], A Grigoriadis[1], P Hill[1]

[1]Orthodontics and Craniofacial Development, Floor 27 Guy's Hospital, London, SE[1]9RT, United Kingdom

Introduction

The molecular mechanisms underlying altered osteoblast activity in metabolic bone diseases can be explained, in part, by changes in osteoblast programmed cell death (PCD). Ceramide, the precursor to all complex sphingolipids, is an important secondary messenger that has been demonstrated to play a role in PCD in a number of tissues. However, the potential role of ceramide in PCD in bone and its mechanisms of action have not yet been studied.

Materials and Methods

The MTT assay was used to test the effects of ceramide and TNF-alpha on viability, FACS analysis to quantify cell death after staining with Annexin V, FLICA labelling, the caspase inhibitor DEVD-CHO and western blots to evaluate the mechanistic involvement of caspase. We further assessed the activation of autophagy by TEM, immunofluorescence microscopy and western blot analysis.

Results

We previously demonstrated that exogenous C2-ceramide, but not its inactive precursor dihydroceramide, reduced MC3T3-E1 osteoblast viability in a dose-dependent manner, and that primary osteoblasts were 5 times more sensitive to low doses of C2-ceramide. Further analysis of caspase activity and cell death measurement, demonstrated that this reduction in viability was due to induction of caspase-dependent apoptotic cell death. We are currently identifying the specific caspases that are mediating the apoptotic effect, and preliminary data suggest that caspases-3 and -7 are not involved. Furthermore, ceramide elevated the expression of the autophagy proteins beclin1 and ATG5, and cleaved LC3 I to its active form LC3 II. These data suggest that ceramide activates autophagy in osteoblasts. We have also demonstrated that synthesis of endogenous ceramide is required for TNF-alpha and FGF1-induced cell death in both MC3T3-E1 and primary osteoblasts. Pretreatment with chloropromazine (an acid sphingomyelinase inhibitor) and Fuminosine B1 (an inhibitor of de novo ceramide synthesis) showed full rescue of both TNF-alpha and FGF1-induced cell death. In addition, TNF-alpha-treated primary osteoblasts exhibited autophagosomes typical of autophagic cell death.

Discussion

This work has highlighted a novel role for ceramide in inducing caspase-dependent osteoblast PCD. Therefore, the use of ceramide, or inhibitors of its synthesis, could be utilised as a therapeutic tool for modulating osteoblast survival in different metabolic bone diseases.

P025

Protection of osteocytes and osteoblasts from apoptosis by bisphosphonates

X Duan[1], R Locklin[1], FH Ebetino[2], RGG Russell[1], P Hulley[1], et al.

[1]Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford Nuffield Orthopaedic Centre Headington, Oxford, OX3 7LD, United Kingdom; [2]New Drug Development, Procter and Gamble Pharmaceuticals, Mason, OH 45040, USA

Introduction

Studies over the years have shown that the pharmacological potencies of bisphosphonates (BPs) are dependent both on their binding affinities for bone mineral and on their inhibitory actions on osteoclasts. However, other groups have reported protective effects on osteocytes with sub micromolar concentrations of BP, thought to involve novel membrane actions of the BPs on connexin 43 hemi-channels. We examined the ability of risedronate (a nitrogen-containing BP) and etidronate (a simple BP) to exert anti-apoptotic effects on osteocytes and osteoblasts and to activate prosurvival signalling pathway in these cells.

Materials and Methods

Risedronate (10-8M) and 20% Fetal Calf Serum were given to serum-starved osteocytic MLOY4 cells and osteoblastic 2T3 cells. Following a 5, 30 or 60 minute incubation, the cells were harvested and the cell lysates were used for Western blotting to evaluate activation of the ERK and PKB/Akt pathways.

MLOY4 cells and 2T3 cells in normal growth medium were pretreated with risedronate and etidronate (10-8M and 10-7M, respectively), for 1 hour, prior to the addition of etoposide (50muM). After 6 or 24 hours, cells were harvested and Nick Translation assay, TUNEL staining and active caspase 3 immuno-staining were performed to quantify the extent of MLOY4 and 2T3 cell apoptosis. Cell morphology was also examined by the latter two techniques.

Results

Risedronate (10-8M) induced a rapid but subtle increase in phosphorylation of ERK in 2T3 cells within 5 minutes which then decreased to basal levels by 30 minutes. PKB was not activated.

Pretreatment with risedronate and etidronate (10-8M and 10-7M) for one hour before addition of etoposide (50muM) reduced apoptosis of both MLOY4 cells by an average of 20% and 2T3 cells by 37%, as shown by NT assay. Both bisphosphonates also reduced the percentage of cells exhibiting typical apoptotic morphology.

Discussion

Our results show that bisphosphonates protect osteocytes and osteoblasts from apoptosis induced by etoposide, regardless of the structure of the bisphosphonates. Further study is required to determine the best concentration to achieve maximal anti-apoptotic effects, which may involve the rapid phosphorylation of ERK but also some additional signaling pathways.

Funded by P&G and ARC

P026

Lactoferrin protects osteoblasts and osteocytes from apoptosis

R Locklin[1], P Hulley[1], D Naot[2], J Cornish[2]

[1]Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Nuffield Orthopaedic Centre Windmill Road, Headington, Oxford, OX3 7LD, United Kingdom; [2]Department of Medicine University of Auckland Private Bag 92019, Auckland, 1001, New Zealand

Introduction

Lactoferrin, an iron-binding glycoprotein found in milk and other body fluids, is known to have an anabolic effect on bone, accelerating bone formation, reducing osteoclastogenesis and increasing osteoblast proliferation, differentiation and survival. In this study we aimed to establish whether Lactoferrin can protect osteoblast-like and osteocyte-like cells from apoptosis induced by the chemotherapeutic agent Etoposide.

Materials and Methods

Murine osteoblast-like cells (2T3) or osteocytic cells (MLO-Y4) were cultured in monolayer until they reached 80% confluence and then treated for 1 hour with Lactoferrin at a range of concentrations followed by the addition of Etoposide for the next 6 or 24 hours. Cultures were then harvested and fixed for nick translation assay to measure apoptosis. To investigate possible signalling pathways, plates of cultured 2T3 cells were lysed for Western blot analysis at 5, 30 and 60 minutes after stimulation with Lactoferrin, and probed with antibodies to phospho-PKB and phospho-ERK. Results were normalised to levels of the appropriate total protein. In addition the MEK-ERK inhibitor U0126 and the PI3K/Akt inhibitor LY294002 were added in the presence of Lactoferrin and Etoposide to investigate the mechanism of protection.

Results

Pre-treatment with Lactoferrin for one hour, followed by treatment with Lactoferrin together with Etoposide for 24 hours was able to reduce the apoptosis caused by Etoposide by up to 60% in both osteoblastic and osteocytic cells. Short-term stimulation with Lactoferrin increased phosphorylation of ERK but not of PKB, indicating selective stimulation of this pro-survival pathway. However neither inhibitor blocked the protective effect of Lactoferrin in either cell type, although used at concentrations that clearly block ERK and PKB signalling in these cell lines.

Discussion

This is the first instance of Lactoferrin providing protection from apoptosis in osteocytes. It is also the first study showing protection from the effects of Etoposide, which is a very potent and non-selective apoptosis-inducing agent. Signalling studies implicate the ERK pathway, but additional prosurvival pathways may also be involved and further studies are required to establish the protective mechanism(s).

This study was supported by the International Investment Opportunities Fund (IIOF) of the New Zealand Foundation for Research, Science and Technology (JC, PH) and the Arthritis Research Campaign (PH).

P027

A novel in vitro bone callus model for distraction osteogenesis

CJ Chang[1], *JT Triffitt*[1], *PA Hulley*[1]

[1]Botnar Research Centre, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Windmill Road, Oxford, OX3 7LD, United Kingdom

Introduction

Distraction osteogenesis (DO) is a process whereby gradual, controlled displacement of surgically created skeletal fractures results in expansion of bone volume and lengthening of associated soft tissues. It is a special form of bone healing and regeneration that may be used for clinical reconstruction of skeletal deformities. Clinical research and studies with experimental animals in vivo have been very informative in describing the biological events occurring during DO. However, the precise cellular development and signalling effects of biomechanical loading in DO may be dissected out in more detail by using in vitro models. Here we present a novel, in vitro, three-dimensional mineral scaffold-based distraction model.

Materials and Methods

The model consists of two sections of a porous hydroxyapatite/tricalcium phosphate scaffold that are separated by a known, small but variable distance. The whole model is attached to a simple mechanical fixation and distraction device with pins or screws. A cell-seeded fibrin clot bridges the gap between the two scaffold sections to simulate the initial situation at the distraction gap, and this construct can be lengthened at a precise rate during the in vitro distraction process.

Results

By using confocal microscopy and a variety of additional imaging techniques, it is shown that mouse 2T3 osteoblasts proliferate actively in the fibrin clot and on the surfaces of the mineral scaffold. Alkaline phosphatase staining is evident after 3 days and increases over 7 days in culture. The use of a fluorescently labelled bisphosphonate (rhodamine-risedronate) allows imaging of the cell-mineral scaffold interface, and osteogenic cell migration, alignment and differentiation was observed following a series of daily 0.5 mm distractions.

Discussion

The observed cellular interactions following mechanical distraction in vitro show basic elements of cellular arrangement and osteogenic activity that are seen during DO in vivo. It is concluded that the novel model developed here is a promising method for dissecting out certain specific cellular and molecular activities observed in the mechanically activated tissues during distraction.

Funding for this study and the authors was generously provided by the Furlong Research Charitable Foundation, the Arthritis Research Campaign, and the National Institutes of Health (USA).

P028

Effects of chemotherapy, infection, and bisphosphonates on osteocytes in relation to the development of osteonecrosis (ON) during the treatment for childhood acute lymphoblastic leukaemia (ALL)

A Abdussalam[1], *C Elford*[2], *MEM Jenney*[1], *JW Gregory*[1], *BAJ Evans*[1]

[1]Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom; [2]Paediatric Oncology, Cardiff and Vale NHS Trust, Heath Park, Cardiff, CF14 4XW, United Kingdom

Introduction

Osteonecrosis (ON) is known to be a significant complication in children treated for acute lymphoblastic leukaemia (ALL). Its pathogenesis is unclear. We hypothesise that chemotherapy, in the presence of a systemic infective process, modulate osteocyte number and function, and that these changes are related to the development of ON.

Materials and Methods

We evaluated effects of chemotherapy, and the bacterial-derived endotoxin lipopolysaccaride (LPS), on cell numbers (MTS assay) of murine osteocytes (MLO-Y4s). Apoptosis (FACS), and secretion (ELISAs) of IL-6 (inflammatory mediator) and VEGF (angiogenesis factor) were also quantified. Furthermore, we studied the effect of osteocyte conditioned-medium (OCM) on osteoblast numbers. Since bisphosphonates may be used as treatment for ON in childhood, we also evaluated the effects of bisphosphonates on osteocytes in vitro. (*,**,***; $p< 0.05$, <0.01, <0.001).

Results

All chemotherapeutic agents variably decreased osteocyte numbers. The lowest concentrations reducing numbers were: dexamethasone (10-8M**), vincristine (10-8M***), methotrexate (10-7M**) and L-asparaginase; (0.125IU***). LPS alone had no effect, but with dexamethasone (10-8M) and LPS (100 ng/ml) together, the reduction was less than that with dexamethasone alone. All agents induced apoptosis, although different apoptotic patterns were observed with medium±phenol red±ribonucleosides/deoxyribonucleosides. Dexamethasone significantly decreased, whilst LPS significantly increased, IL-6 and VEGF concentrations (e.g. VEGF 8.62, 4.78* 14.66* ng/ml control, dexamethasone, LPS respectively). However, dexamethasone with LPS significantly increased IL-6 but did not modulate VEGF secretion. OCM significantly reduced osteoblast numbers (~30% reduction after 1 day). Alendronate (10-5M) and pamidronate (10-5M) significantly ($p<0.001$) reduced osteocyte numbers after 3 days by ~60%, whereas 10-6M of each significantly increased cell numbers (~10%; $p<0.05$), maybe by inhibiting apoptosis.

Discussion

All chemotherapy reduced osteocyte numbers. This was partly due to apoptosis, although the oestrogenic effect of phenol-red, and the presence of ribonucleosides/deoxyribonucleosides were implicated. Dexamethasone modulated IL-6 and VEGF secretion, but responses were changed in the presence of LPS. OCM assays indicated the presence of osteocyte to osteoblasts cross-talk. Bisphosphonates reduced osteocyte numbers at high concentrations, but increased numbers at a lower concentration. This work is of relevance to the treatment of ON in childhood ALL, but also to the aetiology of ON of the jaw seen in adults treated with bisphosphonates.

P029

The PPAR gamma agonist rosiglitazone reverses the persistent fibrotic phenotype of scleroderma fibroblasts

X Shi-wen[1], M Eastwood[2], CP Denton[1], A Leask[3], DJ Abraham[1]

[1]Centre for Rheumatology University College London (Royal Free Campus) Rowland Hill St., London, NW3 2PF, United Kingdom; [2]School of Biosciences University of Westminster 115 New Cavendish Street, London, W1W 6UW, United Kingdom; 3 Division of Oral Biology University of Western Ontario, ON, N6A 5C1, Canada

Introduction

The transcription factor peroxisome proliferator-activated receptor (PPAR) gamma plays an important role in controlling cell differentiation. The aim of the present study was to examine whether PPAR gamma expression was reduced in scleroderma (SSc) fibroblasts and whether PPAR gamma agonists could suppress the persistent fibrotic phenotype of SSc fibroblasts.

Materials and Methods

Fibroblasts (n=6) were obtained from control and SSc tissue. The effect of PPAR gamma agonist rosiglatizone (20uM) on the phenotype of normal and SSc fibroblasts was also assessed by functional assays. Cell migration was performed by in vitro scratch wounding assays. Proteins including PPAR gamma, alpha-smooth muscle actin (alpha-SMA), vinculing, connective tissue growth factor (CTGF) and collagen type 1 (Col-1) were examined by immunohistochemistry staining and/or Western blot analysis. The ability of rosiglatizone to influence matrix remodelling in 3-D collagen contraction models was also examined.

Results

PPAR gamma protein expression was significantly reduced in SSc fibroblasts compared to normal control ($p<0.05$). The expression of alpha-SMA, Col-1, CTGF and PPAR gamma protein expression were elevated SSc fibroblasts ($p<0.05$). Rosiglitazone treatment caused a suppression in alpha-SMA, type I collagen and CTGF protein expression in SSc fibroblasts, but an increase in PPAR gamma expression. Moreover, rosiglaitazone alleviated the enhanced ability of SSc fibroblasts to generate contractile forces across a fixed collagen gel lattice ($p<0.05$). In contrast Rosiglitazone profoundly decreased in vitro SSc fibroblasts migration following in vitro scratch wounding.

Discussion

Our studies examining the involvement in PPAR gamma in the function of SSc fibroblasts may have profound implications for pathological processes by contributing to our understanding of basic mechanisms regarding fibrogenesis. As a consequence, our results may have future therapeutic implications for the treatment of SSc.

P030

Thrombospondin 1 – a key mediator of matrix contraction depend on MEK/ERK in SSc pathogenesis

M Eastwood[2], Y Chen[2], X Shi-wen[1], CP Denton[1], CM Black[1], DJ Abraham[1]

[1]Centre for Rheumatology University College London, Royal Free Campus Rowland Hill St., London, NW3 2PF, United Kingdom; [2]School of Biosciences University of Westminster 115 New Cavendish Street, London, W1W 6UW, United Kingdom

Introduction

Thrombospondin 1 (TSP1) is a multifunctional matricellular glycoprotein and expressed de novo in many fibrosis disease processes. TSP-1 has been identified as an activator of TGF-beta which is known to promote various states of wound healing from cellular proliferation to chemotaxis and angiogenesis. Scleroderma (SSc) is a generalized disease of unknown etiology characterized by inflammation, autoimmune attack, and vascular damage, and often progressive fibrosis of the skin ad internal organs.

Materials and Methods

Normal and SSc fibroblasts (N=6) were pre-treated with TSP-1 blocking peptides, with or without TGF-beta and then cultured in three-dimensional, tethered floating fibroblast-populated collagen lattice (FPCL), 24 hours as fibroblasts attach, spread, migrate and differentiate into myofibroblasts. Some of cultured fibroblasts in FPCL system also were stimulated by 12 hours of mechanical force loading, which is similar in magnitude to that in skin wounds. After that the fibroblasts in collagen lattice were performed with Western blotting and immunofluoresce stain assay.

Results

TSP-1 blocking peptide significantly reduced the force activities of fibroblasts from normal and SSc groups, and also blocked the TGFbeta induced force activities in fibroblasts from both of groups ($p<0.05$). Western blot shown that TSP-1 blocking peptide reduced some matrix proteins expression, such as CCN2, integrin alpha3, integrin alpha5 and p-ERK kinase activities in fibroblasts from normal and SSc, and impaired TGFbeta induced the above matrix proteins over expression and p-ERK activities for fibroblasts in collagen lattice as well. We also found that after mechanical force loaded stimulating, TSP-1 expression and p-ERK activities were significantly increased in normal and SSc fibroblasts ($p<0.05$). To further inquire whether TSP-1 function is dependent on WEK/ERK signalling pathway, the fibroblasts were also individually pre-treated by antagonizing TGFbeta receptor type I alphaactivin linked kinase (ALK-5), ERK (U0126) with / without TGFbeta. The antagonizing regents blocked TGFbeta induced the cell force ability, and impaired TSP-1 expression and p-ERK activities.

Discussion

Our data suggest that TSP-1 plays a central role in controlling the response of fibroblasts to TGFbeta. TSP-1 is an important mediator for cell contraction in SSc pathogenesis which is dependent on WEK/ERK pathway.

P031

The Wiskott-Aldrich syndrome protein verprolin homologous (WAVE) 1 is essential to localize MT1-MMP at the leading edge of migrating cells

BK Wolters[1], K Sato-Kusubata[2], N Ito[1], S Suetsugu[3], T Takenawa[4], Y Itoh[1]

[1]The Kennedy Institute of Rheumatology, Imperial College London, 65 Aspenlea Road, London, W6 8LH, United Kingdom; [2]Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [3]Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; [4]Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan

Introduction

The Membrane-type 1 matrix metalloproteinase (MT1-MMP) plays a crucial role in promoting cellular invasion by degrading pericellular extracellular matrix (ECM). In this process, the localization of MT1-MMP to the leading edge of the cells is essential to create the migration path. However, its detailed mechanism is not clearly understood. To form the leading edge in migrating cells, the organisation of the actin cytoskeleton is essential and Wiskott-Aldrich syndrome protein verprolin homologous (WAVE) proteins are thought to play an important role in this process. In this study we have investigated a role of WAVE proteins in the localization of MT1-MMP on the cell surface.

Materials and Methods

Wild-type, WAVE1-null and WAVE2-null mouse embryonic fibroblasts (MEF) were transfected with MT1-MMP and its localization as well as the cytoskeletal organisation were analyzed. The WAVE1 gene was also knocked down in human fibrosarcoma, HT1080 cells, using specific siRNA and its effect on the MT1-MMP localization and cytoskeletal organisation were analyzed.

Results

The lack of WAVE1, but not WAVE2, impaired the localization of MT1-MMP to the plasma membrane (PM) in MEF and reduced the gelatine film degradation. Re-introducing WAVE1 into WAVE1-null MEF rescued this phenotype. The siRNA knock down of WAVE1 in the human HT1080 cells also reduced the MT1-MMP expression at the PM and decreased the gelatine film degradation. The analysis of the cytoskeleton in MEF and HT1080 cells lacking WAVE1 showed an altered microtubule organisation with an impaired extension of microtubule to the cell periphery and protrusions while the organisation of the actin cytoskeleton remained indistinguishable to wild-type MEF or control HT1080 cells. The microtubule phenotype was also recovered upon re-expression of WAVE1.

Discussion

Our results show that the localisation of MT1-MMP at the leading edge of migrating cells is dependent on a WAVE1-mediated organisation of the cytoskeleton. We hypothesize that the inhibition of WAVE1, causing a reduced MT1-MMP activity, might offer a new approach to control the MT1-MMP dependent cell invasion in cancer and rheumatoid arthritis.

P032

Spatial regulation of MT1-MMP controls tubulogenesis of epithelial cells in a 3D collagen matrix

S Bird-Weaver[1], B Wolters[1], N Ito[1], Y Itoh[1]

[1]Dept of Matrix Biology, Kennedy Institute of Rheumatology Division, Imperial College London, London, W6 8LH, United Kingdom

Introduction

Epithelial tubes are essential structures in multicellular organisms. However, the fundamental mechanism underlying the formation of such structures is still unclear. Previously it was reported that knock down of the MT1-MMP gene by anti-sense oligo nucleotides inhibited tube structure formation, but overexpression of the enzyme disrupted tube structure, resulting in formation of the large cysts. This suggests that MT1-MMP is essential for tube extension but that it needs to be regulated in order to form organised structures. In this study we investigated the regulation of MT1-MMP during tubulogenesis.

Materials and Methods

Madin-Darby Canine Kidney (MDCK) epithelial cells were cultured in the collagen gel with addition of hepatocyte growth factor (HGF) to see tubulogenesis. To study basal and apical localization of MT1-MMP, cells were cultured on the collagen gel in transwell chamber. Cells were then infected with recombinnat adenovirus and localization of MT1-MMP was analysed by confocal microscopy.

Results

Spatial regulation of MT1-MMP occurs during tubulogenesis in 3D collagen matrices. Polarized MDCK cells cultured on collagen gel exclusively localized MT1-MMP to the apical side of their plasma membrane, and the cells exhibited little collagen degradation at the basal side, although they were expressing MT1-MMP. Upon treating cells with hepatocyte growth factor (HGF), which stimulates tubulogenesis of MDCK cells in 3D collagen, MT1-MMP was localized to the basal side, resulting in efficient collagen degradation. Under this condition, epithelial polarity was maintained as tight junctions were intact, and GPI-anchored proteins, namely uPAR and MT4-MMP, were still exclusively localized to the apical side. In 3D collagen MT1-MMP localized to the basal side of cells that were extending into the collagen gel, but cells in non-extending parts of the structure did not localize MT1-MMP to their basal side.

Discussion

Our results indicate that temporal and spatial localization of MT1-MMP provides an additional dimension to post-translational regulation of the enzyme, and that such regulation is crucial for tubulogenesis of epithelial cells.

P033

CTGF plays a pivotal role in lung fibrosis through the MAPK dependent activation of Collagen Type I

M Ponticos[1], *X Shi-wen*[1], *K Khan*[1], *P Leoni*[1], *GE Lindah*[1], *DJ Abraham*[1]

[1]Department of Inflammation Centre for Rheumatology Royal Free and University College Medical School University College (Royal Free Campus), London, NW3 2PF, United Kingdom

Introduction

Connective tissue growth factor (CTGF, CCN2) is overexpressed in systemic sclerosis (SSc) and has been hypothesized to be a key mediator in this condition. CTGF known to be induced by transforming growth factor-beta (TGF-beta is a mediator of some profibrotic effects of TGF-beta in vitro. Here, we investigate the role of CTGF on enhanced collagen type I expression in bleomycin-induced lung fibrosis, and delineate mechanisms underlying the action of CTGF in this mouse model and in human pulmonary fibroblasts.

Materials and Methods

Bleomycin treated transgenic mice carrying Col1a2 and CTGF promoter/reporter genes, were utilized in combination with CTGF blocking agents. Transient transfection, and electrophoretic mobility shift assays were used to determine mechanisms underlying CTGF action on pulmonary human and mouse fibroblasts.

Results

CTGF promoter activity and expression proceeded Col1a2 expression/promoter activity which peaked two weeks after bleomycin challenge. Fibroblasts from bleomycin treated mice displayed greatly elevated type I collagen and CTGF protein expression and promoter activity. In vitro, inhibition of CTGF by siRNA and neutralizing antibody substantially reduced Col1a2 expression and promoter activity (41%, $p<0.001$). In vivo, anti-CTGF antibody significantly reduced Col1a2 promoter activity by approximately 25% ($p<0.01$). Histological analysis showed improved lung architecture and reduced COL1 immuno-reactivity.

The enhanced Col1a2 promoter activity in fibroblasts from bleomycin treated lungs was partly Smad-dependent, CTGF acted on the Col1a2 promoter by a mechanism independent of the Smad-binding site, but instead dependent on the ERK1/2 and JNK MAPK pathways. The CTGF effect was mapped to the proximal promoter region surrounding the inverted CCAAT-box, possibly involving CREB and c-jun.

Discussion

Our results define clearly a direct profibrotic effect of CTGF, and demonstrate its contribution to lung fibrosis through transcriptional activation of Col1a2. Blocking strategies reveal signaling mechanisms involved and presents CTGF as a rational target for therapy in fibrotic diseases.

P034

Structural and regulatory components of the pericellular matrix of young and mature articular cartilage

C McLean[1], *J Saklatvala*[1], *T Vincent*[1]

[1]Kennedy Institute of Rheumatology, 65 Aspenlea Road, Hammersmith, London, W6 8LH, United Kingdom

Introduction

The pericellular matrix of articular cartilage is a distinct hypodense zone that lies between the chondrocyte and the further removed territorial matrix. By electron microscopy it is devoid of type II collagen fibres and contains fine filamentous structures which probably represent non-fibrillar type VI collagen. The structural organisation of the PCM is thought to allow amplification of mechanical signals through the territorial matrix to the cell. The PCM also plays an important role in sequestering the growth factor FGF2 which is bound to perlecan, and is released from cartilage upon loading (Vincent et al, 2004, 2007).

Materials and Methods

We examined the PCM of mature human articular cartilage using confocal microscopy and electron microscopy. We compared expression of PCM components between young, old and osteoarthritic human tissue.

Results

The pericellular matrix of mature human tissue stained strongly for type VI collagen and heparan-sulphated perlecan. Type II collagen was completely excluded from the PCM, as was aggrecan, assessed both by staining the chondroitin sulphate stubs generated by chondroitinase treatment, and the core protein. Staining for link protein revealed a distinctive band pattern which appeared to lie at the outer margin of the PCM, but which extended into the further removed matrix. In contrast when we studied young cartilage (pre-skeletally mature), we found that link protein and aggrecan were present throughout the PCM and territorial matrices. In the PCM of osteoarthritic tissue perlecan and type VI collagen were increased and there was increased staining for the heparin bound growth factors FGF2 and CTGF.

Discussion

The pericellular matrix has a distinct structural composition which changes with age and in disease. Type II collagen, aggrecan and link protein are all excluded from the PCM of mature human cartilage. The fact that aggrecan and link protein are present in the PCM of younger tissue suggests either that the PCM proper does not develop fully until later in life, or that we are detecting high levels of transitting proteoglycan which is evident in highly synthetic young tissue. The presence of perlecan-bound FGF2 and CTGF in the PCM supports a vital role of the PCM in the sequestration and release of regulatory molecules.

REFERENCES

Vincent TL, Hermansson M, Hansen U et al. Basic FGF mediates transduction of mechanical signals when articular cartilage is loaded. Arthritis and Rheumatism, 2003, 50(2):526-33.

Vincent TL, McLean C, Full L et al. FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer.

Osteoarthritis Cartilage. 2007 Jul;15(7):752-63

P035

Connective Tissue Growth factor: matrix localisation, regulation and possible function in articular cartilage

C McLean[1], *J Saklatvala*[1], *TL Vincent*[1]

[1]Kennedy Institute of Rheumatology, 65 Aspenlea Road, Hammersmith, London, W6 8LH, United Kingdom

Introduction

Our group has found previously that the heparin-binding connective tissue growth factor (CTGF, also known as CCN2) is an abundant secreted protein of porcine and human osteoarthritic cartilage (Hermansson 2004). Its role in articular cartilage is unclear although it is essential in cartilage and musculoskeletal development (Ivkovic, 2003).

Materials and Methods

Confocal microscopy was performed on normal and osteoarthritic human articular cartilage using antibodies raised against perlecan and CTGF. CTGF secretion from cartilage explants and isolated chondrocytes was measured by western blot in the presence or absence of heparin. In some experiments the LDL related-protein (LRP) blocker, RAP was added. Recombinant CTGF (His-tagged) was stably expressed in HEK293 cells and purified. In vitro experiments were performed on human fibroblasts, chondrocytes and mesenchymal progenitor cells to assess the role of CTGF on signalling pathways and gene expression. Some experiments were performed in combination with TGF beta family members.

Results

CTGF was present in the pericellular matrix of articular cartilage where it co-localised with the heparan sulphate proteoglycan, perlecan. Increased CTGF and perlecan staining was seen in explants treated with TGFbeta and in osteoarthritic tissue. Secretion of CTGF by chondrocytes was constitutive but increased in the presence of heparin and the LRP inhibitor RAP suggesting that significant turnover of CTGF occurs at the cell surface. When cells were stimulated with recombinant protein alone little cellular effect was observed. However, when the cells were stimulated with low dose TGF beta in the presence of CTGF a synergistic response was observed in mesenchymal progenitor cells both at the level of Smad2 phosphorylation and gene induction. Both fibroblasts and chondrocytes made high constitutive levels of CTGF and synergism with TGF beta was not observed in vitro in these cells.

Discussion

CTGF is identified as another potentially important regulatory molecule of the pericellular matrix. In vitro experiments in mesenchymal progenitor cells, which have low constitutive expression of CTGF, demonstrate that exogenous CTGF acts synergistically with TGFb3. High constitutive levels of CTGF secretion in chondrocytes suggest a physiological function of this molecule in articular cartilage in vivo, although may also explain why synergy is difficult to demonstrate in vitro.

REFERENCES

Hermansson M, Sawaji Y, Alexander S et al. Proteomic analysis of articular cartilage shows increased type II collagen synthesis in OA and expression of inhibin beta A (activin A), a regulatory molecule for chondrocytes. J Biol Chem. 2004 Oct 15;279(42):43514-21

Ivkovic S, Yoon BS, Popoff SN et al. CTGF coordinates chondrogenesis and angiogenesis during skeletal development. Development 2003 Jun; 130 (12):2779-91

P036

Positional identity gene expression in MSCs isolated from different bones

S Prajaneh[1], *SCF Rawlinson*[1], *M Ghuman*[1], *FJ Hughes*[1], *IJ McKay*[1]

[1]Adult Oral Health, Queen Mary University of London, Barts & The London School of Medicine and Dentistry, London, E[1]2AD, United Kingdom

Introduction

Bone marrow stromal cells that populate the medulary cavities of bones are able to travel to distant sites within the body. Adult tissues are likely to retain defined patterns of gene expression as part of their differentiation. Bone marrow stromal cells which constitute a population of undifferentiated progenitors show considerable plasticity in their development. This study investigated the expression of Hox genes which are associated with the establishment and maintenance of regional identity during embryogenesis in cultures of bone marrow cells. This study aimed to investigate if regional differences between bone marrow cultures, derived from a range of bones, based on expression patterns of Hox and other genes were evident.

Materials and Methods

Bone marrow was removed from the ulna, femur, tibia, humerus and ribs of five rats (100-110 grams), and plated separately into 25cm2 culture flasks. After initial plating the non-adherent cells were removed and cells cultured until they were 90% confluent. Cultures were then lysed and total RNA extracted. Gene expression was assessed by RT-PCR (Taqman) to investigate levels of Hoxa1, Hoxa2, Hoxa4, Hoxa5, Hoxa7, Hoxa10, Hoxa13, AlkP, Osteocalcin, and Tgfbi.

Results

Hox genes were expressed by all cultures. Expression of the Hox genes in the rib was distinct from other sites, with highest expression of Hoxa4 and 5 and low levels of Hoxa7. Patterns of expression of marrow from the femur, tibia and humerus were similar, with highest expression of Hoxa7 and lower levels of Hoxa10. Expression of Hoxa13 was restricted to the ulna. Levels of Hoxa1 were uniformly low whilst levels of Hoxa2 were extremely low - close to detection limits in all cultures.

Discussion

The analysis confirms that there are significant differences in the pattern of genes expressed in cultured marrow populations. The pattern of gene expression in bone marrow matches the pattern of expression seen in osteoblasts derived by collagenase digestion. These findings raise the possibility that bone marrow may be positionally linked to the bone where it is located. Testing the plasticity of bone marrow will show if it can integrate with all parts of the skeleton.

P037

Osteoblasts isolated from different anatomical locations display similar characteristics in vitro

SEB Taylor[1], *M Lander*[1], *ML Key*[1], *IR Orriss*[1], *JJ Patel*[1], *TR Arnett*[1]

[1]Department of Cell and Developmental Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom

Introduction

Bone formation in vivo occurs in two distinct ways. The majority of bones, including the long bones, are created mainly by

endochondral ossification, in which bone forms via an intermediate cartilage template. In contrast, the flat bones of the head are formed directly (i.e. without a cartilage intermediate), a process misleadingly termed 'intramembranous' ossification. Whilst maintenance of endochondrally-formed bone in vivo normally requires regular mechanical loading, maintenance of the bones of the skull appear to be much less strain-dependent (and do not develop osteoporosis). The best-known method for culturing normal osteoblasts uses cells derived from calvaria of neonatal rats or mice. The value of a culture system that uses skull bone-derived cells as a general model for osteoblast behaviour has been questioned.

Materials and Methods

We applied a simple trypsin/collagenase digestion method (the method normally used to harvest osteoblasts from rat calvaria) to washed fragments of neonatal rat long bones. The cells released were pre-cultured for 3-4 days, then grown in multiwell plates at an initial density of ~104 / cm2.

Results

We found that long bone and calvarial osteoblasts behaved in a similar manner. At day 14, collagen synthesis and deposition, alkaline phosphatase activity and formation of 'trabecular' bone structures were all comparable. Both calvarial and long bone osteoblasts required dexamethasone (10nM) for cell differentiation, ascorbate for matrix deposition (50µg/ml) and β-glycerophosphate (2mM) for mineralisation. Neither glycosaminoglycan deposition nor adipocyte formation were seen in cultures from either bone type. RT-PCR analysis of calvarial and long bone osteoblasts revealed comparable expression of key markers, including osterix, alkaline phosphatase, osteocalcin, matrix gla protein, type 1 collagen and osteopontin, at both 7 and 14 days of culture.

Discussion

These data indicated that proliferation, differentiation and function of osteoblasts in vitro occur in a similar manner, regardless of anatomical origin. Our results suggest that prior to enzyme digestion, it is reasonable to pool all the bones that can conveniently be harvested from a neonate, thus minimising animal usage. Such an approach yields at least 107 cells per neonatal rat, sufficient to populate at least 200 cell wells (1.5 cm) with bone-forming osteoblasts.

P038

Positional identity gene expression in osteoblasts isolated from distinct anatomical sites

P Ryan[1], *SCF Rawlinson*[1], *M Ghuman*[1], *IJ McKay*[1], *FJ Hughes*[1]

[1]Adult Oral Health, Queen Mary University of London, Barts & The London School of Medicine and Dentistry, London, E[1]2AD, United Kingdom

Introduction

Bones maintain a distinct identity as evident by their ability to heal following injury. It is unclear if osteoblasts isolated from different adult bones can retain their bone-specific identity. Homoebox containing genes of the Hox cluster are associated with the establishment of positional identity during embryogenesis. In this study, the expression of Hox and other genes in osteoblasts isolated from 4 different adult bones was investigated. Our aim was to assess the extent that osteoblasts isolated from different sites retain distinct patterns of gene expression.

Materials and Methods

Bones (ulna, femur, tibia and ribs) were dissected from five rats (100-110 grams). Attendant soft tissue and epiphyses were removed and the cortical shafts were flushed to remove marrow. Bone tissue was then minced prior to sequential digestion with collagenase. Osteoblast fractions were seeded into 25cm2 culture flasks in alpha-MEM containing 10%FCS. Cells were passaged once into 75cm2 flasks and grown until 90% confluence. Osteoblast cultures were then lysed and total RNA extracted. cDNA was generated from the RNA and subjected to RT-PCR (Taqman) to investigate Hoxa1, Hoxa2, Hoxa4, Hoxa5, Hoxa7, Hoxa10, Hoxa13 expression. The osteoblast related genes AlkP, Osteocalcin, and Tgfβi gene expression levels were also assessed.

Results

Expression of relatively low levels of Hox genes was seen in all cultures. Levels of Hoxa1, a4 were uniformly low in all cultures. Levels of Hoxa5 were generally low but were highest in cells from the rib where it was the abundantly expressed Hox gene. Hoxa7 and 10 were significantly expressed in all cultures except rib. Expression of Hoxa13 was highest in cultured tibial osteoblasts, lower in the ulna and almost undectable in femur and rib. Levels of Hoxa2 were extremely low in all cultures. The expression pattern of other osteoblastic genes did not appear to be site specific.

Discussion

This study confirms previous studies showing that isolated osteoblasts from parietal and ulna retain differential patterns of gene expression in culture. Comparing a range of other skeletal bones reveals significant differences that further confirm fine patterning exists and is retained by cultured cells.

P039

The role of glutamate transporters in osteoblast proliferation and activity

K Brakspear[1], *P Parsons*[2], *DJ Mason*[1]

[1]Cardiff University School of Biosciences, Biomedical Building, Museum Avenue, Cardiff, CF10 3AX, United Kingdom; [2]Smith and Nephew Research Centre, York Science Park, York, YO10 5DF, United Kingdom

Introduction

Mechanical loading is a potent osteogenic stimulus. A screen for genes associated with mechanically-induced bone formation identified the glutamate transporter GLAST-1, implicating the excitatory amino acid glutamate in the mechanoresponse. Five high affinity Na+-dependant excitatory amino acid transporters (EAATs 1-5) regulate glutamatergic signalling. EAAT1 (GLAST-1) is expressed by osteocytes and bone-forming osteoblasts in vivo.

Materials and Methods

Transcripts for EAATs 1-3 and two splice variants (EAAT1a and EAAT1ex9skip) were quantified in human osteoblasts (MG63, SaOS-2 and primary) using real time-PCR. Expression and localisation of EAAT1 and EAAT3 were revealed by immunofluorescence. EAAT function was determined by 14C-labelled glutamate uptake. EAAT1a and EAAT1ex9skip splice variants were overexpressed in osteoblasts using antisense oligonucleotides (AONs) targeted to splice donor sequences of exons 3 and 9 respectively and effects on gene expression, alkaline phosphatase activity, cell number and glutamate uptake compared with scrambled AON. Experiments were performed in 0-500µM glutamate.

Results

EAATs 1-3, EAAT1a and EAAT1exon9skip mRNAs were expressed in MG63, SaOS-2 and primary osteoblasts. EAAT1a expression was very low whilst the dominant negative EAAT1ex9skip was ~20% of full length EAAT1 expression. EAAT1 and EAAT3 proteins were detected by immunofluorescence. Sodium-dependent glutamate uptake revealed functional EAATs in osteoblasts. Overexpression of EAAT1ex9skip increased cell number, decreased cell death, and increased PCNA, Osteonectin and Type I collagen mRNA expression in MG-63, and increased alkaline phosphatase activity in SaOS-2. Overexpression of EAAT1a or EAAT1exon9skip reduced glutamate uptake in MG63 osteoblasts at 1μM but not at 10μM.

Discussion

We have demonstrated expression and function of glutamate transporters in osteoblasts. Our data reveal a role for the EAAT1 splice variants in uptake of low concentrations of glutamate and demonstrated that overexpression of EAAT1ex9skip influences osteoblast proliferation and activity. These data confirm that mechanically regulated glutamate transporters are important in regulating bone homeostasis.

P040

The effect of Cobalt(II) and Chromium(VI) on the proliferation and activity of SaOS-2 osteoblast cells in vitro

RE Andrews[1], *JM Wilkinson*[1], *A Gartland*[1]

[1]Academic Unit of Bone Biology and Academic Unit of Bone Metabolism, Department of Human Metabolism, University of Sheffield, Sheffield, S10 2RX, United Kingdom

Introduction

Metal-on-metal (MOM) hip resurfacing arthroplasty is increasingly used as an alternative treatment for younger, active patients requiring hip replacement. Although these prostheses may have advantages for select patients, the long-term outcome in patients receiving this type of prosthesis is unknown. Increased metal ion release post surgery using this type of bearing has been previously demonstrated, but the local and systemic effects are unclear. It has been suggested that Cobalt(II) (Co^{2+}) and Chromium(VI) (Cr^{6+}) in the short-term have toxic effects on osteoblasts and can reduce cell activity. Long-term effects, representative of chronic exposure post-surgery, have not been extensively investigated.

Materials and Methods

SaOS-2 osteoblast cells were treated both short-term (3 days) and long-term (14 days) with an extensive range of concentrations, 0.0001 - 2000muM, for Co^{2+} and Cr^{6+}. Proliferation was measured using MTS assay, Alkaline phosphatase (ALP) release was measured colourimetrically and standardised against DNA content. Mineralisation of 21 day cultures was measured with Alizarin Red staining and quantified by image analysis using ImageJ.

Results

Short-term treatments of SaOS-2 cells resulted in significantly decreased cell proliferation. The IC50 for Co^{2+} and Cr^{6+} were 207muM and 2.5muM, respectively. Long-term cultures were treated with concentrations up to 5muM and under these conditions no chronic effect was seen for Co^{2+}, however Cr^{6+} significantly decreased cell number at 1muM. ALP release was significantly decreased in both Co^{2+} and Cr^{6+} at 100muM and 10muM respectively, however when normalised against DNA, this reduction was accounted for entirely by decreased cell number. The ability to produce mineralised nodules displayed similar results to ALP activity, with reduced overall mineralisation.

Discussion

Our results indicate that metal ions affect proliferation rates of SaOS-2 osteoblast cells which results in reduced alkaline phosphatase release and mineralisation. This may have a significant impact on bone cell activity in the local environment, where metal ion levels may exceed the IC50. These findings may provide an explanation for reduced prosthetic osseo-integration, femoral neck narrowing, and periprosthetic fracture that are observed in implants using a MOM bearing.

P041

A role for TRPV1 and K+ channels in the regulation of osteoblast transdifferentiation to adipocytes

NC Henney[1], *A Thomas*[1], *BAJ Evans*[2], *KT Wann*[1]

[1]Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3NB, United Kingdom; [2]Department of Child Health, Wales School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom

Introduction

As osteoporosis progresses, osteoblast activity and numbers are reduced, accompanied by a proliferation of bone marrow adipocytes – apparently at the expense of osteoblast maturation. Osteoblasts and adipocytes have a common stem-cell origin, and before terminal differentiation occurs, osteoblasts may be diverted along the adipocyte pathway (Thompson et al., 1998). Whilst the differentiation of preadipocytes to adipocytes appears to be regulated by several factors and receptors, including the ion channels TRPV1 (Zhang et al., 2007) and IKCa (Hu et al., 2009), it is not known if this translates to the osteoblast-adipocyte transdifferentiation scenario. Here we tested the hypothesis that TRPV1 and BKCa channels are involved in regulating the differentiation of osteoblasts into adipocytes.

Materials and Methods

7F2 mouse osteoblasts, displaying all the characteristics of maturity, were treated to undergo adipocyte transdifferentiation (Thompson et al., 1998) with alpha-MEM containing 10% FBS, 50 μg/ml ascorbic acid, 100 nM dexamethasone and 50 μM indomethacin, and exposed to TRPV1 or K+ channel ligands. Controls were cultured in alpha-MEM containing 10% FBS. After 4–7 days, cells were stained for lipid using Oil Red O and lipid stain was extracted and quantified by absorbance measurement. RT-PCR was performed on non-induced and adipocyte-induced cells for TRPV1 and BK channels.

Results

Staining showed that TRPV1 agonists capsaicin (1 muM), resiniferatoxin (100 nM) or the cannabinoid anandamide (1 muM) reduced lipid levels in the adipogenic-induced cells, whilst TRPV1 antagonists capsazepine (1 muM) or SB366791 (100 nM) alone did not modify lipid levels. Additionally, cells incubated with both antagonists and agonists in each combination did not modify lipid production (n = 3). The generic K+ channel blocker tetraethylammonium (10 mM) also produced a large increase in lipid deposition in the adipogenic-induced cells (n = 3). Control cultures did not produce lipids. RT-PCR revealed predicted band sizes for TRPV1 and BK channel subunits.

Discussion

We have shown evidence that TRPV1 and K+ channels may play important roles in the regulation of osteoblast differentiation to adipocytes. Given the importance of this cellular diversion in osteoporosis, these channels may provide targets for future interventions to slow the progress, or reverse the process of this debilitating disease.

REFERENCES

Hu H., He M.L., Tao R. et al. (2009) Characterization of ion channels in human preadipocytes. J. Cell. Physiol. 218(2), 427-435.

Thompson D.L., Lum K.D., Nygaard S.C. et al. (1998) The derivation and characterization of stromal cell lines from the bone marrow of p53-/- mice: new insights into osteoblast and adipocyte differentiation. J. Bone Miner. Res. 13(2), 195-204.

Zhang L.L., Yan Liu D., Ma L.Q. et al. (2007) Activation of transient receptor potential vanilloid type-1 channel prevents adipogenesis and obesity. Circ. Res. 100(7), 1063-1070.

P042

TRPV1b is expressed and may assist in pH 'switching' of human osteoblasts

NC Henney[1], *EJ Kidd*[1], *BAJ Evans*[2], *KT Wann*[1]

[1]Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3NB, United Kingdom; [2]Department of Child Health, Wales School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom

Introduction

The capsaicin-sensitive ion channel TRPV1 is expressed in bone, where it may act as a pH detector, switching osteoblasts and osteoclasts on or off (Arnett, 2008). A previously reported splice-variant of this channel (TRPV1b) in various other cells is known to decrease the protein sensitivity to capsaicin and protons, and is thought to be dominant over TRPV1 when expressed in channel heteromultimers (e.g. Vos et al., 2006). Here we test the hypothesis that TRPV1b renders the channel insensitive to capsaicin in osteoblasts, which is reversible at a lower pH.

Materials and Methods

Single-channel patch-clamp recordings were made from MG63 osteosarcoma cells and HOB-c primary human osteoblasts within ± 150 mV, using external and pipette 'Locke' solutions (mM: NaGluconate 150, KCl 3, CaCl2 2, MgCl2 2, HEPES 10, glucose 10) at pH 7.2-7.4, and applying TRPV1 agonists capsaicin, resiniferatoxin or anandamide. RT-PCR was performed on MG63, SaOS-2 and HOB-c cells, and Western blotting on MG63 cells, for TRPV1 and TRPV1b. Calcium fluo-3 imaging was performed in MG63 cells in 'Locke' solution, challenged with capsaicin and pH changes from 7.4 to 6.3.

Results

Electrophysiology revealed no evidence of TRPV1 currents in MG63 (0/73) or HOB-c cells (0/81) with or without the agonists, and/or by reducing pH to 6.1 with HCl (0/13 and 0/14 respectively). RT-PCR revealed predicted band sizes for TRPV1 and TRPV1b from MG63, SaOS-2 and HOB-c cells (n=3). Western blotting of MG63 cell membranes confirmed expression of both TRPV1 and TRPV1b proteins (n=3). Fluo-3 loaded MG63 cells did not respond to capsaicin challenge (1 - 10 muM) at pH 7.4, but on reducing the pH to 6.3 (no response) with HCl, capsaicin (1 muM) caused a clear increase in fluorescence, which was non-repeatable in the same cells indicating capsaicin desensitisation.

Discussion

We report for the first time the co-expression of splice variant TRPV1b with TRPV1 in human osteoblasts, which appears to reduce the channel sensitivity to capsaicin and resiniferatoxin, whilst showing that sensitivity is regained by reducing pH. This supports the idea that TRPV1 may be a pH switch in bone, and therefore may be a useful target for therapeutic strategies.

REFERENCES

Arnett T.R. (2008) Extracellular pH regulates bone cell function. J. Nutr. 138(2), 415S-418S.

Vos M.H., Neelands T.R., McDonald H.A. et al. (2006) TRPV1b overexpression negatively regulates TRPV1 responsiveness to capsaicin, heat and low pH in HEK293 cells. J. Neurochem. 99(4), 1088-1102.

P043

The expression of ecto-nucleotidases by osteoblasts: the regulatory role of acid

ML Key[1], *IR Orriss*[1], *TR Arnett*[1]

[1]Department of Cell & Developmental Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom

Introduction

Previous work has shown that acidosis (pH=6.9) prevents bone nodule formation in vitro by inhibiting mineralisation of the collagenous matrix. The ratio of phosphate (Pi) to pyrophosphate (PPi) in the bone microenvironment is a fundamental regulator of bone mineralisation. Both Pi and PPi, a potent inhibitor of mineralisation, can be generated from extracellular nucleotides by the actions of ecto-nucleotidases. There are four distinct families of ecto-nucleotidases: 1) E-NTPdases (ecto-nucleoside triphosphate diphosphohydrolase); 2) the E-NPPs (ecto-nucleotide pyrophosphatase/phosphodiesterase); 3) alkaline phosphatases, and 4) ecto-5'nucleotidase. E-NTPdases catalyse the reactions: NTP → NDP + Pi and NDP → NMP + Pi, whereas E-NPPs hydrolyse NTP → NMP + PPi or NDP → NMP + Pi.

Materials and Methods

This study used RT-PCR, qPCR, western blotting and biochemical assays to determine the expression and activity of ecto-nucleotidases by osteoblasts. Osteoblasts were obtained from rat calvariae by trypsin/collagenase digestion; mRNA expression and enzyme activity were studied at days 4, 7 and 14 of culture.

Results

The progression of osteoblast differentiation was shown by 2.5-fold and 5-fold increases in alkaline phosphatase (ALP) mRNA expression at days 7 and 14, respectively. We found that osteoblasts express mRNA for E-NTPdase 1, 2, 3, 4, 5 & 6 and E-NPP1, 2 & 3. Ecto-nucleotidase expression changed with osteoblast differentiation; for example, levels of E-NTPdase 2 and E-NTPdase 3 were increased 7-fold and 6-fold, respectively, in mature bone-forming osteoblasts (day 14 cultures), relative to immature proliferating cells. In contrast, ENPP-1 mRNA and protein expression was decreased in mature osteoblasts. Ecto-nucleotidase expression and activity was also investigated in osteoblasts cultured at pH6.9 relative to pH7.4. We found that acidosis upregulated E-NPP1 mRNA expression at all stages of osteoblast differentiation. Furthermore, total E-NPP activity was increased up to 53% in osteoblasts cultured in acid conditions. E-NPP1 is thought to play a key role in the regulation of PPi levels in bone.

Discussion

These data indicate that increased E-NPP1 expression and activity might contribute to the decreased mineralisation observed when osteoblasts are cultured at pH6.9.

P044

Purinergic agonists synergise with gut hormones, including GIP, to induce expression of c-fos in osteoblastic cells

EL Pacheco-Pantoja[1], JP Dillon[1], L Ranganath[2], PJM Wilson[1], WD Fraser[2], JA Gallagher[1]

[1]HACB, School of Biomedical Sciences, University of Liverpool, Liverpool, L69 3GE, United Kingdom; [2]Clinical Chemistry, School of Clinical Sciences, University of Liverpool, Liverpool, L69 3GE, United Kingdom

Introduction

We have previously reported that osteoblastic cells express receptors for gut hormones including glucose-dependent insulinotropic polypeptide (GIP). Here we show that activation of GIP receptors leads to induction of c-fos, a transcription factor with a pivotal role in osteoblastic activation and bone remodelling. Furthermore we show that when cells are exposed to GIP in the presence of extracellular ATP, there is a synergistic activation of c-fos.

Materials and Methods

Expression of c-fos was measured using a reporter system in which the full c-fos-promoter linked to the firefly luciferase gene was stably transfected into the human osteosarcoma cell line, SaOS-2. Cells were made quiescent by serum starvation for 24 hours and were then treated with nucleotides alone or in combination with GIP.

Results

When cells were exposed to GIP at 0.1–10.0 nM, we observed a modest but consistent dose dependent induction of c-fos expression up to 40% above control values ($P<0.01$). Similarly, when cells were exposed to extracellular ATP at 10uM, there was a small induction of c-fos up to 60% above control values ($P<0.01$). However, when both ligands were applied together, there was a synergistic induction up to 4 fold above control values ($P<0.001$).

Discussion

GIP is a hormone which is released after food intake and has an incretin effect, stimulating insulin secretion. Recent results from several laboratories including ours have demonstrated expression of GIP receptors on osteoblasts, providing a mechanism for the skeleton to be remodelled according to the requirements of the organism during feeding or fasting states. The stimulation of c-fos in response to GIP and extracellular ATP observed in this study provide a further example of how locally released nucleotides can interact with systemic hormones in bone, targeting the sites where these hormones are needed to regulate bone turnover. We postulate that GIP provides a systemic signal that nutrients are available for bone formation whilst locally released ATP targets bone formation to specific loci in the skeleton, perhaps in response to mechanical loading. The findings add further weight to the theory that synergy between extracellular ATP and other hormones is a ubiquitous phenomenon in homeostasis

P045

Adenosine receptor expression and function as mesenchymal stem cells differentiate to osteoblasts

B Gharibi[2], J Ham[2], BAJ Evans[1]

[1]Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom; [2]Centre for Endocrine and Diabetes Sciences, School of Medicine, Cardiff University Heath Park, Cardiff, CF14 4XN, United Kingdom

Introduction

Adenosine is a purine nucleoside that mediates a variety of physiological functions by interacting with four cell surface G protein-coupled receptors: A1, A2a, A2b and A3. We have previously showed the presence of functional adenosine receptors (ARs) in mesenchymal stem cells (MSCs) and osteoblasts. The aim of this study is to investigate AR expression and function in MSCs as they differentiate into osteoblasts.

Materials and Methods

MSCs, derived from rat femurs and tibias, were induced to differentiate to osteoblasts with dexamethasone and ascorbate-2-phosphate; glycerophosphate was added to induce mineralization. AR expression was assessed by QRT-PCR and western blotting. Adenosine mediated cAMP accumulation (radioimmunoassay), osteoblastogenesis (alkaline phosphatase (ALP) expression and activity) and mineralization (alizarin red staining of calcium deposits) were determined during the differentiation process.

Results

In comparison to undifferentiated MSCs, adenosine (10-4M) and NECA (10-4M) (universal AR agonist) stimulated cAMP levels were increased respectively from 10- and 24- to 153- ($P<0.001$) and 275- ($P<0.001$) fold in osteoblasts (after 9 days of differentiation). Adenosine (10-4M) and NECA (10-5M) also significantly stimulated ALP mRNA from 12- to 26- ($P<0.001$) and 27-fold ($P<0.001$) respectively and ALP enzyme activity by up to 70 % ($P<0.001$) during differentiation to osteoblasts. The stimulatory effects on ALP were reversed by the A2bAR antagonist, MRS1706 ($P<0.001$) and partly by the A2aAR antagonist, SCH442416. Osteoblastic differentiation was also associated with increased expression of A2aAR (2.5-fold; $P<0.001$) and A2bAR (2-fold; $P<0.001$) mRNA and protein (7- and 2-fold; $P<0.05$). Mineralization was increased (by up to 300%, $P<0.003$) after 10 days of incubation with adenosine and NECA, an effect which was reversed with MRS1706.

Discussion

These data, together, suggest that expression and function of A2aAR and A2bAR are up regulated during differentiation of MSCs into osteoblasts. Adenosine acting via these receptors may be important in regulating differentiation and mineralization of osteoblast precursors. Targeting adenosine signal pathways may therefore be important for preventing or treating conditions where there is insufficient bone formation.

P046

Modulation of A1, A2a and A2b adenosine receptor expression and function as osteoblasts differentiate to adipocytes

B Gharibi[1], *J Ham*[2], *BAJ Evans*[1]

[1]Department of Child Health, School of Medicine Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom; [2]Centre for Endocrine and Diabetes SciencesSchool of Medicine Cardiff University Heath Park, Cardiff, CF14 4XN, United Kingdom

Introduction

It is thought that trans-differentiation of osteoblasts into adipocytes play a role in the development of osteoporosis. Since adenosine receptors (ARs) are functional in osteoblasts we have used mouse 7F2 osteoblast cells to investigate the involvement of ARs in the trans-differentiation of osteoblasts into adipocytes.

Materials and Methods

7F2 cells were differentiated (ascorbate-2-phosphate, indomethacin, dexamethasone) to adipocytes, and markers of osteoblast and adipocyte phenotypes assessed. RT-PCR, cAMP assays and western blotting were used to assess AR protein expression and signalling with AR agonists and antagonists.

Results

Maximal (50%) conversion to adipocytes (FACS; Nile Red) was achieved after 7 days. Increased expression of adipocyte related markers (PPARgamma, CEBPalpha, LPL, GPDH) and reduced expression of osteocalcin were observed within 2-3 days. Following trans-differentiation there was an increase in A1AR (>1800-fold; P<0.0001) and a reduction in A2aAR and A2bAR (80%, 78% respectively; P<0.0001) mRNA expression. A1AR protein expression was also increased, whereas A2aAR was reduced; A2bAR protein, however, showed a size increase (possibly phosphorylation).

AR agonists stimulated cAMP accumulation in 7F2 osteoblasts - rank order of potency NECA (universal AR agonist)>adenosine>CGS21680 (A2aAR agonist) (16.5- (P<0.0001), 4- (P<0.0001) and 1.6-fold respectively). These effects were down-regulated following 9 days of adipogenesis to 5- (P<0.001), 2.5- (P<0.001) and 1.3- fold respectively.

In osteoblasts, adenosine (10-4M) and NECA (10-6M) activated ERK1/2 phosphorylation that was blocked by the A2bAR antagonist, MRS1706 but not by PSB36 (A1AR antagonist) or SCH442416 (A2aAR antagonist). A1, A2a and A3AR agonists had no effect on ERK1/2 phosphorylation. ERK activation was blocked by a phospholipase C inhibitor (U73122) and a MAPK inhibitor (PD098059) but unaffected by inhibitors of PI3K (wortmannin) and PKC (Ro31-8220). NECA also stimulated CREB phosphorylation that was blocked by U73122, PD098059 and Ro31-8220 but not by wortmannin. NECA, however, had no effect on ERK and CREB phsphorylation following trans-differentiation of osteoblasts into adipocytes.

Discussion

These data suggest that trans-differentiation of osteoblasts into adipocytes is accompanied with increased A1AR expression, and down-regulation of A2aAR and A2bAR expression and function. In osteoblasts but not in adipocytes, A2bAR is coupled to ERK and CREB signal pathways. Targeting ARs may be important in controlling osteoblast differentiation to adipocytes.

P047

Modulation of intracellular trafficking in osteoblasts by TGFbeta

D Ribé[1], *K Akram*[1], *Y-L Liu*[1], *G Stenbeck*[1]

[1]Bone Metabolism Research Group, Centre for Cell and Chromosome Biology, Division of Biosciences, Brunel University, Uxbridge, Middlesex, UB8 3PH, United Kingdom

Introduction

Ordered matrix secretion is dependent on intracellular trafficking, which ensures vectorial delivery of matrix proteins vital for bone remodelling and differentiation of progenitor cells. Osteoblasts show reduced responsiveness to transforming growth factor beta (TGFbeta) in conditions such as osteoarthritis, which are also characterised by aberrant matrix deposition. Our studies aim to identify the effects of TGFbeta on intracellular membrane trafficking and secretion of bone matrix proteins by osteoblasts and the molecular machinery responsible.

Materials and Methods

Primary osteoblast-like cells derived from two day old mouse calvaria were cultured with L-ascorbate (50 mug/ml), beta-phosphoglycerate (2 mM) and dexamethasone (10-8 M). The rat osteosarcoma cell line ROS17/2.8, the mouse osteoblastic cell line MC3T3 and the human osteosarcoma cell line MG63 were used for comparison. Protein expression was detected by Western blotting, immunocytochemistry and total internal reflection microscopy (TIRFM). Changes in the actin cytoskeleton were monitored by rhodamine phalloidin staining.

Results

Western blotting showed that osteoblastic cells express varying levels of endogenous TGFbeta with expression being highest in MG63 cells. To study the immediate early effects of TGFbeta, we monitored cytoskeletal changes, which in other cell systems have been shown to precede transcriptional effects and are known to modulate secretion. Responsiveness of MG63 cells to exogenous TGFbeta addition (up to 25 ng/ml) was low. However, ROS17/2.8 cells responded by forming membrane ruffles within 20 min, an effect which persisted for at least 60 min. Primary mouse osteoblasts showed increased formation of focal adhesions after 20 min treatment with TGFbeta, an effect which was not sustained. To monitor matrix secretion in detail, we transfected ROS17/2.8 cells with a plasmid expressing a fusion of green fluorescent protein (GFP) with osteopontin (OP). Trafficking of GFP-OP was visualised with TIRFM. Kinetic analysis of TIRFM images showed that TGFbeta caused a marked downward shift in vesicle speed over a 30 min period with initial reductions observed after 10 min.

Discussion

These studies indicate that TGFbeta has an immediate effect on the cytoskeleton and membrane trafficking in osteoblasts. Dissecting the signalling mechanisms involved will lead to a better understanding of the early stages of diseases associated with aberrant matrix formation.

P048

AMP-activated protein kinase (AMPK) activation in osteoblasts regulates bone cell differentiation and in vitro bone formation

M Shah[1], *B Kola*[2], *A Bataveljic*[1], *D Sovik*[1], *IR Orriss*[3], *ML Key*[3], *M Korbonits*[2], *C Chenu*[1]

[1]VBS, Royal Veterinary College, Royal College Street, London, NW[1]4SE, United Kingdom; 2 Department of Endocrinology Barts and the London Medical School, London, EC1M 6BQ, United Kingdom; 3 Department of Cell & Developmental Biology University College London, London, WC1E 6BT, United Kingdom

Introduction

Adenosine 5'-monophosphate-activated protein kinase (AMPK), a regulator of energy homeostasis, has a central role in mediating the appetite-modulating and metabolic effects of many hormones and neuromodulators as well as the antidiabetic drugs metformin and glitazones. We previously demonstrated similar neuroendocrine activation of AMPK in osteoblasts. In this study, we tested whether stimulation of AMPK activity in osteoblasts plays a role in their function.

Materials and Methods

ROS 17/2.8 rat osteoblast-like cells were cultured in the presence of two activators of AMPK, AICAR (a cell-permeable AMP analogue) and metformin, as well as the specific AMPK inhibitor compound C. AMPK activity in cell lysates was measured by a functional kinase assay using SAMS, a synthetic peptide substrate of AMPK. AMPK protein phosphorylation was studied by western blotting using an antibody recognizing AMPK-alpha phosphorylated at Thr-172 residue. Osteoblast number was determined by cell counting using the "In Cyto" system. Osteoblast differentiation was evaluated by alkaline phosphatase activity. Primary osteoblasts were obtained from rat calvaria by trypsin/collagenase digestion and cultured for 14-17 days in the presence of AMPK modulators. Formation of 'trabecular-shaped' bone nodules was evaluated following alizarin red staining

Results

We demonstrated that treatment of ROS 17/2.8 cells with AICAR and metformin for 1 hour stimulates Thr-172 phosphorylation of AMPK and dose-dependently increases its activity. In contrast, treatment of ROS 17/2.8 cells with compound C inhibits AMPK phosphorylation and activity. Dose-dependent stimulation by AICAR of AMPK activity in ROS 17/2.8 osteoblastic cells did not affect cell proliferation but decreased alkaline phosphatase activity. The antidiabetic drug metformin had no significant effect on ROS 17/2.8 cell proliferation and alkaline phosphatase activity, although metformin stimulated proliferation of another osteoblastic cell line, the MC3T3-E1. Our preliminary results in primary osteoblasts demonstrated that AICAR dose-dependently decreases trabecular bone nodule formation, whereas metformin stimulates bone formation.

Discussion

Our data are consistent with AMPK playing a role in osteoblast function. While both AICAR and metformin stimulate AMPK activity in osteoblasts, they have different effects on osteoblast function, suggesting alternate signalling pathways. Further studies will determine the role of AMPK in skeletal physiology.

P049

ATPase activity and ATP release in osteoblast cultures

RMH Rumney[1], *N Wang*[1], *G Reilly*[2], *A Gartland*[1]

[1]Academic Unit of Bone Biology, Department of Human Metabolism, The University of Sheffield, Sheffield, S10 2RX, United Kingdom; [2]Department of Engineering Materials Kroto Research Institute Broad Lane University of Sheffield, Sheffield, S[2]7HQ, United Kingdom

Introduction

Constitutive ATP release from osteoblasts increases in response to mechanical stimuli and can signal in an autocrine/paracrine manner via P2 receptors. ATP is hydrolysed to ADP by ATPases expressed on the cell surface. The exact mechanism by which ATP is released from osteoblasts and the contribution of ATPase activity on subsequent purinergic signalling in bone remains unclear.

Materials and Methods

SaOS-2 and Te85 osteoblast cells were cultured in 24 well plates in monolayer and in polyurethane scaffolds in 3D. ATP release in response to mechanical stimuli was measured using an offline luciferase assay and LDH was measured as a control for cell death. Mechanical loading was applied to cultures in monolayer via fluid displacement. 3D cultures were loaded either via compressive loading in a modified Electroforce 3200 powered BOSE biochamber or by fluid displacement. SaOS-2 cells expressing a luciferase reporter construct were used to quantify the activation of the immediate early gene c-fos in response to mechanical stimuli. c-fos activation was quantified using the ONE-Glo luciferase assay. The monolayer fluid displacement model was utilised to study the effects of putative ATP release inhibitors.

Results

Exogenous ATP in the presence of SaOS-2 or Te85 cultures had a half life of 2-3 minutes. The alkaline phosphatase inhibitor levamisole significantly decreased the rate of ATP hydrolysis. Fluid displacement in monolayer and 3D cultures stimulated significantly increased ATP release, whereas we could not detect a significant increase in ATP release from compressive loaded 3D cultures. c-fos activation in SaOS-2 cells increased significantly in response to fluid displacement but not in response to compressive loading. The vesicular inhibitor N-ethylmaleimide and the calcium chelator BAPTA-AM significantly decreased ATP release in response to fluid displacement in monolayer cultures.

Discussion

Together these results indicate that ATP release and c-fos activation, a paradigm for bone remodelling, are induced by fluid displacement but not compressive loading in polyurethane scaffolds. ATP release by fluid displacement may occur in a calcium dependent vesicular manner. This has implications for the understanding of the forces that influence bone remodelling and its underlying mechanisms.

P050

Regulation of osteoblast function and bone mineralisation by extracellular nucleotides: the role of P2X receptors

IR Orriss[1], *G Burnstock*[2], *TR Arnett*[1]

[1]Department of Cell and Developmental Biology, University College London, Gower Street, London, WC1E6BT, United Kingdom; [2]Autonomic Neuroscience Centre, Royal Free and University College Medical School, London, United Kingdom

Introduction

Extracellular nucleotides, signalling through P2 receptors, play significant roles in bone, modulating both osteoblast and osteoclast function. To date, we have demonstrated that osteoblasts express P2X2, P2X5, P2X7, P2Y1, P2Y2, P2Y4 and P2Y6 receptors in a differentiation-dependent manner. We showed that ATP/UTP potently inhibit alkaline phosphatase (ALP) activity and bone mineralisation in vitro, an effect which could be mediated, at least in part, via the P2Y2receptor. MicroCT analysis of P2Y2 receptor-deficient mice demonstrated significant increases in trabecular and cortical bone parameters in the femora and tibae.

Materials and Methods

Using qPCR and immunocytochemistry we have extended our investigation of P2 receptor expression by primary rat osteoblasts. P2 receptor expression was studied at days 4, 7 and 14.

Results

The progression of osteoblast differentiation was shown by a 5-fold increase in alkaline phosphatase (ALP) mRNA expression at day 14 of culture. We found that osteoblasts additionally expressed mRNA and protein for P2X1, P2X3, P2X4, P2X6, P2Y12, P2Y13 and P2Y14 receptors. Receptor expression changed with cellular differentiation: eg, P2X4 receptor mRNA levels were 5-fold higher in mature, bone-forming osteoblasts relative to immature, proliferating cells. To investigate whether receptors other than P2Y2 might influence osteoblast function, osteoblasts were cultured with the P2 agonists α,β-meATP, β,γ-meATP, Bz-ATP and 2-MeSATP (1-100μM). The P2X1 and P2X3 receptor agonists α,β-meATP and β,γ-meATP (1μM) inhibited bone mineralisation by 70% and 90%, respectively, with complete abolition at ≥25μM. Bz-ATP, a potent P2X7 receptor agonist, reduced bone mineralisation by 65% and 90% at 1μM and 100μM, respectively; this inhibition was abolished by the selective P2X7 antagonist, A438079. The P2 agonist 2-MeSATP, which is not active at P2Y2, also reduced bone mineralisation by >50% at 10μM. Osteoblast ALP activity was similarly reduced by these agonists. These responses are consistent pharmacologically with involvement of the P2X1, P2X3, P2X5 and/or P2X7 receptors.

Discussion

Our results highlight the expression of multiple P2 receptors by normal osteoblasts and indicate that extracellular nucleotides could function as local signalling agents that "switch off" bone mineralisation.

P051

Targeting of fibroblast growth factor to hydroxy apatite for bone formation

W Kang[1], *E Jeon*[1], *JH Jang*[1]

[1]7-241shinheung 3ga, Jung-gu, Incheon, 400-712, Korea (Republic of)

Introduction

Among many growth factors regulating bone metabolism, fibroblast growth factor 2 (FGF2 or basic FGF) is recognized as a potent mitogen for a variety of mesenchymal cells. In this study, we aimed to develop a novel FGF2 protein fused with highly conserved bone mineral-binding domain of osteocalcin (OC) for targeting to HA.

Materials and Methods

The binding capacity of FGF2-OC proteins to HA particles was measured by a modified method of a protein adhesion assay. The HA particles were placed in the bottoms of the 6-wells culture dish and immobilized with either FGF2, or FGF2-OC protein (0.5 muM) for 3h at 37°C. After binding, the HA particles were exposed to a horseradish peroxidase (HRP)-conjugated His antibody (Santa Cruz Biotechnology) for 1h at 37 °C. A colorimetric substrate for the HRP was then added (Pierce), and the absorbancy at 450 nm was measured.

Results

The engineering of a novel FGF2 fused with highly conserved bone mineral-binding domain of osteocalcin (OC) for targeting to bone mineral hydroxyapatite (HA) exhibited much stronger HA-binding affinity than native FGF2. FGF2-OC also showed a significant increase of mitogenic activity and cellular differentiation of osteoblastic cells compared with native FGF2.

Discussion

Here we showed that FGF2-OC fusion protein has certain advantages over native FGF2 and may offer a novel strategy by which to potentiate the therapeutic effect of FGF2 in bone repair and regeneration.

REFERENCES

Kim, J.H., Park, S., Kim, H.W. and Jang, J.H. (2007) Recombinant expression of mouse osteocalcin protein in Escherichia coli. Biotechnol Lett 29, 1631-1635.

P052

Osterix is required for bone formation and maintenance in postnatal period

WY Baek[1], *JE Baek*[1], *JY Choi*[2], *ES Cho*[3], *B de Crombrugghe*[4], *JE Kim*[1]

[1]Cell and Matrix Research Institute, Department of Molecular Medicine Kyungpook National University School of Medicine, Daegu, 700-422, Korea (Republic of); [2]Cell and Matrix Research Institute, Department of Biochemistry and Cell Biology Kyungpook National University School of Medicine, Daegu, 700-422, Korea (Republic of); [3]Laboratory for Craniofacial Biology Institute of Oral Bioscience Chonbuk National University School of Dentistry, Jeonju, 561-756, Korea (Republic of); [4]Department of Molecular Genetics University of Texas M. D. Anderson Cancer Center, Houston, TX77030, USA

Introduction

Osterix (Osx) is a zinc-finger-containing transcription factor that is highly specific to osteoblasts in vivo. Because Osx homozygotes die immediately in perinatal period showing a complete absence of bone formation, it is impossible to address the critical question regarding the possible role of Osx in bones that are already formed after birth. To address Osx is essential for the maintenance of osteoblast phenotype and for bone homeostasis postnatally, we plan to inactivate conditionally the

Osx gene using the Cre/loxP system after bone formation and osteoblast differentiation.

Materials and Methods

Previously, 2.3-kb Col1a1-CreERT2 mice expressing a Cre recombinase that is transiently inducible by 4-hydroxytamoxifen (4-OHT) were intercrossed with Rosa26R reporter, thus rendering Cre-expressing cells, osteoblasts detectable by X-gal staining. Using inducible Col1a1-CreERT2 together with conditional Osx mice (Osxflox), Osxflox/-;Col1a1-CreERT2 mice were generated. Osx gene in Osxflox/-;Col1a1-CreERT2 was inactivated in fully differentiated osteoblast of already formed bones by active Cre after 4-OHT administration. Both bones from 4-OHT- and oil-treated Osxflox/-;Col1a1-CreERT2 mice were analyzed using radiography, histology, and histomorphometry.

Results

Even though no significant difference was observed in radiographic imaging of whole mouse skeleton, mineralized trabecular bone volume, thickness, and number in vertebrae remarkably reduced in 4-OHT-treated Osxflox/-;Col1a1-CreERT2 compared to oil-treated control mice. Bone forming rate and mineralized surface was also significantly reduced in 4-OHT-treated Osxflox/-;Col1a1-CreERT2. Osx inactivation in already formed bones during postnatal period caused a functional defect of osteoblasts, following a reduction of bone formation, without any apparent differences in osteoblast proliferation and osteoclast formation.

Discussion

The characterization of mice in which Osx is inactivated after birth should help define the role of Osx in bone physiology. If Osx is indeed essential for the maintenance of the osteoblast function and bone homeostasis after birth, then Osx should be a potential target for drugs that would aim at correcting osteoporosis and other bone diseases.

KRF grant (MOEHRD, Basic Research Promotion Fund) (KRF-2005-204-E00006), KOSEF grant (MOST) (R01-2007-000-20005-0) funded by Korea Government, and the BK21 Project in 2009.

P053

Calcium-induced secretion of DKK-1 in osteoblastic cells

HS McCarthy[1], *JH Williams*[2], *MWJ Davie*[1], *MJ Marshall*[1]

[1]Charles Salt Research Centre RJAH Orthopaedic Hospital, Gobowen, SY10 7AG, United Kingdom; [2]Chester Centre for Stress Research, Parkgate Road, University of Chester, Chester, CH[1]4BJ, United Kingdom

Introduction

Wnt signalling is important for regulating and maintaining a healthy bone mass. Dickkopf-1 (DKK-1) is a secreted inhibitor of Wnt signalling and plays a vital role in the regulation of Wnt signalling. DKK-1 is produced by a variety of cell types including osteoblasts although the mechanisms of its regulation are not clear.

Materials and Methods

The osteoblastic cell line MG63 was used to study the regulation of DKK-1 production. DKK-1 production was measured by ELISA and mRNA expression was analysed by qRT-PCR.

Results

We observed that calcium increased DKK-1 protein production in a dose dependent manner. Pathway inhibitors for ERK (U0126), P38 (SB202190), Pi3K (LY294002), JNK (SP600125), NFκB (BAY11-7082) and PKC (bisindolylmaleamide and staurosporine) failed to inhibit calcium-induced DKK-1 production. Ionomycin (calcium ionophore), nifedipine (L-type calcium channel blocker) and NPS2390 (type I metabotropic glutamate inhibitor) also failed to inhibit calcium-induced DKK-1 production. qRT-PCR analysis for DKK-1, Wnt3a, -4, -5a and -10 revealed no significant change in expression at various time points over 24. Wnt7b was significantly up regulated x10 fold at 4 hours and expression remained x3.5 fold above that of control at 24 hours. Wnt11 was significantly down regulated (x0.3) at 8 hours and had returned to control levels by 24 hours. The ELISA for DKK-1 over this time course showed calcium to have doubled the rate of DKK-1 production compared to control by 1 hour, slowly returning to control levels by 12 hours.

Incubation with cycloheximide demonstrated that the increase in DKK-1 in response to calcium was not a result of diminished receptor-ligand internalisation. When MG63 cells were extracted with 0.1% Triton X-100 after calcium stimulation, significantly less DKK-1 was detected in the cell lysate from calcium stimulated cells than in control cells.

Discussion

In conclusion, these results indicate that calcium affects the secretory rate of DKK-1 as opposed to its actual production. During bone resorption calcium is produced by the osteoclast, often up to 40mM. These results potentially represent a mechanism by which osteoblasts are controlled by osteoclasts during bone resorption.

P054

CCN mRNA expression during osteoclast and osteoblast differentiation

W Wang[1], *A Hughes*[1], *P Crombie*[1], *RM Aspden*[1], *JC Crockett*[1]

[1]Bone and Musculoskeletal Research Programme, Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB252ZD, United Kingdom

Introduction

Although osteoarthritis (OA) is traditionally seen as a disease of articular cartilage, we have hypothesised that generalised OA is a systemic condition and that increased bone formation is part of the primary disease process. We wish to identify factors indicative of tissue growth that correlate with disease progression. CCN proteins (CCN 1-6) are matricellular proteins involved in cellular proliferation, adhesion, migration and survival. Differential expression of CCN proteins has been described during differentiation of mesenchymal stem cells into osteoblasts, chondrocytes and adipocytes. We have shown that CCN1 inhibits osteoclast formation in vitro, but little is known about the other CCN proteins in osteoclasts. Here we report a preliminary study of the relative expression levels of CCN proteins during osteoclast and osteoblast differentiation.

Materials and Methods

Peripheral blood mononuclear cells (PBMC) were obtained from healthy individuals and cultured for 7 days with M-CSF and RANKL to induce osteoclastogenesis. Samples were measured at baseline and daily thereafter. Osteoblasts were expanded from femoral heads removed from OA patients and incubated in beta-glycerophosphate and ascorbic acid to induce mineralisation. Samples were measured every 3 days for 12 days. CCN mRNA levels were assessed by qPCR using gene specific primers and UPL hydrolysis probes and normalised by expression of GAPDH.

Results

PBMCs expressed only CCN1 mRNA but fell by 80% at day 1, after the addition of RANKL, and remained at this level throughout the culture period. Human OA osteoblasts expressed CCN1, CCN2 and CCN4. CCN1 expression remained unchanged, whereas CCN2 increased almost 2-fold over the culture period. CCN4 expression was almost undetectable at day 0 but increased rapidly to become comparable with CCN1.

Discussion

These data demonstrate that osteoclasts and osteoblasts have different CCN mRNA expression profiles that change with time in culture. During osteoclast differentiation, levels of CCN1 fall, whilst in osteoblasts, CCN2 and CCN4 levels increase during mineralisation. We now intend to compare the expression of all CCN proteins in OA-derived and non-OA derived osteoblasts and osteoclasts to determine whether these changes in expression may be characteristic of OA and if this could be exploited in diagnosis.

P055

Mild hypothermia promotes osteoclastogenesis whilst retarding osteoblast differentiation and bone formation

JJ Patel[1], *IR Orriss*[1], *ML Key*[1], *SEB Taylor*[1], *K Karnik*[2], *TR Arnett*[1]

[1]Department of Cell and Developmental Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom; [2]Unilever Discover - Unilever PLC, Sharnbrook, MK44 1LQ, United Kingdom

Introduction

Reductions in core body temperature occur with aging due to a reduction in heat production and peripheral vasoconstriction. In the elderly, core body temperatures of 35.50C, or less are common. The effects of temperature reduction on bone cell function and skeletal homeostasis are not well-studied. The aim of this project was to investigate the effects of mild hypothermia (35.5oC) on osteoblasts and osteoclasts.

Materials and Methods

Primary osteoblast cultures were derived from neonatal rat calvaria by trypsin/collagenase digestion and cultured for 14-21 days in medium supplemented with ascorbate, beta-glycerophosphate and dexamethasone. Osteoclast-forming mononuclear cells were isolated from long bone marrow of 6-8 week old mice and cultured on ivory discs with MCSF and RANKL for 8 days, with acidification to pH 6.9 for the final 2 days to activate resorption.

Results

Osteoblasts maintained at 35.5oC showed modest reductions in cell number (15%) for the first few days of culture, but recovered to control levels by day 7. Expression of osteopontin, type I collagen, osteocalcin and alkaline phosphatase mRNAs in hypothermic osteoblasts showed a similar pattern of initial downregulation followed by recovery; the same trend was also observed for soluble collagen and alkaline phosphatase activity. Formation of 'trabecular' bone nodules, assessed morphometrically with alizarin red staining, was decreased by 75% at 35.50C, compared to 370C after 14 days but showed recovery after 21 days. No differences between hypothermic and control cultures were evident with oil red O and alcian blue staining (to demonstrate adipogenesis and chondrogenesis, respectively). In contrast with osteoblast responses, we found that osteoclast cell number and resorption pit formation were reproducibly increased 1.5 to 2-fold at 35.5oC compared to 37oC.

Discussion

Therefore, mild hypothermia exerts reciprocal effects on bone cell function by retarding bone formation, whilst stimulating osteoclastogenesis (and thus resorption). These results suggest that mild hypothermia could shift bone remodelling in the negative direction, potentially contributing to osteopenia/osteoporosis in the elderly.

P056

Osteoclast function is essential in the hematopoietic stem cell niche

S Lymperi[2], *A Ersek*[1], *F Dazzi*[2], *N Horwood*[1]

[1]Kennedy Institute of Rheumatology Imperial College 65 Aspenlea Road, London, W6 8LH, United Kingdom; [2]Stem Cell Biology Section Department of Haematology Hammersmith Hospital Imperial College, London, W12 0HS, United Kingdom

Introduction

Hematopoietic stem cell (HSC) function and number are tightly regulated in a specific microenvironment - the HSC niche - constituted of several cell types. Amongst them, osteoblasts have a prominent role since their increment is associated with an expansion of HSC. However, we demonstrated that an overall increase in osteoblasts does not necessarily promote hematopoiesis (Lymperi et al.). To date, bisphosphonates are the most wildly used anti-osteoporotic drugs as they inhibit osteoclastic bone resorption. In the present study we have examined the impact of osteoclast inhibition on the bone microenvironment contribution to the maintenance of the HSC niche.

Materials and Methods

6-8 week old C57BL/6 female mice were injected with the bisphosphonate, Alendronate (ALN) (50mug/kg/week), for 4 weeks and the bone parameters were tested by micro-computed tomography and bone histomorphometry. HSC numbers were assessed for colony forming units – cells (CFU-C), FACS for primitive HSC (Lin- Sca1+ c-kit+ FlK2-) and long term culture - initiating cells (LTC-IC). The ability of bone marrow (BM) cells from bisphosphonate treated mice to engraft and reconstitute the hematopoietic system was tested in a competitive transplantation assay.

Results

Following ALN treatment mice exhibited a significant increase in bone volume compared with the non-treated age matched control mice whilst osteoclast number and surface area was dramatically reduced. This was accompanied by a decrease in the proportion and absolute number of HSC in the bone marrow (BM) as assessed by both FACS and LTC-IC. The number of CFU-C in the BM of bisphosphonate treated mice was increased, while more Lin-Sca1+c-kit+ enriched cells were found in the S/M phase indicating that the hematopoietic balance was changed in favour of progenitor differentiation. In competitive transplantation assays, the long-term engraftment of treated BM cells was inferior to control BM, reflecting the decrease in HSC numbers.

Discussion

Inhibiting osteoclast activity with ALN resulted in dramatic changes in bone metabolism and microarchitecture as well as changes in normal haemopoiesis with restricted HSC numbers in the bone marrow. Thus, our data suggests that targeting the HSC microenvironment may be essential for influencing normal and malignant HSC engraftment and survival.

REFERENCES

Lymperi S, Horwood NJ, Marley S, Gordon MY, Cope AP & Dazzi F (2008) Strontium can increase some osteoblasts without increasing haemopoietic stem cells. Blood 111:1173-81

P057

Novel technology to provide an enriched therapeutic cell concentrate from bone marrow aspirate

JN Ridgway[1], *P Chen*[1], *A Butcher*[1], *K Ruth*[1], *SJ Curran*[1]

[1]Smith&Nephew Research Centre, York Science Park, Heslington, York, YO10 5DF, United Kingdom

Introduction

Bone marrow is rich in osteogenic progenitor cells with potential to enhance bone healing. However, clinical use of BMA has highly variable outcomes as aspiration techniques yield marrow diluted with peripheral blood. To address this issue Hernigou et al (2005) performed clinical studies using concentrated BMA, demonstrating that reliable healing occurs when osteoprogenitor cells exceed 1000 colony forming units per ml. Currently BMA concentration depends upon centrifugation or cell capture onto graft material , requiring capital equipment or limiting application to open grafting procedures. To address these issues we have developed a stand alone, single use, disposable automated filtration device allowing rapid concentration and recovery of nucleated cells from BMA.

Materials and Methods

Our BMA concentrator utilizes controlled vacuum pressure and acoustics to maintain steady state filtration. Volumes of Human BMA (Lonza, Rockville MD) ranging from 5ml to 45ml were processed through the device. Nucleated cell number was determined using a Coulter Counter, cell viability using a Guava EasyCyte and osteoprogenitor and stem cell number by CFU assays.

Results

The time for each run, irrespective of starting volume was under 15 minutes. There was a linear relationship between BMA volume reduction and total nucleated cell (TNC) concentration, up to 8 fold volume reduction . Samples with >4 fold volume reduction (our target clinical minimum) showed a mean recovery of 93.2% TNC irrespective of starting TNC concentration. The linear relationship between volume reduction and TNC concentration was lost at >9 fold volume reduction with recovery of TNC falling to 69%. TNC viability in all samples remained in excess of 95% irrespective of volume reduction. The number of CFU-f and CFU-ob/ml of aspirate increased linearly versus volume reduction. Interestingly, CFU/CFUOb recovery remained above 90% at >9 fold volume reduction, the point where TNC recovery was seen to fall.

Discussion

The data show our device rapidly and consistently concentrates the therapeutic cell containing TNC fraction of BMA. Rapid processing times and automated functioning are key features for intra-operative application of this device to enhance bone healing. We propose to advance development of the technology whilst testing efficacy of the concentrated cell fraction in clinically relevant in vivo models.

REFERENCES

Hernigou P., Poignard A., Beajean F. & Rouard H. (2005) Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg. 87-A (7), 1430-1437.

P058

Protection against glucocorticoid-induced ROS generation and Forkhead-mediated damage response in human tenocytes

RC Poulsen[1], *AJ Carr*[2], *PA Hulley*[1]

[1]Botnar Research Centre, Nuffield Dept of Orthopaedics Rheumatology & Musculoskeletal Science, University of Oxford, Oxford, OX3 7LD, United Kingdom; [2]UK NIHR Musculoskeletal Biomedical Research Unit and Botnar Research Centre, Nuffield Dept of Orthopaedics Rheumatology & Musculoskeletal Science, University of Oxford, Oxford, OX3 7LD, United Kingdom

Introduction

Local glucocorticoid injection is commonly used for the management of pain associated with tendon degeneration and rupture. In the UK alone, 500,000 injections are administered annually. However glucocorticoids cause substantial bystander damage in skin, bone and other musculoskeletal tissues and therefore excessive use in tendon is a major clinical concern. The objective of this study was to identify negative effects of glucocorticoids on tenocytes and to explore protective strategies.

Materials and Methods

Human tenocytes, isolated by explant culture of hamstring tendon, were treated with 1μM dexamethasone or carrier (ethanol) for up to 7 days. Reactive oxygen species (ROS) generation was visualised by DCFDA assay and proteins of interest were quantified by Western Blotting.

Results

ROS were rapidly generated in tenocytes following dexamethasone treatment. FOXO3a protein levels were markedly increased and this involved glucocorticoid receptor signalling. The ratio of phosphorylated-PKB: total PKB was lower and the ratio of phosphorylated- Jnk:total Jnk was higher in dexamethasone-treated cells indicating conditions were appropriate for nuclear translocation of FOXO3a. The ratio of phosphorylated-ERK:total ERK and the levels of proliferating cell nuclear antigen (PCNA) were lower in dexamethasone-treated tenocytes. Levels of the pro-apoptotic proteins BNIP-3, Noxa and Bim were higher in dexamethasone-treated cells compared to controls. Co-treatment with vitamin C (0.5mM or 1mM), tempol (0.5mM) or insulin (30μg/ml) prevented the dexamethasone-induced rise in FOXO3a protein levels. Vitamin C (1mM) was the most effective at preventing the dexamethasone-induced reduction in both PCNA protein levels and ERK activation.

Discussion

Dexamethasone, in physiologically relevant concentrations, induces ROS generation and FOXO3a signalling whilst reducing ERK activation in cultured human tenocytes. Dexamethasone treatment also results in lower levels of PCNA, a marker of cell proliferation, in tenocytes. The ROS quencher, vitamin C, ameliorates the dexamethasone-induced increase in FOXO3a protein levels, rescues ERK signalling and prevents the dexamethasone-induced reduction in PCNA protein levels in tenocytes. These findings implicate ROS damage as a novel effector of glucocorticoid therapy. Furthermore the forkhead transcription factors act as damage response switches, triggering negative effects on cell proliferation in tenocytes with probable additional effects on matrix synthesis and cell survival.

Funded by ENDO-Stiftung and the ARC.

P059

Matrix genes show a different pattern of expression in functionally distinct equine tendons

CT Thorpe[1], *PD Clegg*[2], *AE Goodship*[1], *HL Birch*[1]

[1]University College London, Institute of Orthopaedics, Brockley Hill, Stanmore, Middx, HA7 4LP, United Kingdom; [2]University of Liverpool, Department of Clinical Veterinary Sciences, Leahurst, Neston, CH64 7TE, United Kingdom

Introduction

Tendons transfer force from muscle to bone; in addition some tendons such as the equine superficial digital flexor tendon (SDFT) play a role as an energy store. Energy storing tendons need to stretch and recoil for efficient function and have a lower elastic modulus than positional tendons, such as the common digital extensor tendon (CDET). We have previously shown that this difference in mechanical properties relates to a difference in matrix composition. Although the total collagen content does not differ, lower levels of GAG in the positional CDET suggest a difference in proteoglycan content. We hypothesise that the SDFT shows a higher ratio of proteoglycan to collagen gene expression than the CDET.

Materials and Methods

The SDFT and CDET were collected from 32 horses aged 4-30. Cell phenotype was assessed by measuring the expression of genes associated with matrix synthesis and degradation using real-time RT-PCR.

Results

Expression of COL1A1 was significantly higher per cell in the CDET but due to a lower cell number in the CDET, expression per tissue weight did not differ between tendons. Expression of the proteoglycans aggrecan, biglycan, decorin, fibromodulin and lumican was significantly higher in the SDFT than in the CDET. Collagenase (MMP-1 and -13) expression was significantly higher in the CDET, while stromelysin (MMP-3 and -10) gene expression was significantly higher in the SDFT than in the CDET. Gene expression levels did not alter with increasing horse age.

Discussion

The data support our hypothesis; the SDFT shows higher expression of proteoglycans than the CDET. For example the decorin:collagen ratio per tissue weight is 70.0 in the SDFT and 7.6 in the CDET. This difference in the expression profile is matched by a higher expression of proteoglycan degrading enzymes in the SDFT. The higher levels of COL1A1 expression per cell in the CDET suggest that these cells are more metabolically active than those in the SDFT and may be more able to remodel the collagenous matrix. Interestingly, levels of gene expression did not decline with increasing horse age, suggesting that cell senescence is not responsible for age related tendon degeneration.

P060

Mechanical strain modulation of Matrix Metalloproteinases in human tenocytes

ER Jones[1], *GC Jones*[1], *GP Riley*[1]

[1]University of East Anglia, Norwich, Norwich, NR4 7TJ, United Kingdom

Introduction

Tendinopathies are a range of diseases that are a significant cause of morbidity. Major contributing factors are thought to be mechanical strain and dysregulated Matix Metalloproteinase (MMP) expression. This project aims to investigate the effect of mechanical loading on cytokine-stimulated MMP expression by human tenocytes.

Materials and Methods

Explant culture-derived human Achilles tenocytes from tendinopathy patients were seeded into 3D collagen gels and stretched using a sinusoidal waveform of 0-5% at 1Hz using the Flexcell FX4000T™ system. Media and cells were harvested and analyzed using Western blotting and qRT-PCR techniques.

Results

In non-IL1 treated controls, strain decreased expression of MMP1 by an average of 51% (lower cell density) and 77.5% (higher cell density) at 48 hours. Strain decreased MMP3 by 28% (lower cell density) and 47% (higher cell density). At 96 hours, strain decreased expression of MMP1 by 78% and MMP3 by 96%. ADAM12 expression was increased by an average of 93% (low cell density) and 30% (high cell density) at 16 hours. At 24 hours ADAM12 expression increased with strain an average of 148% (lower cell density) and 76% (higher cell density). There was no consistent effect of strain upon MMP23 and MMP2. In un-strained cultures IL1 stimulated MMP1, MMP3 and MMP13 but had no effect on ADAM12, MMP2 or MMP23. The effects of mechanical strain on IL1 stimulated cultures varied according to cell density. At low cell densities strain decreased IL1 stimulated expression of MMP1 by 43%. At higher cell densities strain increased the IL1 stimulated expression of MMP1 by 21%. This data represents a mean of 3 separate experiments.

Discussion

Mechanical strain may be an important factor in tendinopathy development. Previous studies have shown high levels of strain (~15%) can induce the expression of MMPs in 2D culture systems. More recently it has been suggested that these levels of strain are not physiologically relevant, and mechanical under-stimulation of the cells is the major contributory factor. Here we report that MMP protein output by human tenocytes can be modified by cyclic tensile strain levels as low as 5%. Cell density-dependent effects suggest that cell-cell interactions influence cellular response to strain. Further studies are required to confirm these data and to determine the mechanism.

P061

Lovastatin upregulates chondrocyte marker expression in tenocytes

HR Cornell[1], *AJ Carr*[1], *PA Hulley*[1]

[1]Botnar Research Centre and UK National Institute for Health and Research (NIHR) Musculoskeletal Biomedical Research Unit, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, OX3 7RD, United Kingdom

Introduction

Torn rotator cuff tendons show a variety of degenerative matrix changes including chondroplasia - the development of cartilage-like characteristics in tendinous tissue. Healthy tensional tendon is composed of tenocytes with a fibroblastic morphology within a collagen I matrix. In chondroplastic regions cells become rounded and the surrounding matrix contains collagen II.

The actin cytoskeleton has been implicated in the control of chondrogenesis with recent work suggesting a role for small GTPases. Statins, inhibitors of protein prenylation which disturb the intracellular location of small GTPases, upregulate markers of the chondrocytic phenotype in primary adult bovine chondrocytes and in the ATDC5 cell line.

Expression of the chondrocytic Sox trio (Sox5, Sox6 and Sox9) is sufficient to induce chondrogenesis in mesenchymal stem cells. Sox5, Sox6 and Sox9 cooperatively activate expression of collagen II. We therefore examined whether the statins could affect expression of the chondrocytic Sox trio and collagen II in tenocytes.

Materials and Methods

Healthy human hamstring tenocytes were obtained from patients undergoing anterior cruciate ligament reconstruction. Tenocytes were cultured with 0.2-2muM lovastatin, 3muM cytochalasin D, 10muM Y27632 or 10muM PP2 for 24h, 48h, 72h, 1 week or 3 weeks. Appropriate cultures were supplemented with 100muM mevalonate in add-back experiments. Small GTPase prenylation status was assayed by Western blot. Staining for F-actin and collagen II was performed. Sox5, Sox6 and Sox9 mRNA levels were determined by RT Q-PCR.

Results

Tenocyte phenotype was stable in culture until passage 5. Tenocytes below passage 4 were used for experiments. Cytochalasin D and PP2 caused F-actin dissolution and upregulated Sox9 mRNA at 72h. Lovastatin inhibited protein prenylation and led to the dissolution of F-actin, resulting in tenocyte rounding. Sox5, Sox6 and Sox9 mRNA was dose-dependently increased after 1 week lovastatin treatment. Addition of the downstream metabolite mevalonate reversed these effects. The rounded cells showed positive staining for collagen II after 3 weeks lovastatin treatment.

Discussion

Statins induce tenocyte rounding by dissolution of actin stress fibres. This is accompanied by a dose dependent increase in the chondrocytic Sox trio and the expression of collagen II. Our results suggest that modulation of pathways including the actin cytoskeleton may alter tenocyte phenotype.

P062

Evidence for differential regulation of proteoglycans and collagen in response to cyclical strain in tendon

C Avella[1], *J Dudhia*[1], *N Young*[1], *A Goodship*[1], *RKW Smith*[1]

[1]Royal Veterinary College, North Mymms, Hatfield, Herts, AL9 7TA, United Kingdom

Introduction

Repetitive cyclical loading is thought to be a major drive in the degeneration of tendon extracellular matrix that precedes clinical tendinopathy. We hypothesised that cyclical loading of tendon in vitro would result in the release of matrix components into culture media in a strain magnitude dependent manner.

Materials and Methods

Cyclical strain was applied to tendon explants mounted in a custom-designed jig in a servohydraulic materials testing device housed in an incubator (Dudhia et al., 2007). Explants were strained at 2%, 5%, 8% or 12% at 1Hz for 20 hours using a sine wave input. Controls were placed in similar culture conditions but were not cyclically strained. The media was analysed for glycosaminoglycan (GAG) and collagen release by DMMB and Sirius red binding assays respectively.

Results

High strain amplitudes resulted in significantly increased GAG release and decreased collagen release in strained explants compared to controls. The reduction in collagen release was strain magnitude dependent, with low strains (2% n=4 and 5% n=5) showing no significant difference in collagen release between strained and control explants, while high strains (8 % n=9 and 12% n=3) induced a significant reduction in collagen release compared to controls ($p=<0.05$). In contrast, a significant strain magnitude dependent increase in GAG release compared to controls was evident with increased strain ($p=<0.05$) (n=3 per group).

Discussion

Strain had contrasting effects on collagen and GAG release from the tendon matrix. Increased strain appears to displace proteoglycans from the matrix while preserving the collagen network suggesting that these two pools of extracellular matrix proteins are differentially regulated in tendon. Further work is necessary to determine whether the increased release of proteoglycans is related to degradation of previously synthesised matrix proteoglycans, the release of newly synthesised proteoglycans or a combination of the two. However, the increased release would be consistent with an important role for those enzymes involved primarily in proteoglycan turn-over. These findings would be consistent with the high activity of the ADAMTS family of enzymes in tendon (Rees et al., 2007) and suggests that their activity may be increased in response to cyclical strain. The selection of strain amplitudes that minimise both processes may be optimal to protect tendon matrix integrity.

REFERENCES

Dudhia, J., Scott, C. M., Draper, E. R., Heinegard, D., Pitsillides, A. A. & Smith, R. K. (2007) Aging enhances a mechanically-induced reduction in tendon strength by an active process involving matrix metalloproteinase activity. Aging Cell, 6, 547-56.

Rees, S. G., Waggett, A. D., Dent, C. M. & Caterson, B. (2007) Inhibition of aggrecan turnover in short-term explant cultures of bovine tendon. Matrix Biol, 26, 280-90.

P063

Altered gene expression in fibroblasts cultured in 3-D collagen lattices subjected to biomechanical load

M Eastwood[2], X Shi-wen[1], CP Denton[1], E Renzoni[3], DJ Abraham[1]

[1]Department of Inflammation - Centre for Rheumatology, University College London Royal Free Campus Rowland Hill St., London, NW3 2PF, United Kingdom; [2]School of Biosciences University of Westminster 115 New Cavendish Street, London, W1W 6UW, United Kingdom; [3]Interstitial Lung Disease Unit Royal Brompton Hospital National Heart and Lung Institute Imperial College, London, SW3 6LR, United Kingdom

Introduction

Connective tissues from wound repair or fibrotic diseases exhibit excessive production, deposition, and contraction of extracellular matrix (ECM). Contraction processes depend on myofibroblasts that develop during tissue remodelling. Fibroblasts cultured in 3-D collagen matrices can generate contractile forces, analogous to those in scars and in granulation tissue undergoing remodelling during pathological situations. Here we cultured dermal fibroblasts in 3-D collagen matrices and assessed gene expression changes during defined biomechanical stimulation.

Materials and Methods

Human dermal fibroblasts were obtained by punch biopsy of normal skin. Explants were cultured, and at passage 4, fibroblasts (106cells/ml) were seeded into 3-D-collagen gel. Fibroblast populated collagen lattices (FPLC), were allotted to one of three groups. In group one, once set, the FPLC was immediately homogenised and processed. In group 2, contraction of FPCLs against a force transducer was monitored for 24hours. In the third group, after 12hrs in culture FPCLs were mechanically stimulated by loading with a 120 dyne force in a cyclic ramp; 15 minutes loading followed by a 15 min rest. Total cycle time was 1hr repeated for 6 cycles. At the end of the 24 hour period FPCLs in groups 2 and 3 were homogenized in Triazol; biotinylated cRNA probes were generated and expression profiles were assessed by hybridization to gene chips (Affymetrix U133). Data were analysed and differential gene expression was defined as greater than 3.5 fold induction.

Results

Gene expression profiles revealed that of the ~12,000 sequences analysed, approximately 4,000 were expressed in all fibroblast samples examined. Of these almost 10% were differentially expressed between control FPCL and lattices that had undergone a contraction regime. Gene sequences found to be significantly elevated included proteases (serine protease) and structural proteins such as myosin and smoothelin. Vascular endothelial growth factor gene expression was elevated as were genes for transcription factors such as c-fos. Extracellular matrix modifying genes, inhibitors of metalloprotease, plasminogen activator inhibitor-1 and plasminogen activator receptor genes were also over-expressed.

Discussion

These results indicate that biomechanical contractile force-induced activation in fibroblasts within 3-D contracted collagen matrices is analogous to activation by TGFbeta. This approach provides new and valuable insights into the molecular mechanisms mediating the ability of fibroblasts to exert contractile forces within matrix containing lattices.

P064

FGF receptor-dependent, integrin-independent phosphorylation of p38, JNK and ERK MAPKs following mechanical stimulation of human articular chondrocytes in primary monolayer cell culture

Y Zhou[1], C Beadle[1], DM Salter[1], G Nuki[1]

[1]Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, United Kingdom

Introduction

The mitogen activated protein kinases (MAPKs) are a family of intracellular proteins that are involved in the control of many inflammatory and stress responses. We have previously demonstrated Jun N-terminal kinase (JNK)-dependent increases in proteoglycan synthesis and selective, partially integrin-dependent activation of JNK kinases in human chondrocyte cell lines following cyclical mechanical stimulation (MS)(1). Vincent et al(2) have recently shown FGF-2 dependent activation of extracellularly regulated kinase (ERK) in porcine bead cultures following mechanical loading and human articular cartilage following cutting or loading. The aim of this study was to examine the phosphorylation of MAPKs in normal and osteoarthritic (OA) human articular chondrocytes (HAC) following MS, and to determine whether MAPK activation was integrin and/or FGF-2 receptor dependent.

Materials and Methods

Articular cartilage was obtained from patients with OA undergoing knee joint replacement and normal human articular cartilage postmortem. Cartilage was graded macroscopically for OA. After enzymatic isolation, chondrocytes were cultured in monolayer. The cells were mechanically stimulated at 0.33 Hz and 3700 μstrain in the absence or presence of the FGF receptor inhibitor PD173074, integrin blocking antibodies P4C10 (beta1), JB1A (beta1), BIIG2 (alpha5) and P1F6 (alphavbeta5) and gadolinium. Proteins were analysed by Western blotting.

Results

p38, ERK1/2 and JNK were identified in normal and OA HAC. Phosphorylation of p38 increased in normal and OA chondrocytes after 5 minutes MS and ERK1/2 phosphorylation increased in normal chondrocytes following 10 minutes MS. Phosphorylation of ERK1/2 in OA HAC following MS was more variable. Phosphorylation of 46 kDa JNK increased after 10 minutes MS, but no phosphorylated 54kDa JNK could be detected following MS in normal or OA chondrocytes. No inhibition of MAPK phosphorylation induced by MS was observed following pre-incubation with integrin blocking antibodies or gadolinium (10 muM) but MS–induced phosphorylation of p38 and ERK were inhibited by PD173074 (25 nM).

Discussion

Normal and OA HAC express MAPKs in primary monolayer culture and p38, JNK and ERK 1/2 are phosphorylated following cyclical MS. Activation of MAPKs in normal and OA HAC in monolayer cell culture following MS is FGF receptor-dependent and not mediated by integrins or stretch activated ion channels.

REFERENCES

1. Zhou Y, Millward-Sadler SJ, Lin H et al. (2007) Evidence for JNK-dependent up-regulation of proteoglycan synthesis and for activation of JNK1 following cyclical mechanical stimulation in a human chondrocyte culture model. Osteoarthritis and Cartilage,15: 884-893.

2. Vincent TL, McLean CJ, Full LE et al. (2007) FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. Osteoarthritis and Cartilage 2007; 15: 752-763

P065

Mechanical loading of murine knee joints: determining the interplay between genetics and mechanical loading in the development of OA

B Poulet[1], *AA Pitsillides*[1]

[1]The Royal Veterinary College, Royal College Street, London, NW[1]0TU, United Kingdom

Introduction

The mechanisms responsible for the loss cartilage integrity in osteoarthritis (OA) remain elusive. Although both genetic and mechanical factors are important in the OA disease process, their contribution and interaction during induction of OA are, however, ill-defined. This paucity in our understanding of OA aetiology is at least partly due to the absence of appropriate non-surgical in vivo models of mechanically-induced OA. Here, we use a novel non-invasive model to examine whether joint loading induces cartilage lesions and their similarity to those described in OA.

Materials and Methods

Right knees of 24 week-old male CBA mice (n=8) were loaded 3 times each week for 2 weeks (40 cycles, 0.1Hz at 9N; de Souza et al, 2005). Articular cartilage lesions were scored as described by Chambers et al (2002). Multiple sections from the entire knee joint were scored, as were its 4 major sub-compartments (medial/lateral of tibia/femur). Loaded knees were compared to contra-lateral, non-loaded knees by paired t-test. Expression of UGDH (glycosaminoglycan synthesis) and a collagen type II degradation product were visualised immunohistochemically and compared to sections from 24 week-old Str/ort knee joints, which show spontaneous natural OA.

Results

Maximum and mean scores of lesion severity were increased by loading and were restricted to the lateral femur. Cartilage turnover markers showed a marked load-induced reduction in UGDH expression in chondrocytes neighbouring lesions and strong labelling for collagen type II degradation in the matrix bordering lesions. Similar labelling patterns for UGDH and collagen type II degradation were seen in the OA tibial plateau of Str/ort joints.

Discussion

This loading model induces localised articular cartilage lesions in the mouse knee joint and our immunolabelling studies suggest a resemblance to 'natural' murine OA. Although, full model validation is required, this model has many potential advantages, including: non-surgical induction, contra-lateral control joint availability and, uniquely, precise controllability of loads applied. Use of this model in mice with specific genetic backgrounds will help us elucidate the interplay between genetic factors and mechanical loading and the role of specific genes in OA development.

REFERENCES

Chambers MG, Kuffner T, Cowan SK, Cheah KS, Mason RM (2002). Expression of collagen and aggrecan genes in normal and osteoarthritic murine knee joints. Osteoarthritis and Cartilage, 10: 51.

De Souza RL, Matsuura M, Eckstein F, Rawlinson SC, Lanyon LE, Pitsillides AA (2005) Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element. Bone, 37: 810

P066

Molecular response of articular cartilage to injury: Wnt signaling in an in vivo model of joint surface injury

N Eltawil[1], *C Pitzalis*[1], *F Dell'Accio*[1]

[1]Experimental Medicine and Rheumatology Department, Charterhouse Square, London, EC1M 6BQ, United Kingdom

Introduction

The outcome of acute joint surface defects (JSD) varies from spontaneous healing to the development of post-traumatic osteoarthritis (PTOA) (Ding et al. 2006). The molecular mechanisms regulating joint surface repair are still unknown. Failure of this reparative mechanism could be a contributing factor for PTOA development. We have previously shown that adult human articular cartilage deploys a robust response to injury with activation of genes encoding signaling molecules and morphogens including Wnt signaling in which Wnt16 was the only ligand to be regulated (Dell'accio et al. 2008). The aim of this work was to test whether the results obtained in vitro matches the in vivo biology of cartilage injury where cartilage interacts with neighbouring tissues in the joint

Materials and Methods

Full thickness defects were generated in the patellar groove of adult C57BL/6 and DBA/1 mice by microsurgery and control knees were either sham-operated or non-operated. Outcome was evaluated by histological scoring systems. Gene expression of Wnt ligands known to play a role in embryonic joint development and Wnt target genes was assessed in injured and control cartilage by real time PCR and confirmation at protein level was performed by immunohistochemistry.

Results

DBA/1 mice displayed consistent and superior healing of articular cartilage defect whereas; C57BL/6 repaired poorly and developed PTOA. Wnt signaling was activated in both strains one day after injury as evidenced by downregulation of the secreted Wnt inhibitor FRZB, nuclear translocation of beta-catenin and up regulation of the target gene axin2. Wnt16 ligand was up regulated upon injury in both strains with a higher net activation of the pathway in C57BL/6 mice.

Discussion

We have generated a murine model in which the outcome of joint surface injury is strain dependent representing the two possible outcomes of cartilage injury. This model also recapitulates the signaling events observed in human cartilage following injury thus giving the opportunity to address the function of different molecules modulated in response to injury which is a pre request to develop novel molecular therapeutics to support joint surface healing. We are currently exploring the function Wnt signaling modulation in the context of cartilage regeneration and degradation.

REFERENCES

Dell'accio, F., De, B. C., Eltawil, N. M., Vanhummelen, P., & Pitzalis, C. 2008, "Identification of the molecular response of articular cartilage to injury, by microarray screening: Wnt-16 expression and signaling after injury and in osteoarthritis", Arthritis Rheum., vol. 58, no. 5, pp. 1410-1421.

Ding, C., Cicuttini, F., Scott, F., Cooley, H., Boon, C., & Jones, G. 2006, "Natural history of knee cartilage defects and factors affecting change", Arch.Intern.Med., vol. 166, no. 6, pp. 651-658.

P067

The metaphyseal index for assessing development of the distal radius

LC Reddie[1], KA Ward[1], MZ Mughal[2], SM Astley[1], JE Adams[1]

[1]Clinical Radiology, Imaging Science and Biomedical Engineering School of Cancer and Imaging Science University of Manchester Oxford Road, Manchester, M13 9PT, United Kingdom; [2]St Mary's Hospital for Women & Children Department of Paediatric Medicine Hathersage Road, Manchester, M13 0JH, United Kingdom

Introduction

During skeletal growth and maturation remodelling of the ends of long bones, a process occurs known as metaphyseal inwaisting. In a healthy child, metaphyseal inwaisting results in proportional decreases in metaphyseal width (MW) and increases in growth plate width (GPW). The Metaphyseal Index (MI) compares MW to GPW. MI of the distal femur has been examined (Ward et al, 2005; Land et al, 2006). However, the most commonly performed radiograph in children is the hand/forearm for bone-ageing.

Primary Aim: To examine distal radius morphology, measure MI in healthy children and compare these reference data to examples from different disease/athlete groups, specifically Neurofibromatosis type 1 (NF1) and gymnasts.

Hypotheses: The gymnast group would show a similar age distribution of MI to the normal group but with higher MI values. The NF1 group would also show a similar age distribution of MI to the normal group but with lower MI values.

Materials and Methods

Posterior-anterior hand radiographs of 378 (156 male) healthy white Caucasian children, 36 (15 male) healthy gymnasts and 17 (6 male) children with NF1, aged between 5 and 19 years were digitised and semi-automated measurements of GPW and MW were obtained using QWin by Leica Microsystems (Milton Keynes, UK). MI ± 0.5MW / GPW.

Results

The MI increases with age in both sexes and all groups, then levels off in the teenage years. Mean MI results: Normal group: 0.351 (± 0.018 SD); Gymnast group: 0.335 (± 0.015 SD); NF1 group: 0.339 (± 0.023 SD).

Discussion

Unexpectedly, the mean MI of the gymnast group was similar to that of the NF1 group. This may be due to small sample size. Age and gender matching with normal controls is in progress and may shed further light on these results.

REFERENCES

1) Ward, K., Cowell, C.T., & Little, D.G. (2005) Quantification of metaphyseal modeling in children treated with bisphosphonates. Bone. 36, 999-1002.

2) Land, C., Rauch, F. & Glorieux, F.H. (2006) Cyclical intravenous pamidronate treatment affects metaphyseal modeling in growing patients with osteogenesis imperfecta. J. Bone. Miner. Res. 21, 374-379.

P068

Profile analysis of metaphyseal trabecular bone reveals a bimodal dose-dependent response to administered bone active agents

A Pitsillides[2], PL Salmon[1], A Tivesten[3], S Moverare[3], C Ohlsson[3], L Oste[4], G Dixon[4], I Grieg[5], A Idris[5], R Van T'Hof[5], et al.

[1]SkyScan NV, Kartuizersweg 3B, Kontich, 2550, Belgium; [2]Royal Veterinary College, Royal College Street, London, NW[1]0TU, United Kingdom; [3]Sahlgrenska Institute, Goteborg, Sweden; [4]Galapagos plc., Mechelen, Belgium; 5 Institute for Molecular Medicine, Universty of Edinburgh, Edinburgh, United Kingdom

Introduction

At the metaphyses of long bones there is a gradient of decreasing crossectional trabecular bone area with increasing distance from the growth plate. Here we re-analyse micro-CT image data from several OVX rodent studies to examine this "metaphyseal profile".

Materials and Methods

Four OVX studies are reviewed involving combined ovariectomy and drug treatment of (initially) 2-3 month old rodents. The 3D images of the whole metaphyses were analysed to assess the metaphyseal profile. These studies involved treatment with sex steroids, the bisphosphonate alendronate, PTH and the novel antiresorptive compound, ABD295.

Results

Where metaphyseal trabecular bone volume in OVX rodents was restored by a specific drug treatment to levels similar to sham control levels, this gave a uniform elevation of the metaphyseal profile, but without change to its slope. In contrast, treatments which produce an elevation in trabecular volume to levels substantially greater than in sham control bones ("high-responding" groups), are found to engender an upturn in the metaphyseal profile in the region closest to the growth plate. Drugs causing such high responses included sex steroids and alendronate.

Discussion

The studies disclosed two distinct metaphyseal profiles that appear to represent a bimodal dose-related response to treatment. Partial or complete restoration of trabecular volume in OVX groups was shown to occur via a uniform alteration of the remodeling balance throughout the metaphysis. By contrast, in high-responding groups the upturned metaphyseal profile implicated an additional positive effect upon the rate of trabecular bone formation at the growth plate.

P069

The osteogenic response to applied mechanical loading is restricted to distal regions of the fibula cortex

RC Collinson[1], R de Souza[1], AA Pitsillides[1]

[1]Department of Veterinary Basic Sciences, The Royal Veterinary College, London, NW[1]0TU, United Kingdom

Introduction

We have shown, using our non-invasive hind limb murine model, that tibial loading culminates in increases in cortical bone volume in distal regions1. The adjoining fibula also serves a load-bearing function, yet its response to loading has not been examined. We have examined regional load-induced modifications in fibula

structure and their association with responses in the neighbouring tibia.

Materials and Methods

Right female C57/Bl/6 mouse limbs (n=6) were loaded for 2 weeks (9N, 3 times/week, 1200με peak strain on tibial midshaft); magnitudes known to be osteogenic in tibiae. High resolution μCT scans were acquired and proximal (25%), mid-diaphyseal (50%) and distal (75%) segments of fibula cortex, between the proximal head and tibio-fibula junction, analysed.

Results

Our findings show no significant load-induced changes fibula cortex at either proximal or mid-diaphyseal regions, but significant increases in periosteal ($p<0.01$) and endosteal perimeter ($p<0.05$) in distal regions. Absence of any changes in total bone area in the fibula suggests that local architecture is modified predominantly by bone re-distribution and that these are most pronounced, as in the conjoined tibia, in distal regions.

Discussion

These data indicate a proximo-distal association in load-induced changes in tibiae and fibulae, suggesting that distal regions experience greatest local strain change or most marked adaptive responses. Whilst loading induces increased tibial bone volume, the distal fibula adapts predominantly by radial redistribution of bone volume to increase bending strength. This indicates that different bones in the same limb achieve mechanoadaptation by potentially differing mechanisms.

REFERENCES

De Souza RL, Matsuura M, Eckstein F, Rawlinson SC, Lanyon LE, Pitsillides AA (2005) Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element. Bone, 37: 810

P070

Short bouts of dynamic compressive loading stimulate mineralized matrix production by human mesenchymal stem cells (hMSC) on 3-D polyurethane scaffolds

A Sittichokechaiwut[1], *A Scutt*[2], *GC Reilly*[1]

[1]Kroto Research Institute, Department of Engineering Materials, University of Sheffield, Broad Lane, Sheffield, S10 7HQ, United Kingdom; [2]School of Medicine and Biomedical Sciences, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX, United Kingdom

Introduction

Mechanical forces regulate bone growth in vivo (Klein-Nulend et al., 2005). Our previous studies have shown that applying compressive loading, for 2 hours every 5 days of culture, to fully differentiated osteoblastic cells (MLO-A5, MC3T3-E1, MG63) cultured in three dimensional (3D) polyurethane foams increases bone matrix production (Sittichokechaiwut et al., 2009). The effects of mechanical loading on hMSC differentiation are not well understood. Although dexamethasone (DEX) is used widely as a differentiation agent of hMSCs, using a pharmaceutical agent to stimulate hMSC differentiation is not the best choice for engineered tissue transplantation due to cost and potential side-effects. The goal of the present study is to investigate the effects of dynamic compressive loading on differentiation and mineralized matrix production of hMSCs in 3D polyurethane scaffolds.

Materials and Methods

500,000 hMSCs were seeded in polyurethane scaffolds (10 mm diameter and 5 mm height) and cultured in standard culture media with or without DEX. Cell-seeded scaffolds were compressed at 5% global strain for 2 hours on day 9 and then every 5 days in a media-filled sterile biodynamic chamber (BOSE). Samples (N=6) were tested for cell viability by MTS assay, collagen by Sirius red and calcium by alizarin red at day 24 of culture.

Results

Neither DEX nor loading had significant effects on cell viability. Collagen production was significantly increased ($p< 0.01$) in the loaded group compared with the non-loaded group in all conditions. There was no difference in the amount of collagen and calcium produced between the non-loaded group supplemented with DEX and the loaded group without DEX.

Discussion

We have demonstrated the potential of short bouts of mechanical loading to stimulate osteogenic differentiation of hMSCs in 3D in a commercially available polyurethane scaffold. Dynamic loading has the ability to stimulate osteogenic differentiation of hMSC in the same way as pharmaceutical treatments. We suggest that mechanical loading has the potential to improve production of bone matrix in 3D culture of hMSC either as a substitute for DEX treatment or to further improve matrix production over DEX treatment.

REFERENCES

Klein-Nulend, J., Bacabac, R. G. & Mullender, M. G. (2005). Mechanobiology of bone tissue. Pathol. Biol. (Paris) 53, 576-80.

Sittichockechaiwut, A., Scutt .A.M., Ryan, A.J., Bonewald L.F., & Reilly G.C. (2009). Use of rapidly mineralising osteoblasts and short periods of mechanical loading to accelerate matrix maturation in 3D scaffolds. Bone (In press, doi:10.1016/j.bone.2008.12.027).

P071

eNOS null osteoblasts produce Nitric Oxide in response to fluid flow but do not translocate beta-catenin

C Huesa[1], *RM Aspden*[1], *MH Helfrich*[1]

[1]Bone and Musculoskeletal Programme School of Medicine and Dentistry Institute of Medical Sciences University of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom

Introduction

The constitutively expressed enzyme endothelial nitric oxide synthase (eNOS) is activated in endothelial cells in response to mechanical stimuli leading to increased NO synthesis. Mice lacking active eNOS show a mild and transient low bone mass phenotype induced by defects in osteoblast function and differentiation. An early cellular response seen in osteoblasts subjected to pulsatile fluid flow (PFF) is the translocation of beta-catenin to the nucleus. Here we tested the hypothesis that in osteoblasts eNOS is the only enzyme responsible for NO production following PFF and that some of the resulting cellular responses may be mediated by beta-catenin.

Materials and Methods

Osteoblasts from 4 day-old mouse calvaria were obtained from eNOS null and wildtype mice and subjected to PFF resulting in 1.3 Pa of shear stress. NO production was measured in cells loaded with the fluorescent probe DAR4M-AM, which allowed calculation of the relative increase in NO synthesis in real time. Localisation of beta-catenin was determined by quantitative immunofluorescence using ImageJ software.

Results

Under static conditions wildtype osteoblasts showed a constant increase of NO and this was significantly reduced in eNOS null

osteoblasts. Wildtype and eNOS null osteoblasts showed an increase in the rate of NO production shortly after the start of PFF (within 10-35 s). NO production in response to PFF was inhibited by the general NOS inhibitor L-NAME (1 mM), but also by the selective inducible NOS (iNOS) inhibitor 1400W (5 muM) in both eNOS null and wildtype osteoblasts. Translocation of beta-catenin was seen in wildtype, but not eNOS null, osteoblasts after mechanical (PFF) or chemical (inhibition of GSK3beta) stimulation and was inhibited by L-NAME. In eNOS null osteoblasts beta-catenin translocation in response to GSK3beta, but not PFF, was restored by the NO donor SNAP.

Discussion

Our data indicate that in mouse osteoblasts eNOS is not solely responsible for NO production following PFF. Instead they reveal that the low basal levels of NO in eNOS null osteoblasts may be responsible for the lack of beta-catenin translocation, suggesting a novel link between NO and beta-catenin, which may underlie the osteoblast phenotype in the eNOS null mouse.

P072

Mechanical and material properties of bone in cannabinoid receptor knockout mice

AB Khalid[1], SR Goodyear[1], MJ Rogers[1], RA Ross[2], RM Aspden[1]

[1]School of Medicine and Dentistry University of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom; [2]School of Medical Sciences University of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom

Introduction

There are two classical cannabinoid receptors, CB1 and CB2. Recent studies in mice using µCT have shown that loss of CB1 leads to more bone formation whereas loss of CB2 resulted in a low bone mass phenotype, with apparent differences between strains. This study analysed whole CB1-/- or CB2-/- bones to measure their mechanical and material properties.

Materials and Methods

Tibiae and femora were obtained from 6-month old male CD1 mice (7 wildtype (WT),5 CB1-/-) and 3-month old C57Bl/6 mice (4WT, 6 CB1-/-, 5 CB2-/-). Mechanical properties were measured using 3 point bending. Geometrical properties of the cortical shaft and BV/TV in the trabecular bone were measured using µCT. Density was measured using Archimedes' principle and composition by ashing. The elastic modulus (E) was measured by an ultrasonic technique.

Results

CB2-/- tibiae were stiffer (78.6 vs 61.8 N mm-1, P= 0.013) and stronger (15.3 vs 11.9 N, P= 0.014) than wildtype. This was due to an increased second moment of area (I= 0.638 vs 0.442 mm4, P= 0.002) as the material properties were unchanged. Data from femora showed similar trends although the differences were less marked. In contrast, there was no apparent difference in stiffness between CB1-/- bones in both strains of mice. In the older, CD1 mice, I was smaller in CB1-/- (0.55 vs 0.69 mm4, P= 0.069) while the elastic modulus was greater (E= 20.8 vs 18.7 GPa, P= 0.019). The C57B1/6 showed a trend to a greater modulus although I was unchanged. Femoral and tibial trabecular bone volumes were greater in both CB1-/- and CB2-/- mice although this only reached significance in the CD1 strain in both tibia and femur.

Discussion

These pilot data suggest that ablating CB2 in C57Bl/6 mice increases the stiffness and strength of the cortical bone and the amount of trabecular bone.Deleting CB1 left the whole bone properties unchanged; a reduction in cross-section was balanced by an increase in modulus. Trabecular bone volume, however, was increased. Differences described were significant in the CD1 strain, possibly because they had reached skeletal maturity, but similar trends were found in the younger C57Bl/6 strain.

P073

Visualization of blood vessels using high definition X-ray microtomography

K Tang[1], G Davis[1], F Ahmed[1], S Mohsin[1]

[1]Institute of Dentistry Barts & The London Queen Mary University London UK, Mile end, E[1]4NS, United Kingdom

Introduction

Osteoporosis is a disease characterized by exaggerated loss of bone mass and changes in microarchitecture of the bone tissue that compromise bone quality. A universally accepted definition of bone quality does not exist. Several factors may be involved; the most important is probably the bone microarchitecture (Legrand et al. 1999). Haversian systems are an integral part of compact bone but much is still not known about their three dimensional structure.Previous studies have used serial histological sections followed by three dimensional reconstructions using various computer methods (Mohsin et al. 2002) but they are all time consuming and invasive techniques. The aim of this study was to understand bone microstructure by reconstructing vascular channels in three dimensions using a non-invasive technique, high definition x-ray microtomography (XMT).

Materials and Methods

Ten specimens were prepared from five different bovine femurs. Blocks of 5 x 5 x 5 mum were removed from the medial aspect of proximal diaphysis of the femur for compact bone. These specimens were then scanned using a unique XMT, MuCat at Queen Mary, University of London. The scanner used for the study is free from ring artefacts, allows for long exposure times giving a good signal to noise ratio with an impact source. Bone specimens were scanned at 10mum resolution and images were captured using a slow scan CCD camera (Astracam Ltd, UK, now part of Perkin Elmer Inc) with 1:1 lens-coupling to a 70 mum thick columnar CsI scintillator (Applied Scintillation Technologies Ltd, UK) for maximum dynamic range and linearity. The Haversian canals were reconstructed in three dimensions and measurements were taken using a specialised software, Drishti.

Results

The study suggests that the Haversian canals form highly complex networks, running in a longitudinal manner, interconnected to each other via Volkmann canals. Haversian canals are longer than Volkmann canals. The mean length and diameter of Haversian canals were 1.3mm ± 0.13mm and 0.072mm ± 0.014mm respectively. Volkmann canals were 0.23mm ± 0.11mm and 0.035mm ± 0.01mm respectively.

Discussion

The role of microtomography is the key to this study and will become a major tool in future medical diagnosis. We aim to use this technique to detect microdamage and its interaction with bone microstructure.

REFERENCES

Legrand E, Chappard D and Pascaretti C. (1999) Trabecular bone architecture and male osteoporosis. Morphologie. 83(261), 35-40.

Mohsin S, Taylor D and Lee T.C. (2002) Three-dimensional reconstruction of Haversian system in ovine compact bone. European Journal of Morphology. 40(5), 309-315.

P074

Molecular interactions of ADAMTS-4 in the VEGFR2 signalling complex

YP Hsu[1], CA Staton[2], DJ Buttle[1]

[1]Academic Unit of Molecular Medicine and Rheumatology School of Medicine and Biomedical Sciences University of Sheffield, Sheffield, S10 2RX, United Kingdom; [2]Microcirculation Research Group Academic Unit of Surgical Oncology School of Medicine and Biomedical Sciences University of Sheffield, Sheffield, S10 2RX, United Kingdom

Introduction

Angiogenesis is the process of new blood vessel formation from existing vessels. Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor identified to date and stimulates endothelial cell proliferation, migration and tube formation. VEGF mediates its effects by binding to receptor kinases VEGF-R1/ R2. Neuropilin-1(NP1) is a co-receptor for VEGF that binds to both VEGF and VEGFR2 to increase VEGFR2 regulated angiogenesis.

A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1 and -4 are two aggrecanases. ADAMTS-1 has been shown to be anti-angiogenic through binding to VEGF. Because ADAMTS-4 is closely related to ADAMTS-1 and is produced by endothelial cells, it may also be an anti-angiogenic molecule. ADAMTS-1 and ADAMTS-4 bind to heparin, and heparin sulphate may modulate their acitivity and localisation. Therefore the aim of this project is to investigate the potential role of ADAMTS-4 in angiogenesis by investigating its binding to components of the VEGF receptor complex in both the presence and absence of heparin, and its effects on endothelial cell tubule formation.

Materials and Methods

Recombinant human (rh) proteins (ADAMTS-1, ADAMTS-4, VEGF, VEGFR2 and NP1) were mixed to form potential complexes and then co-immunoprecipitation was used to pull down complexes with/without heparin. Western blotting was used to detect the proteins, the amounts of which were semi-quantified by densitometry. The Matrigel tubule formation assay was used to assess the effects of recombinant ADAMTS-4 on angiogenesis in vitro.

Results

rhADAMTS-1 and rhADAMTS-4 bound to rhVEGF and rhNP1, with or without heparin. Neither ADAMTSs bound to VEGFR2 without heparin. ADAMTS-4 inhibited the numbers of tubes formed on Matrigel in both the presence and absence of VEGF.

Discussion

These new interactions between ADAMTS-1, ADAMTS-4 and VEGF signalling complexes may suggest new mechanisms of regulating angiogenesis. Heparin was found to further modulate these interactions. ADAMTS-4 inhibited tubule formation in both the presence and absence of VEGF suggesting that it is a potential anti-angiogenic agent.

P075

Neo-cartilage grafts derived from deep and surface zone chondrocytes display unique matrix characteristics yet secrete a consistent profile of angiogenic regulatory proteins

C Clarkin[1], B Poulet[1], C Hughes[2], J Edwards[3], C Wheeler-Jones[1], B Caterson[2], A Pitsillides[1]

[1]Department of Veterinary Basic Sciences The Royal Veterinary College, London, NW1 0TU, United Kingdom; [2]Cardiff School of Biosciences Biomedical Building Museum Avenue, Cardiff, CF10 3US, United Kingdom; [3]University College London, Gower Steet, London, W1P 9PG, United Kingdom

Introduction

Articular cartilage exhibits zonation in chondrocyte behaviour and matrix composition and this zonation must provide smooth articulating surfaces, dissipate load and oppose vascular ingress. To investigate the contribution of chondrocytes from each zone to these functions we measured sulphated-glycosaminoglycan (sGAG) production, markers of extracellular matrix and angiogenic factor release profiles from apical and basal regions of neo-cartilage grafts originating from superficial or deep zones.

Materials and Methods

Chondrocytes were isolated from 7-day old bovine articular cartilage superficial (SZ) or deep zones (DZ) and neo-cartilage grafts grown in transwell cultures (Hayes et al, 2007). Conditioned media were collected weekly from inner (apical) and outer (basal) wells for 3 weeks and sGAG content and pro/anti-angiogenic factor release profiles analysed by DMMB assay and protein array (RayBio), respectively. Following 3 weeks in culture cartilage grafts were fixed and immunolabelled for cartilage oligomeric matrix protein (COMP), collagen type II and UDP- glucose dehydrogenase protein expression.

Results

Grafts elaborated by SZ, but not DZ, chondrocytes showed increased sGAG secretion from their basal aspect during the 3-week culture period and exhibited high levels of immunodetectable type II collagen expression. In contrast, grafts produced by DZ chondrocytes were thicker, and expressed higher levels of COMP in their ECM than SZ chondrocytes, suggestive of a higher capacity for sGAG retention. In contrast, apical and basal secretion, as well as 'switching' of predominant angiogenic factors over time in culture, was consistent in both SZ and DZ grafts. EGF, bFGF and RANTEs were the predominant factors secreted from basal and apical aspects during week one of culture, and this switched to mainly ENA-78 IL-6 from apical and ENA-78 and PIGF from basal aspects following 3 weeks of culture.

Discussion

These studies highlight a potential influence of chondrocyte zonation in regulating cartilage growth potential in vitro. In addition chondrocyte origin does not appear to influence the predominant patterns of angiogenic factors released temporally from neocartilage grafts in vitro. This work was funded by the Barbara Mawer travel fellowship.

REFERENCES

Hayes et al (2007) Macromolecular organization and in vitro growth characteristics of scaffold free neocartilage grafts. Journal of Histochemistry and Cytochemistry 55 pp853-866.

P076

Anti-angiogenic properties of proteoglycan in cartilage explant cultures

JJ Bara[1], S Roberts[1], WEB Johnson[1]

[1]Spinal Studies/ISTM Keele University, ARC/TORCH Building, RJAH Orthopaedic Hospital, Oswestry, SY10 7AG, United Kingdom

Introduction

It has been shown previously that vascularisation of osteoarthritic cartilage is associated with a loss of proteoglycan. In addition, human aggrecan has been demonstrated to inhibit the adhesion and migration of endothelial cells in monolayer culture. Here we have investigated the effects of proteoglycan depletion of cartilage explants (caused by enzymatic digestion) on the susceptibility of the explants to invasion by endothelial cells.

Materials and Methods

Bovine cartilage explants were cultured for three days (to equilibrate) and then treated for 24 hours with testicular hyaluronidase. The treated and untreated (control) explants were seeded with fluorescently tagged human endothelial cells (HMEC-1). Endothelial cell adherence was quantified 4 hours and 7 days after seeding. Explant proteoglycan content was quantified immediately prior to HMEC-1 seeding on parallel cultured explants and after 7 days using the DMMB assay. Chondrocyte viability was assessed using live/dead scoring post-hyaluronidase treatment (vs. controls) and after 7 days of culture with HMEC-1 cells.

Results

Hyaluronidase treatment induced a 57±4% loss of proteoglycan from cartilage explants compared to controls. This decreased proteoglycan content was associated with a significant increase in the number of endothelial cells that attached to the treated explants (12±3 cells/mm) compared with controls (3±1 cells/mm) at 4 hours (p=0.01). The endothelial cells adhered to the surface and deeper/calcified zones of the cartilage, but they did not appear to proliferate or migrate into the tissue over the next 7 days. Chondrocyte viability was significantly lower in proteoglycan depleted explants compared to control explants, i.e. 67±2% vs. 86±2% respectively. Conversely, there was no significant difference in chondrocyte viability after a further 7 days of culture with HMEC-1 cells.

Discussion

We found an inverse relationship exists between the proteoglycan content of cartilage explants and their susceptibility to endothelial cell adherence. This supports previous work suggesting that cartilage proteoglycans have anti-angiogenic properties. The relationship between chondrocyte viability (observed to decrease after hyaluronidase treatment) and the presence of endothelial cells requires further investigation.

Acknowledgments: Funded by EU "MyJoint" (FP6:28861(NEST)).

P077

The effect of serum and IGF-1 on pH homeostasis in articular chondrocytes in hypoxia

PI Milner[1], RJ Wilkins[2], JS Gibson[3]

[1]Faculty of Veterinary Clinical Science, Chester High Road, Neston, Cheshire, CH64 7TE, United Kingdom; [2]Department of Physiology Anatomy and Genetics Parks Road, Oxford, OX[1]3PT, United Kingdom; [3]Department of Veterinary Medicine University of Cambridge, Cambridge, CB3 0ES, United Kingdom

Introduction

Extracellular matrix production by articular chondrocytes, and hence joint integrity, is affected by changes in intracellular pH (Wilkins and Hall 1995). A number of pathophysiological factors alter pH homeostasis, including ambient oxygen tension and abnormal growth factor levels. Importantly, we have shown that hypoxia inhibits acid efflux from chondrocytes resulting in intracellular acidification (Milner et al 2006). Since both may be altered in joint disease, the interaction between oxygen levels and exposure to serum factors on pH homeostasis were investigated.

Materials and Methods

Articular chondrocytes were isolated from the metacarpophalangeal joints of healthy, mature horses, killed for other reasons, in the presence or absence or serum (10% FCS) or insulin-like growth factor-1 (IGF-1; 1-100ng/ml). Cells (1x10^6/ml) were incubated in 1%, 5% or 20% O2 for 3 hours. For the final 30 minutes of incubation inhibitors of protein phosphorylation (PD98059, SB202190 and wortmannin) were added. Acid efflux (JH) was then measured fluorimetrically (pH-sensitive dye BCECF-AM; Ex 490/439nm; Em 535nm) using the ammonium prepulse method. Amiloride (1mM) was used to inhibit the Na+/H+ exchanger (NHE).

Results

Addition of serum and higher concentrations of IGF-1 (100ng/ml) increased acid efflux, mainly via stimulation of NHE ($P<0.05$). Although hypoxia (1% O2) inhibited NHE, serum and IGF-1 prevented this effect. Kinase inhibitors indicated a role for protein phosphorylation in these responses. The IGF-1 induced increase in H+ extrusion was significantly reduced by inhibitors of MAPK kinase (25μM PD98059) and PI-3 kinase (200nM wortmannin) at normoxic (5%O2) levels of oxygen, whereas the PI-3 kinase pathway was implicated in the modulation of pH homeostasis by IGF-1 in hypoxia (1% O2).

Discussion

These findings emphasise the complexity with which changes in the physicochemical environment of articular chondrocytes interact at a cellular level to influence ion homeostasis, thereby potentially modulating matrix production. It shows that the presence of growth factors such as IGF-1 can modulate important physiochemical parameters, such as oxygen tension, on cell function and this mechanism is likely to involve protein phosphorylation.

REFERENCES

Milner PI, Fairfax TP, Browning JA, et al (2006) The effect of O2 tension on pH homeostasis in equine articular chondrocytes. Arthritis Rheum 54:3523-3532

Wilkins RJ, Hall AC (1995) Control of matrix synthesis in isolated bovine chondrocytes by extracellular and intracellular pH. J Cellular Physiol 164:474-481

P078

Functional imaging of tendon extracellular matrix

AK Harvey[1], *HR Cornell*[2], *PA Hulley*[2], *LE Cochlin*[3], *U Tirlapur*[1], *Z Cui*[1], *M Brady*[1], *MS Thompson*[1]

[1]Department of Engineering Science, University of Oxford, Parks Road, Oxford, OX[1]3PJ, United Kingdom; [2]Botnar Research Centre, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences Headington, Oxford, OX3 7LD, United Kingdom; [3]Department of Physiology Anatomy and Genetics, University of Oxford, Oxford, OX[1]3QX, United Kingdom

Introduction

Tendons contain predominantly collagen (70-80% dry weight), a structural protein, and enable movement by transmitting forces generated in muscles to bones. They suffer from a wide range of degenerative and mechanical disorders, therefore driving a growing demand for methods to assess tissue quality in vivo. To realise the potential of magnetic resonance imaging (MRI) in examining tendon, it is necessary to establish precisely the source of MR signal. This study describes an unprecedented combination of state of the art techniques from across imaging modalities, matrix biology and image analysis, in order to functionally evaluate tendon. Specific analysis of second harmonic generation (SHG) by determining collagen orientation and intrinsic tissue geometry enables interpretation of MR signal intensity in terms of tissue quality.

Materials and Methods

Normal and enzyme-digested (papain, trypsin, collagenase) bovine tendon samples were imaged using ultra-high field (7 Tesla) MRI and near infrared-multiphoton laser scanning microscopy (NIR-MPLSM), at longitudinal and transverse orientations. For NIR-MPLSM, paraffin-embedded sections were incubated in primary antibody chondroitin sulphate (CS)-56 (Sigma), and secondary antibody, AlexaFluor-488 (Invitrogen). SHG images were quantified by estimating tissue curvature, order, crimp waveform amplitude and wavelength using custom-written image analysis algorithms employing differential geometry techniques.

Results

MR images revealed high signal intensity corresponding to the fatty surrounding sheath, and intra-tendinous signal corresponding to longitudinal fibre directionality and transverse fascicular bundling in normal tendon. Papain and trypsin induced moderate damage captured by MRI and collagenase caused severe identifiable damage. SHG image analysis demonstrated that fibre direction and the crimp waveform banding are orthogonal to each other in healthy tissue. Crimp waveform, matrix organisation and amount of CS were reduced by digestion.

Discussion

Both MRI and NIR-MPLSM were able to distinguish between normal and damaged tendon. MR imaging revealed macroscopic changes and evidence of tissue disruption at the microscopic level, and NIR-MPLSM provided local parameters relating to the ECM. These findings have major implications for clinical assessment of tendon, potentially leading towards novel in vivo MRI protocols for quantifying tendon tissue parameters relating to damage and repair, and show a novel and exciting role for differential geometry in characterising tissue quality.

P079

In situ ultrastructural imaging of native extracellular matrix macro-molecules

HK Graham[1], *CEM Griffiths*[1], *NW Hodson*[2], *JA Hoyland*[1], *J Millward-Sadler*[1], *AW Trafford*[1], *REB Watson*[1], *MJ Sherratt*[1]

[1]Faculty of Medical and Human Sciences, University of Manchester, Manchester, M13 9PT, United Kingdom; [2]Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, United Kingdom

Introduction

Conventional approaches to the visualisation of extracellular matrix (ECM) assemblies, whether via in situ fixation and staining, or via chemical and/or mechanical extraction regimes, have the potential to irreversibly denature biomolecules. Here, we describe an atomic force microscopy (AFM) approach which is capable of resolving nm-scale features of native proteins in tissue cryo-sections.

Materials and Methods

Biopsies of young human skin, human osteoarthritic articular cartilage and ferret left ventricle were snap frozen in OCT prior to cryo-sectioning, adsorption onto 13mm diameter glass coverslips and air-drying. These unstained, unfixed, 5 micron thick cryo-sections were visualised by intermittent contact mode AFM. In subsequent experiments, tissue cryo-sections were visualised by AFM before and after exposure to Tris-buffered saline in the presence or absence of type IA bacterial collagenase.

Results

Fibrillar collagens were readily identifiable in the AFM amplitude images of all three tissues by their characteristic 67nm periodicity. Within skin, collagen fibrils were arranged as large bundles and sheets in the reticular dermis and as woven bundles and individual fibrils in the papillary dermis. In cartilage the distribution of type II collagen fibrils was dependent on the both the proximity to embedded chondrocytes (interterritorial versus pericellular matrices) and the disease state (healthy versus osteoarthritic tissue). In addition to mapping micron-scale variations in tissue organization the technique resolved nm-scale telopeptide ridges within type I collagen fibrils from ferret epicardium and finely structured banding patterns within the 67nm repeat in human skin fibrillar collagens.

In parallel experiments, rehydration with a physiological buffer and subsequent air drying had no discernible effect on ECM ultrastructure but exposure to bacterial collagenase profoundly altered tissue organisation.

Discussion

This study describes the non-destructive AFM imaging of native bio-molecules in situ. Combining AFM with cryo-sectioning can provide high-resolution quantitative data on the same ECM assemblies, before and after, exposure to biologically active agents.

P080

Imaging early molecular alterations in articular cartilage degeneration by Raman spectroscopy: diagnostic applications

J Dudhia[1], *S Firth*[2], *PF McMillan*[2], *ERC Draper*[1]

[1]Department of Veterinary Clinical Sciences, Hawkshead Lane, North Mymms, Herts, AL9 7TA, United Kingdom; [2]Department of Chemistry and Materials Chemistry Centre, University College, London, WC1H 0AJ, United Kingdom

Introduction

No reliable and sensitive non-destructive measures exist for quantifying early molecular alterations in osteoarthritis (OA). We have taken advantage of the earliest observed molecular changes to quantify sulphated GAGs, collagen and tissue hydration by Raman spectroscopy (RS) in intact cartilage. The aim was to apply an assessment strategy that correlated with the gold standard of histological grading.

Materials and Methods

Full-depth cartilage was obtained under Institutional guidelines from normal specimens (5-80yrs, n=14), OA cartilage (54-76yrs, n=4) and macroscopically normal specimens (46-73yrs, n=4) with a mild lesion considered early OA (Lorenzo, P et. al. 2004). Raman spectra were obtained using a Raman InVia spectrometer laser diode (150mwatt, ë785nm) in-line with a microscope. Cartilage was graded by safranin O histology: 0 (normal), I (unfibrillated, some GAG loss), II (surface roughening, obvious GAG loss) and III (thinning cartilage, poor GAG staining, cell loss).

Results

Common Raman spectral peaks included O-H (H2O) (2500-3700cm-1) and organic components including SO42- (~1063cm-1) and collagen proline (~856cm-1) and hydroxyproline (~877cm-1). To quantify spectral output with disease grade, we established a reproducible measure of the integrated intensity ratio between characteristic peak areas. Thus, the SO42- peak significantly diminished in mean relative intensity with degeneration, consistent with the known loss of aggrecan both in early and established disease. Similarly, tissue hydration and the ratio of proline:hydroxyproline were significantly increased. Grades I, II & III disease was unambiguously identified with this quantification. No age-related differences in spectra of healthy cartilage were discernable indicating that changes were associated with disease stage. Furthermore, spectra of IL-1beta treated cartilage correlated with early proteoglycan loss (grade I-II) and later by increased hydration and proline:hydroxyproline ratios (grade II-III).

Discussion

We have established an objective assessment strategy using Raman spectroscopy that unambiguously measures very early changes in SO42-, tissue hydration and collagen content. Spectral measurements at sub-millimetre spatial resolution presents the means to map articular 'molecular-lesions' in assessing disease progression in vivo at exploratory arthroscopy or in cartilage tissue engineering applications. The technology, unlike MRI, is amenable to low costs, portability and minimal technical skill, allowing for use in the clinic and bench.

REFERENCES

1. Lorenzo, P. Bayliss, M.T. and Heinegard, D. (2004) Altered patterns and synthesis of extracellular matrix macromolecules in early osteoarthritis. Matrix Biol 23, 381

P081

Apoptosis in anteromedial gonarthrosis - evidence for the role of reactive oxygen species

R Rout[1], *S Mcdonnell*[1], *S Snelling*[1], *X Dao*[1], *AJ Price*[1], *PA Hulley*[1]

[1]Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal science, University of Oxford, Botnar Research Centre, Oxford, OX3 7LD, United Kingdom

Introduction

Anteromedial gonarthrosis (AMG) is a distinct phenotype of osteoarthritis (OA), with a specific progressive pattern of disease that can be considered to be a spatial model of OA progression.

Chondrocyte cell death has been shown to be a feature of OA cartilage, however the triggers are poorly understood; similarly, reactive oxygen species (ROS) have been implicated in OA.

This study characterises the regional levels of cell death and implicated ROS in AMG using a number of immunohistochemical studies.

Materials and Methods

Ten tibial resection specimens, from patients undergoing unicompartmental knee arthroplasty and eight above knee amputations, used as age matched controls, were paraffin embedded. Sections underwent histology and immunohistochemistry for Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), Active Caspase 3, Cytochrome C, Active Bax, Bim, 3-Nitrotyrosine and Forkhead box O3A (FOXO 3A).

Results

Cell death (TUNEL) appeared predominantly in the surface layer of chondrocytes of damaged cartilage ($p<0.001$). Median values were 23% in superficial cartilage (range 0 – 51) compared to 0% in deeper cartilage (range 0 – 15). There was a significant difference in TUNEL staining between regions ($p=0.001$). This ranged from 26% (most damaged) to 4% (undamaged) with a good correlation with histological grade. ($r2=0.66$, $p<0.001$). TUNEL was significantly higher ($p<0.001$) in AMG compared to the control samples which showed an average of 2% TUNEL overall.

Active Caspase 3, Cytochrome C, Active Bax, assessed qualitatively, were present in a similar distribution to that of TUNEL staining.

3-Nitrotyrosine was also a predominantly surface phenomenon. There was a significant difference ($p<0.001$) between regions, ranging from 58% (most damaged) to 10% (undamaged). This was significantly higher that the control samples ($p<0.001$). In line with indicators of ROS mediated damage, Bim and FOXO3A were also detected.

Discussion

The mechanism of apoptosis in OA cartilage has not been studied in depth, and understanding the biochemical and molecular responses of 'stressed' chondrocytes may provide invaluable information about the specific causes of cell death. Such cellular responses may provide targets for disease modification, thus delaying or preventing the need for joint arthroplasty.

Apoptosis involves the intrinsic mitochondrial pathway and ROS appear to be implicated. Further work is needed to provide evidence of what lies further upstream of markers demonstrated in this study.

P082

Knockout of P58IPK, a known inhibitor of PKR, in mice results in a degenerative phenotype in the knee joint

SJ Gilbert[1], *M Patel*[1], *H Toumi*[1], *VC Duance*[1], *DJ Mason*[1]

[1]School of Biosciences, Museum Avenue, Cardiff, CF10 3AX, United Kingdom

Introduction

Our work has implicated the TNF-activated protein kinase, PKR in cartilage degradation (Gilbert et al. 2004). PKR mediates various signal transduction pathways regulating apoptosis, cell proliferation and pro-inflammatory responses, all of which are important in the pathology of arthritic diseases. Our in vitro studies support a role for PKR in arthritis since TNF-alpha-induced degradation of cartilage is prevented by PKR inhibition. Here we report that absence of P58IPK, an inhibitor of PKR, in mice (Ladiges et al. 2005) is associated with a degenerative joint phenotype.

Materials and Methods

Degenerative changes were measured in the knees of male P58IPK-/- mice and wild-type littermates at 12, 18 and 24 months (2 wild-type and 2 P58IPK-/- at each age). Coronal, decalcified sections were taken from several points throughout the knee joint and scored for degenerative changes using our modified Mankin score. Tibial cancellous bone volume was quantified using image analysis software (Toumi et al. 2006) to assess degenerative changes of the bone.

Results

P58IPK-/- mice developed significantly more severe cartilage damage than wild-type littermates. The total Mankin score in the knockout mice ranged from 55 to 91 (68.3±16.10, n=6), and in the wild-type mice it ranged from 24 to 44 (32.4±7.96, n=6). One-way ANOVA indicated that the difference between the two groups of mice was significant (P=0.002). The difference in Mankin score between the two groups increased with age, indicating accelerated progression of cartilage degeneration in the knockout mice. Bone volume analysis did not show significant differences between the two groups but the mean bone volume in the knockouts was lower than in wild type mice (68.7% vs. 71%). Bone volume in knockout mice decreased by 4.5% as the mice aged from 12 months to 24 months, whereas in wild type mice, bone volume remained fairly constant.

Discussion

This study shows that P58IPK-/- mice develop significantly more severe cartilage damage than wild-type littermates, and that this difference increased with age, indicating accelerated progression of cartilage degeneration. These findings support our recent studies implicating PKR in arthritis and provide a new animal model for further studies on the pathological mechanisms of arthritic disease.

REFERENCES

Gilbert S.J., Duance V.C., & Mason D.J. (2004) Does protein kinase R mediate TNF-alpha- and ceramide-induced increases in expression and activation of matrix metalloproteinases in articular cartilage by a novel mechanism? Arthritis Res. Ther. 6(1), R46-R55.

Ladiges W., Morton J., Blakely C., Gale M. (2000) Tissue specific expression of PKR protein kinase in aging B6D2F1 mice. Mech. Ageing Dev. 114(2), 123-32.

Toumi H., Higashiyama I., Suzuki D. et al. (2006) Regional variations in human patellar trabecular architecture and the structure of the proximal patellar tendon enthesis. J. Anat. 208(1), 47-57.

P083

Glutamatergic signalling in the osteoarthritic knee

DJ Mason[1], *K Brakspear*[1], *C Wilson*[2], *R Williams*[2], *RS Kotwal*[2]

[1]School of Biosciences Cardiff University., Cardiff, CF10 3US, United Kingdom; [2]University Llandough Hospital, Cardiff, United Kingdom

Introduction

Pain is the major burden in osteoarthritis. Synovial fluid concentrations of the neurotransmitter glutamate are increased in arthritis (1, 2), and contribute to nociception, inflammation and degradation via receptors in nerves and musculoskeletal tissues (3). We investigated whether glutamate signalling components are expressed in human osteoarthritic knees.

Materials and Methods

Subchondral bone was removed after total knee arthroplasty (n=2, TKR, Kellgren Lawrence grade 3) or from tibial drill hole sites after high tibial osteotomy (n=2, HTO, KL grades 2 and 3) for osteoarthritis. Meniscus samples, obtained after TKR (n=3), were sub-divided according to anatomical site (anterior horn, body or posterior horn; inner vascular or outer avascular). RNA was extracted, reverse transcribed and RT-PCR performed for GAPDH, the glutamate transporter EAAT-1, and glutamate receptors (NR2A, GluR3 and KA1). Quantitative RT-PCR assessed differences in the expression of EAAT-1, a dominant negative splice variant called EAAT-1ex9skip, type I collagen and osteocalcin.

Results

EAAT-1, NR2A and KA1 mRNAs were expressed in subchondral bone, alongside high osteocalcin expression, indicating RNA derived from osteoblasts and osteocytes. EAAT-1 expression was significantly reduced in the anterior versus the middle or posterior zones (ANOVA, $p<0.001$) in one patient. RT-PCR indicated differential expression of EAAT-1 between medial and lateral bone, however these differences were not significant by quantitative RT-PCR. EAAT-1ex9skip mRNA appeared less abundant in HTO than TKR patients.

Type 1 collagen, EAAT-1, EAAT-1ex9skip, NR2A, AMPA GluR3 and KA1 were expressed in human meniscus. EAAT-1 expression normalised to GAPDH did not vary with anatomical site in the meniscus but EAAT-1ex9skip was significantly more common within the outer zones (ANOVA, P=0.040) and in the posterior horns (ANOVA, p=0.038).

Discussion

We have shown for the first time that glutamate transporters and receptors are abundant in the meniscus and subchondral bone of patients with osteoarthritis and that EAAT-1 and EAAT-1ex9skip expression may vary with anatomical location and pathology. Activation of these receptors and transporters by the increased synovial fluid concentrations of glutamate that occur in osteoarthritis may contribute to pathological changes and nociception.

REFERENCES

1.McNearney (2000) Excitatory amino acid profiles of synovial fluid from patients with arthritis. J Rheumatol 27; 739-745.

2.Jean (2005) Increased concentrations of neuro-excitatory amino acids in rat anterior cruciate ligament transacted knee joint dialysates: a microdialysis study. J Orthop Res 23, 569-575.

3.Flood S, Parri R, Williams A, Duance V and Mason D (2007) Modulation of interleukin-6 and matrix metalloproteinase 2 expression in human fibroblast-like synoviocytes by functional ionotropic glutamate receptors. Arthritis and Rheumatism 56(8): 2523-2534.

P084

A gene expression profile of damaged versus undamaged cartilage in anteromedial gonarthrosis

R Rout[1], S Snelling[1], X Dao[1], AJ Carr[1], PA Hulley[1], AJ Price[1]

[1]Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal science, University of Oxford, Botnar Research Centre, Oxford, OX3 7LD, United Kingdom

Introduction

Anteromedial gonarthrosis (AMG) is a distinct phenotype of osteoarthritis (OA), with a specific progressive pattern of disease that can be considered to be a spatial model of OA progression. Gene expression differences between regions have previously been shown and microarray technology presents the opportunity to compare the gene expression of thousands of genes.

Materials and Methods

Ten tibial resection specimens, obtained from patients undergoing unicompartmental knee arthroplasty, had regions of damaged and undamaged cartilage dissected and hand ground under liquid nitrogen. RNA was extracted using RNeasy kits, reverse transcribed, and quality control undertaken using a bioanalyser. Each paired sample was labelled using Agilent's two-colour microarray-based gene expression analysis protocol and run on a whole genome microarray plate. Samples were kept back for realtime Polymerase chain reaction validation.

Results

Bioanalyser data showed adequate quantity and quality: the average RNA integrity number was 7.3 (range 6.5–8.1). Initial analysis revealed 820 genes that were significantly up-(416) or down-(404) regulated in damaged cartilage, with at least a 2-fold change.

Searching for differences in gene ontologies showed significant functional clusters in groups including, cell communication, collagen process and proteinaceous extracellular matrix. There was an increase in undamaged cartilage of Type I Collagen expression. There was a decrease in several Matrix metalloproteinase (MMP) expression in damaged cartilage (the most downregulated were MMPs 1, 2, 3, 9, and 13). There was also decreased expression of the Wnt antagonists FRZB, sFRP1 and sFRP4 (including the OA-associated FRZB gene) and increased expression of the BMP antagonist Noggin in damaged cartilage.

Discussion

This study shows a number of changes in gene expression that warrant further investigation. The matrix change of Type I collagen expression corroborates previous findings of increased content in the undamaged cartilage of AMG using immunoassays. MMP results were somewhat unexpected, but raises potential questions regarding where OA cartilage is obtained from in cellular studies.

In AMG the signalling microenvironment is altered, with results implicating both Wnt and BMP signaling in cartilage damage regulation. Further validation of selected genes is ongoing, and ultimate aims are to identify a series of therapeutic targets for disease modifying interventions.

P085

Two types of chondro-osseous developmental defects in adjacent sites within the same joint in young horses

EC Firth[1], M Doube[2], A Boyde[2]

[1]National Research Centre for Growth and Development Institute of Veterinary Animal and Biomedical Sciences Massey University, Palmerston North, 4442, New Zealand; [2]Queen Mary University of London, Barts and The London Scool of Medicine and Dentistry, Centre for Oral Growth and Development, Dental Institute, New Road, London, E[1]1BB, United Kingdom

Introduction

The aetiology of equine metacarpal (Mc3) condylar fracture is assumed to lie in athletic training/racing, because fractures occur then, and clinically undetectable lesions occur in trained racehorses at the fracture origin. Because developmental abnormality (osteochondrosis) is known to occur from an early age in horses, we surveyed the distal aspect of Mc3 in two groups of untrained horses.

Materials and Methods

Distal Mc3 was available from 12 pasture-raised Thoroughbreds aged 17 months; one group of 6 had been randomly assigned to additional exercise from 3 weeks of age. Bone slices (2mm thick, mediolateral longitudinal plane) in the centre of the epiphysis (B slice), and 30–35° palmar oblique (C) and 25° dorsal oblique (A) slices were imaged by point projection digital x-ray (26kV, linear resolution 10µm).

Results

In some C slices, linear or ovoid radiolucency was in articular calcified cartilage (ACC) and subchondral bone (SCB) at only the sagittal groove (exact site of origin of condylar stress fracture); in only the apex of the sagittal ridge of A slices ovoid radiolucency was present, with or without fragmentation or disturbance (flattening, concavity) of the immediate subchondral mineralised tissue. Prevalence of abnormalities was similar in the two groups. There were no abnormalities in other sites, or in B slices. BSE SEM of selected specimens confirmed C slice ACC and SCB changes, and A slice retained cartilage in SCB.

Discussion

C slice changes are early forms of more severe changes, both with unknown healing potential, which apparently lead to catastrophic fracture. The findings imply that aetiology of condylar stress fracture includes abnormality in bone/joint development, before athletic activity occurs. A slice lesions were typical of equine osteochondrosis, known to heal without implications to normal joint function in equine performance athletes. The presence of two developmental defects in specific sites less than 20mm apart in the same joint, with different healing capabilities and orthopaedic health implications is intriguing and points to the role of local biomechanical influences.

P086

Histone deacetylase inhibitors as chondroprotective agents

KL Culley[1], *S Fenwick*[2], *IM Clark*[1]

[1]University of East Anglia, Norfolk, Norwich, NR4 7TJ, United Kingdom; [2,2]Smith and Nephew Group Research Centre, York, YO10 5DF, United Kingdom

Introduction

Cartilage destruction in osteoarthritis is thought to be mediated mainly by the action of proteinases from the matrix metalloproteinase (MMP) and 'a disintegrin and metalloproteinase domain with thrombospondin motifs' (ADAMTS) families. The expression of these enzymes can be altered through changes in protein acetylation mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Cell-based and cartilage explant assays show that HDAC inhibitors can block proinflammatory cytokine induction of key MMPs and ADAMTSs resulting in inhibition of cartilage resorption. This project aims to define the molecular pathways by which HDAC inhibitors mediate their chondroprotective affects.

Materials and Methods

The activity of trichostatin A (TSA), valproic acid (VPA) and MS-275 on histone and alpha-tubulin acetylation within SW1353 cells was assessed via western blot. Quantitative RT-PCR was used to determine the effect of HDAC inhibitors on cytokine induced metalloproteinase expression. Bovine nasal cartilage (BNC) explant assays were used to determine the effect of HDAC inhibitors on cartilage destruction.

Results

Western blot analysis concluded all inhibitors cause a concentration dependent increase in histone 3 and histone 4 acetylation, with only trichostatin A causing an increase in alpha-tubulin acetylation. Quantitative RT-PCR analysis of cytokine stimulated SW1353 cells showed that MMP1 and MMP13 expression is significantly reduced by TSA and VPA but not MS-275, and all three compounds significantly reduce cytokine induced MMP3 expression. BNC explant assays indicate all three compounds inhibit cytokine induced cartilage resorption and we are currently screening gene expression in this model.

Discussion

All three inhibitors protect against cytokine induced cartilage resorption despite their differential ability to inhibit classical HDACs. MS-275 blocks cartilage resorption despite having no effect on the expression of key collagenase genes. In common with the other HDAC inhibitors, it does abrogate cytokine-induced MMP3 expression, potentially preventing collagenase activation, and this may therefore be a key mode of action for such compounds.

P087

Dietary histone deacetylase inhibitors as chondroprotective agents

RK Davidson[1], *KL Culley*[1], *Y Bao*[1], *IM Clark*[1]

[1]Biomedical Research Centre, University of East Anglia, Earlham Road, Norwich, NR4 7TJ, United Kingdom

Introduction

Organosulfur compounds such as sulforaphane and allicin occur naturally in plants and are part of the normal diet. Sulforaphane can inhibit tumourigenesis in animal models, has anti-inflammatory properties and of particular interest, is reported to inhibit histone deacetylase activity (Myzack et al; 2004). Allicin, the pungent compound found in garlic, and related allyl sulfur compounds have been shown to inhibit the proliferation of several human tumour cell lines but not normal cells. These compounds have been shown to modify DNA methylation and acetylation (Lea et al; 2002). Histone deacetylase inhibitors are potential chondroprotective agents (Young et al; 2005, Xu et al; 2006).

Materials and Methods

Sulforaphane (SFN) and diallyl disulfide (DADS) were tested for their relative efficacy in modulating cytokine-induced metalloproteinase expression and histone acetylation in chondrocytes using quantitative RT-PCR and Western blotting. Furthermore, these compounds were tested for their ability to prevent cartilage destruction in the bovine nasal cartilage assay. Lactate dehydrogenase assays were used to test for toxicity.

Results

Both SFN (5-10muM) and DADS (4-32muM) significantly attenuated IL-1beta/oncostatin M-induced MMP1, MMP3, MMP13, and ADAMTS4 expression in SW1353 chondrosarcoma cells. MMP2 and MMP28 were not affected. Basal ADAMTS5 expression was repressed by SFN at 5-15muM. Global and histone H3 acetylation was not affected by SFN or DADS treatment in the SW1353 cell line. Cytokine-induced cartilage destruction was abrogated in a dose dependent manner by SFN (5-30muM) and DADS (8-32muM), measured by collagen and glycosaminoglycan release.

Discussion

Type II collagen and aggrecan are major structural components of cartilage. MMP-1 and MMP-13 are key collagenolytic metalloproteinases in arthritic disease since they can degrade collagen type II. ADAMTS-4 and ADAMTS-5 have aggrecanase activity. SFN and DADS can attenuate the induction of these genes in a dose dependent manner but do not appear to function through the inhibition of histone deacetylases. SFN and DADS are potential chondroprotective agents.

REFERENCES

Myzak MC, Karplus PA, Chung FL, Dashwood RH. (2004) A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. Cancer Res. 64(16):5767-5774.

Young DA, Lakey RL, Pennington CJ, Jones D, Kevorkian L, Edwards DR, Cawston TE, Clark IM. (2005) Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. Arthritis Res Ther. 7(3):R503-512.

Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, Conney AH, Kong AN. (2006) Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer Res. 66(16):8293-6.

P088

Involvement of NMDAR in chondrocyte cell death and matrix degeneration

L Ramage[1], *GE Hardingham*[2], *DM Salter*[1]

[1]Centre for Inflammation Research, Queens Medical Research Institute, The University of Edinburgh, 47 Little France Crescent, Edinburgh, Edinburgh, EH16 4TJ, United Kingdom; [2]Centre for Neuroscience Research, Hugh Robson Building, The University of Edinburgh, George Square, Edinburgh, Edinburgh, EH8 9XD, United Kingdom

Introduction

NMDA receptors (NMDAR) are expressed by chondrocytes in articular cartilage although their function is yet to be fully understood. NMDAR are ionotropic glutamate receptors which when excessively stimulated in neurones induce cell death and neuronal degeneration. In osteoarthritis (OA) chondrocyte death is recognised to be important and associated with matrix loss and cartilage degeneration. The aim of this study was to identify if activation of chondrocyte NMDAR influences cell survival and influences matrix production or breakdown.

Materials and Methods

Rat femoral heads. normal and OA human articular chondrocytes (HACs) were used. Femoral heads treated with NMDA (50μM and/or 1mM) for 72 hours, were fixed and stained to assess cell death (live/dead and TUNEL assays) and proteoglycan content (toluidine blue). HACs were cultured in monolayer before treatment with 50μM NMDA for up to 24 hours, RNA extracted and cartilage matrix and protease gene expression analysed by RT-PCR.

Results

Excessive stimulation of NMDAR led to an increase in cell death and loss of proteoglycan in rat femoral heads. Stimulation of HACs with NMDA resulted in changes in gene expression. In normal chondrocytes the expression of MMP-3 was reduced, TIMP-1 was increased and aggrecan, collagen II, and MMP-13 were unaffected. In OA chondrocytes collagen II, MMP-13, and TIMP-1 were reduced while aggrecan and MMP-3 gene expression were unaffected.

Discussion

The results in this study indicate that stimulation of NMDAR in chondrocytes has the potential to modify cartilage structure. Excessive stimulation of normal cartilage was able to induce cell death and reduction of proteoglycan expression. Analysis of matrix molecule gene expression in human chondrocytes after NMDAR stimulation from both normal and OA cartilage indicates that there is a shift from a more anabolic (↓ MMP-3, ↑ TIMP-1) to a more catabolic (↓ collagen, TIMP-1) gene expression profile in OA chondrocytes.

P089

The MEK-ERK signalling pathway plays a role in SOX9 gene expression in human articular chondrocytes

MJ Peffers[1], *SR Tew*[1], *PI Milner*[1], *PD Clegg*[1]

[1]University of Liverpool, Liverpool, Liverpool, L69 7ZJ, United Kingdom

Introduction

Chondrocytes can respond to external signals including local osmotic environment to regulate extracellular matrix (ECM) production (Mow at al.1999). The expression of many of the genes encoding cartilage ECM is controlled by the transcription factor SOX9. The mechanisms of regulation of SOX9 are not fully understood but may involve post-transcriptional regulation by a p38 MAPK - mediated mechanism (Tew and Hardingham 2006). Interestingly, p38 MAPK activity and post transcriptional regulation of SOX9 can be controlled by changes in chondrocyte osmotic environment (Tew S.R., et al. unpublished). Here we examine if ERK1/2 signalling is also required for osmotic regulation of SOX9 in human primary and passaged chondrocytes.

Materials and Methods

Osteoarthritic human articular chondrocytes obtained following total knee arthroplasty were grown as monolayers and used as either primary or passaged cells. Cells were exposed to control (380mOsm) or hyperosmotic (550mOsm) medium for 5 hours containing either DMSO, or the MEK1/2 inhibitor U0126. SOX9 mRNA levels were measured using qRT-PCR following lysis of cells with Tri-reagent and RNA isolation

Results

Hyperosmotic (550mOsm) conditions significantly increased SOX9 mRNA in both primary and passaged chondrocytes (P=0.0027 and P=0.003) in comparison to controls. Overall in primary cells U0126 significantly increased SOX9 gene expression (P= 0.016). This equated to a 2 fold increase at 380mOsm. An interaction was also seen between 550mOsm and U0126 as the increase in SOX9 at 550mOsm is lower in the presence of MEK1/2 inhibitor. In contrast in passaged cells there was a reduction in SOX9 mRNA gene expression when U0126 was present (P=0.0081).

Discussion

In primary cells it would appear that ERK1/2 signalling has a negative effect on-the expression of SOX9 mRNA. Although this alteration in fold change of SOX9 is small, changes in SOX9 mRNA gene expression in chondrocytes are restricted and the importance of small changes is evident from mouse Sox9 knock-out/knock-in studies (Akiyama et al. 2004). In contrast in passaged cells, which have lost their chondrocyte phenotype and become more fibroblastic in appearance, it seems that the presence of ERK1/2 signalling has a positive effect on SOX9 induction. These differences would suggest that the mode of regulation is dependent upon the differentiation state of the cell.

REFERENCES

Akiyama H., Lyons J.P., Mori-Akiyama Y., et al. (2004). Interactions between Sox9 and beta-catenin control chondrocyte differentiation. Genes Dev. 18, 1072-1087.

Mow V.C., Wang C.C., Hung C.T. (1999). The extracellular matrix, interstitial fluid and ions as a mechanical signal transducer in articular cartilage. Osteoarthritis Cartilage 7, 41-58.

Tew S.R.& HardinghamT.E (2006) Regulation of SOX9 mRNA in Human Articular Chondrocytes Involving p38 MAPK Activation and mRNA Stabilization. J. Biol. Chem. 281, 39471-39479

P090

Hyperosmotic stimulation of chondrocytes leads to differential post transcriptional regulation of SOX9 and COL2A1 mRNA: potential role for the RNA binding proteins TTP and AUF1?

SR Tew[1], *PD Clegg*[1]

[1]Faculty of Veterinary Sciences, Leahurst, Neston, Cheshire, CH64 7TE, United Kingdom

Introduction

Post transcriptional control of SOX9 mRNA has been shown to occur following exposure of human articular chondrocytes (HAC) to stress conditions (cycloheximide, hyperosmolarity) (Tew &

Hardingham 2006, Tew et al. 2008). These conditions lead to stabilisation of the mRNA and an increase in its steady state levels. In this study we have started to examine how sequences within the SOX9 3`UTR may control this process and how the chondrocyte specific collagen gene COL2A1 responds under similar conditions.

Materials and Methods

Freshly isolated HAC were plated as high density monolayer cultures and used within 48 hours. Cells were cultured for 5 hours under 380mOsm (control) or 550mOsm (hyperosmotic) conditions before RNA was isolated. To measure t1/2 of mRNA, actinomycin D chase experiments were performed under these conditions. SOX9 and COL2A1 mRNA levels were determined by qPCR. SOX9 3'UTR was amplified by RT-PCR and cloned into pGEMT-Easy. In vitro transcription was performed to create biotinylated probes for RNA electromobility shift assays (EMSAs) or SOX9 3`UTR was subcloned into the pBBB reporter vector (Loflin et al. 1999) and transfected into NIH/3T3 cells.

Results

Hyperosmotic stimulation of HAC led to increased SOX9 mRNA levels which was associated with an increase in the mRNA t1/2. Conversely, COL2A1 mRNA levels were slightly decreased and this was associated with a decrease in the mRNA t1/2. Molecular analysis of the SOX9 3`UTR by RNA EMSA revealed a number of complexes formed following incubation with HAC cell lysates and addition of antibodies to the proteins TTP or AUF1 (but not to HuR) caused supershifts. Introduction of the SOX9 3`UTR sequence into the pBBB vector destabilised the encoded beta-globin transcript (t1/2- pBBB = 17.7 hours, pBBB_S9UTR = 2.8 hours).

Discussion

Despite increased SOX9 levels, COL2A1 mRNA levels decrease in HAC exposed to hyperosmotic conditions. Changes in both genes appear to involve a post transcriptional mechanism but display opposite responses. Elements within the SOX9 3`UTR act to destabilise transcripts and can interact with the RNA regulatory proteins TTP and AUF1. Understanding this post transcriptional control mechanism could help us to regulate cartilage gene expression during tissue regeneration and disease.

REFERENCES

Loflin P.T., Chen C.A., Xu N. And Shyu A (1999) Transcriptional Pulsing Approaches for Analysis of mRNA Turnover in Mammalian Cells. Methods 17,11-20.

Tew S.R. & Hardingham T.E (2006) Regulation of SOX9 mRNA in Human Articular Chondrocytes Involving p38 MAPK Activation and mRNA Stabilization. J. Biol. Chem. 281, 39471-39479

Tew S.R., McKay T., Lowe E., Clegg P.D. & Hardingham T.E. (2008) Osmotic regulation of SOX9 in human articular chondrocytes through p38 MAPK mediated mRNA stabilization. Int J Exp Path. 89, A26

P091

Mir-675 regulates collagen II levels in human articular chondrocytes

K Dudek[1], *JE Lafont*[1], *CL Murphy*[1]

[1]Kennedy Institute of Rheumatology, 65 Aspenlea Road, Hammersmith, London, W6 8LH, United Kingdom

Introduction

MicroRNAs (miRNAs) are endogenous RNAs that have recently emerged as a novel class of important gene-regulatory molecules. They have been found to take part in many biological processes, however almost nothing is known about their function in cartilage at present. Nevertheless, Kobayashi and colleagues have proven the importance of miRNAs in skeletal development by showing Dicer-null mice to have reduced amounts of proliferating chondrocytes, which caused severe skeletal growth defects (Kobayashi et al, 2008). In our laboratory, microarray studies performed using human articular chondrocytes revealed a highly abundant non-protein coding RNA transcript (H19) whose expression was comparable with the main cartilage matrix genes: collagen II (Col2) and aggrecan (Agc) (Lafont et al., 2008). H19 has been found as a primary miRNA transcript encoding miR-675. The function of H19/miR-675 is currently being investigated.

Materials and Methods

The function of miR-675 was studied by its overexpression (using miRNA mimics) and inhibition (using antisense oligonucleotides). The primary transcript itself (H19) has been investigated for its possible role in Col2 expression changes independently from miR-675 by performing its knock-down (using siRNAs) with concurrent miR-675 overexpression. The phenotypic effects were assessed by measuring Col2a1 at mRNA (real time PCR-based assays) and protein levels (western blotting).

Results

RNA interference experiments showed that both H19 and miR-675 are hypoxia-inducible and SOX9 dependent. The overexpression of miR-675 increased Col2a1 levels (mRNA and secreted protein). Whereas the H19 knock-down and the use of miR-675 antisense caused a decrease in its expression levels. Finally, in experiments where H19 was knocked-down while miR-675 was concurrently overexpressed the expression of Col2a1 was restored.

Discussion

We show that Col2a1 expression in human articular chondrocytes is highly dependent on the miRNA derived from H19 primary transcript. MiR-675 may be a new regulator taking part in promotion of differentiated chondrocyte phenotype.

REFERENCES

Kobayashi T., Lu J., Cobb B. S., Rodda S. J., McMahon A. P., Schipani E., Merkenschlager M., Kronenberg H M. (2008) Dicer-dependent pathways regulate chondrocyte proliferation and differentiation. PNAS 105, 1949-1954

Lafont J.E., Talma S., Hopfgarten C. & Murphy C.L. (2008) Hypoxia promotes the differentiated human articular chondrocyte phenotype through SOX9-dependent and -independent pathways. J. Biol. Chem. 283, 4778-4786.

P092

CXCR1/2 signalling in the phenotypic stability of in vitro expanded adult human articular chondrocytes

J C Sherwood[1], *C Pitzalis*[1], *F Dell'Accio*[1]

[1]Centre for Experimental Medicine & Rheumatology, Barts & the London Queen Mary's School of Medicine, John Vane Building Charterhouse Square, London, EC1M 6BQ, United Kingdom

Introduction

The capacity of in vitro expanded chondrocytes to form stable cartilage in vivo is one mechanism of action of autologous chondrocyte transplantation for the repair of joint surface defects, but is progressively lost with culture expansion needed to obtain sufficient chondrocytes for implantation. The expression of some ELR+ CXC chemokines is associated with this capacity and is lost with culture expansion. This study tests the hypothesis that CXCR-1 and CXCR-2 signalling is required for the phenotypic stability of adult human articular chondrocytes.

Materials and Methods

Human adult articular chondrocytes (AHAC) were enzymatically released and expanded in monolayer culture under standard conditions. CXCR-1 and -2 were blocked in vitro using validated

blocking antibodies. Cells treated with aspecific mouse IgG antibodies represented negative controls. CXCR1/2 downstream signalling was blocked using the Galpha-i inhibitor pertussis toxin. The expression of molecular markers associated with the capacity of AHAC to form stable cartilage in vivo was assessed using real time PCR (Dell'Accio et al. 2001, A&R), and the ability of the cells to produce proteoglycans was analysed using Alcian blue staining.

Results

Blocking signalling through CXCR1 and CXCR2 in early passage AHAC resulted in a downregulation of some but not all molecular markers associated with the cartilage forming capacity of AHAC, including type II collagen, aggrecan, BMP2 and SOX9 (a transcription factor required for cartilage formation). Downstream blockade of CXCR1/2 receptors using the Galpha-i inhibitor pertussis toxin also resulted in downregulation of type II collagen and aggrecan. Inhibition using both methods resulted in a decrease in the proteoglycan synthesis of cells cultured in micromass.

Discussion

CXCR1 and CXCR2 blockade results in downregulation of some but not all molecular markers associated with the capacity of AHAC to form stable cartilage in vivo.

In vivo blockade in transplantation experiments are ongoing to investigate whether these phenotypic changes are result in a loss of cartilage-forming capacity.

These data suggest that CXCR signalling may be required for articular cartilage homeostasis.

REFERENCES

Dell'accio,F.; De,Bari C.; Luyten,F.P. (2001) Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. Arthritis Rheum. (44) 7; 1608-1619.

P093

Effects of the modulation of Wnt-canonical pathway on the phenotypic stability of human articular chondrocytes

G Nalesso[1], *NM Eltawil*[1], *C Pitzalis*[1], *F Dell'Accio*[1]

[1]Barts and the London Medical School William Harvey Research Institute, London, EC1M 6BQ, United Kingdom

Introduction

Wnt-canonical pathway has been demonstrated to have a role in adult cartilage homeostasis, however its precise function is still controversial as both activation or disruption result in osteoarthritis (Zhu et al. 2008; Zhu et al. 2009). We have focused our study in investigating the effect of the activation of Wnt-canonical pathway on chondrocyte phenotypic stability because this property is important for the successful outcome of Autologous Chondrocytes Implantation (ACI) (Luyten et al. 2001).

Materials and Methods

Cartilage explants and primary chondrocytes were isolated from preserved areas of human cartilage of patients undergoing knee arthroplasty for osteoarthritis. Cartilage caps were dissected from adult mouse femoral heads. To activate the canonical pathway, cells, explants, and femoral heads were cultured in presence of Wnt3a, LiCl, or vehicle control for variable times. WNT activation was evaluated by reporter assay, nuclear accumulation of beta-catenin, or expression of the target gene Axin-2. The chondrocyte phenotype was evaluated by real time PCR testing the expression of cartilage markers including Col2A1 and aggrecan. The phenotypic stability of articular chondrocytes has been evaluated by using an ectopic implantation assay in nude mice.

Results

Activation of the canonical pathway by Wnt3a and LiCl in chondrocytes, explants and murine cartilage caps was confirmed by upregulation of Axin-2 mRNA, activation of the TOP-FLASH reporter assay, and nuclear accumulation of beta-catenin. Wnt-beta-catenin activation also resulted in a marked and irreversible downregulation of Col2A1 and aggrecan mRNA in monolayer cells but in their upregulation in human cartilage explants and in the murine cartilage caps.

Discussion

We have shown that the activation of Wnt canonical pathway under different experimental conditions may result in an opposite regulation of markers of chondrocyte differentiation. Therefore, we are currently testing whether activation of the canonical Wnt signalling can modulate the capacity of cultured articular chondrocytes to form stable cartilage in vivo. This capacity is proposed as a crucial quality control for efficacy of chondrocyte preparation. for ACI (Luyten et al. 2001). If successful in vivo, we anticipate that the modulation of Wnt–canonical pathway in chondrocytes may represent a tool to enhance efficacy and consistency of ACI.

REFERENCES

Luyten F.P., Dell'Accio F. & De Bari C. (2001) Skeletal tissue engineering: opportunities and challenges Best Pract Res Clin Rheumatol. 15, 759-69

Zhu M., Chen M., Zuscik M., Wu Q., Wang Y.J., Rosier R.N., O'Keefe R.J. & Chen D. (2008) Inhibition of beta-catenin signaling in articular chondrocytes results in articular cartilage destruction. Arthritis Rheum. 58, 2053-64.

Zhu M., Tang D., Wu Q., Hao S., Chen M., Xie C., Rosier R.N., O'Keefe R.J., Zuscik M. & Chen D. (2009) Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. J Bone Miner Res. 24, 12-21.

P094

Promotion of chondrocyte function by inhibition of HIF-specific hydroxylases

BL Thoms[1], *JE Lafont*[1], *CL Murphy*[1]

[1]Kennedy Institute of Rheumatology, 65 Aspenlea Road, London, W6 8LH, United Kingdom

Introduction

Recent work in our laboratory has demonstrated that hypoxia inducible factor 2alpha (HIF-2alpha), not HIF-1alpha, is necessary for hypoxia-induced SOX9 synthesis and the subsequent cartilage matrix production (1). Under normoxic conditions the HIF-alpha subunit is hydroxylated on specific conserved proline residues by proyl-hydroxylase domain enzymes (PHDs). This hydroxylation signals the alpha-subunit's polyubiquitination and subsequent degradation. While all HIF-targeting hydroxylases have demonstrated the ability to hydroxylate HIF-alpha, recent work has shown some PHD enzymes display a preference for either HIF-1alpha or HIF-2alpha. We have investigated the regulation of HIF-alpha isoforms by hydroxylases using siRNA against PHD1/PHD2/PHD3/PHD4 with the aim of stabilising HIF-2alpha, up-regulating SOX9 and increasing cartilage matrix deposition.

Materials and Methods

Human articular chondrocytes (HACs) were cultured as a monolayer at 20% or 1% oxygen. Hydroxylases were broadly inhibited using either DMOG or DFO, or specifically inhibited using siRNA. The resulting HIF-alpha stabilization was assessed using western blot and the expression of chondrocyte markers

(SOX9) and key cartilage extracellular matrix proteins (COL2A1, COL9A1 and AGGRECAN) using real-time PCR and western blot.

Results

We report the knockdown of PHD1/2/3/4 and FIH1 by between 82%-97% at the mRNA level in HACs. Knockdown of PHD2 alone was sufficient to stabilise both HIF1-alpha and HIF-2alpha even under normoxic conditions (when HIF-alpha is normally degraded). PHD1 may preferentially regulate HIF-2alpha but this deserves further investigation. To date, results indicate that inhibition of PHD2 can stabilise HIF-2alpha, up-regulate SOX9 and promote matrix gene expression in human articular chondrocytes.

Discussion

We have identified that the inhibition of PHD2 stabilizes both HIF-1alpha and HIF-2alpha even under normoxic conditions. Importantly, SOX9 and COL2A1 levels were enhanced by PHD2 depletion in both 20% and 1%. This work may therefore have therapeutic applications since directly inactivating one or more of the PHD enzymes could stabilise HIF-2alpha, promote cartilage synthesis and stimulate repair.

REFERENCES

LaFont J.E., Talma, S. and Murphy C.L. (2007) Hypoxia-Inducible Factor 2 alpha is essential for the human articular chondrocyte phenotype. Arthritis & Rheumatism. 56, 3297-3306.

P095

TGFbeta1-mediated collagen biosynthesis in articular chondrocytes is dependent on an intact microtubular network

EJ Blain[1], *SJ Gilbert*[1], *VC Duance*[1]

[1]CTBL, Cardiff University, Museum Avenue, Cardiff, CF10 3AX, United Kingdom

Introduction

In chondrocytes, an intact tubulin network has previously been shown to be required for secretion of collagen and sulphated glycosaminoglycans. The spatial organisation of these networks is disrupted in human osteoarthritic chondrocytes (Fioravanti et al, 2003) and absent in a rat model of osteoarthrosis (Capín-Gutiérrez et al, 2004). The aim of this study was to elucidate the mechanism(s) involved in the differential regulation of matrix biosynthesis in articular chondrocytes containing a disassembled tubulin network.

Materials and Methods

Tubulin microtubules were disrupted with 1muM colchicine over a 7 day period in articular chondrocytes and disassembly confirmed by confocal microscopy. De novo collagen synthesis was measured by [3H]-proline labelling. Expression levels of beta-tubulin and type II collagen mRNA and protein were assessed by quantitative PCR and Western blotting respectively. TGFbeta1 expression was assessed by ELISA. Chondrocytes were subsequently pre-treated with colchicine for an hour prior to the addition of 10ng/ml TGFbeta1, and mRNA and protein levels of type II collagen, TGFbeta1 and TGFbeta1 receptor assessed. The signalling pathway(s) responsible for TGFbeta1-mediated effects was assessed by pre-treating chondrocytes with selective MAPK and/or SMAD inhibitors.

Results

Disruption of the chondrocyte tubulin networks not only affected spatial organisation but also significantly inhibited beta-tubulin mRNA and protein expression over the 7 day period. De novo collagen synthesis was inhibited by tubulin disruption. In the absence of intact tubulin networks, there was a significant reduction in TGFbeta1 synthesis. TGFbeta1 is a potent inducer of type II collagen synthesis in chondrocytes. We have found that if the tubulin networks are disrupted, exogenous TGFbeta1-induced collagen mRNA and protein synthesis is prevented, whilst expression levels of TGFbeta1 and TGFbeta1-receptor mRNA are significantly elevated. Our preliminary studies suggest that an intact tubulin network, SMAD 2 and p38 kinase are involved in TGFbeta1-mediated signalling in chondrocytes.

Discussion

Modulation of tubulin dramatically affects chondrocyte biosynthesis. Clearly there is a strong link between the correct assembly/turnover of the microtubule network and tissue homeostasis. These studies will aid in elucidating the role of the chondrocyte cytoskeleton in signal transduction and provide an insight into how an abnormal cytoskeleton may contribute to arthritic diseases.

REFERENCES

1 Fioravanti A, Nerucci F, Annefeld M, Collodel G & Marcolongo R. (2003). Morphological and cytoskeletal aspects of cultivated normal and osteoarthritic human articular chondrocytes after cyclical pressure: a pilot study. Clin Exp Rheumatol. 21, 739-46.

2 Capín-Gutiérrez N, Talamás-Rohana P, González-Robles A, Lavalle-Montalvo C & Kourí JB. (2004). Cytoskeleton disruption in chondrocytes from a rat osteoarthrosic (OA) -induced model: its potential role in OA pathogenesis. Histol Histopathol.19, 1125-32.

P096

Identification and characterisation of microRNAs involved in chondrogenesis and osteoarthritis

TE Swingler[1], *T Dalmay*[1], *DA Young*[2], *RP Boot-Handford*[3], *E Crawford*[3], *IM Clark*[1]

[1]Dept of Biological Sciences, University of East Anglia, Earlham rd, Norwich, NR4 7TJ, United Kingdom; [2]University of Newcastle, Newcastle, United Kingdom; [3]University of Manchester, Manchester, United Kingdom

Introduction

The majority of skeletal bones develop through the process of endochondral ossification. During development mesenchymal cells aggregate where future bones will develop. These early chondrocyte cells begin a series of differentiation events, including proliferation, hypertrophy, terminal differentiation, mineralization and programmed cell death. This process controls longitudinal growth of endochondral bones with eventual closure of the growth plate leaving only articular cartilage. Chondrocytes in the articular cartilage are constrained from completing this developmental programme allowing maintenance of a functional cartilage layer, essential for normal joint function. The pathology of osteoarthritis (OA), at least in part, involves replaying developmental signals leading to aberrant differentiation of chondrocytes with expression of hypertrophic genes such as collagen X and MMP13. MicroRNAs (miRNAs) have emerged as a new class of gene expression regulators. MiRNAs are 20–24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. Little is known about miRNA expression in chondrocyte differentiation.

Materials and Methods

We have used a murine cell model of chondrocyte differentiation to examine miRNA expression. ATDC5 cells are a murine embryonic line that can differentiate in vitro through chondrogenesis. To confirm differentiation, markers such as matrix deposition (alcian blue staining for GAG) and collagen X expression were examined. We then performed a microRNA microarray (Exiqon) to profile the expression of 609 miRNAs during chondrocyte differentiation.

Results

Differentiation of ATDC5 cells was confirmed by progressively intense matrix staining with alcian blue in the induced cells over the 42 day culture period. Quantitative RT-PCR confirmed increased expression of collagen X at the later time points. MicroRNA microarray analysis identified a number of miRNAs differentially expressed during chondrocyte differentiation. We have examined the expression pattern of some of the identified miRNAs by in situ hybridisation (ISH) on mouse embryos at varying stages of development.

Discussion

We are currently undertaking experiments identifying targets of miRNAs expressed during chondrocyte differentiation. MiRNAs may function to regulate the expression of keys genes during normal and abberant chondrocyte differention.

P097

The organization of microfibrils and elastin fibres within the canine cruciate ligament complex

KD Smith[1], A Vaughan-Thomas[1], PD Clegg[1], D Spiller[2], JF Innes[1], EJ Comerford[1]

[1]University of Liverpool, Leahurst Campus, Chester High Rd, Neston, CH64 7TE, United Kingdom; [2]Centre for Cell Imaging, University of Liverpool, Liverpool, United Kingdom

Introduction

Cruciate ligaments (CLs) are dense bands of collagenous tissue which stabilise the knee joint. Failure of the anterior cruciate ligament (ACL) is a major source of morbidity in the dog. Histological changes including loss of collagen architecture, loss of ligament cells and chondrogenic change, are considered degenerative (Vasseur et al. 1985). While elastin fibres and microfibrils have important mechanical, biochemical and cell-regulatory functions (Kielty et al. 2002) neither their distribution nor role in maintaining integrity of the canine ligament have been investigated. We report the distribution of elastin fibres and microfibrils in the cruciate ligaments of two breeds of dogs with a differing risk of ligament rupture: the Greyhound (GH, low risk) and the Labrador Retriever (LR, high risk).

Materials and Methods

Cruciate ligaments were obtained from dogs euthanatized for reasons other than musculoskeletal disease. Samples were prepared for histology and confocal laser scanning microscopy (CLSM). Elastin was identified using Van Gieson's and Verhoeff's staining. Fibrillin-1 and -2 were localised using specific primary antibodies and fluorescent secondary antibody detection by CLSM. Hydrated tissue was additionally analysed using Nomarski differential interference microscopy.

Results

Microfibrils and elastin fibres were located predominantly within ligament fascicles, parallel to collagen bundles and with little regional variation. Interfascicular fibres were more heterogenous in size and orientation. Elastin fibre distribution was retained within areas of degenerative matrix, but a marked difference in microfibril staining was noted in the GH but not the LR. Fibrillin 1 and fibrillin 2 were found in microfibrils, pericellularly and between collagen bundles.

Discussion

The distribution of elastin fibres is suggestive of a mechanical role in bundle reorganization following ligament deformation. Microfibrils and smaller elastin fibres may affect low strain stiffness or have additional roles such as cell adhesion or growth factor regulation. Variations in lateral connections between collagen bundles may reflect complex intraligament mechanics. In regions of matrix degeneration, no differences in elastin fibre distribution were noted between the GH and LR CLs. However, as degenerative matrix change increased, increased microfibril staining was noted in the GH, but not the LR. This may represent a fundamental difference between these breeds, reflecting the differing incidence of CL disease.

REFERENCES

Kielty C.M., Sherratt M.J. & Shuttleworth C.A. (2002) Elastic fibres. J. Cell. Sci. 115, 2817-2828.

Vasseur, P.B., Pool, R.R., Arnoczky, S.P. & Lau, R.E. (1985) Correlative biomechanical and histologic study of the cranial cruciate ligament in dogs. Am. J. Vet. Res. 46, 1842-1854

P098

The elastic network in human articular cartilage: an immunohistochemical study of elastin fibres and microfibrils

J Yu[1], S Roberts[2], EH Evans[2], JP Urban[1]

[1]Department of Physiology Anatomy and Genetics, Sherrington Building, University of Oxford, Parks Road, Oxford, OX[1]3PT, United Kingdom; 2 Centre for Spinal Studies, Robert Jones and Agnes Hunt Orthopaedic Hospital & ISTM Keele University, Oswestry, Shropshire, SY10 7AG, United Kingdom

Introduction

Articular cartilage (AC) covers the surfaces of long bones and provides a compression-bearing, low friction surface within synovial joints. The role of AC ultimately depends on its macromolecular composition and organisation. Collagen II and proteoglycans are the main components of the AC but many other components play important roles in its behaviour. Recently we observed extensive and well-organised elastin fibres and microfibrillar networks in bovine cartilage [1]. Here we report our observations on the organisation of the elastic network in human knee articular cartilage.

Materials and Methods

Human healthy cadaveric knees (21- 50 years) were obtained from the UK Human Tissue Bank within 24 hours of death (approved by the Trent Research Ethics Committee, UK). Biopsies were taken from three regions including the trochlear groove and medial and lateral femoral condyles and were snap-frozen and then stored in liquid nitrogen until use. Sagittal sections of 20µm in thickness were cut with a cryomicrotome. Elastin fibres and microfibrils were revealed by dual immunostaining elastin and fibrillin-1 labeled with two different fluorescent dyes. A conventional fluorescent microscope was used to study the network organisation and its colocalisation.

Results

In general a dense network of elastin fibres and microfibrils was observed in the uppermost superficial layer. In the middle and deep layers, little elastin was found in the matrix but there was immunostaining for it within the cell nuclei. Immunostaining for microfibrils appeared to be more dense pericellularly and also within the inter-territorial matrix. There was, however, variation of both elastin and microfibrillar networks seen in samples from different regions of the knee and different aged individuals.

Discussion

Articular cartilage deforms under load and then recovers completely. The surface layer is more compliant than the deeper layers and shows the most deformation under compressive loading [2]. A dense elastic network in the superficial layer could play an important role in reinforcing the network during the loading and enabling network recovery after the load is released.

REFERENCES

1.Yu, J. and J.P. Urban, The Elastic Network of Bovine Articular Cartilage:An Immunohistochemical Study of Elastin Fibres and Microfibrils. J Anat, 2009 submitted.

2.Gore, D.M., G.R. Higginson, and R.J. Minns, Compliance of articular cartilage and its variation through the thickness. Phys Med Biol, 1983. 28(3): p. 233-47.

P099

Echinoderms display key features of vertebrate decoran-collagen fibril interactions despite their lack of genomic decoron

JE Scott[1], J Bella[2]

[1]Chemical Morphology Stopford Building, University of Manchester, Manchester, M13 9PT, United Kingdom; 2 WellcomeTrust Centre for Cell-Matrix Research Smith Building University of Manchester, Manchester, M13 9PT, United Kingdom

Introduction

Proteoglycan (PG, e.g. decorin, now decoran [IUPAC/ IUBMB recommended nomenclature]) anionic glycosaminoglycan (AGAG) interfibrillar bridges are found in all vertebrate extracellular matrices, associated at sites in the collagen fibril d & e bands (DS rich) and a & c bands (KS rich), in which pentapeptide-motives (e.g GDRGE) lie close to decoran (Scott, 2008). Apparently similar structures are found in echinoderms (Erlinger et al, 1993), although decoron is absent from their genomes (Park et al, 2008). We now compare vertebrate and echinoderm interfibrillar structures for homologies.

Materials and Methods

PGs/AGAGs were located electron microscopically after staining with Cupromeronic blue in the critical electrolyte concentration (CEC) mode. Positive staining of collagen banding patterns used uranyl acetate. Negative staining for gap and overlap zones used phosphotungstate. Aminoacid sequences of collagen molecules were from Swissprot.

Results

Echinoderm PGs/AGAGs regularly associated with specific bands in the collagen D-period repeat pattern. These patterns differed too much to allow comparisons with vertebrates. However, PGs/AGAGs were always attached at the the gap zone, as in vertebrates. Collagen pentapeptide motives were in equivalent positions after alignment.

Discussion

Key elements of the PG interfibrillar shape modules (Scott 2008) are present in the echinoderms featherstar, holothurians, sea urchins. Echinoderm AGAGs are chondroitin sulphate-based (Erlinger, Welsch & Scott, 1993), as in vertebrates. Iduronate is present in tunicates (in DS). Since decoron is absent from echinoderm genomes (Park et al, 2008) we speculate that other protein(s) may substitute for decoron in echinoderms.

REFERENCES

Scott, J.E.(2008) Cartilage is held together by elastic glycan strings. Physiological and pathological implications. Biorheol. 45, 209-217.

Erlinger, R., Welsch, U., & Scott, J.E. (1993) Ultrastructural and biochemical observations on proteoglycans and collagen in the mutable connective tissue of the feather star Antedon bifida (Echinodermata, Crinoidia). J.Anat., 183, 1-11.

Park H., Huxley-Jones, J., Boot-Handford, R.P., Bishop P.N., & Bella, J. (2008) LRRCE: a leucine-rich repeat cysteine capping motif unique to the chordate lineage. BMC Genomics 9, 599.

P100

Behaviour of enamel following a short duration acid exposure

P Laurance-Young[1], L Bozec[1], JC Knowles[1], G Rees[2], F Lippert[2]

[1]Dept Of Biomaterials & Tissue Engineering, UCL Eastman Dental Institute, 256 Gray's Inn Road, London, WC1X 8LD, United Kingdom; [2]GlaxoSmithKline Consumer Healthcare St George's Avenue, Weybridge, KT13 0DE, United Kingdom

Introduction

Dental erosion is a rising phenomenon in developed countries, especially amongst children (53% of children under 5 and 25% of 12-15 year olds in the UK were reported to have at least one incisor demonstrating tooth surface loss). Although many studies have been documented regarding dental erosion, the behaviour of enamel following early duration acid challenges - within a time frame similar to social drinking habits – remains poorly understood. A majority of dental experiments utilise times not commensurate with drinking behaviour to study dental erosion, frequently employing erosion durations of between 10-30 minutes or longer.

In order to more accurately simulate drinking behaviour we performed a series of studies to examine the behaviour of enamel following a short duration acid exposure.

Materials and Methods

Two models were developed using erosion windows created on samples of bovine enamel as well as human. The first model, commonly used in research, involved exposing the enamel to acid at single, defined time points. The second model involved subjecting a single enamel sample to repeated acid challenges. The resultant bulk tissue loss was measured using optical profilometry

Results

Our results indicated a considerable difference in erosion rates following initial erosion events compared to later, longer durations. Erosion depths ranged from 7.84 µm (10sec) to 60.13µm (600sec) were observed in the repeated challenge model, whereas depths of 0.82 (10sec) to 10.20 (600sec) where observed in the first model. This data demonstrated significantly deeper erosion lesions compared to the single acid challenge suggesting two distinct behaviours. It was also noted that the continuous model had a much higher rate of erosion compared to the single: 0.32 for the repeated acid challenge compared to 0.19 for the single acid challenge.

Discussion

Whilst performing microhardness measurements, we found no statistical variation between the two models; however we suspect the presence of the softened enamel surface layer in the case of the single acid challnege which may not be detectable using this approach. Another particular point of interest was the presence of "pillars" within the erosion widows that proved to be resistant to short acid exposure. The nature and mechanical implications of those have been characterised using atomic force microscopy. Taking this into account, it is anticipated that erosive loss of the softened surface layer is responsible for the discrepancy observed between the two erosion models. Using an AFM-based indentation in conjunction to X-ray diffraction, we aim to correlate the variability between the two models and present a more accurate model of acid – enamel erosion mimicking social drinking habits.

REFERENCES

Mellberg JR, (1992), Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity in situ, J. Den. Res. 71(Special Issue), 913-919

Attin T, Wagehaupt F, Gries D, Wiegand A, (2007), The potential of deciduous and permanent bovine enamel as substitute for deciduous and permanent enamel human enamel: Erosion-abrasion experiments, J. Dent. 35,773-777

P101

Breast cancer-bone marrow stromal cell interactions modify expression of proteolytic enzymes and regulators of bone turnover

J Woodward[1], I Holen[1], D Lefley[1], P Ottewell[1], D Buttle[2]

[1]Academic Unit of Clinical Oncology, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, S10 2RX, United Kingdom; [2]Academic Unit of Molecular Medicine, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, S10 2RX, United Kingdom

Introduction

Breast cancer cells frequently metastasise to bone, disrupting normal bone turnover, favouring bone breakdown. Proteolytic enzymes are implicated in several key steps in this process. The enzymes involved, as well as their endogenous inhibitors and the factors that regulate bone turnover are produced both by tumour cells and by the surrounding non-malignant cells, and expression and enzyme activity is regulated by a number of complex cell-cell interactions, amongst other factors. We have investigated whether interactions between non-metastatic (MDA-MB-436) and bone-metastatic (B02) breast cancer cells and human bone marrow stromal cells (HS-5) affect gene expression of proteolytic enzymes and their endogenous inhibitors, as well as regulators of bone turnover, by these cells.

Materials and Methods

MDA-MB-436 or B02 breast cancer cells transfected with GFP, and HS-5 cells were grown individually or as co-cultures for 72h, and cells from the mixed population were subsequently separated using flow cytometry. Changes in expression of 96 genes (43 proteolytic enzymes, 50 regulators of bone turnover and 3 house-keeping genes) were assessed using custom made low-density arrays with real-time RT-PCR.

Results

Co-culture of the B02 cells with HS-5 cells caused predominant increased expression of genes involved with bone metastasis including GM-CSF, uPA and inhibitors of the Wnt-pathway by B02 cells. In contrast, co-culture of MDA-MB-436 cells with HS-5 cells decreased expression of cathepsin K and PAI-1 and inhibitors of the Wnt pathway by the HS-5 cells. Genes that were commonly up-regulated in both systems following co-culture included a number of MMPs and neprilysin by the tumour cells and cystatin 4 by the HS-5 cells.

Discussion

Our data demonstrate that direct contact between tumour cells and the cells of the bone microenvironment modulate expression of genes potentially involved in tumour spread to bone. The modulation of gene expression by cancer cells that freely metastasise to bone was different from the effects of co-culturing non-metastatic cancer cells. These experiments therefore identify genes that may affect the ability of particular breast cancer cells to metastasise to grow and expand in bone.

P102

Regulation and functional analyses of MMP-28

U Rodgers[1], L Kevorkian[1], A Parker[2], I Clark[1]

[1]School of Biological Sciences, Earlham Rd, University of East Anglia, Norwich, NR4 7TJ, United Kingdom; [2]AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Macclesfield, SK10 4TG, United Kingdom

Introduction

MMP-28 (epilysin) is a recently discovered member of the matrix metalloproteinase family. We have previously reported expression of MMP28 in normal human articular cartilage and synovium with significant induction of expression in osteoarthritis (Davidson et al., 2006). The regulation of the MMP28 gene and the physiological function and substrates of the MMP-28 protein are currently unknown.

Materials and Methods

Expression constructs for wild-type MMP28, an inactive mutant (EA mutant) and a truncated form containing only the pro and catalytic (Pro-Cat) domains were cloned into pcDNA4-FLAG. Protein expression after transfection into a variety of cell types was assessed by western blotting and immunocytochemistry. Stable cell lines were generated using SW1353 chondrosarcoma cells and clonal lines were then generated. Functional analyses included cell proliferation, adhesion and migration.

Results

Lysates from SW1353 cells over-expressing the full-length MMP28 cDNA contained pro- and active forms of MMP-28, conditioned medium contained pro-MMP-28 and a cleaved C-terminal domain, and ECM fractions contained some pro-MMP-28 and also the active form. Using a furin inhibitor, we found that the activation of MMP-28 was furin dependent. Overexpression of MMP-28 leads to increased adhesion on both fibronectin and type II collagen and a decrease in migration on type II collagen. An increase in MMP-2 expression and activity is also seen. No change in proliferation or apoptosis was observed.

Discussion

In initial studies to characterise the regulation and function of MMP-28, we observed that the activation of pro-MMP-28 is furin dependent. Interestingly, the active enzyme preferentially associates with the ECM. This may suggest a matrix substrate for MMP-28. We have successfully generated SW1353 cell lines stably transfected with MMP28 (full length, EA mutant and pro-cat). Phenotypic changes to adhesion and proliferation have been measured, along with altered expression of other metalloproteinase genes. A more complete analysis including candidate protein and proteomic screens of potential substrates is currently underway.

REFERENCES

Davidson, R. K., Waters, J. G., Kevorkian, L., Darrah, C., Cooper, A., Donell, S. T. & Clark, I. M. (2006). Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. Arthritis Res Ther, 8, R124.

P103

S100A4 expression in fibrostenosing crohn's disease: evidence for a role in T-lymphocytes and fibroblasts but not in epithelial-mesenchymal transition

NG Docherty[1], *MF Cunningham*[1], *T Toivonen*[2], *DC Cottell*[2], *JP Burke*[1], *PR O'Connell*[1]

[1]UCD Conway Institute of Biomolecular and Biomedical Sciences UCD School of Medicine and Medical Sciences and Surgical Professorial Unit St. Vincent's University Hospital, Dublin, Irish Republic; [2]Electron Microscopy Unit, University College Dublin, Dublin, Irish Republic

Introduction

Obstructive, fibrotic stricturing of the terminal ileum is a common phenotype in Crohn's disease (CD) patients. S100A4 overexpression is implicated in progression in gastrointestinal tumours. While S100A4 has been identified as a marker of activated T-lymphocytes and as a fibroblast derived factor in tumour-stroma interplay, most studies concentrate on its role in epithelial-mesenchymal transition (EMT). As EMT and fibroblast activation may be implicated in stricture pathogenesis, we examined the expression of S100A4 in specimens of resected ileum from patients with fibrostenosing CD.

Materials and Methods

Mucosal samples from strictured (S) and macroscopically normal areas of adjacent terminal ileum (NAdj) from patients undergoing intestinal resection for fibrostenosing CD (n=10) were processed for histochemistry, electron microscopy, immunohistochemistry (S100A4, alpha-smooth muscle actin and CD-3) and real-time RT-PCR and Western blotting of TGF-beta1 and S100A4. In parallel, mRNA and protein were prepared from ileal fibroblast explant cultures of S and NAdj specimens and from Caco-2 and HT-29 colonic epithelial cells exposed to 10ng/ml of TGF-beta1 for 48 hours (n=3). Levels of S100A4 mRNA were determined using real-time RT-PCR

Results

Mucosal peels from S specimens showed lamina propria inflammatory hyperplasia and villous blunting. Electron microscopy revealed loss of epithelial junction structure. No evidence of epithelial invasion of the basement membrane was observed. S specimens showed significant increases in TGF-beta1 and S100A4 mRNA and protein expression, paralleling findings in cultured CACO-2 and HT-29 cells in response to TGF-beta1. Immunohistochemical studies demonstrated that mucosal S100A4 expression in patient samples derived from intraepithelial and lamina propria T-lymphocytes

Immunohistochemistry revealed prominent S100A4 staining in alpha-smooth muscle actin negative fibroblastic cells within fibrosed regions of S specimens. Significantly increased S100A4 mRNA expression was also observed in fibroblast explant cultures from S samples.

Discussion

These data do not support a role for intracellular S100A4 in mediating EMT in the mucosal epithelium in fibrostenosing CD. However, increased expression of S100A4 in stricture fibroblasts in situ and in explant culture does support a role for S100A4 in fibrosis. Experiments characterising the effect of S100A4 knockdown on pro-fibrotic responses in intestinal fibroblasts are underway.

P104

Proteolytic fragments of tenascin-C promote cartilage degradation

N Sofat[1], *M Kashiwagi*[1], *K Midwood*[1], *H Nagase*[1]

[1]The Kennedy Institute of Rheumatology, Dept of Matrix Biology, 65 Aspenlea Road, London, W6 8LH, United Kingdom

Introduction

Tenascin-C is a normal constituent of the extracellular matrix in cartilage. It has a modular structure and shares close structural homology to fibronectin in the type III repeats region. We have previously found that fibronectin expression is highly upregulated in osteoarthritic cartilage compared with normal, and fragments of fibronectin induce cartilage matrix degradation. We now investigate the role of tenascin-C and its fragments in cartilage degradation.

Materials and Methods

We used trypsin/chymotrypsin-digested tenascin-C to investigate the role of full-length and fragmented tenascin-C in the induction of aggrecan loss in porcine articular cartilage. Experiments used the demethylmethylene blue dye assay to detect glycosaminoglycan (GAG) release from cartilage cultures and the anti-ARGSV neoepitope assay to detect aggrecanase activity by tenascin-C fragments. A specific inhibitor of aggrecanase activity, N-TIMP-3 (Kashiwagi et al. 2001), was also used to investigate the specificity of the cartilage degradation induced. Further experiments using recombinant tenascin-C fragments expressed in HEK (human embryonic kidney cells) and E.coli were used to identify which regions of tenascin-C are implicated in cartilage loss.

Results

Fragmented tenascin-C, but not full-length tenascin-C, promoted GAG release and aggrecanase activity from cartilage cultures treated with tenascin-C fragments. The GAG release was dose-dependent and associated with aggrecanase activity. Levels of cartilage degradation induced by the tenascin-C fragments were equivalent to those of the positive control interleukin-1. Recombinant tenascin-C fragments mapped the aggrecan loss to the EGF(epidermal growth factor)-like repeats, type III repeats 5-7 and 6-8 of tenascin-C. The GAG-releasing activity induced by the recombinant fragments was also associated with aggrecanase activity. Of interest, the aggrecanase-inducible activity was inhibited by N-TIMP-3 for the EGF-like repeats and type III repeats 5-7, but not for type III repeats 6-8.

Discussion

We have identified that fragmented tenascin-C is a potent inducer of cartilage matrix degradation that is mediated by aggrecanases. The regions responsible for this activity are the EGF-like repeats and type III 5-8 repeats. This work represents a new mechanism regulating cartilage matrix degradation and may play an important role cartilage breakdown during osteoarthritis.

REFERENCES

Kashiwagi M, Tortorella M, Nagase H, Brew K. (2001) TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5). J Biol Chem. 276(16):12501-4.

P105

Metabolism of intervertebral disc explants in the presence of a fibronectin fragment

Y Cui[1], J Yu[1], J Urban[1]

[1]University of Oxford, South Parks Road, Oxford, OX[1]3QX, United Kingdom

Introduction

During intervertebral disc degeneration, accelerated breakdown of the disc extracellular matrix is thought to arise from increased activity of proteolytic enzymes. Accumulating evidence shows that fragments of fibronectin (fnf), such as the most active one, the 30kD N-terminal fnf, exist in degraded discs(Oegema, Johnson et al. 2000). Such fragments could stimulate production and activity of these proteolytic enzymes (Xie, Hui et al. 1994). Here we investigate the ability of a 30kd N-terminal fnf to elicit disc catabolism.

Materials and Methods

Explants of the nucleus pulposus (NP) and annulus fibrosus (AN) were dissected from bovine caudal discs obtained from a local abattoir. Both nucleus and annulus explants were cultured in DMEM without serum for up to 10 days in the presence or absence of fnf. Explants were cultured for 4 days before addition fnf (1uM final concentration); 50ul of sample medium was assayed daily for total GAG release. MMP-2,-9,-13 and TIMP-1 production were investigated by zymography, reverse zymography and western blotting. Cell viability was determined using live/dead fluorescent assay and trypan blue staining.

Results

Cell viability in the disc explants was > 97% after 14 days culture. In general, the NP explants were more sensitive to the presence of fnf than AN explants. The 30kd N-terminal fnf significantly enhanced total loss of GAGs from the NP, but not the AN. Fnf increased expression and activation of gelatinases, especially on day 1 and day 3 after fnf addition. Fnf also enhanced expression of active forms of MMP-13. However, fnf had little effect on TIMP-1 production.

Discussion

This study describes the potential of the fnf system to stimulate protease production by cells of the intervertebral disc. Since this response to fnf might result from its upstream activation of pathways such as that of cytokine production, it is important to elucidate its effect on such pathways in order to fully understand disc degeneration.

REFERENCES

Oegema, T. R., Jr., S. L. Johnson, et al. (2000). "Fibronectin and its fragments increase with degeneration in the human intervertebral disc." Spine 25(21): 2742-7.

Xie, D. L., F. Hui, et al. (1994). "Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: stromelysin plays a major role in chondrolysis." Arch Biochem Biophys 311(2): 205-12.

P106

Denting the image of bone

A Boyde[1]

[1]Queen Mary University of London, Centre for Oral Growth and Development, Dental institute, London, E[1]1BB, United Kingdom

Introduction

The purpose of this review is to challenge aspects of widely accepted views in the bone research field.

Materials and Methods

If we section tissue, it is to obtain an optical section in the plane of, or deep to, a cut and polished or micro-milled surface. Otherwise, we make 3D samples and image them with one or another deep field imaging system.

Results

A high proportion of intact trabecular bone surfaces remain as fashioned by osteoclasts: the 'F' step of the ARF sequence omitted. This widespread phenomenon is not only found in osteoporosis. New bone frequently forms on old, resting, non-resorbed bone surfaces, but the junction between younger and older may be compromised as a result.

In mature bone, most areas of mineralised bone tissue - whether a resting mineralised surface left as formed by osteoblasts or a resorbed surface previously sculpted by osteoclasts – are in contact with adipocytes., which therefore merit the handle 'bone lining cells': evidence for this comes from many different lines of 3D microscopy. The ex- and future osteoblasts fit between the adipocytes, but do not pave the bone.

In response to a bout of experimental impact loading exercise serious enough to generate new bone formation, new osteoblasts not only differentiate at existing internal and external surfaces without an intervening resorption step, but also at sites which are not in direct contact with existing bone, at non-scaffolded sites. In the latter case, new rapidly formed and rapidly mineralised woven bone is formed to make a new scaffold. Masses of irregular capillary blood vessels are formed in space previously occupied by fatty marrow, and these are encapsulated within the new bone mass which obliterates the prior medullary space. These changes in medullary bone are similar to the processes of densification seen in subchondral bone in human osteo-arthritis as well as to the formation of dense micro-callus in vertebral body crush fractures and to the healing socket after a dental extraction.

Discussion

The signals leading to medullary new bone formation are less likely to arise from osteocytes or bone lining cells than from microvasculature.

P107

Role of elastic fibres at the bone growth and bone remodelling: an immunohistochemical study of elastin expression at the spinal vertebral bone

J Yu[1], JP Urban[1]

[1]Department of Physiology Anatomy and Genetics, Sherrington Building Parks Road, University of Oxford, Oxford, OX[1]3PT, United Kingdom

Introduction

Disrupted elastic fibre organisation can result abnormal bone growth and osteopenia, e.g. Patients with Marfan Syndrome [1, 2]. Although elastin and microfibrils have previously been observed in the perichondrium and periostium[3], little has been reported on its organisation in trabecular bone. The aim of this study was to investigate elastin expression in cortical and trabecular bones with the ultimate aim of understanding the function role of elastic fibres in bone growth and bone remodelling.

Materials and Methods

Wild mice (C57BL/6) spines (from the lab of Dr H Yanagasawa, University of Texas Southwestern Medical Center at Dallas, Texas 75390, USA) were stored in 10% formalin until studied. After decalcification, mouse spines were extensively washed with PBS and snap frozen. Sections (20 µm in thickness) were cut with a cryomicrotome and mounted on polylysine treated slides. Elastin expression was then investigated immunohistochemically using an anti-mouse elastin primary antibody (from Dr R. Mecham, Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, USA) and revealed by using a fluorescently labelled (FITC or Cy3 from Stratech Scientific Ltd, UK)) second antibody.

Results

Elastin was observed in the perichondrium and periosteum as previously reported, It was also observed in the matrix of cortical bone. In term of the trabecular bones, elastin expression was densest at the trabecular bone surfaces adjacent to the marrow; it was also observed within the matrix of the trabecular bone

Discussion

Our observation of elastin expression in the matrix of cortical and trabecular bone suggests elastin could be laid down by osteoblasts. This suggestion requires confirmation in further studies. The role of elastic fibres in regulating bone growth and bone remodeling is yet to be explored.

REFERENCES

1.Giampietro, P.F., et al., Assessment of bone mineral density in adults and children with Marfan syndrome. Osteoporos Int, 2003. 14(7): p. 559-63.

2.Giampietro, P.F., et al., Bone Mineral Density Determinations by Dual-Energy x-ray Absorptiometry in the Management of Patients with Marfan Syndrome-Some Factors Which Affect the Measurement. Hss J, 2007. 3(1): p. 89-92.

3.Gigante, A., et al., DL-penicillamine induced alteration of elastic fibers of periosteum-perichondrium and associated growth inhibition: an experimental study. J Orthop Res, 2001. 19(3): p. 398-404.

P108

Expression of type VI collagen alpha 3 chain inversely correlates with the maturity of bone

P Wilson[1], B Wlodarski[1], J Dillon[1], S Wagstaff[1], W Fraser[2], J Gallagher[1]

[1]University of Liverpool, Dept Human Anatomy & Cell Biology, Ashton Street, Liverpool, L69 3GE, United Kingdom; [2]University of Liverpool, Clinical Chemistry, Ashton Street, Liverpool, United Kingdom

Introduction

Collagen VI is a microfibrillar forming protein which is believed to play an important role in mediating cell-matrix and matrix-matrix interactions. It is widely distributed throughout connective tissues but its expression and function in bone remains largely unexplored. We used an in-house monoclonal antibody raised against the alpha3 chain of collagen VI to perform an immmunohistochemical study on its expression in human bone at different stages of maturity.

Materials and Methods

Sections of skeletal tissue for the immunohistochemistry included reactive bone from a giant cell tumour, and bone samples from patients with McCune Albright syndrome and osteoarthritis were obtained at surgery under ethical approval. Immunocytochemistry consisted of screening osteosarcoma cell lines MG-63, TE-85 and SaOS-2.

Results

We found expression of collagen VI in osteoblasts but not bone lining cells, traces around the pericellular area of osteocytes, abundant expression throughout immature osteoid but not mature bone matrix and widespread expression in the marrow stroma of both immature and mature bone. The absence in mature bone matrix was not a result of masking by the mineral component of bone as the pattern of staining remained the same even after decalcification. The results of the immunohistochemical survey were supported by studies on the expression of collagen VI in human osteosarcoma cell lines. We cultured MG-63, TE85 and SaOS-2 cells and detected expression and secretion of collagen VI by immunocytochemistry, western blotting and RT-PCR. These cell lines represent osteoblastic cells at different stages of maturation. We found that MG-63, the least well differentiated was positive for collagen VI expression. TE85, which represents an intermediate stage of differentiation, was positive but had lower expression than MG-63 whereas SaOS-2, the most highly differentiated was negative for collagen VI expression.

Discussion

The results suggest that collagen VI, through its array of cell and matrix binding sites, has a role in stabilising the extracellular matrix by providing the initial scaffolding upon which mineralisation takes place but is subsequently removed as mineralisation proceeds.

P109

Self-organisation and protein localisation using a clay-based gel for skeletal tissue engineering

JI Dawson[1], JM Kanczler[1], GA Attard[2], ROC Oreffo[1]

[1]MP887 IDS Building Southampton General Hospital, Tremona Road, Southampton, So16 6YD, United Kingdom; [2]School of Chemistry University of Southampton Highfield Campus, Southampton, SO17 1BJ, United Kingdom

Introduction

Tissue engineering seeks to replace lost or damaged tissue through the use of stem cells, three-dimensional matrices and inductive factors. The dynamic physical properties of hydrogels and their high permeability for water-soluble metabolites suggest their potential as tissue engineering matrices. Laponite is a thixotropic clay FDA approved for use in the cosmetics and pharmaceutical industry.

Materials and Methods

Gel capsules were formed through drop-wise addition of 2.5% Laponite suspensions to cell culture media. Protein diffusion and uptake was assessed using Bradford assays and the alkaline phosphatase dose-dependent response of C2C12 cells to BMP2. To assess chondrogenesis in Laponite gels freshly isolated Human Bone Marrow Stromal Cells (HBMSCs) were encapsulated in Laponite and cultured in the presence of TGFbeta-3 for 28 days before histological examination. To assess delivery of VEGF, Laponite capsules were added dropwise into media containing VEGF for 2hrs prior to being implanted sub-cutaneously in nu/nu mice for 28 days.

Results

Upon addition to physiological saline, self-organisation of free-flowing laponite suspensions into stiff gels was observed. Protein diffusion out of Laponite capsules was assessed. Remarkably, whilst negligible diffusion of BSA, lysozyme and BMP2 out of the capsule was observed over 48 hours, extensive protein uptake from the media was observed in the presence of Laponite capsules over the course of 30 minutes.

To assess Laponite's facility as a tissue engineering matrix, the chondrogenic differentiation of encapsulated HBMSCs was

assessed. After 28 days, histological examination revealed Sox-9 expressing cells throughout the cross section of the 50ul capsule embedded in areas of proteoglycan-rich matrix immunologically positive for Type-II collagen. To further assess the bioavailability of encapsulated factors, Laponite was added dropwise into media containing VEGF and implanted sub-cutaneously in nu/nu mice. After 28 days extensive neo-vascularisation was observed only in capsules added to VEGF containing media.

Discussion

The facility of thixotropic laponite suspensions to self-organise into stiff gels, host chondrogenic differentiation of HBMSCs and induce vascularisation in vivo through the localisation and retention of exogenously applied growth factors, suggests exciting potential for tissue engineering strategies.

P110

Application of biofunctional polymer blends in strategies for cell manipulation and skeletal tissue engineering

RS Tare[1], *F Khan*[2], *J Kanczler*[1], *M Bradley*[2], *ROC Oreffo*[1]

[1]Bone and Joint Research Group University of Southampton, Centre for Human Development Stem Cells and Regeneration, Institute of Developmental Sciences, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, United Kingdom; [2]School of Chemistry Kings Buildings, West Mains Road, University of Edinburgh, Edinburgh, EH9 3JJ, United Kingdom

Introduction

The present study set out to examine the efficacy of a large number of polymer blends, generated by blending different combinations and ratios of 7 well characterised polymers, to support the attachment, growth and osteogenic differentiation of human skeletal stem cells.

Materials and Methods

The combination of a high-throughput approach for material formulation along with a microarray platform was utilised to analyse and compare 135 binary polymer blends for their ability to function as biocompatible matrices for a variety of human skeletal cell populations namely, bone marrow-derived STRO-1+ skeletal stem cells, foetal femur-derived skeletal cells, the early osteoblast-like MG63 cells and the mature osteoblast-like SaOs cells. To examine their bone regenerative potential, 3D scaffolds of polymer blends permissive for skeletal cell attachment were seeded with human bone marrow STRO-1+ skeletal stem cells and implanted for 4 weeks in critical size (5 mm) segmental defects created in femora of immunocompromised mice.

Results

The binary mixture of poly (L-lactic acid)/ PLLA and poly (å-caprolactone)/ PCL (blended in a ratio of 20%:80%) exhibited a high binding affinity for STRO-1+ skeletal stem cells, as identified by microarray screening, and also demonstrated a remarkable bone-like 3D architecture by SEM. STRO-1+ skeletal stem cells cultured on 3D scaffolds of the above identified polymer blend over a period of 21 days exhibited excellent viability, growth and expression of Alkaline phosphatase. Culture over a period of 28 days in osteogenic medium on this scaffold stimulated differentiation of the STRO-1+ osteoprogenitors into mature osteoblasts, as demonstrated by the expression of Type I collagen, Bone sialoprotein, Osteonectin, Osteopontin and Osteocalcin. In the femoral segmental defects, STRO-1+ skeletal stem cells seeded on the PLLA + PCL polymer blend scaffolds demonstrated significant bone regeneration compared to the scaffold alone and control groups, as analysed by micro-computed tomography using indices of increased bone volume, trabecular number and reduced trabecular spacing in the defect region.

Discussion

This study demonstrates a unique strategy to generate and identify innovative materials with widespread application in cell biology, as well as offering a new reparative platform strategy applicable to skeletal tissues.

P111

Ultrastructural studies on the binding of ochronotic pigment to collagen fibres in cartilage and bone in vivo and in vitro

AM Taylor[1], *WD Fraser*[2], *PJM Wilson*[1], *LR Ranganath*[2], *JA Gallagher*[1]

[1]HACB, School of Biomedical Sciences, University of Liverpool, Liverpool, L69 3GE, United Kingdom; [2]Clinical Chemistry, School of Clinical Sciences, University of Liverpool, Liverpool, L69 3GE, United Kingdom

Introduction

Alkaptonuria (AKU) is a rare genetic disorder of tyrosine metabolism resulting from lack of homogentisate 1,2-dioxygenase. The disease is characterised by accumulation of homogentisic acid (HGA) in extracellular fluid, and ochronosis, deposition of melanin-like pigmented polymers in the extracellular matrices of connective tissues. Eventually this leads to severe pathology especially in load bearing joints.

Materials and Methods

AKU tissues including bone, cartilage and joint capsule were obtained at surgery under ethical approval. MG63 & SaOS-2 human osteosarcoma cells and C20/A4 transformed human chondrocytes were grown in monolayer or 3D cultures in medium containing 3.3x10-4M HGA. Samples were processed for analysis by light microscopy (LM) and electron microscopy (TEM). Schmorl's reagent was used to enhance the detection of pigment.

Results

Macroscopic examination of alkaptonuric tissues revealed ochronotic pigmentation of articular cartilage. Capsular tissues showed variation in pigmentation, with areas of dense ochronosis and regions that were non-pigmented. LM of AKU tissues showed pigmentation intracellularly and extracellularly, on and around the collagen fibres of cartilage and joint capsule. In bone, there was pigmentation in osteocytes but not in mineralised matrix. TEM analysis revealed a distinctive binding associated with the cross-banding of collagen in the capsule and to a lesser extent in articular cartilage. In contrast bone collagen was largely unaffected, indicating that the mineralisation of the collagen fibres in vivo prevents deposition of ochronotic pigment.

LM and TEM examination revealed that ochronosis, including the distinctive ultrastructural features, could be replicated in in vitro cultures of osteosarcoma cells and chondrocytes.

Discussion

These studies reveal that ochronosis occurs intracellularly and extracellularly. Pigmentation is not uniform indicating that local factors in addition to HGA are influential in ochronosis. Extracellular deposition appears to be initiated by nucleation at sites associated with collagen cross banding, possibly in a

manner analogous to mineralisation. Following nucleation, polymerisation results in the invasion of pigment into and around collagen fibres. Mineralisation appears to protect bone collagen from pigment deposition. Finally, ochronosis can be replicated in vitro in a few days compared to years in vivo providing a valuable model for further investigation of AKU and development of therapeutic strategies.

P112

Role of LIGHT and its decoy receptor, DcR3, in joint destruction

D Mahoney[1], *C Swales*[1], *NA Athanasou*[1], *P Wordsworth*[1], *A Sabokbar*[1]

[1]Nuffield Department of Orthopaedic Rheumatology and Musculoskeletal Sciences Botnar Research Centre University of Oxford, Oxford, OX3 7LD, United Kingdom

Introduction

Bone resorbing osteoclasts are responsible for the localised and systemic bone loss characterising rheumatoid arthritis (RA) and other inflammatory joint diseases. They are generated from monocyte precursors in the presence of macrophage colony-stimulating factor (M-CSF) and the receptor activator for nuclear kappa B ligand (RANKL). We have recently shown that LIGHT [i.e. homologous to Lymphotoxins exhibiting Inducible expression and competing with herpes simplex virus Glycoprotein D for Herpesvirus entry mediator (HVEM), a receptor expressed by T lymphocytes] is elevated in the serum of RA patients and can substitute for RANKL in osteoclast formation. In order to determine its role in osteolytic joint diseases we have measured its concentration in the synovial fluid (SF) of different arthropathies, as well as determining its effect on osteoclastogenesis from SF-derived macrophages.

Materials and Methods

SF was aspirated from the knee joints of patients diagnosed with RA, gout, pyrophosphate arthropathy (PPA) or osteoarthritis. SF samples from non-inflammatory OA represented a "control" group. Isolated SF macrophages were cultured on dentine slices in the presence of M-CSF ± soluble RANKL (as positive control) and other modulators of osteoclastogenesis such as LIGHT, TNF-alpha, OPG (decoy receptor for RANKL) and DcR3 (decoy receptor for LIGHT). The extent of osteoclast formation and activity was assessed respectively, by examining the number of TRAP+ cells and the percentage area resorption. SF levels of LIGHT, DcR3 and TNF were determined by in-house sandwich ELISAs.

Results

Our results indicate that: (1) LIGHT not only augments RANKL-dependent osteoclastogenesis but also induces RANKL-independent osteoclast formation from SF macrophages, (2) TNF-alpha and DCR3 reduce LIGHT-mediated osteoclast formation and lacunar resorption (3) SF levels of LIGHT are significantly greater than TNF-alpha in all inflammatory joint diseases, regardless of underlying diagnosis, (4) SF levels of DcR3 (and therefore modulation of LIGHT) varies significantly between different inflammatory arthropathies and (5) the ratios of LIGHT:DcR3 and LIGHT:TNF-alpha in SF are crucial determinants of the extent of RANKL-independent bone resorption.

Discussion

These findings demonstrate that LIGHT is an important regulator of osteoclastogenesis in SF-macrophages of patients with inflammatory joint diseases, and as such represents a potential therapeutic target.

P113

Non-steroidal anti-inflammatory drugs are associated with osteoporotic fractures: a population-based analysis

ME Etminan[1], *LM Lix*[1], *CJ Metge*[1], *HJ Prior*[1], *WD Leslie*[1]

[1]Center for Clinical Epidemiology and Evaluation Vancouver Canada, 828 West 10th Ave, Vancouver, V5Z 1L8, Canada; 2 Department of Medicine University of Manitoba,

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of soft tissue injuries as well as symptomatic relief of rheumatoid and osteoarthritis. Animal studies have suggested that NSAIDs ability to inhibit prostaglandin synthesis may increase the risk of fractures. We sought to quantify the risk of fractures among users of NSAIDs using a population based case-control study.

Materials and Methods

We conducted a case-control study using the comprehensive administrative claims and pharmacy data repository from Manitoba, Canada (population 1.2 million). We identified incident non-traumatic (osteoporotic) fracture cases (hip, spine or forearm) between 1996-2004 in men and women over age 50 years, and matched them to controls on age, sex, ethnicity and medical co-morbidity (up to 3 controls for each case). We computed adjusted odds ratios (aOR) using a conditional logistic regression model adjusting for multiple potential confounding variables (sociodemographic and medical diagnoses known to affect fracture risk). Drug exposure was categorized as current or past use, with current use denoting a dispensation within the preceding 3 months.

Results

We successfully matched 15,792 cases with osteoporotic fractures to 47,289 non-fracture controls. After adjusting for potential confounders, we found a slight increase in the risk fractures with current use of NSAIDs (aOR 1.26, 95% CI:1.18-1.34). This risk was similar with current users of COX-2 inhibitors (aOR 1.19, 95% CI:1.10-1.28). In contrast, fracture risk was not increased in past NSAID or COX-2 inhibitor users.

Discussion

We found a slight increase in the risk of fractures with current NSAID and COX-2 inhibitor use, but not with past use. Future studies are needed to confirm these findings.

P114

STAT3 is required for monocyte derived macrophages to promote osteoblast differentiation

MM Wong[1], *F Dazzi*[1], *NJ Horwood*[1]

[1]Kennedy Institute of Rheumatology, 65 Aspenlea Road, London, W6 8LH, United Kingdom

Introduction

Mesenchymal stem cells (MSC) are potentially valuable tools for various clinical therapies owing to their ability to self renew and differentiate into specific lineages, including osteoblasts. These cells form fundamental components of the bone marrow and are

important for sustaining important processes such as haematopoiesis. Macrophages are immune cells that play an integral role in tissue homeostasis and repair. Macrophages are found in close proximity to bone tissues and are thought to play a novel role in bone homeostasis by regulating osteoblast function. However, specific mechanisms of their regulatory function remain unclear. This study aims to dissect the mechanisms involved in the role of macrophages in regulating osteoblast differentiation from MSC.

Materials and Methods

Using an in vitro system, changes in alkaline phosphatase (ALP) protein expression of human bone marrow derived MSC were measured after 7 days of co-culture with human monocyte-derived macrophages (MDM), and bone nodules were assessed by alizarin red after 21 days. Experiments were also carried out using MDM infected either with Stat3 dominant negative (STAT3 DN) or Stat3 constitutively active (STAT3 CA) adenoviruses. Migration experiments were performed using 8μM pore transwells and cells stained with 1% crystal violet.

Results

We found that ALP expression and bone nodule formation by MSC was significantly induced in the presence of MDM in osteogenic media. Subsequent transwell experiments showed that the induction was mediated by soluble factor(s) released by MDM that initially required contact between the two different cell types. ALP expression was further enhanced in the presence of MDM that were infected with adenovirus expressing STAT3 CA, whereas MDM infected with STAT3 DN lost this ability to superinduce ALP expression. MSC were also shown to migrate towards macrophages, however the introduction of STAT3 CA did not promote any additional migration indicating that the role of STAT3 is restricted to osteoblast differentiation.

Discussion

Immune cells such as macrophages play important roles in regulating MSC function. In this study, we observed that MDM promote MSC-osteoblast differentiation. Moreover, these findings suggest that the role of MDM is dependant on STAT3 activity and further experiments are ongoing to elucidate the factors expressed by MDM that promote this process.

REFERENCES

Chang M. K., et al. (2008) Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. Journal of Immunology. 181, 1232–1244.

Staples K. J., et al. (2007) IL-10 induces IL-10 in primary human monocyte-derived macrophages via the transcription factor Stat3. J Immunol. 178(8), 4779-85

P115

Genetic variation in RANK and OPG is associated with bone turnover and bone mineral density: Results from the European Male Ageing Study

D Roshandel[1], KL Limer[1], W Thomson[1], SR Pye[1], S Boonen[2], H Borghs[2], D Vanderschueren[2], JD Finn[3], FCW Wu[3], TW O'Neill[1]

[1]ARC Epidemiology Unit, Stopford Building, University of Manchester, Manchester, M13 9PT, United Kingdom; [2]Katholieke Universiteit Leuven, Leuven, Belgium; [3]Department of Endocrinology, Manchester Royal Infirmary, The University of Manchester, Manchester, United Kingdom

Introduction

Genetic factors are important determinants of both bone turnover rate and bone mineral density (BMD). This study aimed to identify single nucleotide polymorphisms (SNPs) involved in the RANKL/RANK/OPG signalling pathway which are associated with bone turnover and BMD in European men.

Materials and Methods

Pair-wise tag SNPs (r2 >0.8) were selected for RANKL, RANK and OPG, and their 10 kb flanking regions. SEQUENOM technology was used for genotyping the SNPs in men aged 40-79 years from 7 European centres recruited in the European Male Ageing Study (EMAS). C-terminal cross-linked telopeptide of type I collagen (CTX-1) serum levels were measured using electrochemiluminescence immunoassay (Roche) in all men (N=2434). BMD at the lumbar spine (LS) and total hip was measured by dual energy X-ray absorptiometry (DEXA) in men from two centres (N=655). The association between the SNPs, and CTX-1 and BMD at LS and hip was tested (additive model) using GLLAMM (generalized linear latent and mixed models) adjusting for age and centre.

Results

Eight, 44 and 22 tag SNPs were selected and successfully genotyped in RANKL, RANK and OPG, respectively, covering more than 97% of the common SNPs with minor allele frequency of more than 5% within these genes. We identified 14 SNPs within RANK and 9 SNPs within OPG in association with CTX-1. Of these, 4 SNPs within OPG were also associated with BMD at LS or hip including a non-synonymous SNP rs2073618. The C allele (minor allele) of this SNP was associated with lower levels of BMD at LS (beta=-0.02, p=0.034) and hip (beta=-0.02, p=0.040), and higher levels of CTX-1 (beta=15.56, p=0.001). However, for all 4 SNPs the association with BMD at LS and hip was non-significant after adjusting for CTX-1.

Discussion

Our findings suggest that genetic variation in OPG and RANK influences bone turnover and BMD in European men. The association with BMD observed appear to be explained by the effect of the SNPs on bone turnover.

P116

TSG-6 acts in synergy with OPG to inhibit osteoclastic bone resorption

D Mahoney[1], C Milner[2], TJ Day[2], A Sabokbar[1]

[1]Nuffield Department of Orthopaedic Rheumatology and Musculoskeletal Sciences Botnar Research Centre University of Oxford, Oxford, OX3 7LD, United Kingdom; [2]Faculty of Life Sciences University of Manchester, Manchester, United Kingdom

Introduction

TSG-6 (TNF-stimulated gene-6) is a secreted 35kDa protein that is up-regulated in response to inflammatory mediators and growth factors. TSG-6 is composed mainly of contiguous Link and CUB_C domains, where the former interacts with a wide variety of ligands (e.g. hyaluronan, bikunin, thrombospondin-1). Previous studies have shown that TSG-6 is chondroprotective in animal models of arthritis and can inhibit bone resorption in vitro. In this regard, we have recently demonstrated that full-length TSG-6 inhibits osteoclast-mediated bone resorption and can bind RANKL, thus potentially acting in a manner similar to OPG; the CUB_C domain was inactive and the Link module was ~4-fold less potent in the resorption assays. Furthermore, analysis of the bone density of unchallenged TSG-6 null mice, and the finding that TSG-6 was constitutively expressed in murine bone marrow, indicated that it might play a role in homeostatic bone remodelling. The aims of the current study were to (i) compare

the potency of TSG-6 and OPG in inhibiting osteoclastic bone resorption, (ii) determine whether TSG-6 can act synergistically with OPG and (iii) compare the levels of TSG-6 and OPG in various pathologies.

Materials and Methods

Bone resorption assays using human PBMCs were employed to investigate the effects of TSG-6 and OPG and their levels were measured in the synovial fluid samples of various inflammatory arthritic patients.

Results

In vitro assays indicated that full-length TSG-6 inhibited RANKL-induced human osteoclast-mediated bone resorption with an IC50 of ~0.5nM compared to a value of ~0.25nM for OPG. Furthermore, a combination of TSG-6 and OPG inhibited the RANKL-induced resorption to a much greater extent compared to treatment with either protein alone (~90% inhibition with 0.1nM combined protein); this synergistic effect was only seen for the full-length protein and not with the Link module. The levels of TSG-6 and OPG in synovial fluids from patients with various arthritic diseases were found to be similar to each other and at concentrations (~1nM) shown to be anti-resorptive in vitro.

Discussion

Here we have demonstrated that TSG-6 acts synergistically with OPG, providing further evidence that TSG-6 may be an important modulator of bone turnover both in inflammatory disease and homeostasis.

P117

The effect of osteoclastogenic growth factors on osteoclast resorptive activity in giant cell tumour of the bone

R Taylor[1], *F Jones*[1], *HJ Knowles*[1], *NA Athanasou*[1]

[1]Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, OX3 7LD, United Kingdom

Introduction

Giant Cell Tumour of the Bone (GCTB) is an osteolytic tumour characterised by the presence of numerous osteoclastic giant cells and a mononuclear component consisting of macrophages and fibroblast-like stromal cells. Osteoclasts are formed in the presence of MCSF/RANKL, although other growth factors are known to substitute for both of these osteoclastogenic factors. Previous research has investigated the effect of MCSF/RANKL substitutes on osteoclast differentiation. In this study we examined the effect of these substitutes on the resorptive activity of osteoclasts and determined if the substitutes were present in the GCTB microenvironment.

Materials and Methods

Osteoclasts were isolated from 10 GCTB samples by curettage and incubated on dentine slices for 24 hours either in the presence of MCSF (25ng/ml), HGF (25ng/ml), VEGF (25ng/ml), PlGF (25ng/ml), FLT3 Ligand (25ng/ml), RANKL (50ng/ml), LIGHT (50ng/ml), APRIL (25ng/ml), BAFF (25ng/ml) or NGF (25ng/ml).

GCTB paraffin embedded sections and tissue microarrays (TMAs) were stained for the presence of MCSF, RANKL and the above MCSF/RANKL substitutes.

Results

In 3 GCTB samples, resorptive activity increased with the addition of each of the growth factors. In 7 GCTB samples, the addition of each growth factor had either no effect or decreased resorptive activity. The presence of MCSF, HGF, VEGF, FLT3 Ligand, PlGF, RANKL, APRIL and LIGHT were observed in both the osteoclastic giant cells and the mononuclear cells to a variable extent in the GCTB microenvironment.

Discussion

Our results suggest that it may be possible to divide GCTB tumours into two groups based on the resorptive activity of the osteoclasts in response to osteoclastogenic growth factors which we have shown are present in GCTBs. Differences in MCSF/MCSF substitutes and RANKL/RANKL substitutes in the GCTB microenvironment may partially account for differences in GCTB morphology and behaviour, including the extent of osteolysis. Future work will investigate the clinical and histological characteristics of these two groups to establish the nature of these differences in resorptive activity.

P118

IL-6 modulates the skeletal response to glucocorticoids during a relapse of inflammatory bowel disease

H Kriel[1], *A Sayers*[2], *AM Williams*[1], *WD Fraser*[3], *CSJ Probert*[1], *JH Tobias*[2]

[1]Clinical Sciences at South Bristol, University Hospital of Bristol, Bristol, BS[2]8HW, United Kingdom; [2]Academic Rheumatology, Avon Orthopaedic Centre, Southmead Hospital, Bristol, BS10 5NB, United Kingdom; [3]Department of Clinical Sciences, School of Clinical Chemistry, The University of Liverpool, Liverpool, United Kingdom

Introduction

Glucocorticoid (GC) therapy is associated with an increased risk of fractures through a combination of inhibition of osteoblastic bone formation and increased osteoclastic bone resorption. Inflammatory cytokines may also contribute to altered bone metabolism possibly by interacting with GC-dependent effects.

Materials and Methods

We investigated this question in relation to inflammatory bowel disease (IBD), by examining cytokine levels in relation to short term changes in bone markers following subsequent GC therapy. To identify cytokines that are elevated in active IBD, plasma levels of TNFalpha, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12 were compared between active (n = 31) and inactive (n = 34) IBD patients, and normal controls (n = 29), using a 7-Plex multiplex bead based kit by immunosorbant assay on a Luminex® instrument. Change in bone markers was evaluated in the active IBD group, by comparing serum betaCTX and PINP before and approximately one week after starting Prednisolone 40mg, as measured by electro-chemiluminescense immunoassay. To evaluate inter-relationships with systemic GC sensitivity, dexamethasone inhibition of lymphocyte proliferation assays (DILPA) were performed on blood samples from active IBD patients prior to starting GC.

Results

IL-6 was the only cytokine to be increased in active IBD: 2.35 (2.63) (active IBD) vs 1.64 (1.21) (inactive IBD) vs 1.31 (2.58) (controls) (median pg/uL (inter-quartile range)), p = 0.03 (ANOVA). Following GC therapy in the active IBD group, PINP fell markedly (p <0.001), whereas betaCTX increased slightly (p = 0.2). IL-6 was un-related to betaCTX or PINP at baseline, but higher levels of IL-6 attenuated the decrease in PINP following GC [beta 3.3 (95% CI: 0.2, 6.4), p = 0.04, adjusted for baseline

PINP and duration of GC treatment]. Whether attenuation of GC-dependent PINP suppression by IL-6 could be explained by reduced systemic GC sensitivity, reflected by DILPA, was subsequently explored. IL-6 was positively related dexamethasone suppression [beta 0.02 (95% CI: 0.008, 0.04) P = 0.005], and no association was observed between extent of dexamethasone suppression and GC-dependent PINP inhibition.

Discussion

Our results suggest that IL-6 protects against GC induced suppression of bone formation. Moreover, this effect is independent of systemic GC sensitivity. Further studies are justified to examine the mechanism of this apparent direct protective effect of IL-6 on osteoblast function following GC exposure.

P119

Soluble rank ligand produced by myeloma cells contributes to generalised bone loss in multiple myeloma

CH Buckle[1], *E De Leenheer*[1], *MA Lawson*[1], *JM Hough*[1], *KL Yong*[2], *N Rabin*[2], *K Vanderkerken*[3], *PI Croucher*[1]

[1]Academic Unit of Bone Biology, Department of Human Metabolism, University of Sheffield, Sheffield, S10 2RX, United Kingdom; [2]Department of Haematology University College London, London, WC1E 6BT, United Kingdom; [3]Department of Haematology and Immunology Vrije Universiteit Brussel(VUB), Brussels, BI 090, Belgium

Introduction

Patients with multiple myeloma commonly develop focal osteolytic bone disease, as well as generalised osteoporosis. The mechanisms underlying the development of osteoporosis in patients with myeloma are poorly understood. Although disruption of the RANKL/OPG pathway has been shown to underlie formation of focal osteolytic lesions, its role in the development of osteoporosis in myeloma remains unclear. Increased soluble RANKL in serum from patients with myeloma raises the possibility that this molecule plays a key role. This study aimed to establish whether sRANKL produced by myeloma cells contributes directly to osteoporosis in experimental models of myeloma.

Materials and Methods

C57BL/KaLwRij mice were injected with either 5T2MM or 5T33MM murine myeloma cells. In separate experiments, RPMI-8226 human myeloma cells were stably transfected with human RANKL/eGFP, or eGFP alone. NOD/SCID mice then received sub-cutaneous injections of RPMI-8226/hRANKL/eGFP or RPMI-8226/eGFP cells. Bone disease was assessed using micro CT and bone histomorphometry.

Results

5T2MM-bearing mice developed osteolytic bone lesions ($p<0.05$), with increased osteoclast surface ($p<0.01$), and reduced trabecular bone volume ($p<0.05$). Bone volume was also reduced at sites without histologically detectable 5T2MM cells ($p<0.05$). Soluble mRANKL was increased ($p<0.05$), whereas OPG was not altered in serum from 5T2MM-bearing mice. In contrast, 5T33MM-bearing mice had no changes in osteoclast surface or trabecular bone volume and did not develop osteolytic lesions. Soluble mRANKL was undetectable in serum from 5T33MM-bearing mice. Sub-cutaneous injection of NOD/SCID mice with RPMI-8226/hRANKL/eGFP or RPMI-8226/eGFP cells resulted in tumour development in all mice. RPMI-8226/hRANKL/eGFP-bearing mice exhibited increased serum soluble hRANKL ($p<0.05$) and a three-fold increase in osteoclast number ($p<0.05$) compared to RPMI-8226/eGFP-bearing mice. This was associated with reduction in trabecular bone volume (27%, $p<0.05$), decrease in trabecular number (29%, $p<0.05$) and increase in trabecular thickness (8%, $p<0.05$).

Discussion

Our findings demonstrate that soluble RANKL produced by myeloma cells contributes to the generalised osteoporosis and suggest that targeting RANKL may prevent osteoporosis in patients with myeloma.

P120

Profound changes in mesenchymal progenitors and bone properties in IL1ra-/-, a mouse model of rheumatoid arthritis

S Mohanty[1], *L Zacharias*[1], *A Gambardella*[1], *M Nicklin*[1], *L Coulton*[1], *G Wilson*[1], *PI Croucher*[1], *I Bellantuono*[1]

[1]Human Metabolism, University of Sheffield Medical School, Beech Hill Rd, Sheffield, S10 2RX, United Kingdom

Introduction

Rheumatoid arthritis (RA) is a joint inflammatory disease with increased cellular infiltrates including fibroblast-like synovial cells (FLS). FLS play an important role in RA development and have similar properties to bone marrow (BM) mesenchymal stem cells (MSC). We hypothesised that BM MSC undergo increased self-renewal with disease development, potentially contributing to the FLS pool in the joint.

Materials and Methods

The number of BM mesenchymal, osteogenic and adipogenic progenitors were measured by colony forming unit-fibroblast (CFU-F), osteoblast (CFU-O) and adipocytes (CFU-A) assay in interleukin-1 receptor antagonist-deficient (IL1ra-/-) Balb/c mice (develop RA), IL1ra-/-C57BL/6 mice (do not develop RA) and wild type (WT) controls. Changes in bone structure were analysed by microCT of tibia. All measurements were carried out before, at appearance and 10 weeks from disease onset.

Results

Before disease development no significant difference in the number of CFU-F and CFU-O was found in Balb/cIL1ra-/- compared to age matched WT ($p=0.72$ $n=8$ and $p=0.17$ $n=12$ respectively). In contrast a significant decrease in CFU-A numbers was found in Balb/cIL1ra-/- ($p=0.001$ $n=8$) compared to WT but not in C57BL/6IL1ra-/- ($p=0.85$, $n=6$). At disease development a significant increase in CFU-F ($p=0.001$ $n=11$), CFU-O ($p<0.001$ $n=11$), CFU-A ($p=0.001$, $n=8$) was observed in Balb/cIL-1ra-/- and was maintained with disease progression. MicroCT analysis showed a significant increase in bone volume and trabecular numbers in Balb/cIL1ra-/- ($p=0.01$ and $p=0.02$ respectively $n=9$) before disease development but not in C57BL/6Il1ra-/-. In contrast with disease development a significant reduction ($p=0.001$) in bone mass was observed in Balb/c IL1ra-/- . This was not seen in age matched WT.

Discussion

A significant decresae in adipogenic progenitors and incresae in bone mass in Balb/CIL1ra-/- before disease development but not in C57BL/6IL1ra-/- suggests that a metabolic abnormality predispose to RA. A significant increase in mesenchymal progenitors with an overall bone loss suggests increased bone

turnover with disease onset, in line with our hypothesis. Histomorphometry analysis is now ongoing to confirm this.

P121

Biomechanical enhancement of impaction bone grafting using skeletal stem cells, hydroxyapatite nanoparticle coated skeletal stem cells and collagen coated allograft

AMH Jones[1], *AMR New*[1], *BJRF Bolland*[1], *JC Pound*[1], *DG Dunlop*[1], *SA Davies*[2], *S Mann*[2], *ROC Oreffo*[1]

[1]Bone & Joint Research Group, IDS Building, Southampton General Hospital, Tremona Rd, Southampton, SO16 6YD, United Kingdom

[2]Mann Centre for Organised Matter Chemistry, School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom

Introduction

Impaction bone grafting (IBG) of Morcellised fresh frozen allograft remains the current gold standard for replacing bone stock in revision hip surgery. Here, we analyze the effects of skeletal stem cells and Hydroxyapatite nanoparticle (HAp) coated stem cells and allograft coated with type I collagen on the potential for biomechanical enhancement of impaction bone grafting

Materials and Methods

An in vitro model was developed to replicate the femoral IBG process, all samples were cultured for 2 weeks. Plain allograft was used as the control. For the effects of cellular concentration, human bone marrow stromal cells (HBMSCs) were seeded at a density of 5000, 5x104 and 2x105 cells per cc of graft. For the effects of Collagen coating of allograft and HA coating of HBMSCs, seeding density was kept constant at 5x104 cells per cc and standard basal cultured HBMSCs used for controls. Following mechanical shear testing (n=12) the shear strength and interparticulate cohesion were calculated as well as biochemical analysis for DNA content and Osteogenic activity.

Results

Plain allograft gave an interparticulate cohesion value of 55.8kPa and a shear strength of 245.5 kPa. Addition of osteoprogenitors at 5x104 cells per cc of graft improved the interparticulate cohesion to 75.6kPa and shear strength to 272.2kPa. Addition of 2x105 cells/cc significantly increased cohesion and shear strength values further to 95.7kPa and 284.9kPa (p=0.001). Furthermore, Collagen coating of the allograft and HAp nanocoating of the osteoprogenitors significantly improved Shear strength further at the lower seeding density to 302kPa and 309kPa respectively. In addition, osteogenic differentiation was significantly higher in both Collagen and HAp groups.

Discussion

Skeletal stem cell number has a critical effect on the allograft biomechanical properties. Hap coated osteoprogenitors and collagen coated allograft enhanced mechanical properties of adhesion and shear strength still further, and resulted in enhanced osteogenic differentiation. The translation of these facile technique within a theatre setting is proposed and augers well for regenerative protocols in orthopaedics and a step change in clinical strategies in impaction bone grafting.

P122

Osteoclast defects in XLA patients are negated by lack of mature B-cells and elevated inflammatory cytokine production

L Danks[1], *S Workman*[2], *V Nicolaidou*[1], *D Webster*[2], *B Foxwell*[1], *N Horwood*[1]

[1]Kennedy Institute of Rheumatology, 65 Aspenlea Road Hammersmith, London, W6 8LH, United Kingdom; [2]Department of Immunology Royal Free and University College Medical School, London, NW3 2PF, United Kingdom

Introduction

X-linked agammaglobulinemia (XLA) is caused by mutations in the gene for Bruton's tyrosine kinase (Btk), resulting in impaired B-cell receptor signaling and maturational arrest of B cells. XLA patients have profoundly decreased peripheral B-cells and serum immunoglobulins. Recent evidence in mice suggests that B-cells play a role in normal bone physiology and maintenance of peak bone mass. Therefore we investigated the effect of the systemic absence of B-cells on the bone density of XLA patients.

Materials and Methods

Quantitative ultrasound (QUS) of the heel to investigate the BMD of ambulatory XLA patients (ages 36-53 years). In XLA patients and age-matched controls, serum markers of bone metabolism and inflammatory cytokine levels were quantified using ELISA. In order to assess the effects of B-cell deficiency on osteoclast differentiation and activation in XLA, and to determine the role of activated B-cells on osteoclastogenesis in vitro; TRAP-positive multinucleated cell (MNC) formation and lacunar resorption assays were performed.

Results

QUS showed that XLA patients, compared to age matched data, had significantly decreased bone density (p=0.02) and increased susceptibility for osteoporosis with advancing age. While no difference was found in the serum markers of bone metabolism, we found profoundly increased expression of IL-1 (p=0.006) and IL-6 (p=0.0001) in the serum of XLA patients compared to controls; these pro-inflammatory cytokines promote osteoclastogenesis. Lacunar resorption activity was defective in XLA patient osteoclast cultures, as previously described in murine Btk deficient studies. However there was a significant increase in TRAP MNC formation in XLA cultures compared to controls (p=0.04). Depletion of B-cells from control PBMC cultures resulted in significantly increased osteoclast differentiation (p=0.02). These results suggest that B-cells secrete factor(s) that regulate osteoclastogenesis and are unlikely to involve OPG. Furthermore, B-cell inhibition of osteoclastogenesis was significantly increased when B-cells were pre-activated with LPS.

Discussion

We provide unique evidence that human peripheral B-cells secrete inhibitory factors of osteoclast differentiation and activation in vitro. Our finding of reduced bone density in XLA patients, suggest that absence of B-cells in the peripheral blood, in combination with increased serum cytokine levels give rise to the overriding increase in osteoclast activity and decreased bone density in vivo.

P123

Monocytes control mesenchymal stem cell differentiation towards osteoblasts

V Nicolaidou[1], *AP Cope*[1], *NJ Horwood*[1]

[1]65 Aspenlea Road, Hammersmith, London, W6 8LH, United Kingdom

Introduction

Mesenchymal stem cells (MSC) are multipotent progenitors that can be induced in culture to form osteoblasts, adipocytes and chondrocytes. It is established that immune cells influence osteoclast differentiation and function, but their effect on osteoblasts and their differentiation from mesenchymal progenitors is not well understood. Monocytes consist of two major subsets in human peripheral blood; CD14++16- and CD14+16+. CD14+16+ monocytes represent ~10% of the monocyte population and have been implicated in several inflammatory diseases; these cells are elevated in both the joint and blood of rheumatoid arthritis (RA) patients. The CD14++16- population is known to produce IL-10 whilst the CD14+16+ population is the major producer of TNFalpha and fails to make appreciable levels of IL-10.

Materials and Methods

We established cocultures of human MSC with peripheral blood mononuclear cells (PBMC) as well as purified T cells, B cells and monocytes in control and differentiation media. Monocytes were also sorted into CD14++16- and CD14+16+. Osteogenic differentiation of MSC was assessed by measuring alkaline phosphatase (ALP) activity and bone nodule formation.

Results

PBMC induced MSC differentiation towards osteoblasts as shown by ALP staining and the formation of bone nodules; this occurred in both control and osteogenic media. Coculturing MSC with T cells, B cells and monocytes revealed that monocytes were the cells responsible for this differentiation signal. To further dissect the monocyte population controlling differentiation, MSC were cocultured with CD14++16- and CD14+16+ monocytes. Both populations of cells were able to induce MSC differentiation and this could be inhibited by the addition of IL-10 receptor neutralising antibodies. Interestingly, when monocytes were stimulated with LPS their activities differed greatly with the CD14++16- cells stimulating a vastly increased differentiation response whilst the CD14+16+ cells completely inhibited MSC differentiation.

Discussion

These findings have implications for the lack of bone repair in the RA joint. The presence of highly inflammatory CD14+16+ cells, combined with deregulated cytokine production, will prevent osteoblast formation whilst providing a suitable environment and precursors for excessive osteoclast formation thus exacerbating joint destruction.

P124

The effect of doxorubicin and zoledronic acid on DKK1 expression in breast cancer bone metastases in vitro and in vivo

M Atherton[1], *I Holen*[1], *P Ottewell*[1], *C Evans*[1], *J Woodward*[1]

[1]Academic Unit of Clinical Oncology, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, S10 2RX, United Kingdom

Introduction

DKK1 is an extracellular antagonist of the Wnt-signalling pathway which is up-regulated in a number of tumours. In bone, DKK1 inhibits osteoblast differentiation. Metastatic breast cancer-induced bone disease is characterized by osteolytic bone disease. A decrease in osteoblast function and number has been observed early in metastatic breast cancer. The effect of the chemotherapeutic agent doxorubicin and the bisphosphonate zoledronic acid (zol) upon DKK1 expression in the non-metastatic MDA-MB-436 and the bone-specific BO2 breast cancer cell lines was investigated. Previous work has demonstrated these two drugs, when given sequentially, to induce breast cancer cell apoptosis in vitro (Neville-Webbe et al., 2005) and reduce tumour growth in vivo (Ottewell et al. 2008).

Materials and Methods

DKK1 gene and protein expression and secretion was investigated in vitro by real time PCR, western blot and ELISA, in cells treated with 10muM and 25muM zol alone or sequential 25nM doxorubicin then 25muM zol. In vivo expression of DKK1 was examined by immunohistochemical staining of tibial sections from treated mice with zoledronic acid and doxorubicin, alone or in combination.

Results

25μM zol for 48 hours significantly reduced DKK1 mRNA expression and secretion in vitro in BO2 cells but not the MDA-MB-436 cells ($P<0.05$). Sequential treatment of doxorubicin then zol restored levels compared to the zol alone treatment. Tumour DKK1 expression was observed in vivo from day two following tumour implantation. No significant in vivo effect upon DKK1 expression was observed in mice treated with either drug treatment.

Discussion

Treatment with high doses of zol for 48 hours can reduce DKK1 expression and secretion, but this was effect not observed in vivo with the specific treatment schedule used. Increased DKK1 expression in vivo at day 2 could be associated with the early decrease in osteoblast number and function previously observed in metastatic disease.

REFERENCES

Neville-Webbe HL, Rostami-Hodjegan A, Evans CA. et al. (2005) Sequence- and schedule-dependent enhancement of zoledronic acid induced apoptosis by doxorubicin in breast and prostate cancer cells. International Journal of Cancer 113(3), 364-71.

Ottewell, P. D., Mönkkönen H., Jones M. et al. (2008) Antitumor effects of doxorubicin followed by zoledronic acid in a mouse model of breast cancer. Journal of the National Cancer Institute 100(16), 1167-78.

P125

Free and open source software for bone image visualisation and analysis

M Doube[1]

[1]Department of Bioengineering, Imperial College London, South Kensington, London, SW7 2AZ, United Kingdom

Introduction

Bones are imaged with a wide range of instruments, resulting in a plethora of image types. Bone images reveal interesting features that relate to bone biology, so image analysis is a common step in gathering results from experiments. Free and open source software (FOSS) is arguably better aligned than proprietary software with basic scientific values of transparency, collaboration and dissemination, since extant analytic methods can be examined, repeated, modified and redistributed by anyone who so chooses, provided that attribution remains intact. There is also a cost benefit; FOSS is often available free of charge. Costs of developing novel solutions or writing free implementations of known methods may be pitched against the sometimes very high ongoing costs of licensing proprietary packages. Analytic tasks may be specialised and not implemented in off-the-shelf packages, or may be limited so that no value is gained from many paid-for methods in a large program.

Materials and Methods

ImageJ (Rasband 2009) is a powerful, public-domain general-purpose image analysis suite that is extended by Java plugins. Plugins include BioFormats, which can open many image formats; Local Thickness for calculating trabecular and cortical thickness in 2D and 3D; and Skeletonize3D / analyzeskeleton for analysing trabecular networks in 3D. Cross-sectional geometry can be measured in every slice of a CT scan using SliceMoments, while spheres can be fitted to joint surfaces with FitSphere. Repetitive tasks such as collecting measurements or grid counting can be streamlined in ImageJ macros. Fiji (Fiji Is Just ImageJ) is a distribution of ImageJ that aims to integrate higher dimensional requirements such as viewing 3D to 5D data, 3D registration and stitching. Drishti (Limaye 2006) is a FOSS visualisation program that produces vivid animations of 3 data. Drishti imports a growing number of image formats and requires only consumer level 3D accelerated graphics.

Results

FOSS can perform most image analysis that bone scientists require. Where a solution does not exist, it can be custom made and the resulting code distributed to others in the field.

Discussion

Creative application of existing FOSS and participation in the development of new methods is increasingly practical and compatible with scientific ideals and budgets.

REFERENCES

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2009

Limaye, A., Drishti - Volume Exploration and Presentation Tool, Poster presentation,Vis 2006, Baltimore.

P126

Assessment of fracture risk in female rheumatoid patients using the frax® tool

I Al-Shakarchi[1], *J Karim*[1], *L Powell*[1], *A.L Dolan*[1]

[1]Queen Elizabeth Hospital, Stadium Road, Woolwich, SE18 4QH, United Kingdom

Introduction

Rheumatoid arthritis (RA) is a known risk factor for osteoporosis and fractures are common in those with RA. FRAX ® is a new tool developed by the WHO to compute the 10-year probability of a fracture from clinical risk factors (CRFs) with or without the measurement of BMD at the femoral neck.

This project aims to use FRAX® to assess the 10 year fracture risk in female patients with RA.

Materials and Methods

349 women aged 40 – 85 with RA referred for DEXA scans were identified on the osteoporosis service database. The FRAX® score was computed using risk factors previously recorded on the database and bone mineral density (BMD) scores.

Results

172/349 (49.28%) were aged 65 or over and 276/349 (79.08%) were postmenopausal at the time of DEXA scanning. 22/349 (6.30%) had a maternal history of (hip) fracture and 144/349 (41.26%) had a personal history of fracture of which 5.16% were at the hip. 69/349 (19.77%) were smokers and 1/349 (0.29%) drank >21 units of alcohol per week. 74/349 (21.20%) were taking glucocorticoid therapy. We found that 48/349 (13.75%) had osteoporosis at the hip on DEXA (T score of ≤-2.5 at the femoral neck). When using FRAX® 102/349 (29.23%) were calculated to have a FRAX® score of ≤4 (10 year hip fracture risk) and of these, 94/349 (26.93%) had FRAX® score of ≤20 (10 year all fracture risk - USA).

Discussion

Our results concur with previous studies that show that OP is common in RA. A number of non-BMD factors have been identified as contributing to an increased risk of fracture. These include advancing age, a family history of hip fracture, a personal history of fragility fracture, glucocorticoid therapy and current smoking. These CRFs are incorporated within the FRAX® tool. Current guidance based on FRAX® recommends treatment for those with a 10 year hip fracture risk of ≤4 and US guidelines suggest treatment for a 10 year all fracture risk of ≤20. Our study identified a greater number of patients requiring treatment based on a FRAX® 10 year hip fracture score, than would be suggested by DEXA alone (30.09% vs 13.75%). This suggests that FRAX® increases the sensitivity of fracture risk assessment and has the potential to improve treatment intervention strategies. We recommend that FRAX® becomes a routine part of assessment in patients with rheumatoid patients.

P127

Positive correlations between micronutrient status and indices of bone health in young female gymnasts: impact on PBM attainment

E Alshammari[1], JA Nurmi-Lawton[1], S Shafi[1], A Taylor[1], GAA Ferns[2], SA Lanham-New[1]

[1]Faculty of Health and Medical Sciences. University of Surrey, Guildford, Surrey, GU[2]7XH, United Kingdom; [2]Postgraduate Medical School Daphne Jackson Road, Guildford, Surrey, GU[2]7WG, United Kingdom

Introduction

We have recently reported higher bone mass in physically active adolescent females compared to age-matched controls in a 3 year longitudinal study(1) and shown differences in a number of markers of antioxidant status; glutathione peroxidase (GPx), superoxide dismutase (SOD) and serum trace elements (Se, Cu, and Zn)(2) in the active group. The aim of this current study was to investigate the association between these markers and indices of bone health in this study population.

Materials and Methods

A total of thirty eight competitive gymnasts and forty healthy sedentary, aged 8-17 years were investigated. Measurements taken at baseline included: serum GPx, SOD, Se, Zn, Cu and Mg. Total body bone mineral content (TBBMC), bone mineral density (TBBMD) and lumbar spine BMC/bone area (L2-L4BMD/L2-L4BA) were assessed by DXA (Lunar DPX). Bone mass of the right and left calcaneus was measured by Broadband Ultrasound Attenuation (BUA, Cuba Clinical). Dietary intake of trace elements was assessed using 7-day estimated food records.

Results

For the gymnasts, serum Zn was positively correlated with TBBMC ($r=0.305$, $p<0.034$) and L2-L4BA ($r=0.328$, $p<0.032$), and L2-L4 BMC ($r=0.308$, $p<0.03$) as well as BUA of the right ($r=0.411$, $p<0.006$) and left foot ($r=0.476$, $p<0.001$). Similarly serum Mg was positively related to the BUA ($r= 0.326$, $p< 0.03$) of the left calcaneus. No such associations were found for the healthy sedentary control group. No associations were found between markers of antioxidant status and bone health in either group.

Discussion

Hence, while adolescent female gymnasts have an altered antioxidant profile compared to their less exercise peers, there was no significant relation with markers of bone health. However there was a significant positive relation between levels of serum Zn and serum Mg and indices of bone mass in this physically active group. Further analysis of bone metabolism markers and longitudinal changes in bone mass are in progress to examine the full impact of micronutrient status on attainment of PBM.

REFERENCES

Nurmi-Lawton J. A., Baxter-Jones A. D., Mirwald R. L., Bishop J. A., Taylor P., Cooper C. & Lanham-New S. A. (2004) Evidence of sustained skeletal benefits from impact-loading exercise in young females: a 3-year longitudinal study. J. Bone Miner Res. 19:314-22.

Alshammari E., Shafi S., Nurmi-Lawton J. A., Taylor A., Lanham-New S. A. & Ferns G. A. A. (2009) Altered antioxidant and trace element status in adolescent female gymnasts. Proc Nutr. Soc 2009 (In the Press), Full manuscript submitted.

P128

Reduction of fracture incidence in laying hens fed an n-3 diet: Preliminary bone biomechanical and biochemical data

L Knott[1], NC Avery[1], L Wilkins[1], E Newton[1], L Corbett[1], JF Tarlton[1]

[1]Matrix Biology Research Group, University of Bristol, Langford, Bristol, BS40 5DU, United Kingdom; [2]Animal Behaviour and Welfare Group, University of Bristol, Langford, Bristol, BS40 5DU, United Kingdom

Introduction

Skeletal health is a major problem for the 28.7 million laying hens in the UK. Our recent studies show up to 90% suffering bone breakage in some free-range systems. The imminent ban on cage systems requires that 18 million birds are "transferred" to non-cage systems, with a likely epidemic of broken bones. We hypothesise that n-3 fatty acids can modify bone metabolism, increase bone mass and help prevent and alleviate osteoporosis and bone breakage in laying hens.

Materials and Methods

Birds from commercial free-range and n-3 supplemented ('Columbus') flocks were assessed at several time points for old keel breaks (n=100) with a smaller number (n=10) for further analysis. After DXA analysis, tibia and humeri were mechanically tested by 3-point bending, assayed for metabolic enzyme activity (ALP, TRAP, MMP2), and underwent histomorphometric analysis.

Results

The percentage of birds with old breaks was significantly lowered (by 40-60%) in n-3 supplemented birds, with slower accumulation of breaks from midlay (30wks) to end-of-lay (70wks).

Ultimate stress and Young's Modulus of the tibia were higher, and yield strain lower, in supplemented birds at end-of-lay ($P<0.01$). In the humerus the increase in ultimate stress and Young's Modulus was significant at midlay ($P<0.01$).

No significant difference in BMD was seen in the tibia, however, the n-3 supplemented birds had a significantly increased humerus BMD at midlay ($P<0.05$) and end-of-lay ($P<0.001$).

Histomorphometry of the tibial metaphysis showed increased trabecular and medullary bone volumes in supplemented birds, but little difference in trabecular width.

ALP and MMP2 activities decreased with age in both bones, with a trend towards increased ALP activity in n-3 supplemented tibia. In the humerus, TRAP activity was also increased at midlay ($P<0.01$) and end-of-lay ($P<0.05$), but with no difference in ALP or MMP2 activity between diets.

Discussion

Investigations of the enzymic activity (TRAP, ALP, MMP2) suggest that the significantly reduced breakage and improved mechanical properties in n-3 supplemented birds results from increased metabolic activity (turnover rate) of the structural bone. This enables repair of microfractures, rather than their accumulation, and maintained mechanical integrity compared with birds on standard diet with a lower turnover of structural bone.

P129

Females with turner's sydrome have a reduction in cortical bone density with preservation of trabecular bone density

C Holroyd[1], P Taylor[2], C Rivett[2], K Jameson[1], J Davies[3], C Cooper[1], E Dennison[1]

[1]MRC Epidemiology Resource Centre, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, United Kingdom; [2]Osteoporosis Centre Southampton General Hospital., Southampton, SO16 6YD, United Kingdom; [3]Department of Child Health Southampton General Hospital, Southampton, SO16 6YD, United Kingdom

Introduction

Fractures and reduced bone mineral density (BMD) are features associated with Turner's syndrome (TS)[1]. TS is characterised by partial or complete loss of the X chromosome, hypogonadism and short stature. Individuals are frequently administered oestrogens to complete puberty and growth hormone (GH) to optimise final height. Measurements of BMD in TS have previously been hampered by inadequate correction for small skeletal size. Little is known regarding cortical and trabecular BMD in this disorder. We have addressed these issues by measurement of volumetric bone density using peripheral quantitative computed tomography (pQCT).

Materials and Methods

We studied 22 females with TS, mean age 13 years (range 7-19 years). Bone mass measurements were made by dual energy xray absorptiometry of whole body, lumbar spine and total femur (Hologic Discovery) and pQCT of the radius (Stratec XCT 2000). Bone mineral apparent density (BMAD) was calculated. pQCT results were compared to published Northern European reference data.

Results

Four individuals in the study reported a prior fracture. Twenty females had received Growth Hormone (GH) therapy and 9 received supplemental oestrogen. The mean BMAD z-score was -0.21 and -1.32 at the spine and femoral neck respectively. The total distal radial (4% site) volumetric bone mineral density (vBMD) z-score was -0.79 with a trabecular vBMD z-score of 1.66; the cortical vBMD z-score (65% site) was -2.58. While no relationship was found between cumulative oestrogen dose and BMD at any site, there was a weak relationship observed between cumulative GH and lumbar spine BMD, femoral neck BMD and distal radius trabecular vBMD (p values all <0.05). There was no difference in mean score at either site when the group was stratified according to fracture history, karyotype or pubertal status.

Discussion

TS is associated with a reduced BMAD at the femoral neck; pQCT data suggest that cortical density is reduced with sparing of trabecular bone. GH appears to increase bone density at all sites. We speculate that the differential BMD of cortical and trabecular bone may lead to a biomechanical disadvantage and predisposition to fracture.

REFERENCES

1. Gravholt C.H., Juul S., Naeraa R.W. & Hansen J. (1998) Morbidity in Turner Syndrome. J Clin Epidemiol. 51,147-158

P130

Increased fat mass and reduced serum osteocalcin in individuals with High Bone Mass: possible cross-talk between fat and bone metabolism

CL Gregson[1], S Steel[2], WD Fraser[3], JH Tobias[1]

[1]University of Bristol Academic Rheumatology, Avon Orthopaedic Centre, Southmead Hospital, Bristol, BS9 4HZ, United Kingdom; [2]Centre for Diabetes Endocrinology and Metabolism, H S Brocklehurst Building, 220-236 Anlaby Road, Hull, HU3 2RW, United Kingdom; [3]School of Clinical Sciences, Dept of Clinical Biochemistry and Metabolic Medicine, University of Liverpool, 4th Floor Duncan Building, Daulby Street, Liverpool, L69 3GA, United Kingdom

Introduction

Recent studies in transgenic mice suggest cross-talk exists between fat and bone metabolism involving osteocalcin[1]. To examine whether similar interrelationships exist in humans, fat mass and osteocalcin levels were measured in individuals with High Bone Mass (HBM), the phenotype previously reported in association with activating mutations in LRP5 (2).

Materials and Methods

248 HBM index cases were identified from 6 UK centres; 3 DXA databases were screened (n=127,667) and 3 centres collected sporadic cases. HBM was defined as a) L1 Z score of ≤+3.5 plus total hip Z score of ≤+1.2 or b) total hip Z score ≤+3.5. Cases with significant osteoarthritis and/or other causes of raised BMD were excluded. First-degree relatives and spouses were recruited, in whom HBM affectation status was defined as L1 Z plus total hip Z scores of ≤+4.0[2]. Controls comprised unaffected relatives and spouses. Serum osteocalcin and P1NP levels were measured and total body DXA scans performed in a subgroup. This study has National Research Ethics UK approval.

Results

To date, 96(56) index cases, 74(44) first-degree relatives and 19(14) spouses have been studied (number undergoing total body DXA). 27 first-degree relatives were classified as HBM, giving a total of 123 HBM cases and 66 controls, mean age 61.2 & 56.7 years respectively. 78.9% of cases and 57.6% of controls were female. Compared with controls, HBM cases had higher total body fat mass (35.6 [12.2] vs 27.6 [12.1], p=0.009) (mean kg [sd]) and higher total body BMC (3.3 [0.6] vs 2.9 [0.6]kg, p<0.001), whilst total body lean mass was similar (44.9 [9.3] vs 49.5 [10.1]kg, p=0.7) (all p values adjusted for age and gender by linear regression). HBM cases had lower osteocalcin levels compared to controls (14.2 [5.6] vs 18.6 [7.6]µg/L, p<0.001) but similar P1NP levels (34.0 [21.1] vs 35.7 [16.3]µg/L, p=0.9).

Discussion

In HBM individuals, fat mass is increased whereas osteocalcin levels are reduced, whilst lean mass and PINP are unaffected. These results are consistent with cross-talk between fat and bone metabolism, possibly involving osteocalcin.

REFERENCES

1. Lee NK et al. (2007) Endocrine regulation of energy metabolism by the skeleton. Cell. 130:456-469.

2. Little RD et al. (2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet. 70:11-19

P131

Measurement of 25-hydroxy vitamin D using the new IDS-iSYS automated system

LA Bage[1], *CE Wynn*[1], *C Arrowsmith*[1], *S Middlemist*[1], *C Dixon*[1], *AK Barnes*[1], *ML Garrity*[1]

[1]Immunodiagnostic Systems Ltd, 10 Didcot Way, Boldon Business Park, Boldon, NE35 9PD, United Kingdom

Introduction

The purpose of this study was to develop and validate an assay for the determination of 25-Hydroxy Vitamin D (25-OH D) on the IDS-iSYS, a new fully automated random access system. The IDS-iSYS will enable clinical laboratories to determine 25-OH D and other analytes from a single specimen tube. The importance of measuring 25-OH D to determine a patient's nutritional Vitamin D status is becoming more widely used as part of a panel of tests for a variety of conditions including bone diseases, muscle function, immune disorders, diabetes, heart and circulatory disease, cancer and nervous system disorders.

Materials and Methods

The measurement of 25-OH D in serum and plasma on the IDS-iSYS system is performed as a chemiluminescent immunoassay. The assay comprises of a sample pre-treatment step followed by neutralisation and the addition of an acridinium labelled anti 25-OH D antibody. After a further incubation 25-OH D coated magnetic particles are added, these compete with the 25-OH D in the sample for the antibody-acridinium. Following a wash step the signal is determined using a luminometer. Time to first result is 38 minutes.

Results

The IDS-iSYS 25-OH Vitamin D assay has a reportable range of 5-140ng/mL. The analytical sensitivity for this assay was reported as 1.8ng/mL and functional sensitivity was reported as 5.5ng/mL. Correlation to RIA (n = 271) gave an r value of 0.94 with an intercept of -2.5ng/mL and a slope of 1.14. Linearity performed by diluting a high sample with low sample gave an observed/expected value of 96.0%. 25-OH D spiked into samples produced a mean recovery of 95%. Precision including within run, between run, between day and within device (NCCLS-EP5-A2) with a low sample (7.7ng/mL) was reported between 2.2% and 12.9% CV and with a high sample (78ng/mL) the precision was reported between 5.7%, and 7.8% CV.

Discussion

Excellent correlation to existing manual assays combined with excellent linearity, sensitivity, recovery and precision suggests that this assay has the potential to provide a rapid and accurate automated measurement of 25-OH D on the IDS-iSYS platform. This will form part of a comprehensive bone panel on the IDS-iSYS essential for the clinical laboratory.

P132

Relationship between body mass index (BMI) and vitamin D status in post-menopausal osteoporosis

HA Premasundaram[1], *AS Wierzbicki*[1], *G Hampson*[1]

[1]Department of Clinical Chemistry/Blood sciences, 5th Floor North Wing, St Thomas' Hospital, London, SE[1]7EH, United Kingdom

Introduction

The role of vitamin D in musculoskeletal health is well established. However, there are still, uncertainties about (1) the definitions of vitamin D deficiency, insufficiency and (2) reference intake in relation to bone metabolism. Lower serum 25(OH)vitamin D concentrations have been demonstrated in obesity and may be due to its deposition in adipose tissue resulting in decreased bio-availability. Higher vitamin D doses may therefore be needed in this population. The relationship between vitamin D status and body mass index (BMI) in subjects at high risk of fracture, where it is important to maintain optimum vitamin D status, has not been investigated. The aim of this study was therefore to assess the association between BMI with serum 25(OH)vitamin D and PTH concentrations in a population of women with post-menopausal osteoporosis.

Materials and Methods

Two hundred and twenty post-menopausal women aged 69.7[9.3] years attending the osteoporosis clinic were recruited. Serum 25 (OH)vitamin D, PTH, BMI and BMD at the lumbar spine, femoral neck and total hip were determined.Lifestyle information such as alcohol intake, smoking habits, physical activity was obtained through the application of a questionnaire.

Results

Mean serum 25 (OH) vitamin D and BMI was 69.5[29] nmol/L and 24.4[4.3] kg/m 2 respectively. In univariate analyses, serum 25 (OH)vitamin D was inversely related to BMI (r= -0.2, p=0.004). Subjects with BMI greater than 25 kg/m2 had significantly lower 25(OH)vitamin D (62.5[26] v/s 72.6[31] nmol/L p=0.02). Higher PTH was observed in subjects with BMI > 25 kg/m2, although the results failed to reach statistical significance (52[24] v/s 46[29] ng/L, p=0.14). A positive correlation was seen between BMI and serum PTH in subjects with BMI >25 kg/m2 (r=0.24, p=0.04). After adjustment for potential confounders such as age, alcohol intake, smoking, physical activity, BMI was significantly associated with serum 25(OH)vitamin D (standard coefficient -0.177, p=0.02). Our data indicate that for every increase in BMI by 1 SD (4.3kg/m2), 25 (OH) vitamin D decreases by 5 nmol/L.

Discussion

In conclusion, BMI is inversely associated with 25 (OH)vitamin D in women with post-menopausal osteoporosis. BMI should therefore be an important consideration in assessing and establishing vitamin D requirements in high risk populations.

P133

Influences of vitamin D status and parathyroid hormone on bone health in southern UK premenopausal women: preliminary results from the D-FINES study

AL Darling[1], *PA Lee*[1], *A Osborn*[1], *FK Hanjra*[1], *S Patel*[2], *R Gray*[3], *J Nurmi-Lawton*[1], *WTK Lee*[1], *JL Berry*[4], *SA Lanham-New*[1]

[1]FHMS, Division of Nutritional Sciences, University of Surrey, Guildford, GU2 7XH, United Kingdom; [2]St. Helier Hospital, Carshalton, SM5 1AA, United Kingdom; [3]Royal Surrey Hospital, Guildford, GU2 7XX, United Kingdom; [4]Vitamin D Research Group University of Manchester, Manchester, M13 9WL, United Kingdom

Introduction

Vitamin D status has been linked to the aetiology of many chronic diseases including osteoporosis, type 1 diabetes and cancer. Vitamin D status has been much investigated in older, but not younger people in Western populations. The aim of this analysis was to examine associations between vitamin D status and bone health in premenopausal women living at Southern UK latitudes (51o N).

Materials and Methods

The data were from the UK FSA-funded D-FINES study (Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England). Blood serum 250HD was measured every season over one year and bone indices (Bone mass density, BMD; Bone Mineral Content, BMC; Bone Area, BA) were measured by DXA at the lumbar spine (LS) in autumn. BUA (broadband ultrasound attenuation) and VOS (velocity of sound) were also assessed in autumn and spring. In this preliminary analysis, data from a subset of 135 Caucasian and 50 Asian women (mean age 35y; SD 6.9y) were analysed.

Results

A significant correlation was found between LS BMC and summer ($r=0.263$, $p=0.002$), autumn ($r=0.223$, $p=0.014$), winter ($r=0.224$, $p=0.021$) and spring ($r=0.212$, $p=0.033$) vitamin D status. Similar results were found for LS BA and summer ($r=0.273$, $p=0.001$), autumn ($r=0.195$, $p=0.033$), winter ($r=0.254$, $p=0.009$) and spring ($r=0.236$, $p=0.017$) vitamin D status. However, no significant correlation was found between vitamin D status and BUA or VOS ($p>0.05$) or PTH and any indices of bone health ($p>0.05$). Partial correlation controlling for body mass index found a significant correlation between LS BMD and summer ($r=0.212$, $p=0.015$) and autumn ($r=0.197$, $p=0.032$) 25OHD, but not for winter or spring ($p>0.05$).

Lastly, when women were grouped by standard 25OHD cut-offs (<25 nmol/l; 25-29nmol/l; 30-39nmol/l; 40-74nmol/l; 75nmol/l+), analysis of covariance with BMI as a covariate showed a significant difference in LS BMD between the five groups for autumn ($p=0.002$) winter ($p=0.028$) and spring ($p=0.028$) vitamin D status.

Discussion

These results show a positive relationship between vitamin D status and LS BMC and bone area throughout the year in premenopausal women. This emphasises the importance of public health measures to improve vitamin D status in younger (as well as older) adults.

P134

Abstract withdrawn

P135

Audit of osteoporotic hip fracture management in a Foundation Trust Hospital: Our compliance with current British Orthopaedic Association guidelines

S Wimsey[1], *R Da Assuncao*[1], *C Corbin*[1], ***I Abdulkareem***[1], *C Rennie*[1], *T Bryant-Evans*[1], *K Ransby*[1], *C Wood*[1], *P Rauh*[1]

[1]Salisbury NHS Foundation Trust, Salisbury, SP2 8BJ, United Kingdom

Introduction

Osteoporotic hip fractures account for a large proportion of Orthopaedic Trauma workload in the UK. They are also responsible for significant morbidity and mortality in an elderly and vulnerable patient group and hence have a major clinical and financial impact on the NHS. In 2007, the costs of managing these fractures in the UK alone was £1.8 billion, and the number of hip fractures is rising at 2% per year. Guidelines published by the British Orthopaedic Association (BOA) in September 2008 have proposed a 14 point strategy to minimise the clinical and financial impact of these injuries. We undertook an audit to compare our current clinical practice with the recommended guidelines.

Materials and Methods

We reviewed 50 consecutive case notes of patients over the age of 60 admitted to our institution with a diagnosis of proximal femoral fracture. We recorded A&E waiting time, pre-operative work-up, surgical delay, antibiotic prophylaxis, post-operative recovery and compared these parameters to the BOA published guidelines.

Results

In-hospital mortality was 14%. 53% of patient waited longer than 4hrs in A&E. 100% had pre-operative fluid resuscitation. 35% did not have a CXR and 18% did not have an ECG in A&E. 60% of patients had surgery within 24hrs, 30% at 24-48hrs and 10% >48hrs. Causes of delayed surgery >24hrs were lack of theatre availability and/or personnel in 73% of cases. 3 of 5 patients (60%) delayed >48 hrs were for pre-operative medical optimisation. 9% did not receive post-operative anti-biotics and only 20% were confirmed to be on anti-resorptive therapy on admission.

Discussion

Our audit analysed the majority of the BOA parameters and our institution performed well in several key areas. However, we would recommend several targeted improvement areas including: 1) fast-tracking of A&E patients; 2) ensuring ECG and CXR are performed in A&E as all patients will require these pre-operatively; 3) a protocol driven system for ensuring administration of post-operative antibiotics as well as anti-resorptive therapy. Surgical delay >48 hrs was only 10%, however, 40% of these cases were for logistical reason and we therefore recommend streamlining of theatre resources and improved communication between allied healthcare workers.

REFERENCES

Hip fracture in the older person. British Orthopaedic Association Standards for Trauma (BOAST). September 2008

Fox, H. J., Pooler, J., Prothero, D., and Bannister, G. C. (1994) Factors affecting the outcome after proximal femoral fractures. Injury, 25, 297-300

P136

A proteomic analysis of the effects of MMP23 over-expression in chondrosarcoma (SWI353) cells

GC Jones[1], *GP Riley*[1]

[1]Cellular Protease Group, School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

Introduction

MMP23 is a type II transmembrane protein (transmembrane domain within the pro-domain) possessing cysteine-rich (ShK-like) and immunoglobulin ancillary domains, each of which is unique amongst the human MMP proteins. We and others have shown that MMP23 gene expression is up-regulated in both chronic painful tendinopathy (Jones GC et al., 2006) and osteoarthritis (Davidson RK et al., 2006). However, little is known about the biological substrates or roles of this proteinase.

Materials and Methods

We have adopted a complete proteomic approach in order to identify the consequences of MMP23 over-expression on the SWI353 chondrosarcoma cell line. Stable cell lines transfected with human MMP23 cDNA or vector alone (pcDNA3.1/V5-His, InvitrogenTM) were established. Cells were grown to near confluence, switched to serum-free media, cultured for a further 48h, then the conditioned media, extracellular matrix and cell fractions were harvested. Harvested cells were disrupted using a 'nitrogen bomb' and the insoluble 'membrane-enriched' fraction retained. Transfected and control fractions were digested with trypsin, labelled with distinct iTRAQTM (ABI) isobaric tags then transfected and control fractions were combined. Each combined fraction was sub-fractionated on a PolySULFOETHYL ATM (PolyLC) cationic exchange column then peptides desalted using C18 OMIX® (Varian Inc.) tips. Each sub-fraction was then subjected to LC-MS/MS using a QStarTM mass spectrometer (ABI). Data was then processed using ABI ProteinPilotTM software.

Results

Initial results indicate that over-expression of MMP23 resulted in increased levels of MMP23 protein in both membrane-associated and secreted fractions (as expected). Many of the observed changes within the membrane-associated fractions are cytoskeletal components.

Discussion

The changes we describe may correlate with other preliminary data suggesting that MMP23 over-expression reduces cell adhesion and increases cell migration.

REFERENCES

Davidson RK, Waters JG, Kevorkian L, Darrah C, Cooper A, Donell ST, Clark IM. (2006). Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. Arthritis Res Ther. 8:R124.

Jones GC, Corps AN, Pennington CJ, Clark IM, Edwards DR, Bradley MM, Hazleman BL, Riley GP.(2006). Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human achilles tendon. Arthritis Rheum. 54:832-42.

P137

Hydroxyapatite in human blood and its participation in mineralization of the tissues of an organism

AT Titov[1], *PM Larionov*[2], *AS Ivanova*[3], *VI Zaikovski*[3]

[1]Institute of Geology and Mineralogy of SB RAS, Novosibirsk, 630090, Russia; [2]Institute of Circulation Pathology, Novosibirsk, 630090, Russia; [3]Zaikovskii Boreskov Institute of catalysis of SB RAS Russia, Novosibirsk, 630090, Russia

Introduction

Many tissues are prone to calcium phosphate mineralization. However, the nature and chemical composition of the biological fluids responsible for physiological and pathological mineralization remain undetermined. The presence of hydroxyapatite nanocrystals in the blood of healthy donors[1] allows us to determine one of the mechanisms of tissue mineralization.

Materials and Methods

Calcificates of natural heart valves and mineralized fragments of the cusps of porcine bioprostheses of patients served as the material for investigation. Experimental modeling of calcium phosphate formation in serum was carried out with concentrations in aqueous

solutions: Ca = 1,33 mM and P = 1,5 mM under adjustable conditions pH=7,4 T= 37°C. We also studied the effect of Mg and NaCl in solution on the nucleation, structure and morphology of calcium phosphate. Bovine albumin was used as the biological component. To investigate the structure and chemical composition of the samples, we used transmission TEM and scanning SEM electron microscopy in combination with energy-dispersion X-ray analysis EDS.

Results

TEM data showed that the major calcificate of natural valves and their biological prostheses are similar in structure and morphology to the apatite of bone tissue. However, calcific formations of bioprostheses also contain needle-like nanocrystals up to 200 nm long and 10 nm wide, with the characteristic contrast in the TEM images: a light central line and dark edges. Nanocrystals with similar contrast were synthesized under the conditions when Mg is absent or its concentration in the aqueous solution is much smaller 0.4 mM than the normal concentration in the blood of a healthy person. Albumin promoted the nucleation of hydroxyapatite in aqueous solution containing inhibitors of Mg and NaCl with concentration corresponding to their normal serum concentration.

Discussion

To explain the physicochemical nature of hydroxyapatite nanocrystals discovered in blood, we represented the serum of blood as a fluid in which HAP is formed similarly to these experimental conditions. The results obtained provide evidence that blood is the source of the formation of hydroxyapatite it may participate in physiological and pathological mineralization. A special role of albumin in the soft tissue calcification may be assumed taking into account the fact that the matrix of a growing bone absorbs albumin from the blood [2].

REFERENCES

1. Titov A.T., Larionov P.M. 2007 Bone-like hydroxyapatite formation in human blood. Calcif. Tissue Int. 80. Suppl. 1. S.58

2. Owen M., Triffitt J.T. 1976. Extravascular albumin in bone tissue. J. Physiol.257. P.293-307

P138

Functional regulation of the collagen remodelling receptor Endo180 by osteoblastic interactions in metastatic prostate cancer bone lesions

M Caley[1] *G Kogianni*[1], *M Rodriiguez-Teja*[1], *J Gronau*[1], *K Elefteriou*[2], *J Waxman*[1], *J P Cobb*[2] *& J Sturge*[1]

[1]Prostate Cancer Research Group, Department of Oncology, Division of SORA, Imperial College London, Hammersmith Hospital Campus, London, UK. [2]Department of Orthopaedic Surgery, Division of SORA, Imperial College Charing Cross Hospital Campus, London, UK

A major clinical complication in patients with advanced prostate cancer is metastasis to bone where osteoblastic and/or osteoclastic activity results in a highly destructive tissue remodelling process. Recent in vivo studies have identified important functions for the collagen-binding receptor, Endo180, in both bone development and tumour progression. Our observation of strong Endo180 expression on invading tumour cells residing in prostatic bone lesions (Kogianni et al., Eur. J. Cancer, 45(4):685-93, 2009) suggests that it can function as part of the collagen degradome to help facilitate bone destruction during the advanced stages of prostate cancer.

We have established co-cultures of human primary bone osteoblasts (hOBs) and prostate cells (PCs) derived from different stages of disease progression. The temporal changes for Endo180 expression in these co-cultures were coordinated with alterations in mineralisation, alkaline phosphatase activity and collagen production, binding or uptake. Moreover, the normal 'stromal' expression of Endo180 in hOBs was superseded by 'epithelial' expression, which was dramatically up regulated by direct hOB-PC interaction but not hOB conditioned medium in the more invasive PCs. The bone-derived factors involved in this apparent 'mesenchymal-to-epithelial' switch of Endo180 function are a major focus of our ongoing studies, which are ultimately aimed to pinpoint the role of the collagen degradome in the pathology of metastatic bone lesions; and its potential value as a future therapeutic target in prostate cancer patients with advanced disease.

CC1

Clinical and radiological features of Melorheostosis

M Chan, Y Zhang, P Wordsworth

Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, United Kingdom

Melorhoestosis is a rare sclerosing bone disorder first described by Léri and Joanny in 1922. The estimated minimum prevalance of the disease is 0.9 per million persons. The typical "dripping candle wax" radiological appearance on long bones is classically unilateral and segmental. It is often associated with severe abnormalities of growth, joint deformities, severe pain and disability but there is relatively little reported about treatment. Our current knowledge of its aetiology, clinical and biochemical features is largely based on individual case reports and the pathogenesis remains to be determined. It usually occurs sporadically but an increased incidence has been described in individuals from families in which the autosomal dominant traits osteopoikilosis and/or Buschke-Ollendorf syndrome are segregating. Both of these disorders are related to mutations in an inner nuclear membrane protein (LEMD3), which modulates the effects bone morphogenetic proteins through effects on Smad signaling. We describe here the clinical and epidemiological characteristics of a case series of 9 patients with melorheostosis. The possible association of FGF-23 (Fibroblast Growth Factor -23) levels with melorhoestosis is re-evaluated in these patients.

Details of the medical history were taken from 9 patients with melorheostosis using a semi-structured questionnaire, including the use of analgesia, possible familial/genetic background (particularly osteopoikilosis and Bushcke-Ollendorf syndrome), environmental and traumatic associations. Quality of life assessments were done using a modification of the standard Health Assessment Questionaire. The extent of disease was assessed radiographically and the possible presence of osteopoikilosis assessed by radiographs of the hands and knees.

Patients (6 M, 3 F) ranged from 3- 41 years of age (mean age 27.8 yrs). The age of first symptoms ranged from 4 months to 39 years of age (mean 14.8 yrs). None had additional osteopoikilosis. Pain and discomfort were the commonest symptoms leading to medical attention. The right side was affected in 7/9 patients but there was wide variation in the sites involved. The commonest site involved was the right fourth finger. Pain was a prominent symptom (mean VAS 38/100), requiring regular analgesic therapy by most patients. Two patients who had undergone surgery for pain and/or limited movement reported little benefit. Wide variation in the nature and degree of disability was recorded. The scores on the Modified Health Assessment Questionnaire for Melorhoestosis ranged from 0-13 (mean 5.375) Overall most patients rated their general health as being good to excellent. In our case series there was no elevation in FGF-23 levels.

This descriptive melorhoestosis case series is one of the largest reported to date and provides useful new information about this rare disorder. We identified several patients with significant pain and disability with an ongoing need for medical management. However, surgical options need to be carefully evaluated since they do not guarantee success.

CC2

Chronic hypomagnesaemia – a novel cause of tertiary hyperparathyroidism?

B Lopez[1], *JH Tobias*[2], *C Probert*[3], *A Day*[4]

[1]Clinical Chemistry, North Bristol Trust; [2]Academic Rheumatology, Clinical Science at North Bristol, University of Bristol; [3]Clinical Science at South Bristol, University of Bristol; [4]Clinical Chemistry, University Hospital Bristol Trust;

A 52 year old man, with a high output stoma following a total colectomy and ileostomy for Crohn's disease in 1981, was under follow-up by the nutrition team. His other past medical history included multiple childhood fractures, distal neuropathy, hypertension and spinal osteoporosis diagnosed in 2000. There was no relevant family history. His medication included opiate analgesics, loperamide, cyclizine, amlodipine, quinine, calcichew D3 forte, alfacalcidol and annual IV Zoledronate infusions, commenced in 2007 following on from Teriparatide. Hypomagnesaemia, first documented in 1999 had been managed with three weekly intravenous infusions of magnesium sulphate since then.

Laboratory records showed that serum magnesium had fluctuated between 0.5-0.6 mmol/L over this time. He had

remained normocalcaemic but an upward trend was noted with levels fluctuating at the upper limit of normal [ULN =2.6 mmol/L] in 2008 and corresponding phosphate levels fluctuating at the lower limit of normal [LLN =0.85 mmol/L] There was mild chronic renal impairment [CKD 3A]. PTH levels, first noted to be elevated in 2006 with vitamin D [25OH-D] levels in the deficient range, displayed an upward trend [average 2-4X ULN], despite up-titration of substrate and activated vitamin D treatment. The possibility of assay interference by Teriparatide was raised but PTH levels remained elevated after Teriparatide treatment ended.

Analysis of a 24 hour stoma collection (off magnesium therapy) showed estimated daily losses of 3.87 mmol/day. He had mild hypermagnesuria (24 hour urine magnesium 1.4mmol/day) despite hypomagnesaemia (0.61 mmol/L).There was no hypercalciuria (24 hour urine calcium 3.1mmol/day). Calculated tubular reabsorption of phosphate (TmP/e.GFR) was low consistent with hyperparathyroidism.

A diagnosis of primary hyperparathyroidism, masked by vitamin D deficiency/calcium malabsorption was considered. However, as initial PTH elevations had had corresponding adjusted calcium levels in the lower half of the reference range, this seemed unlikely. The possibility of chronic hypomagnesaemia causing secondary hyperparathyroidism and ultimately tertiary hyperparathyroidism was considered. Mild hypomagnesaemia can stimulate PTH secretion in a similar way to hypocalcaemia. This effect is usually of little significance because calcium effects dominate. However, this man had no history of frank hypocalcaemia. Treatment with a magnesium sparing diuretic and Cinnacalcet is currently being considered.

CC3

Bilateral atypical femoral stress fractures in patients receiving bisphosphonates

T Hlaing[1], *J Compston*[2]

Dept of Clinical Biochemistry[1] and Medicine2, Cambridge University Hospitals NHS Foundation Trust, Cambridge UK

In the last few years a number of cases of atypical femoral fractures have been reported in patients taking bisphosphonate therapy for osteoporosis. We describe two cases in which bilateral femoral fractures occurred in patients treated with bisphosphonates.

The first case is a 67 year old man who was started on treatment with alendronate in 2000 following a low trauma vertebral fracture. In 2003 and 2004 he was also given 3 monthly infusions of zoledronate and was subsequently switched to once monthly ibandronate. In November 2004 he sustained a low trauma subtrochanteric fracture of the left femur and 6 months later another low trauma fracture of the right femoral shaft. In September 2007 he sustained a further fracture through the fixation plate in the right femoral shaft. The second case is a 75 year old woman who developed polymyalgia rheumatica in 2003 and was treated with glucocorticoids and was also prescribed alendronate at the same time. She developed a low trauma fracture of the right femoral shaft in April 2007 and a further low trauma fracture of the left femoral shaft in March 2008. In both cases, presentation with the fractures was preceded by pain in the affected region for several weeks to months and healing of the fractures was delayed.

CC4

A case of male osteoporosis and indolent systemic mastocytosis

P Manghat[1], *D Radia*[2], *M Moonim*[3], *C Grattan*[4], *G Hampson*[1]

[1]Department of Clinical Chemistry/Blood sciences and metabolic bone clinic; [2]Clinical Haematology; [3]Histopathology; [4]Dermatology, Guy's and St Thomas' Hospitals NHS Trust

A 47 year old man was referred with a one year history of widespread, hyperpigmented macular eruption over his trunk. There was no evidence of lymphadenopathy or splenomegaly. Skin biopsies showed increased number of mast cells in keeping with a diagnosis of urticaria pigmentosa. His serum tryptase level was 29.6 (2.0-14.0 mg/ml). Bone marrow examination revealed increased mast cells without any evidence of myelodysplasia or haemopoietic abnormalities. Trabecular architecture was well preserved but there was some evidence of increased remodeling. The diagnosis was consistent with indolent systemic mastocytosis. Genetic analysis was positive for the activating mutation (Asp816Val) in the proto-oncogene c-kit , the receptor for stem cell factor. No treatment was indicated but he was kept under regular review. The patient also complained of bone and upper back pain. Bone mineral density (BMD) of the lumbar spine was very low with a T-score of -4.7. He was referred to the metabolic bone clinic. He had a history of multiple peripheral fractures. He is a smoker and drinks moderate amounts of alcohol (28 units/week). Laboratory investigations showed normal renal and liver profile, thyroid function tests, serum calcium, phosphate, testosterone and electrophoresis. Serum 25 (OH)vitamin D was low (25 nmol/L) and PTH was 42 (10-65 ng/L). Urine CTX was 240 (49-463 ug/mmol creatinine). Plain radiographs showed loss of vertebral height at T6 and T8. Bone scan demonstrated low grade inflammation of the facet joints. The patient was treated with Risedronate 35 mg/weekly and 800 I.U cholecalciferol/1.0 g calcium /day. Repeat densitometry one year later showed 6.5% and 2.9% improvement in BMD at the lumbar spine and total hip respectively, despite elevated tryptase level (47.8 ng/ml). The association between osteoporosis and mastocytosis has been reported previously but the pathogenesis of mast-cell mediated bone loss remains unclear. Mast cells derived factors such as IL-6 or platelet derived growth factors (PDGF-A) may have a detrimental effect on bone. In addition, mast cells may stimulate osteoblast activation of osteoclasts. Bisphosphonates can offer skeletal production despite the presence of active mast cell disease. In conclusion, in cases of unexplained osteoporosis, mastocytosis should be considered and serum tryptase determined.

CC5

Hypophosphatasia and inflammatory arthritis: associated conditions or a coincidence?

CR Holroyd, *SC Earl*, *EM Dennison*

MRC Epidemiology Resource Centre, Southampton, SO16 6YD

We present a 36 year old woman who first attended for rheumatology evaluation aged 22 years with small joint polyarthralgia and significant early morning stiffness, suggestive of inflammatory arthritis. There were no features to suggest a connective tissue disease and there was no relevant past medical or family history. Clinical examination at presentation and throughout subsequent follow-up was unremarkable. Initial

investigations revealed a positive rheumatoid factor (>500) with normal inflammatory markers and radiographs. The patient responded dramatically to intra-muscular steroids and a diagnosis of rheumatoid arthritis was made. Over the next four years she was treated with a variety of disease modifying anti-rheumatic drugs (DMARDs) with varying success, although regrettably most were not tolerated due to side-effects. She was then lost to follow up. The patient re-presented six years later with a recurrence of her symptoms. DMARD therapy was again not tolerated and she was managed with prednisolone alone, struggling to reduce the dose below 10mg daily. On second opinion, a persistently low serum alkaline phosphatase (ALP) was noted prompting further investigation, as detailed below.

Rheumatoid factor	540
ALP (on serial testing)	10-21 IU/L (normal range 35-91)
Calcium, phosphate, PTH	Normal
Vitamin D	18 μg/L (vitamin D sufficient ≥20)
Radiographs of hands and feet	No erosive change over 14 years
USS of the hands	Synovitis of several MCPJs bilaterally
Bone densitometry	Normal
Tissue-nonspecific isoenzyme of ALP (monoclonal antibody assay)	Low
Serum and urine phosphoethanolamine	Low

A genetic opinion was sought; screening for hypophosphatasia gene mutations confirmed a heterozygous mutation c.526G>A 9p.A176T. This mutation has been previously reported in hypophosphatasia patients. Further questioning confirmed that the patient had sustained no fractures and that there was no family history of recurrent fracture.

Hence we report a patient with the correct genetic defect and biochemical results to support a diagnosis of hypophosphatasia, but without any apparent skeletal effects. We are unable to directly ascribe this patient's joint symptoms to hypophosphatasia but would welcome thoughts from other clinicians who may have experience of this condition.

CC6

Acute bone pain after commencing strontium ranelate and raloxifene for osteoporosis

N Sofat, C Mackworth-Young

Department of Rheumatology, Charing Cross Hospital, Imperial College London

A 59-year old woman was referred to the Rheumatology unit by her General Practitioner with an 8-month history of back pain. She had sustained a fall 4 years previously resulting in multiple rib fractures. Her past medical history included chronic renal impairment due to an IgA nephropathy. She was also on thyroid replacement therapy following a subtotal thyroidectomy for hyperthyroidism. She had her menopause aged 55 and was on a normal diet. Her medications included thyroxine, alfacalcidol and codydramol.

Plain X-rays of the thoraco-lumbar spine revealed vertebral collapse at thoracic vertebra 11 with degenerative changes in the lumbar spine. A DEXA scan showed a lumbar spine T-score of -2.9 and femoral neck T-score of -1.8. Her creatinine clearance was 51 mL/min.

A diagnosis of osteoporosis at the lumbar region and osteopenia at the femoral neck were made. She was commenced on calcitonin nasal spray 200 Units daily and calcichew D3 forte 2 tablets daily. Bisphosphonates were not used due to renal impairment. Her spinal pain improved. However, one year after the diagnosis her pain returned and a repeat DEXA scan showed a 7.8% fall in bone density at the lumbar spine and a 2% fall at the femoral neck. Her calcitonin was stopped and she was commenced on strontium ranelate 2g and raloxifene 60 mg daily. She remained on calcichew D3 forte and thyroxine. Seven days after starting her new treatment, she developed generalised muscle pains and acute pain in both knees and ankles. There was no evidence of fractures of these joints on plain X-rays. Repeat blood tests showed an ESR 20 mm/hr, CRP 12, negative rheumatoid factor, ANA and anti-CCP antibodies. Her strontium ranelate and raloxifene were stopped. MRI scans of her left ankle showed microfractures at the talus with soft tissue and bone oedema at both knees.

She was cautiously commenced on 3 monthly intravenous pamidronate infusions after discussion with the renal physicians. Eighteen months after commencing this treatment, there was a 5% improvement in her T-score at the lumbar spine and femoral neck respectively. Her renal function remained stable.

This case highlights the challenges in treating patients with osteoporosis drugs in renal impairment and the importance of metabolic risk factors for the development of osteoporosis. We also describe the unusual side effect of bone pain and myalgia on strontium ranelate and raloxifene.

CC7

Cyclical intravenous Pamidronate therapy improved symptoms of bone pain in a girl with Geroderma Osteodysplasticum.

MZ Mughal[1], JE Adams[2], J Clayton-Smith[3] & AJ Freemont[4]

1Department of Paediatric Medicine, Saint Mary's Hospital, Manchester; [2]Clinical Radiology, Imaging Science & Cancer Studies, University of Manchester; [3]Medical Genetics, St Mary's Hospital & University of Manchester; [4]Laboratory & Regenerative Medicine, University of Manchester, UK.

Geroderma osteodysplasticum (GO) is a rare autosomal recessively inherited disorder characterised by wrinkly skin, joint hypermobility, congenital dislocation of hips and increased propensity to vertebral & long-bone fractures. Recently, GO has been shown to be caused by inactivating mutations in the SCYL1BP1 gene (Nat Genet. 2008;40(12):1410-2.), which is expressed in skin and osteoblasts. We present clinical, radiological, bone mineral density (BMD) and bone biopsy findings in an 11½ year old girl of Pakistani origin with GO, who presented with history of low trauma fractures (clavicle, nose and phalanges) and back pain.

Radiographs of the spine showed generalised osteopenia and wedge compression fractures of T7, T8 and T12 vertebral bodies. Her Z-scores of lumbar spine bone mineral apparent density (LS BMAD) measured by dual energy X-ray absorptiometry and volumetric trabecular BMD (vTBMD) measured by quantitative computed tomography were –3.7 and –5.2, respectively. The distal radius total (RTot-vBMD) and trabecular vBMD (RTr-vBMD) Z-scores, measured by peripheral quantitative computed tomography, were 0 and –2.0, respectively. An iliac crest bone biopsy showed cortical and trabecular osteopenia, with increased osteoblastic activity over osteoblastic activity. Osteoblast

morphology and function appeared to be abnormal, with a deposition of both lamellar and woven bone, which was labelling (and therefore mineralising) poorly.

She was treated with cyclical pamidronate therapy at the dose of 1 mg/kg /day, administered IV, on three consecutive days, at three monthly intervals. This resulted in amelioration of her bone pain and she did not suffer any further long-bone or vertebral (assessed on follow-up spinal radiograph) fractures. At the age of 15½ years, her LS BMAD, RTot-vBMD and RTr-vBMD Z-scores were –1.5, –0.7 and –1.7, respectively. The improvement in her symptoms and BMD might be due to the treatment or her progression through puberty.

Severe cortical and trabecular osteopenia observed in our patient with GO appears to have been caused by impaired bone formation and increased bone resorption. Cyclical IV pamidronate therapy, which suppresses osteoblastic bone resorption, may have a role in the management of osteoporosis associated with GO.

CC8

Pregnancy, lactation and crumbling columns

T.Dugal[1], *S. Griffiths*[2], *B. Hameed*[3]

[1]St Peter's Hospital, Chertsey; [2]East Surrey Hospital, Redhill; [3]Epsom General Hospital, Epsom

JM, 38 yr old female delivered her first child at age 37. She was an ex-smoker and her previous two pregnancies had resulted in first trimester miscarriages. Eight weeks post partum she developed unprovoked thoracic and lumbar spinal pain. She was treated conservatively with analgesics physiotherapy. She continued to fully breast feed for seven months and then partially. Her symptoms failed to improve and ten months later when she had plain radiographs of her spine they showed fractures from L1-L5 and wedging of T6-8 and 10. Her serum Ca+2,PO4 , PTH and Vit D levels were normal. 24 hr Urinary Ca+2 was slightly elevated at 8.5 mmol (N.V=2.5-7.5). Screens negative for Coeliac disease and Myeloma. DEXA scan done showed lumbar spine T score at -2.3 and hip at -1.1. This obviously was not a true reflection in view of multiple fractures and the time lapse of one year after onset of symptoms. A diagnosis of Pregnancy and Lactation associated Osteoporosis (PLO) was made. She was started on 35mg Risedronate weekly and Calcium/Vit D (1000/800). A subsequent DEXA scan done 5 months later showed an improvement in T score in lumbar spine to -1.7, corresponding to an increase of 7.3%. However she continued to have kyphosis and episodes of back pain.

Osteoporosis is a natural consequence of lactation (3-10% loss) after 2-6 months, with mostly trabecular loss. The mechanism postulated is a relative oestrogen deficiency while breast feeding with a rise in PTHrP levels. Losses show spontaneous reversal after weaning.[1] The aetiology is less clear in pregnancy, it appears to be more common in first pregnancy. Possibly is related to increased bone resorption in late pregnancy, low peak bone mass preceding pregnancy due to genetic or recognisable secondary risk factors.[2]

PLO is an uncommon cause of rapidly progressive osteoporosis. This case highlights the importance of early recognition to prevent pain, deformity and disability.

REFERENCES

1. Kovacs CS, Kronenberg HM 1997 Maternal Foetal Calcium and Bone Metabolism during Pregnancy, Puerperium and Lactation. Endocrine Reviews 18(6):832-872
2. Dunne F, Walters B, Marshall T 1993 Pregnancy Associated Osteoporosis. Clin Endocrinology (Oxf) 39(4):487-490

Nick Athanasou

Nick Athanasou is Professor of Musculoskeletal Pathology in the University of Oxford. He has written widely on osteoclast pathobiology and cellular and humoral mechanisms of pathological bone resorption, including tumour osteolysis. Other interests include the pathogenesis and diagnosis of primary bone tumours, particularly giant cell tumour of bone, and the development of new markers to diagnose neoplastic and non-neoplastic diseases of bone, joint and soft tissue.

Paolo Bianco

Paolo Bianco is Professor of Pathology and Director, Anatomic Pathology, at Sapienza Universita' di Roma, Italy, and Chief, Stem Cell Laboratory at San Raffaele Biomedical Science Park of Rome. He works on skeletal diseases and on non-hematopoietic stem cells found in the bone marrow stroma. His earlier work focused among other things on the crucial role of stem cell for modeling genetic diseases of the skeleton, in particular fibrous dysplasia (FD, OMIM#174800), in vitro and in vivo. These studies provided significant advances in the understanding of the disease pathogenesis. His more recent work is directed at identifying and characterizing postnatal progenitors in the human bone marrow and skeletal muscle as subendothelial cells (see Sacchetti et al, Cell 2007, Dellavalle et al Nature Cell Biology 2007, Bianco et al Cell Stem Cell 2008), and on their subsequent use in i) genomic studies of the phenotype-genotype correlation in FD, ii) preclinical models of cell therapy and gene therapy both in vitro and in ad hoc generated murine models of disease, iii) models of cell therapy in bone and skeletal muscle diseases. Dr Bianco has published over 130 peer-reviewed articles.

Ray Boot-Handford

Ray Boot-Handford received his PhD from University College, London and was a postdoctoral fellow with Mike Grant in Manchester and Darwin Prockop in Philadelphia. He is a Professor of Biochemistry in the Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences in Manchester. His current research focuses upon characterising disease mechanisms associated with connective tissue disorders and the evolution of the metazoan extracellular matrix.

Alan Boyde

Alan Boyde is a Professor in the Biophysics Section of the Centre for Oral Growth and Development at Barts and The London School of Medicine and Dentistry, Queen Mary University of London where he graduated (when it was called The London Hospital Medical College) BDS 1958 and PhD in Anatomy 1964. He joined the Bone and Tooth Society – now the Bone Research Society - when the subscription was £1 for four years and was such a loyal supporter that he opted to remain a member when this increased to £1 per year, and so *ad infinitum*. He was rescued from resignation on the grounds of destitution by being offered honorary membership. He served a long spell in the Anatomy Department at UCL. Over the years, he has developed many new approaches in several branches of microscopical science of special relevance to skeletal tissue research.

Ann Canfield

I graduated with a degree in Biochemistry from Manchester and then completed a PhD under the supervision of Professor Mike Grant in Medical Biochemistry. My Post-doctoral studies took me to the Paterson Institute for Cancer Research, where my research focussed on the regulation of angiogenesis by specific matrix molecules. I joined the Wellcome Trust Centre for Cell-Matrix Research at the University of Manchester as a Junior Research Fellow in 1993 and was appointed as a Lecturer in 1996, Senior Lecturer in 2000 and Reader in 2007. My main research interests are in the biology and pathology of blood vessels. In particular, I have a long-standing interest in the multi-lineage potential of vascular pericytes and smooth muscle cells and in elucidating the mechanisms by which the uncontrolled oste/chondro differentiation of these cells can result in several pathologies including the calcification of blood vessels. In addition, as a member of the UK Centre for Tissue Regeneration, my research is also focussed on using specific matrix molecules to generate small diameter vascular grafts with a non-thrombogenic coating of endothelial cells.

Yuti Chernajovsky

Yuti Chernajovsky is ARC Professor of Rheumatology and Head of Department, Bone and Joint Research Unit at Barts and The London, Queen Mary's School of Medicine and Dentistry, University of London. He trained as a molecular biologist, at The Weizmann Institute of Science in Israel, his research activities have included the cloning of several interferons, interleukins and their receptors. He has contributed to the cloning and development of interferon beta as a therapeutic agent and the use of suicidal genes for cancer gene therapy. Current research interests include the development of gene transfer strategies for the treatment of rheumatoid arthritis and other autoimmune diseases via molecular design and cellular engineering. He is member of the editorial board of Gene Therapy and Arthritis Research and Therapy.

Ian Clark

Ian Clark graduated from Bristol University, UK with a BSc in Biochemistry. He went on to complete a PhD in the Rheumatology Research Unit at Addenbrooke's Hospital, Cambridge, UK developing immunoassays to measure matrix metalloproteinases (MMPs) and their inhibitors, the TIMPs. With help from the Arthritis Research Campaign (arc), he spent a year in Dartmouth Medical School, USA learning about MMP gene regulation, then returned to the UK as an arc Postdoctoral Fellow to apply these skills to the TIMP-1 gene. In 1996 he moved to UEA, Norwich UK to build his own research team in this general area. In 2001, he spent a year in the Respiratory and Inflammation Dept. of AstraZeneca Pharmaceuticals, working on osteoarthritis and gaining an understanding of the industrial side of science. He now holds the Chair of Musculoskeletal Biology with research interests primarily concerned with the function and regulation of metalloproteinases in arthritis and other musculoskeletal diseases.

Thomas Clemens

Dr. Thomas Clemens is currently a Professor in the Department of Pathology at the University of Alabama at Birmingham. Dr. Clemens received his Ph.D. in Biochemistry from the University of London in London, England and completed postdoctoral training as a Research Fellow in Medicine at Massachusetts General Hospital. He was an Associate Professor in Medicine at Cedars Sinai Medical Center where he founded a Masters program in Biomedical Sciences. He was a Professor of Medicine at the University of Cincinnati where he directed the Pathobiology and Molecular Medicine graduate program. Dr. Clemens' research is focused on identification of the cellular and molecular mechanisms, which control osteoblast activity. He has authored 119 original publications, and a number of editorials and book chapters. He has served as a council member of American Society of Bone and Mineral Research and was the program co-chair for the 2002 national meeting. He is the current Editor-in-Chief of the Journal of Bone and Mineral Research.

Juliet Compston

Juliet Compston is Professor of Bone Medicine and Honorary Consultant Physician at the University of Cambridge School of Clinical Medicine. Among other activities, she is currently a Member of the Board of the International Osteoporosis Foundation, Member of the Board of the International Bone and Mineral Society, Chair of the UK National Osteoporosis Guidelines Group (NOGG), Chair of the Publications Committee of the International Bone and Mineral Society, Project Leader of European Commission/International Osteoporosis Foundation Call to Action for Osteoporosis and Chair of European Union Osteoporosis Consultation Panel. She was awarded the National Osteoporosis Society Kohn Foundation Award 2006 and International Bone and Mineral Society John G Haddad Jr Award in 2009.

Cyrus Cooper

Cyrus Cooper is Professor of Rheumatology and Director of the MRC Epidemiology Resource Centre at the University of Southampton, and Norman Collisson Chair of Musculoskeletal Science at the University of Oxford. He leads an internationally competitive programme of research into the epidemiology of musculoskeletal disorders, most notably osteoporosis. His key research contributions have been: 1) discovery of the developmental influences which contribute to the risk of osteoporosis and hip fracture in late adulthood; 2) demonstration that maternal vitamin D insufficiency is associated with sub-optimal bone mineral accrual in childhood; 3) characterisation of the definition and incidence rates of vertebral fractures; 4) leadership of large pragmatic randomised controlled trials of calcium and vitamin D supplementation in the elderly as immediate preventative strategies against hip fracture. He is currently President of the Bone Research Society of Great Britain, and Chairman of the Committee of Scientific Advisors, International Osteoporosis Foundation. He has published extensively (over 350 publications) on osteoporosis and rheumatic disorders and pioneered clinical studies on the developmental origins of peak bone mass.

Peter Croucher

Professor Peter Croucher graduated with a BSc in Zoology from University College Cardiff in 1987 and completed a PhD at the University of Wales College of Medicine, Cardiff, in 1990. He undertook post-doctoral training in the Department of Medicine at the University of Cambridge and in the Department of Human Metabolism and Clinical Biochemistry at the University of Sheffield before being awarded a five-year Bennett Senior Fellowship by the Leukaemia Research Fund in 1997. In 2001 he joined the University of Oxford Institute of Musculoskeletal Sciences as a Senior Research Fellow before being appointment in 2003 to his current position as Professor of Bone Biology at the University of Sheffield. He is currently Head of the Academic Unit of Bone Biology and Head of the Department of Human Metabolism. Professor Croucher's principal interests are in understanding the cellular and molecular mechanisms responsible for regulating tumour induced bone diseases such as multiple myeloma.

Leif Dahlberg

Leif Dahlberg is Associate Professor at the Faculty of Medicine, Lund University and Staff Surgeon at the Department of Orthopaedics, Malmö University Hospital, Sweden. His research interests are in Joint Cartilage Monitoring in Health and Disease and Osteoarthritis and Cartilage.

Adam Engler

Adam J Engler is an Assistant Professor of Bioengineering at the University of California, San Diego. His research focuses on how the intrinsic properties of the extracellular matrix regulate embryonic and adult stem cell differentiation as well as heart development. Dr. Engler earned his B.S.E. and Ph.D. degrees in bioengineering and mechanical engineering at the University of Pennsylvania before completing a postdoctoral fellowship at Princeton University's Department of Molecular Biology, funded by the National Cancer Institute. Dr. Engler is the 2008 recipient of the Rupert Timpl and Rita Schaffer Young Investigator Awards from the International Society for Matrix Biology and the Biomedical Engineering Society, respectively.

Tim Hardingham

Professor Tim Hardingham was the founding Director of the UK Centre for Tissue Engineering and is Professor of Biochemistry in the Wellcome Trust Centre for Cell-Matrix Research (www.wtccmr.man.ac.uk), Faculty of Life Sciences, University of Manchester, UK.

Professor Hardingham was previously the Head of Biochemistry at the Kennedy Institute of Rheumatology in London and is the current Chairman of the Arthritis Research Campaign (UK) Research Grants Committee. He is a past member of the Molecular and Cellular Medicine Board of the Medical Research Council (UK) and also immediate past Chairman of the British Society for Matrix Biology and member of Council of the British Society for Rheumatology and has several awards and honours, including the Colworth Medal of The Biochemical Society (1978), the Roussel International Award for Basic Research in Osteoarthritis (1989) and the Carol Nachman International Prize for Research in Rheumatology (1991). Professor Hardingham has been a member of ad hoc NIH study sections on Tissue Engineering and Bioengineering Research Partnerships (1999-2003). He is a founding Trustee of the newly formed International Society for Hyaluronan Sciences and currently co-heads the Tissue Regeneration Section of the Faculty of 1000 Medicine. He was elected (2001) to the Governing Board of Tissue Engineering Society International (TESi) and was European Vice-President of TESi (2003-2005) and on TERMIS-EU Chapter board (2005-2008).

Professor Hardingham's research interests are in the biology and pathology of cartilage and musculoskeletal tissues and the degenerative processes in osteoarthritis and other joint diseases. He has long standing research interests in the physical properties and biological functions of extracellular matrices. Current research is focussed on the biology of chondrocytes and the differentiation of adult and embryonic stem cells and their application in tissue engineering of cartilage and other musculoskeletal tissues. Professor Hardingham also takes a lead in promoting research in the broader applications of tissue engineering in medicine.

Marie-Hélène Lafage-Proust

Pr MH Lafage-Proust, MD, PhD teaches Cell Biology at the Medical School of the University of Lyon, at Saint-Etienne, France. She joined the INSERM 890 team in 1994, after a postdoctoral Fellowship with Gideon Rodan at Merck RL. Her main research interests are the effects of mechanical load on bone. Recently, she focused on the involvement of bone microvascularisation in bone remodelling. She was trained as a rheumatologist at the Medical School of Bordeaux University. She studied bone histomorphometry in Pr Meunier's laboratory in Lyon. Ever since, she has been responsible for the histological diagnosis of metabolic bone diseases. As a clinician, she currently takes care of patients with metabolic bone diseases, in the Rheumatology Department of St-Etienne University Hospital.

Nigel Loveridge

Nigel Loveridge gained his PhD (Biochemistry) from Brunel University in 1981. He has had a lifelong background in the biological sciences, particularly musculoskeletal research and has worked in London (Kennedy Institute), Montreal (McGill University), Zurich (University of Zurich), Aberdeen (Rowett Research Institute) before settling in Cambridge in 1994 where he is a Principal Research Associate (Reader) in the Department of Medicine and Editor in Chief of Cell Biochemistry and Function. He has over 100 publications in areas such endocrinology, longitudinal growth and most recently the microstructure, material properties and cellular activities associated with fragility in the proximal femur. His current interests include the role of osteocytes in bone remodeling in health and disease.

Karen Lyons

Karen Lyons is Professor of Molecular Cell and Developmental Biology at the University of California, Los Angeles. She holds a joint appointment in the Department of Orthopaedic Surgery at UCLA. Prior to joining UCLA, she completed postdoctoral studies in the

laboratory of Brigid Hogan at Vanderbilt University. Her major interests are in signaling pathways controlling growth plate and bone formation. In particular, her studies have focused on the roles of Bone Morphogenetic Proteins (BMPs) in the developing growth plate. A second major focus has been on the roles of the members of the matricellular proteins connective tissue growth factor (CTGF/CCN2), Cyr61/CCN1, and Elm1/CCN4 in cartilage, bone and vascular development. Dr. Lyons' studies are funded by the National Institutes for Health (NIAMS), Arthritis Foundation, and Scleroderma Foundation. She is a member of the Board of Counselors for the American Society for Matrix Biology.

Bjorn Olsen

Dr. Bjorn R. Olsen, Hersey Professor of Cell Biology at Harvard Medical School and Dean for Research and Professor of Developmental Biology at the Harvard School of Dental Medicine, is internationally recognized for his contributions to the fields of extracellular matrix biology and skeletal genetics, cell and developmental biology.

His research has furthered our understanding of diseases from dwarfism to congenital vascular anomalies, osteoporosis, osteoarthritis, corneal dystrophy and retinal degeneration. His studies have uncovered fundamental roles of collagens, transcription factors and receptors that affect not only skeletal development, but also angiogenesis and blood vessel morphogenesis.

His honors include election to the Norwegian Academy of Sciences and ScanBalt Academy, honorary doctoral degrees from the University of Oslo and University of Medicine and Dentistry of New Jersey, the Humboldt Research Award from Germany, the H.C. Jacobæus Prize, and the Senior Research prize of the American Society of Matrix Biology.

Mats Paulsson

Mats Paulsson was trained in medicine and biochemistry at the University of Lund and performed his doctoral work on cartilage matrix proteins in the group of Dick Heinegard. 1983 he moved as a postdoc to Rupert Timpl´s lab at the Max-Planck-Institute for Biochemistry in Munich and was there introduced to the basement membrane field. He started his first own group at the Biocenter of the University of Basel in 1986, was later at the University of Berne and was appointed to a chair of biochemistry at the University of Cologne in 1995. His work has focussed on structure and assembly of the extracellular matrix in cartilage and basement membranes, in recent years with an increasing focus on the role of extracellular matrix proteins in inherited disease. He has been a member of the editorial board of the *Biochemical Journal* and, at present, the *Journal of Biological Chemistry*. At the University of Cologne he serves on the executive boards of the Center for Molecular Medicine, the Graduate School for Biological Sciences and the Collaborative Research Center on Molecular Mechanisms Regulating Skin Homeostasis.

David Reid

Professor David M Reid holds a personal chair of Rheumatology at the University of Aberdeen and is also Head of the Division of Applied Medicine at the School of Medicine & Dentistry.

He has over 250 original papers and reviews, largely on his current research interests which include the utility of bone mass assessment, assessment of risk of fracture, secondary osteoporosis and the assessment of long-term disease activity and drug adverse effects in rheumatic diseases.

He is the Chairman of the Board of Trustees of the UK National Osteoporosis Society (NOS). He was a member of the Writing Groups for the Scottish Integrated Guidelines Network on the Management of Osteoporosis (publication in 2003) and Royal College of Physician's Guidelines on Glucocorticoid-Induced Osteoporosis published in December 2002. Recently he chaired groups who have published the Arthritis and Musculoskeletal Alliance *Standards of Care for Metabolic Bone Diseases* and a UK expert group who have produced Guidance on the *Management of Cancer Treatment Induce Bone Loss*.

Henry Roehl

As a graduate student, Henry Hamilton Roehl worked with C.elegans and made several important discoveries into the mechanism of Notch signalling that resulted in publications in Nature and EMBO Journal. As a postdoc he continued to analyse signalling pathways, switching to the FGF pathway and using zebrafish. He identified two new components of the FGF pathway resulting in a paper being published in Current Biology. While still a postdoc, he performed a large-scale forward genetic screen and isolated more than 40 mutants that affect different aspects of cranial musculoskeletal development. In 2002, he started his own laboratory as a lecturer in the Centre for Developmental and Biomedical Genetics at the University of Sheffield. He initially analysed three zebrafish skeletal mutants required for heparin sulphate synthesis resulting in publications in Neuron and PLoS Genetics. More recently, he has focused on how developmental signalling pathways coordinate differentiation of osteoblasts.

Irving Shapiro

Dr. Shapiro, received a degree in Dental Surgery (BDS) from the University of London, and the degree of Ph.D. in Biochemistry from the University of London in 1968. He

joined the faculty of the University of Pennsylvania (Penn) in 1969 and rose to the rank of Full Professor in 1976. He served as Chairperson of the Department of Biochemistry in the School of Dental Medicine for 9 years; in 1997, with faculty from the Department of Bioengineering at the School of Engineering at Penn, he was instrumental in forming the Center for Bioactive Material Research. During this time period Dr. Shapiro had the honor of chairing two separate Gordon Conferences (Bones and Teeth and Biomineralization) and organizing the First International Conference on the Growth Plate. In 2001, Dr. Shapiro was recruited by the Department of Orthopaedic Surgery at Thomas Jefferson University to lead the Division of Orthopaedic Research. Currently, he is Professor of Orthopaedic Surgery, Biochemistry and Molecular Biology and Director of the Tissue Engineering and Regenerative Medicine Graduate Training Program at Thomas Jefferson University.

Current research activities now being pursued by Dr. Shapiro can be summarized as follows: (a) Fate of the hypertrophic chondrocyte in the endochondral growth plate. The current project is part of a long term interest in the mechanism of chondrocyte hypertrophy and mineralization. Having shown that cells in the epiphysis end their life history through the induction of apoptosis, he has recently shown that prior to apoptosis the chondrocytes assume a new state, autophagy. (b) Creating bioactive surfaces for repair of fractured and infected bone. The goal of this work is to develop a new generation of "smart" implants that promote osteogenesis and prevent bacterial infection. This study relies heavily on the development of new chemical techniques to generate linkages between metals and bioactive molecules and uses imaging techniques such as mass spec analysis, micro-tomography light, electron and X-ray imaging to evaluate efficacy of healing. (c) exploring the origin, form and function of cells of the intervertebral disc. Following a bout of lower back pain, Dr. Shapiro directed his research to determining if stem cells exist in the disc, and whether they can be used to repopulate the nucleus pulposus. His more recent work is aimed at learning how the notch signaling system regulates intervertebral disc proliferation and differentiation.

When not working (a rare occurrence), he enjoys playing with wife, daughter and grandchildren, cooking very hot curries and tap dancing.

Jonathan Tobias

Jonathan Tobias is Professor of Rheumatology at the University of Bristol, and consultant rheumatologist at North Bristol Trust. Following undergraduate studies in medicine at Cambridge University and London University from where he qualified in 1984, he completed MD and PhD theses in bone biology in 1990 and 1994, at St George's Hospital in London. He was appointed Consultant Senior Lecturer in Bristol in 1995, and since 2008 has headed up the Academic Rheumatology Unit at the Avon Orthopaedic Centre. He manages a diverse research program into the causes and treatment of osteoporosis. He has extensive clinical experience in treating patients with osteoporosis, and in running DXA-based osteoporosis diagnostic services. He serves on the editorial board of Calcified Tissue International, the program committee of the National Osteoporosis Society, and the research committee of the Arthritis Research Campaign. He is currently president elect of the Bone Research Society.

Rob van 't Hof

Rob van 't Hof originally studied Biology at the University of Utrecht in the Netherlands. During his PhD at the University of Leiden, he developed an interest in the regulation of osteoclast formation and activity, with a special interest in the cross-talk between osteoblasts and osteoclasts. After his PhD, he moved to the University of Aberdeen to study the effects of Nitric Oxide (NO) on bone cells in the group of prof. Ralston. In July 2005 Dr. van 't Hof moved to the University of Edinburgh to take up his current post as senior lecturer in the Rheumatic Diseases Unit. His main projects here involve the regulation of bone turnover by the central nervous system, and the development of small molecule inhibitors of bone resorption and inflammation.

Amgen

www.amgen.com

Amgen discovers, develops, manufactures and delivers innovative human therapeutics. A biotechnology pioneer since 1980, Amgen was one of the first companies to realize the new science's promise by bringing safe and effective medicines from lab, to manufacturing plant, to patient. Amgen therapeutics have changed the practice of medicine, helping millions of people around the world in the fight against cancer, kidney disease, rheumatoid arthritis, and other serious illnesses. With a deep and broad pipeline of potential new medicines, Amgen remains committed to advancing science to dramatically improve people's lives. To learn more about our pioneering science and our vital medicines, visit www.amgen.com.

Advanced Molecular Vision

www.AMV-Europe.com

We at Advanced Molecular Vision Ltd are specialists in the fields of in vitro and in vivo optical imaging. As the European distributor for Kodak/Carestream in vitro and in vivo small animal imaging systems we offer you a complete service; from the analysis of simple western blots to full multimodal analysis of small animals. Our high resolution scientific grade in vitro systems can form the core basis of any scientific investigation and we offer a range to cover simple gel documentation to complex fluorescence imaging. Our in vivo imaging range includes the Kodak DXS and Multispectral systems which offer the highest resolution digital planar x ray system currently available complete with bone density analysis software. Furthermore, with the addition of fluorescent and bioluminescence imaging modalities, you have the ability to couple high resolution structural images with true functional data. So come and visit our display and see what we have to offer you in your research area.

Company of Biologists

www.biologists.com

The Company of Biologists is run by biologists for biologists, and supports innovation in all aspects of biological research. A not-for-profit publisher of the well established, internationally renowned journals; Development, Journal of Cell Science and The Journal of Experimental Biology, the company has also recently launched a significant new journal, Disease Models & Mechanisms, to create a collaborative forum for scientists and clinicians involved in the use of model organisms for the understanding, treatment, and diagnosis of human disease.

Each journal is printed in house enabling exclusive attention to quality, accuracy and speed of publication and the Company has a policy of free colour reproduction saving biologists and their grants approximately US$1,000,000 per annum.

We are delighted to be able to support the second joint meeting of the Bone Research Society and the British Society for Matrix Biology, as the keynote speakers are of international standing and come from a wide range of disciplines, in line with our commitment to support diversity in the scientific community.

Founded in 1925, the Company continues to make an important contribution to the advancement of scientific discoveries, providing grants, travelling fellowships and sponsorship to noteworthy scientists, meetings, societies and collaborative projects around the world. The Company also provides undergraduates and early stage career scientists with travel bursaries to facilitate attendance at international meetings.

The Company's Directors are leading biologists, librarians and computer scientists, who receive no remuneration for their services, and are dedicated to supporting the interests of the community in the advancement of uncovering new scientific discoveries. For more information on our range of charitable activities please visit our website at www.biologists.com.

e2v/SkyScan

www.e2v.com www.skyscan.be

SkyScan is a fast growing company and one of the world's leading producers of micro-CT systems for a wide range of applications. SkyScan aims to bring to customers the newest technology, the best instrument quality and the highest level of support with a dedicated team of specialists in the biological applications of MicroCT.

SkyScan can genuinely claim to be at the fore-front of the development of high performance micro-CT technology. Our research and development of 3D x-ray microscopy started in the early 1980s leading to the first micro-CT imaging results being obtained in 1987 and published in scientific journals and international conferences proceedings. Building on this early work, SkyScan was founded in 1996, and within a year we were manufacturing a commercially available micro-CT scanner with spatial resolution in the micron range. In 2001 we produced the first high-resolution in vivo micro-CT scanner for small animal imaging. We are continually developing both our hardware and our quantitative software analysis suite. Responding to demand from the growing community of micro-CT users, we are continually active in research and development into new methods for non-destructive 3D microscopy.

Eli Lilly

www.lilly.com

Eli Lilly and Company is one of the world's largest research-based pharmaceutical companies, dedicated to creating and delivering innovative pharmaceutical healthcare solutions that enable people to live longer, healthier and more active lives. Our research and development efforts constantly strive to address urgent unmet medical needs.

Eli Lilly and Company was founded in 1876 in Indianapolis, USA, and has had a long history of producing endocrine products, dating all the way back to the collaboration with Banting and Best and the introduction of the world's first insulin product in 1922.

Another element of Lilly's endocrine portfolio is growth hormone replacement. Lilly manufactures recombinant human growth hormone (somatropin) at Speke near Liverpool, UK. A full range of products and services is provided for the healthcare professional to use with their patients on growth hormone replacement therapy for both adults and kids.

To assist in the therapeutic management of Osteoporosis, Lilly has developed Teriparatide which is available in over 65 countries worldwide.

Finally Lilly continues to focus significant resources on research into the endocrine area. For additional information about any of our products or services please come and talk to us at the Lilly stand or log on to the company website – www.lilly.com.

Immunodiagnostic Systems (IDS)

www.idsplc.com

Immunodiagnostic Systems (IDS) offers a range of esoteric immunoassay kits for clinical and research use, combined with our NEW fully automated IDS-iSYS analyser.

The company focuses in Bone & Mineral Metabolism, Growth Factor research and Cartilage diagnostics.

IDS is a leader in the field of Vitamin D analysis, offering both manual and automated 25(OH) Vitamin D methods, an award-winning 1,25-Dihydroxy Vitamin D RIA system, 1,25(OH)2 Vitamin D, an EIA employing the proven immunocapsule sample preparation.

IDS offers a full range of Bone, Growth and Cartilage products as outlined in the menu below:

- Calciotropic Hormones
- 25-Hydroxy Vitamin D
- 1,25-Dihydroxy Vitamin D
- Intact PTH
- Bone Turnover
- Ostase® BAP
- Rat/Mouse PINP
- N-MID® Osteocalcin
- RAT-MIDTM Osteocalcin
- BoneTRAP® (TRACP 5b)
- RatTRAPTM (TRACP 5b)
- MouseTRAPTM (TRACP 5b)
- Serum CrossLaps® (CTX-I)
- Urine CrossLaps® (CTX-I)
- Alpha CrossLaps® (CTX-I)
- Urine Beta CrossLaps® (CTX-I)
- RatLapsTM(CTX-I)
- Deoxypyridinoline (DPD)
- CrossLaps® for Culture (CTX-I)
- Osteoprotegerin (OPG)
- Ampli Free sRANKL
- Cartilage Markers
- Serum Pre-Clinical CartiLaps®(CTX-II)
- Urine Pre-Clinical CartiLaps®(CTX-II)
- Urine CartiLaps®(CTX-II)
- Total Aggrecan for Culture
- Growth Disorders
- Growth Hormone (hGH) IRMA
- IGF-I
- IGFBP-3
- Rat/Mouse IGF-I
- Mouse High Sensitivity IGF-I HS
- Equine IGF-I
- Placental Growth Hormone (hPGH)

For a complete list of IDS offices and distributors worldwide, visit our website at www.idsplc.com.

Novartis

www.novartis.co.uk

Novartis is one of the world's leading pharmaceutical companies and works to discover, develop and deliver new and innovative products to treat patients, ease suffering and to enhance the quality of life.

With its UK headquarters in Frimley, Surrey, the Novartis group of companies in the UK employs over 3,500 staff across 11 sites working in the pharmaceuticals, consumer health, generics and vaccines divisions. Globally, Novartis is a Swiss based company employing over 100,000 people across 140 countries.

Novartis aspires to contribute to society through our economic contribution, the positive benefits of our products and through open dialogue with our stakeholders.

Think what's possible. For more information visit www.novartis.co.uk.

Procter & Gamble Pharmaceuticals

www.pg.com

Procter & Gamble has a rich heritage in health care that extends back more than 150 years. Then and now, P&G is driven by our mission to improve the lives of people around the world every day. P&G's health care products include prescription medicines, over-the-counter medications and oral care products. P&G began developing and marketing prescription products in the late-1960s.

Roche

www.rocheuk.com

Roche aims to improve people's health and quality of life with innovative products and services for the early detection, prevention, diagnosis and treatment of disease. Part of one of the world's leading healthcare groups, Roche in the UK employs nearly 2,000 people in pharmaceuticals and diagnostics. Globally Roche is the leader in diagnostics, and a major supplier of medicines for the treatment of cancer, transplantation, virology, bone and rheumatology, obesity and renal anaemia. Find out more at www.rocheuk.com.

Scanco

www.scanco.ch

Scanco Medical is the leading manufacturer of a full range of state-of-the-art μCT scanners for research and clinical use (high-resolution specimen, in vivo animal and human extremity scanners). The scanners, together with built-in analysis and visualization software provide for 3-dimensional, non-destructive and comprehensive quantitative measurements. The in vivo scanners are uniquely engineered for high-resolution, rapid scanning with very low radiation exposure. Scanco Medical brings over 20 years of experience in micro-CT and has close to 250 installations worldwide. Scanco Medical also offers contract based scanning services for academic and industrial groups at facilities in the USA or in Switzerland.

Servier

www.servier.com

Servier Laboratories is the UK subsidiary of The Servier Research Group, the leading independent French research based pharmaceutical company. The key franchises of the Servier Research Group are rheumatology, cardiovascular disease, diabetes, central nervous system and oncology. Servier regularly invests more than 25% of its annual turnover into research, discovering and delivering therapeutic innovations to patients through their healthcare professionals.

BRS/BSMB 2009 RESTAURANTS

There are hundreds of restaurants and pubs within a few minutes walk of UCL. Here is a personal selection of restaurants that offer decent food at reasonable prices. Most are tried and tested. Estimated prices are for 2 courses, drinks, coffee & service. It is always advisable to phone ahead to check opening times and availability. The Farringdon / Exmouth Market / Clerkenwell restaurants are in an area that is well-known for good food; the UCL residences (Frances Gardner & James Lighthill) are near by.

The Anchor & Hope – possibly London's best gastropub; modern European; very busy; good value. Food ★★★☆ Wine ★★☆ Beer ★★★. £20-30 / head. 36 The Cut, SE1 (easy to reach from UCL - tube to Southwark or Waterloo) Tel: 020 7928 9898. No bookings. Not Sun.

Andrew Edmunds – modern European; good value. Food ★★★★ Wine ★★★★☆ (low markups). £30-40 / head. 46 Lexington St, Soho, W1. Tel: 020 7437 5708. Booking essential (it's very small - upstairs is best).

ASK – reliable pizza / pasta chain. Food ★★ Wine ★. £15-25 / head. 48 Grafton Way (near corner with Tottenham Court Rd), W1. Tel: 020 7388 8108. Open Sun.

Barrafina – top tapas; small, trendy, very busy, queues inevitable. Food ★★★ Wine ★★★. £25-35 / head. 54 Frith St, Soho, W1. Tel: 020 7813 8010. No bookings.

Côte – French bistro; good value. Food ★★★ Wine ★★. £20-30 / head. 124-6 Wardour St, Soho, W1. Tel: 020 7287 9280. Booking advised.

Diwana Bhelpoori – budget Indian veg. Food ★★ not licensed – BYO from shop next door. £10-20 / head. 121 Drummond St, Euston, NW1. Tel: 020 7387 5556.

The Eagle – brilliant, noisy gastropub; modern European; good value. Food ★★★☆ Wine ★★ Beer ★★★. £20-30 / head. 159 Farringdon Rd, Farringdon, EC1. Tel: 020 7837 1353. No bookings. Not Sun.

Fish Bone – fish & chips. New restaurant / takeaway; not tested but it's supposed to be good. £15-20 / head (restaurant). 82 Cleveland St, Fitzrovia, W1. Tel: 020 7580 2672. Not Sun.

Great Queen Street – noisy modern British; good value. Food ★★★☆ Wine ★★. £25-35 / head. 32 Great Queen St, Holborn / Covent Garden, WC2. Tel: 020 7242 0622. Booking essential. Not Sun.

Great Nepalese – well-known & reliable. Food ★★ Beer. £15-25 / head. 48 Eversholt St, Euston, NW1. Tel: 020 7637 0222. Booking advised.

The Japanese Canteen – budget fast food. Food ★ not licensed. £7-10 / head. Corner of University St & Tottenham Court Rd, W1. Tel: 020 7387 5556.

Marine Ices – legendary S. Italian café with some of the best pizza and seafood pasta (and ices) in London; great value (& cheap wine), friendly, efficient service. Food ★★★ Wine ★★. £15-25 / head. 8 Haverstock Hill, NW3 (opp. Chalk Farm station - a few mins from UCL by tube). Tel: 020 7482 9003. Open Sun (closed Mon).

Mestizo – Mexican (Mex more than Tex-Mex). Food ★★ Beer, cocktails. £20-30 / head. 103 Hampstead Road, NW1 (just N of Warren St Station). Tel: 020 7387 4064. Booking advised.

Moro – Spanish / Moorish cuisine / tapas; Time Out's top restaurant of 2009. Food ★★★★ Wine ★★★★. £30-40 / head. 34-36 Exmouth Market, Farringdon, EC1. Tel: 020 7833 8336. Booking essential. Not Sun.

The Newman Arms – tiny, trad pub with famous pie room upstairs. Food ★☆ good beer. £12-15 / head. 23 Rathbone St, Fitzrovia, W1. Tel: 020 7636 1127. Food served until 9 pm; not Sun.

The Norfolk Arms – gastropub with Spanish flavour; good range of tapas bar snacks. Food ★☆ Wine ★ Beer ★. £10-25 / head. 28 Leigh St, Bloomsbury / Kings Cross WC1. Tel: 020 7388 3937. Open Sun.

North Sea Fish Restaurant – fish & chips. Long-established; not the cheapest but fish is usually good. Food ★☆ Wine ★. £20-25 / head (restaurant). 7-8 Leigh St, Bloomsbury WC1. Tel: 020 7387 5892. Not Sun.

Number Twelve – modern Italian on ground floor of Ambassador's Hotel. Suitable for larger groups. Food ★★★ Wine ★★★☆. £35-40 / head. 12 Upper Woburn Place, Euston, WC1. Tel: 020 7693 5425. Booking advised.

Prince Albert – busy gastropub, good atmosphere / décor; upstairs dining room. Food ★☆ Wine ★☆ Beer ★. £20-30 / head. 163 Royal College St, Camden, NW1. Tel: 020 7485 0270. Booking advised.

Quality Chop House – modern / trad British; preserved early 20C eatery with top nosh. Food ★★★☆ Wine ★★★. £25-35 / head. 92-94 Farringdon Rd, Farringdon, EC1. Tel: 020 7837 5093. Booking essential. Not Sun.

Queen's Head & Artichoke – decent gastropub; European / British cooking, plus good tapas from bar. Food ★☆ Wine ★☆ Beer ★☆. £25-35 / head. 30 Albany Street, Regents Park NW1 020 7916 6206. Open Sun.

Ragam – quality S. Indian / veg / non-veg. Food ★★☆ (licensed). £15-25 / head. 57 Cleveland St, Fitzrovia, W1. Tel: 020 7636 9098 Booking advised. Not Sun.

Rasa Samudra – quality Keralan/S. Indian/seafood/veg. Food ★★★ Wine ★★. £25-35 / head. 5 Charlotte St, Fitzrovia, W1. Tel: 020 7637 0222. Booking essential. Not Sun.

Ravi Shankar – budget Indian veg. Food ★★ (licensed). £10-20 / head. 133-135 Drummond St, Euston, NW1. Tel: 020 7388 6458.

Salt Yard – modern tapas / charcuterie. Food ★★★ Wine ★★★. £25-35 / head. 54 Goodge St, Fitzrovia, W1. Tel: 020 7637 0657. Booking essential. Not Sun.

Sardo – Sardinian; can be pretty good. Food ★★★ Wine ★★★. £30-45 / head. 45 Grafton Way, W1 (near Warren St Station) . Tel: 020 7387 2521. Booking essential. Not Sun.

Somers Town Coffee House – French-run bistro / gastropub; garden & terrace. Food ★☆ Wine ★★. £20-30 / head. 60 Chalton St, Euston, NW1. Tel: 020 7691 9136. No food on Sun.

St John – classic gastrobar / restaurant with full-on British menu. Food ★★★☆ Wine ★★★☆. £25-40 / head. 26 St John Street, Farringdon, EC1. Tel: 020 7251 0848. Booking advised.

TAS Restaurant – modern Turkish / Mediterranean; well-known small chain; very good vaue. Food ★★ Wine ★★. £15-25 / head. 22 Bloomsbury St, WC1. Tel: 020 7637 4555; also at 37 Farringdon Rd, EC1. Tel: 020 7430 9721 & 33 The Cut, SE1. Tel: 020 7928 1444. Booking advised.

Vinoteca – Spanish-Moorish; wine / tapas. Food ★★★ Wine ★★★★☆. £25-40 / head. 7 St John Street, Farringdon, EC1. Tel: 020 7253 8786. Not Sun.

Wahaca – trendy Mexican run by 2005 Masterchef winner; high-quality, high-speed street food. Food ★★☆ top margaritas. £20-30 / head. 66 Chandos Place, Covent Garden WC2. Tel: 020 7240 1883.

The York & Albany – ultimate posh gastropub (Angela Hartnett / Gordon Ramsay); top nosh, top prices. Food ★★★★☆ Wine ★★★☆. £45+ / head. 127-129 Parkway, Camden, NW1. Tel: 020 7388 3344. Booking essential.

Cheap and cheerful

Eating:

Belgo – 72 Chalk Farm Road, London, NW1 8AN. (Approx £15)

La Porchetta – 74/77 Chalk Farm Road, London, NW1 8AN. (Approx £10 for drink and pizza)

Drinking/Clubbing:

CAMDEN TOWN /CHALK FARM

Lockside Lounge – 75-89 West Yard, Camden Lock, Camden. Outside drinking area. Camden Town.

Made in Brasil – 12 Inverness Street. Outside drinking area. Camden Town.

Edinboro Castle – 57 Mornington Terrace, NW1 7RU. Large Beer Garden. Camden Town.

Gigamesh (2am) – The Stables, Chalk Farm Road. Camden/Chalk Farm.

BarTok (3am) – 78-79 Chalk Farm Road, Primrose Hill. Camden Town/Chalk Farm.

SOHO/COVENT GARDEN

Freuds – 198 Shaftesbury Avenue. Leicester Square /Covent Garden.

Bar Soho (1am) – 23-25 Old Compton Street, Soho. Leicester Square.

Guanabara (2am) – Parker Street, Holborn, London, WC2B 5PW. Leicester Square/Holborn.

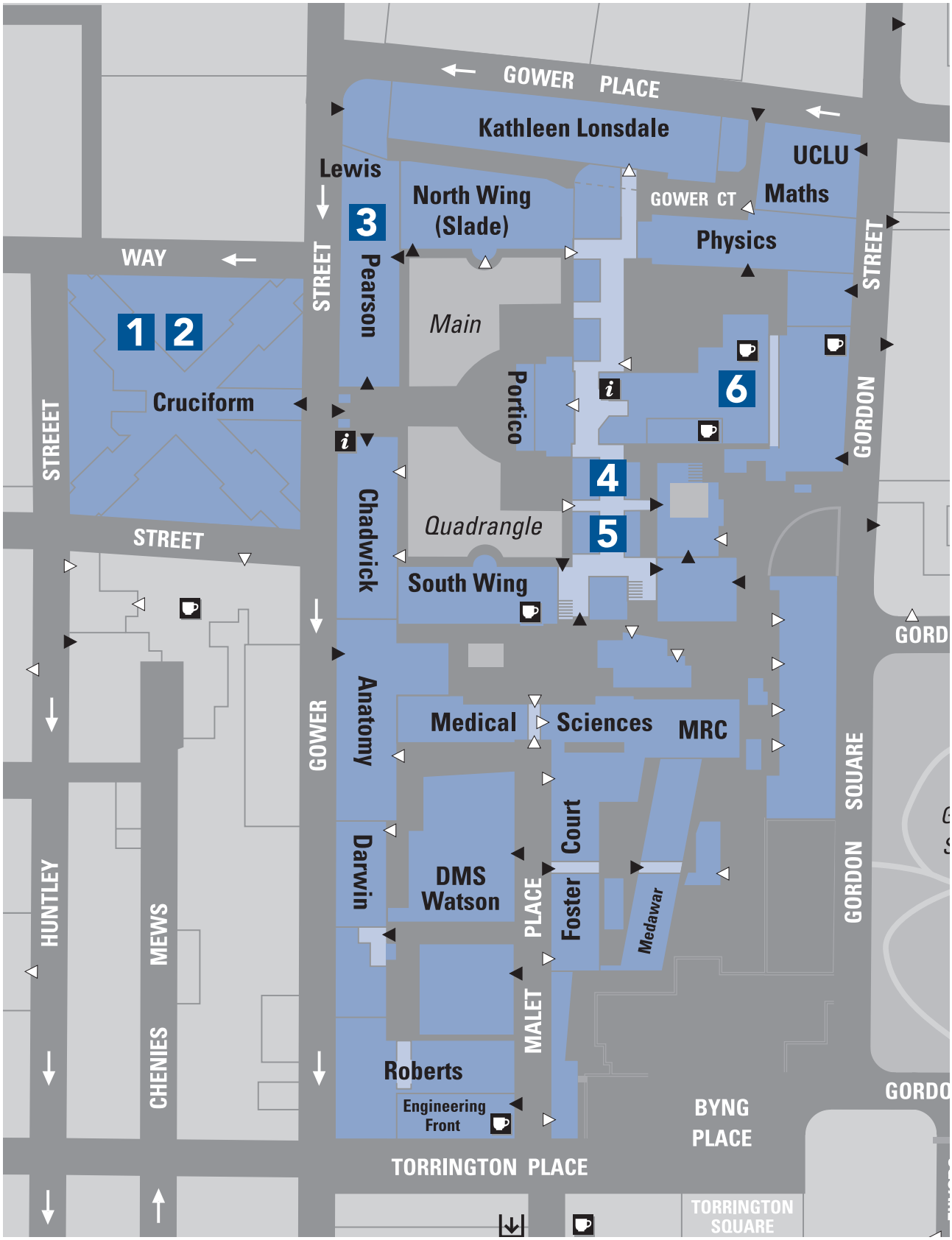
Light Bar Covent Garden (3am) – St Martins Lane Hotel, 45 St Martins Lane. Covent Garden.

KINGS CROSS

Ruby Lounge – 33 Caledonian Road. King's Cross.

The Big Chill House – 257-259 Pentonville Road. King's Cross.

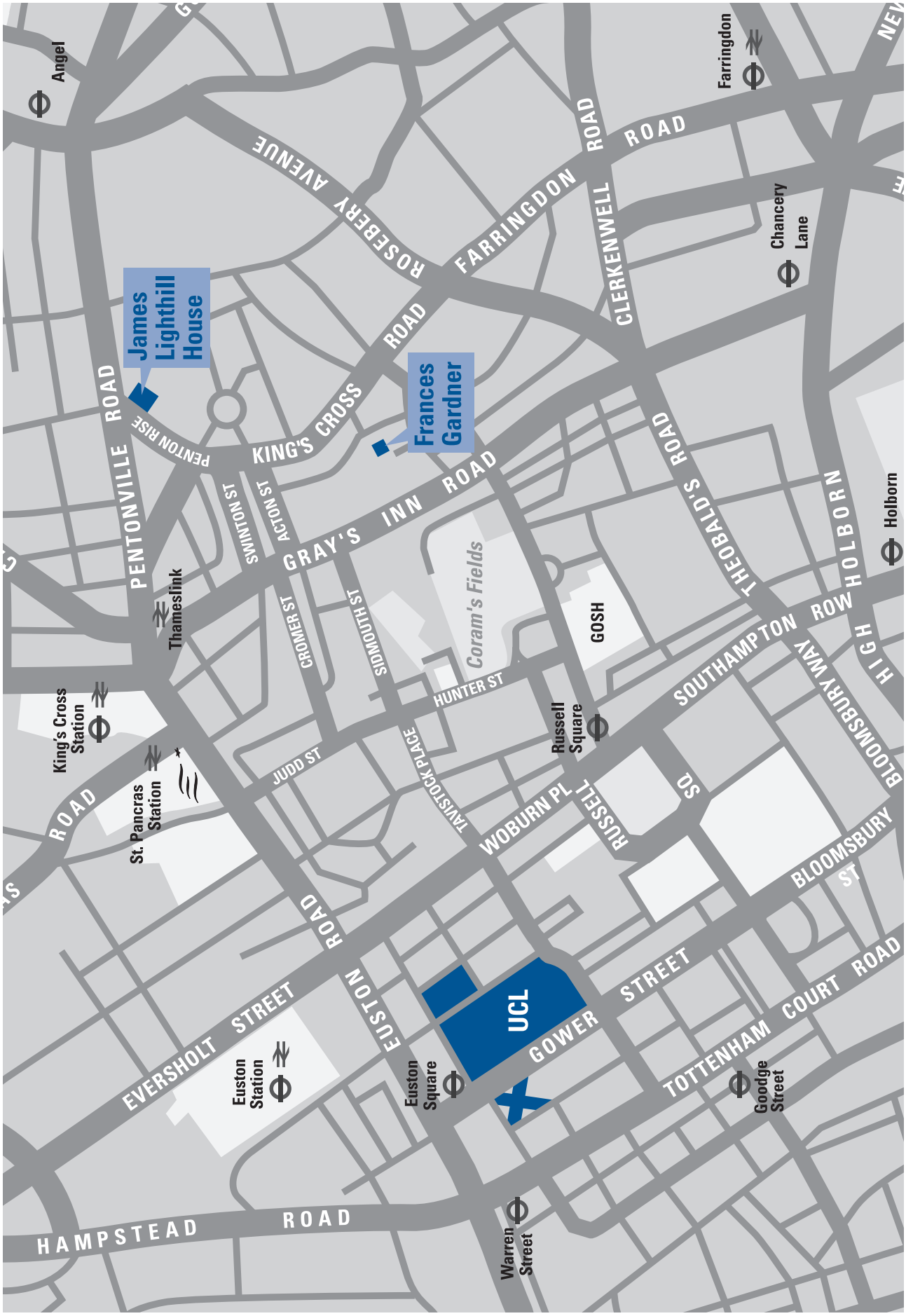
The Cross – 126 York Way. King's Cross.



KEY

1,2 Cruciform Lecture Theatres
3 Pearson Lecture Theatre
4 Registration
5,6 Exhibition, Posters and Food

ACCOMMODATION MAP



BONE
RESEARCH SOCIETY

www.brsoc.org.uk

Next Year's BRS Meeting

Held in association with the
European Calcified Tissue Society

Glasgow, 26-30 June 2010

British Society for
Matrix Biology

www.bsmb.ac.uk

Next Year's BSMB Meeting

Manchester, 29-30 March 2010