TABLE OF CONTENTS
Biennial Meeting of the
American Society for Matrix Biology

CO-SPONSORED BY

THE AMERICAN SOCIETY FOR MATRIX BIOLOGY

&

THE INTERNATIONAL SOCIETY FOR MATRIX BIOLOGY

ACKNOWLEDGEMENTS
Biennial Meeting of the
American Society for Matrix Biology

The American Society for Matrix Biology gratefully acknowledges the financial support of its sponsors:

Amira Pharmaceuticals

Baxter Bioscience

Elsevier

FibroGen

Genzyme

Genentech

Halozyme

IriSys Inc.

LifeCell

Lilly Research Laboratories

Millipore Corporation

National Institutes of Health (NIH)

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

New England BioLabs

Shriners Hospitals for Children

Thomas Jefferson University

Trevigen

OFFICERS AND COUNCIL
Biennial Meeting of the
American Society for Matrix Biology

OFFICERS
Renato Iozzo, President
Bill Parks, President Elect
Linda Sandell, Past-President
Joanne Murphy-Ullrich,
Secretary/Treasurer

SCIENTIFIC PROGRAM COMMITTEE
Bill Parks, Chair
Jamie Fitzgerald
Karen Lyons
Joanne Murphy-Ullrich
Ambra Pozzi
Ralph Sanderson
Marian Young
Peter Yurchenco
Donald R. Senger (SIGs)
Renato Iozzo

FUNDRAISING COMMITTEE
Renato Iozzo
Bill Parks
Roy Zent
Jeff Davidson
Karen Lyons
James Quigley, Local Chair

COUNCILORS
Sherrill Adams (2008)
Scott Argraves (2008)
Jeffrey Esko (2009)
Mark Ginsberg (2008)
Josephine Grima (2009)
Veronique Lefebvre (2009)
Karen Lyons (2009)
Alan Rapraeger (2008)
Bryan Toole (2009)
Tom Wight (2008)
Ken Yamada (2009)
Marian Young (2008)
Amy Bradshaw (2011)
Jean Schwarzbauer (2011)
Jeff Davidson (2011)
David McQuillan, ex officio

NEWSLETTER EDITORS
Marian Young
Ambra Pozzi

***MATRIX BIOLOGY* EDITOR**
Bjorn Olsen

EXECUTIVE DIRECTOR
Jennifer Holland

NATIONAL OFFICE
American Society for Matrix Biology
9650 Rockville Pike
Bethesda, MD 20814-3998
Voice: 301.634.7810
Fax: 301.634.7455
Email: asmb@faseb.org
Web: www.asmb.net

GENERAL INFORMATION
Biennial Meeting of the
American Society for Matrix Biology

Registration Hours

The meeting registration desk is located in the Randle Foyer of the Manchester Grand Hyatt, San Diego, CA. Name badges and conference materials can be picked up at this location. The desk will be staffed and available to provide assistance during the following hours.

Sunday, December 7 ..... 12:00 pm – 6:00 pm
Monday, December 8 ..... 7:30 am – 5:00 pm
Tuesday, December 9..... 7:30 am – 5:00 pm
Tuesday, December 9..... 7:30 am – 12:00 pm

Registration

Registration fees include continental breakfast each morning, Welcome Reception on Sunday night, refreshment breaks, Poster Session I/II Receptions, the Gala Reception on Tuesday evening, scientific sessions and one program book. Registration fees exclude hotel costs. On-site registration will be accepted. Checks and credit cards are accepted.

Accommodations

The Manchester Grand Hyatt boasts a spectacular waterfront location in downtown San Diego. It is ideally located; allowing you to explore Seaport Village, the Gaslamp Quarter, and numerous other San Diego attractions with its easy trolley access for downtown transportation. The hotel is located 3 miles from the San Diego International Airport with airport transfer rates around $10.
The hotel has blocked a group of rooms for our event at a discounted rate.
Individuals are responsible for making their own hotel reservations by contacting:

Manchester Grand Hyatt
One Market Place
San Diego, California 92101
USA

Website: www.manchestergrand.hyatt.com
ASMB Group Reservation URL:
http://manchestergrand.hyatt.com/groupbooking/sanrsasmb2008
Toll Free: 1-800-233-1234
Phone: 619-232-1234
Fax: 619-233-6464
Mention Group Code: "ASMB"
Group Rate: $199 plus tax for single and double occupancy.
Note: Reservations made after November 1, 2008 cannot be guaranteed for price or availability

Accompanying Persons

Guest tickets for the Tuesday evening gala to be held in the Randle Ballroom are available for purchase. Cost per ticket, available at the registration desk, is $50.

Meeting Objective/Target Audience

The American Society for Matrix Biology (ASMB) was founded in 2000 to promote and develop studies in the field of matrix biology and to publicize research. This discipline includes the structure and function of components of the extracellular matrix (ECM), the interactions of these components with cells, the consequences of these interactions for intracellular signaling mechanisms and gene expression, the orderly progression of these processes during embryonic and postnatal development, and the disruption of these processes in hereditary and acquired diseases of animals and humans.
The field of matrix biology is also relevant to many clinical disciplines including oncology, orthopedics, rheumatology, dermatology, and to the fields of bioengineering and tissue engineering.

The purpose of the ASMB Biennial National Meeting 2008 is to:

- Introduce new technologies for advancing the field of matrix biology
- Focus on new extracellular matrix research directions
- Maximize the exposure of new ideas and young scientists

Membership

All members are encouraged and invited to attend the annual Member Business Meeting on Monday, December 8th from 5-6 pm in Randle AB. The advice and guidance of the membership on current society issues and the journal are

welcome in this "open forum" meeting. If you are not currently a member, membership applications are available at the Registration Desk.

Special Needs

Registrants with special needs are invited to contact the Registration Desk for assistance. General information regarding the accessibility of San Diego can be found at **www.accesssandiego.com**.

ASMB-Sponsored Awards

Paul Bornstein, Founder's Award
Robert Mecham, Senior Investigator Award
Hiromi Yanagisawa, Junior Investigator Award
Awarded Onsite, Travel Awards

ISMB-Sponsored Awards

Reinhard Fässler,
Distinguished Investigator Award
Silvia Rossi, Jason Zoeller, Janice Vranka,
Travel Award Winners

Speakers

Presenters are asked to arrive in the session room at least 15 minutes prior to your start time to set-up. If you would like to practice your presentation prior to the day of presentation, please visit the speaker ready room in Gibbons during normal business hours. ASMB will provide laptops, LCD projectors/screens and microphones. If you require assistance loading your presentation, please contact one of the AV Technicians at the Registration Desk. Equipment is compatible with both Mac and MS products.

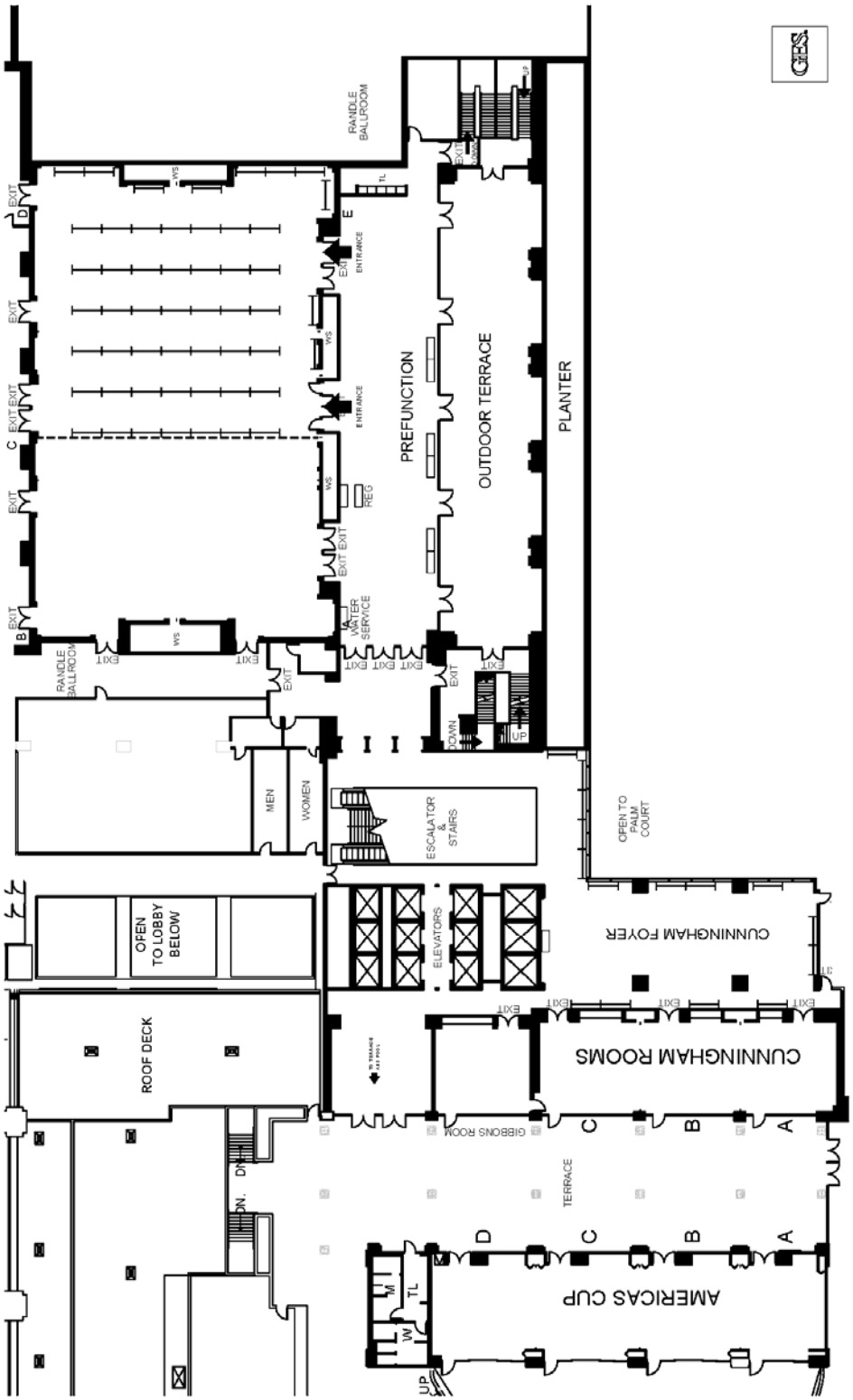
Cyber Café and Speaker Ready Room

A Cyber Café for all attendees and Speaker Ready Room is being provided by ASMB. These services are located in the Gibbons conference room and are available during regular business hours. Please be considerate of your fellow attendees while using these facilities.

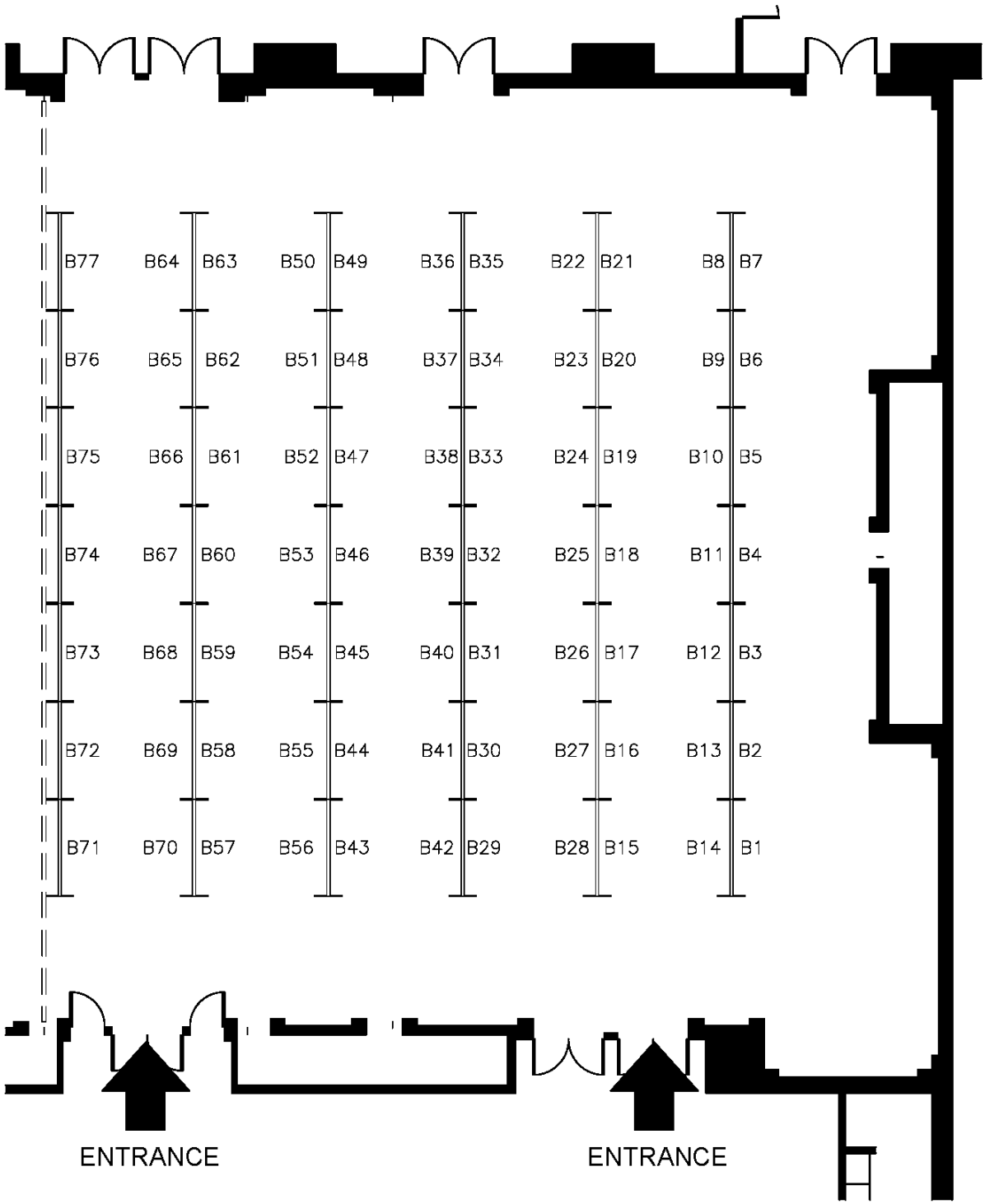
Poster Set-up

Poster boards will be set-up in Randle DE. Please post and remove your posters as described in your acceptance letters. Poster boards are available beginning at noon on Sunday, December 7th and will be removed by noon on Wednesday, December 10th. ASMB is not responsible for any materials posted. If you have a late-breaking abstract poster, please use your assigned board in the Cunningham Foyer.

MASTER FLOOR PLAN
Biennial Meeting of the
American Society for Matrix Biology



POSTER BOARD FLOOR PLAN
Biennial Meeting of the
American Society for Matrix Biology
Randle D/E



SCIENTIFIC PROGRAM
Biennial Meeting of the
American Society for Matrix Biology

◆

December 7-10, 2008
Manchester Grand Hyatt
San Diego, California
Organizers: **Renato Iozzo, Bill Parks**

(Note: First number denotes abstract number where applicable)

Sunday, December 7

12:00-6:00pm **Registration**
(Randle Foyer)

1:00-3:00 pm **Special Interest Groups Session I**

SIG 1: Extracellular Matrix-Driven Regulation of Innate Immune Response and Inflammation

(Cunningham A)
Organizers: Shukti Chakravarti and Liliana Schaefer

1 **Biglycan and Toll-like Receptor 2 Signaling**
Liliana Schaefer; Allgemeine Pharmakologie und Toxikologie, Frankfurt, Germany

2 **Lumican Mediated Neutrophil Migration**
Seakwoo Lee, Johns Hopkins University, Baltimore, MD

Syndecan1 Shedding: An Innate Immune Response to Infection
Pyong Woo Park, Childrens Hospital at Harvard Medical School, Boston, MA

Versican in Regulating Tumor Macrophages
Michael Karin, University of California, Los Angeles, CA

SIG 2: Integrins in Cancer

(Cunningham B)
Organizers: Filippo Giancotti and Valerie Weaver

Clonal Expansion and Adhesive Signaling
Mark Ginsberg, UCSD, San Diego, CA

Integrin alpha-4 Activation Promotes Tumor Inflammation, Angiogenesis, and Lymphangiogenesis
Judith Varner, UCSD, San Diego, CA

Distinct Connections of FAK to Cell Survival and Directional Motility
David Schlaepfer, UCSD, San Diego, CA

Regulation of ECM Remodelling and Angiogenesis by Homeobox Genes
Nancy Boudreau, UCSF, San Francisco, CA

SIG 3: Basement Membranes and the Kidney

(Cunningham C)
Organizer: Roy Zent

Laminins and Kidney Function
Jeff Miner, Washington University, Saint Louis, MO

3 **Role of gamma1-Laminins in Renal Collecting Ducts**
Peter Yurchenco, RWJMS, Piscataway, NJ

Asynchronous Laminin and Type IV Collagen Deposition in Developing Glomerular Basement Membranes
Dale Abrahamson, University of Kansas Medical Center, Kansas City, KS

Collagen IV and the Kidney
Billy Hudson, Vanderbilt Medical Center, Nashville, TN

3:00-3:30 pm **Coffee Break**
(Cunningham Foyer)

3:30-5:30 pm **Special Interest Groups Session II**

SIG 4: Matrix Remodeling in Cancer Invasion

(Cunningham A)
Organizers: Alissa Weaver and Andries Zijlstra

Introduction
Andries Zijlstra, Vanderbilt University, Nashville, TN

Invadopodia and Mechanotransduction
Alissa Weaver, Vanderbilt University, Nashville, TN

Anti-Podosome Therapy
Sara Courtneidge, Burnham Institute, La Jolla, CA

The Intersection of Planar Cell Polarity and ECM Remodeling During Collective Migration and Invasion
Jason Jessen, Vanderbilt University, Nashville, TN

Visualizing Human Tumor Cell Metastasis Using Transparent Zebrafish and High Resolution Intravital Microscopy
Konstantin Stoletov, UCSD, San Diego, CA

SIG 5: ECM Regulation of the Osteoblast-Lineage

(Cunningham B)
Organizers: Marian Young and Kurt Hankenson

Matrix Gla Proteins in Regulating Osteoblast Function
Raj Gopalakrishnan, University of Minnesota, Minneapolis, MN

Role of Thrombospondins in Regulating Osteoblast Progenitor Maintenance and Osteoblast Function
Andrea Alford, University of Michigan, Ann Arbor, MI

HtrA1: A Novel Matrix Modulator that Regulates Osteoblast Differentiation and Matrix Mineralization
Colette Inkson, University of Manchester, Manchester, UK

How Type III Collagen Influences Osteoblast Function through the Modulation of TGFbeta Signaling
Sherri Adams, University of Pennsylvania, Philadelphia, PA

Post-Transcriptional Processing of Osteonectin in the Regulation of Osteoblast Differentiation
Anne Delany, University of Connecticut, Farmington, CT

SIG 6: Heritable Diseases of Elastic Structures: The Paradigms of Pseudoxanthoma Elasticum (PXE) and Cutis Laxa (CL) Supported by PXE International and Cutis Laxa Patient Advocacy Organizations

(Cunningham C)
Organizers: Jouni Uitto and Sharon Terry

4 **The Role of Patient Advocacy Organizations in Genetic Research**
Sharon Terry, PXE International, Washington, DC

PXE: A Multi-System Metabolic Disease Affecting the Elastic Structures

5 **Molecular Genetics of PXE**
Jouni Uitto, Thomas Jefferson University, Philadelphia, PA

Elastin Aberrations in PXE
Olivier Le Saux, University of Hawaii, Manoa, Hawaii

CL: The Disease of Elastic Fibers

Elastin Mutations in Cutis Laxa
Zsolt Urban, Washington University, Saint Louis, MO

Fibulin in Diseases
Mon-Li Chu, Thomas Jefferson University, Philadelphia, PA

6:30-7:30 pm **Welcome Reception**
(Randle Foyer)

7:30-7:45 pm **President's Welcome *and* Founder's Award Presentation to Paul Bornstein**
(Randle ABC)
Renato Iozzo, Thomas Jefferson University, Philadelphia, PA

7:45-8:30 pm **Keynote Lecture: MicroRNAs, the Immune System and Cancer**
(Randle ABC)
Carlo Croce, The Ohio State University College of Medicine, Columbus, OH

Monday, December 8

7:30-8:30 am **Breakfast**
(Randle Foyer)

7:30 am-5:00pm **Registration**
(Randle Foyer)

7:30 am-7:00 pm **Exhibits**
(Randle Foyer)

8:30-10:00 am **Plenary I**
ECM in Stem Cell Niches
(Randle ABC)
Session Chair: *W. Scott Argraves*

6 **8:30 am** **Extracellular Matrix Control of Stem Cell Niches**
Marian Young, NIH/NIDCR, Bethesda, MD

7 **9:05 am** **Glycomics of Proteoglycan Biosynthesis in ESC Differentiation**
Robert Linhardt, Rensselaer Polytechnic Institute, Troy, NY

9:40 am **Synthetic Biomimetic Matrices that Promote Osteoblastic Differentiation of Mesenchymal Stem Cells**
Susan Bellis, University of Alabama at Birmingham, Birmingham, AL

10:00-10:30 am **Coffee Break**
(Randle Foyer)

10:30-12:00 pm **Plenary II**
Matrix Structure
(Randle ABC)
***Sponsored by:* Shriners Hospitals for Children**
Session Chair: *Linda Sandell*

10:30 am **Senior Investigator Awardee: Extracellular Matrix and Vascular Development**
Robert Mecham, Washington University School of Medicine, St. Louis, MO

11:05 am **Delayed Turnover of FGFR3: Pathogenetic Mechanism and Therapeutic Target in Achondroplasia**
William Horton, Shriner's Research Center, Portland, OR

213 **11:40 am Molecular based Structure and Ligation of the Collagen Fibril**
Joseph Orgel, Illinois Institute of Technology, Chicago, IL

12:00-1:30 pm **Lunch Break**
(On Your Own)

1:30-3:00 pm **Concurrent Sessions**

Concurrent A: Angiogenesis

(Randle ABC)
Session Chair: *James Quigley*

8 **1:30 pm Induction of Physiologic and Pathologic Angiogenesis by TIMP-free *pro*MMP-9 Derived Uniquely from Inflammatory Neutrophils**
James Quigley, Scripps Research Institute, La Jolla, CA

9 **2:00 pm Fibulin-5 Inhibits Integrin-Induced ROS Production**
Rolf Brekken, UT-Southwestern, Dallas, TX

10 **2:20 PM Angiogenesis and Intravasation of PC-3 Dissemination Variants**
Erin Conn, The Scripps Research Institute, La Jolla, CA

11 **2:40 PM Endorepellin evokes SHP1 activity in endothelial cells**
Alexander Nystrom, Thomas Jefferson University, Philadelphia, PA

Concurrent B: Proteoglycans

(Cunningham ABC)
Session Chair: *Jeff Esko*

12 **1:30 pm Hypertriglyceridemia caused by mutation of the basement membrane proteoglycan Type XVIII collagen**
Jeffrey Esko, University of California, San Diego, La Jolla, CA

13 **2:00 pm Syndecan-1 Shedding Facilitates Airway Re-epithelialization**
Peter Chen, Center for Lung Biology, University of Washington; Seattle, WA

14 **2:20 pm Synstatin, a Sdc1 peptide, blocks αvβ3-dependent angiogenesis**
DeannaLee Beauvais, University of Wisconsin-Madison, Madison, WI

15 **2:40 pm Decorin binds to and downregulates the MET receptor**
Silvia Goldoni, Thomas Jefferson University, Philadelphia, PA

Concurrent C: ECM Turnover

(America's Cup ABCD)
Session Chair: *Kenn Holmbeck*

16 **1:30 pm Biological Functions of Membrane-Type Matrix Metalloproteinases**
Kenn Holmbeck, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD

17 **2:00 pm Extra- and intracellular collagen degradation linked by uPARAP**
Daniel Madsen, Finsen Laboratory, Rigshospitalet, Denmark

18 **2:20 pm Joint Degeneration in Mice Lacking Adequate Perlecan Levels**
Kathryn Rodgers, University of Pennsylvania, Philadelphia, PA

19 **2:40 pm The role of caveolin-1 in pulmonary matrix remodeling**
Olivier Le Saux, University of Hawaii School of Medicine, Honolulu, HI

3:00-3:30 pm **Coffee Break**
(Randle/Cunningham Foyers)

3:30-5:00 pm **Concurrent Sessions**

Concurrent D: Matricellular Proteins

(Randle ABC)
Session Chair: *Josephine Adams*

20 **3:30 pm Retention of Thrombospondins in ECM, and views of TSP Evolution**
Josephine Adams, Lerner Research Institute, Cleveland Clinic, Cleveland, OH

21 **4:00 pm COMP-specific Ribozyme: A New Strategy for Gene Therapy**
Joseph Alcorn, University of Texas Medical School, Houston, TX

22 **4:20 pm Regulation of fibronectin matrix assembly by tenascin-C**
Wing To, Kennedy Institute of Rheumatology, Imperial College, London, UK

23 **4:40 pm DMP1 Isoforms Promote Differential Cell Attachment and Migration**
Zofia von Marschall, NIDCR, NIH, DHHS, Bethesda, MD

Concurrent E: Molecular & Computational Modeling

(Cunningham ABC)
Session Chair: *Arthur Lander*

3:30 pm Control challenges in tissue growth and patterning
Arthur Lander, University of California Irvine, Irvine, CA

24 **4:00 pm Modeling single and collective cell movements in 3D matrices**
Muhammad Zaman, The University of Texas at Austin, Austin, TX

25 **4:20 pm A Discrete Mathematical Model Simulates In Vitro Angiogenesis**
Scott Sibole, University of Utah, Salt Lake City, UT

26 **4:40 pm The glycosaminoglycan-binding domain of CXC-chemokines controls neutrophil migration into the lungs**
Charles Frevert, University of Washington Seattle, WA

Concurrent F: Inflammation

(America's Cup ABCD)
Session Chair: *Farrah Kheradmand*

3:30 pm Lung Inflammation: Old Concepts with Fresh Perspectives
Farrah Kheradmand, Baylor College of Medicine, Houston, TX

27 **4:00 pm EGF-like domains of stabilin-2 recognizes PS during phagocytosis**
Soyoun Kim, Kyungpook National University, Daegu, Korea

28 **4:20 pm Intact hyaluronan promotes maintenance of immune tolerance.**
Paul Bollyky, Benaroya Research Institute, Seattle, WA

29 **4:40 pm FN-α4β1 Interactions Regulate MMP-9 Expression in Liver IR Injury**
Sergio Duarte, Dumont - UCLA Transplant Center, Los Angeles, CA

5:00-6:00 pm **ASMB Business Meeting**
(Randle ABC)

5:00-7:00 pm **Poster Session I and Exhibitor Reception**
(4th Floor Foyers/Randle DE)

Topics:

Angiogenesis
Basement Membrane
Control of Gene Expression
Development
ECM-Cytokines Interactions
ECM Turnover
Invasion/Migration
Vascular Biology

Tuesday, December 9

7:30-8:30 am **Breakfast**
(Randle Foyer)

7:30 am-5:00pm **Registration**
(Randle Foyer)

7:30 am-7:00 pm **Exhibits**
(Randle Foyer)

8:30-10:00 am **Plenary III**

ECM Influences in Disease Pathogenesis

(Randle ABC)
Session Chair: *Joanne Murphy-Ullrich*

8:30 am **Junior Investigator Awardee: Fibulins: Implications in Vascular Development and Disease**
Hiromi Yanagisawa, UT Southwestern Medical Center at Dallas, Dallas, TX

8:50 am **Location Matters: Matrix Regulation of Lung Inflammation and Remodeling**
Paul Noble, Duke University, Durham, NC

105 **9:25 am** **The Bone Marrow Microenvironment in Neuroblastoma Progression**
Yves DeClerck, Children's Hospital Los Angeles, Los Angeles, CA

10:00-10:30 am **Coffee Break**
(Randle Foyer)

10:30-12:00 pm **Plenary IV**

International Society for Matrix Biology (ISMB) Guest Symposium

(Randle ABC)
Session Chair*: Reinhard Fässler*

Distinguished Investigator Awardee

10:30 am **Genetic Analysis of Integrin Signaling in Mice**
Reinhard Fässler, Max Planck Institute of Biochemistry, Martinsried, Germany

Travel Awardees

106 **11:00 am** **The proteoglycan metastatic signature of a cancer cell**
Silvia Rossi, University of Parma, Parma, Italy

107 **11:20 am** **A central role for decorin during vertebrate convergent extension**
Jason Zoeller, Thomas Jefferson University, Philadelphia, PA

108 **11:40 am** **Prolyl 3-hydroxylase 1 null mice have abnormal bones and tendons**
Janice Vranka, Shriners Hospitals for Children, Portland, OR

12:00-1:30 pm **Lunch Break**
(On Your Own)

1:30-3:00 pm **Concurrent Sessions**

Concurrent G: Proteolytic Pathways

(Randle ABC)
Session Chair: *Rama Khokha*

1:30 pm **TIMPs and Tissue Homeostasis**
Rama Khokha, Princess Margaret Hospital, Toronto, Ontario

109 **2:00 pm** **MT1-MMP, vascular guidance tunnels, and EC-pericyte tube assembly**
George Davis, University of Missouri School of Medicine, Columbia, MO

110 **2:20 pm** **Epilysin (MMP-28) functions in promoting epithelial cell survival**
Anne Manicone, University of Washington, Seattle, WA

111 **2:40 pm** **TGFβ+EGF promotes PAI-1 sensitive collagen gel lysis and invasion.**
Cynthia Wilkins, Albany Medical College, Albany, NY

Concurrent H: Development

(Cunningham ABC)
Session Chair: *Ray Keller*

1:30 pm **Signaling and Biomechanical Roles of Matrix During Early Xenopus Morphogenesis**
Ray Keller, University of Virginia, Charlottesville, VA

112 **2:00 pm** **Scleraxis is required for normal heart valve formation in vivo**
Joy Lincoln, University of Miami, Miami, FL

113 **2:20 pm** **Skeletal abnormalities in quad-KO mice (TSP1, 3, 5, and Col 9)**
Karen Posey, University of Texas Medical School at Houston, Houston, TX

114 **2:40 pm** **Dystroglycan is required for neural maintenance and guidance.**
Robert Johnson, Northwestern University, Chicago, IL

Concurrent I: Glycosaminoglycans

(America's Cup ABCD)
Session Chair: *Gordon Laurie*

1:30 pm **Heparanase Off/On Switch for Syndecan Core Protein-Mediated mTOR/NFAT Signaling**
Gordon Laurie, University of Virginia, Charlottesville, VA

115 **2:00 pm** **Proteoglycan desulfation regulates endochondral ossification**
Carmine Settembre, Columbia University, NY, NY

116 **2:20 pm** **Heparan sulfate function in limb synovial joint formation**
Maurizio Pacifici, Thomas Jefferson University, Philadelphia, PA

117 **2:40 pm** **Role of heparan sulfate in limb skeletal development**
Kazu Matsumoto, Burnham Institute for Medical Research, La Jolla, CA

3:00-3:30 pm **Coffee Break**
(Randle/Cunningham Foyers)

3:30-5:00 pm **Concurrent Sessions**

Concurrent J: Integrins

(Randle ABC)
Session Chair: *Dean Sheppard*

118 **3:30 pm** **Integrin-mediated activation of TGFβ**
Dean Sheppard, Department of Medicine, University of California, San Francisco, CA

119 **4:00 pm** **Merlin Blocks Tumorigenesis by Inhibiting a Nuclear E3 Ligase**
Filippo Giancotti, Memorial Sloan-Kettering Cancer Center, New York, NY

120 **4:20 pm** **Pyk2 FERM regulates p53 in the absence of FAK**
Nichol Miller, UCSD Moores Cancer Center, La Jolla, CA

121 **4:40 pm** **The alpha5beta1 integrin as mechanotransducer in chondrocytes**
Linda Kock, Eindhoven University of Technology, The Netherlands

Concurrent K: Basement Membrane

(Cunningham ABC)
Session Chair: *Matthew Hoffman*

3:30 pm **Basement Membrane Remodeling During Branching Morphogenesis: The Dynamic Interplay of Proteolysis and Proliferation**
Matthew Hoffman, NIH/NIDCR, Bethesda, MD

122 **4:00 pm** **Mice deficient in the ECM protein WARP have nerve defects**
Justin Allen, Children's Hospital Boston, Boston, MA

123 **4:20 pm A Laminin-Nidogen Chimera Facilitates Basement Membrane Assembly**
Stephanie Capizzi, Robert Wood Johnson Medical School, Piscataway, NJ

124 **4:40 pm Mutant COL4A1 triggers oxidative stress in a genetic model of AMD**
Yi-Chinn Weng, University of California, San Francisco, San Francisco, CA

Concurrent L: Vascular Biology
(America's Cup ABCD)
Session Chair: *Anne Canfield*

125 **3:30 pm Molecular regulation of vascular calcification**
Ann Canfield, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

126 **4:00 pm MAPKp38 determines Smad2/3 signaling in the fbn1 null mouse aorta**
Luca Carta, Mount Sinai School of Medicine, New York, NY

127 **4:20 pm C-terminal Domain Modification in Mature Elastin**
Thomas Broekelmann, Washington University School of Medicine, Saint Louis, MO

128 **4:40 pm Granzyme B and Perforin in atherosclerosis and skin pathologies**
Wendy Boivin, University of British Columbia, Vancouver, BC, Canada

5:00-7:00 pm **Poster Session II and Exhibitor Reception**
(4th Floor Foyers/Randle DE)

Topics
Glycosaminoglycans
Inflammation
Integrins
Matricellular Proteins
Matrix Protein Biology
Proteoglycans
Proteolytic Pathways

7:00-10:00 pm **Gala Reception**
(Randle ABC)

Wednesday, December 10

7:30-8:30 am **Breakfast**
(Randle Foyer)

7:30-12:00pm **Registration**
(Randle Foyer)

7:30-12:00 pm **Exhibits**
(Randle Foyer)

8:30-10:00 am **Plenary V**

Mechanical Influences
(Randle ABC)
Session Chair: *Pyong Park*

203 **8:30 am Matrix Elasticity Directs Differentiation & De-Differentiation**
Dennis Discher, University of Pennsylvania, Philadelphia, PA

9:05 am Integration of Actin Dynamics and Adhesion in Cell Migration
Clare Waterman-Storer, NIH, Bethesda, MD

9:40 am Matrix Cross Linking, Mechanotransduction and Tumor Progression
Valerie Weaver, UCSF, San Francisco, CA

10:00-10:30 am **Coffee Break**
(Randle Foyer)

10:30-12:00 pm **Concurrent Sessions**

Concurrent M: Invasion/Migration
(Randle ABC)
Session Chair: *Alex Strongin*

10:30 am Biochemistry of Cell Migration Magic
Alex Strongin, Burnham Institute for Medical Research, La Jolla, CA

93 **11:00 am Novel role of Cdk4 in leukocyte adhesion and trafficking**
Lynn Schnapp, University of Washington, Seattle, WA

205 **11:20 am Cell polarity regulated by a FAK-p120RasGAP-p190RhoGAP complex**
Alok Tomar, University of California, San Diego, La Jolla, CA

206 **11:40 am SPARC is required for collective cell migration during oogenesis**
Nathalie Martinek, Peter MacCallum Cancer Centre, East Melbourne, Australia

Concurrent N: Control of Gene Expression

(Cunningham ABC)
Session Chair: *Michael Granato*

10:30 am Pre and Postsynaptic Development in Zebrafish
Michael Granato, University of Pennsylvania, Philadelphia, PA

207 **11:00 am The response of articular chondrocyte microRNAs to stress**
Gary Gibson, Henry Ford Hospital, Detroit, MI

208 **11:20 am Factors affecting splicing of scleroderma-associated LH2 mRNA**
Seth Puneet, Duke University Medical Center, Durham, NC

209 **11:40 am Postnatal Ablation of Sox9 in Mouse Cartilage**
Stephen Henry, University of Texas MD, Houston, TX

Concurrent O: ECM-Cytokines Interactions

(America's Cup ABCD)
Session Chair: *Sarah Dallas*

10:30 am LTBP1-Fibronectin Interactions in Regulation of Transforming Growth Factors
Sarah Dallas, University of Missouri, Kansas City, MO

210 **11:00 am TGFβ1 Enhances Pancreatic Tumor Progression in SPARC-Null Mice**
Shanna Arnold, UT-Southwestern, Dallas, TX

211 **11:20 am Extracellular regulators of TGFβ signaling in bone.**
Harikiran Nistala, Mt Sinai School of Medicine, New York, NY

212 **11:40 am Collagen X is required for proper hematopoietic development**
Elizabeth Sweeney, University of Pennsylvania, Philadelphia, PA

POSTER PRESENTATIONS
Biennial Meeting of the
American Society for Matrix Biology

(NOTE: The first number is the abstract number used to locate the abstract later in this program book. The second number is the board number used to locate the Poster in Randle DE)

Poster Session I
Monday, December 8th
5:00-7:00pm

Angiogenesis

30 B1 **Influences on the Angiogenic Response in ECM-Based Biomaterials**
Amy M. Overby, Chad E. Johnson, Research Department, Cook Biotech, Inc., West Lafayette, IN

31 B2 **TGFBIp/ βig-h3, an Endogenous Anti-angiogenic Molecule**
Hye-Nam Son, Ju-Ock Nam, In-San Kim, Cell and Matrix Research Institute, Department of Biochemistry and Cell Biology, Kyungpook National Uni. School of Medicine, Daegu, Korea

32 B3 **Histidine-rich Glycoprotein Modulation of Vasculostatin**
Philip A. Klenotic, Maria Febbraio, Roy L. Silverstein, Erwin G. VanMeir, Department of Cell Biology, Cleveland Clinic, Cleveland Ohio; Emory University, Atlanta, Georgia

33 B4 **Chondrostatin Inhibits Angiogenesis**
Zhepeng Wang, Jennifer Bryan, Brian J. Ell, Allan C. Rapraeger, Linda Sandell, Washington University; University of Wisconsin, Madison, WI

34 B5 **Small molecule inhibitors of collagen-induced angiogenesis**
Kari Habursky, Renato V. Iozzo, Kevin Turner, Michelle Burrows, Sung-wook Choi, Soni Basra, Joel S. Bennett, William F. DeGrado, James D. San Antonio, Dept Med, Thom Jeff U, Phil, PA; Dept Path, Anat, Cell Biol, Thom Jeff U, Phil, PA; Depts Chem, Biochem & Biophys, U PA, Phil, PA; Dept Med, U PA, Phila., PA

35 B6 **MMP14 and TIMP regulation of angiogenesis in aortic ring cultures**
Alfred C. Aplin, Eric Fogel, Roberto F. Nicosia, Department of Pathology, University of Washington, Seattle, WA; VA Medical Center, Seattle, WA

Basement Membrane

36 B7 **ECM-ligand functionalized fibrillar peptides**
Jangwook P. Jung, Joel H. Collier, Department of Surgery, University of Chicago, Chicago, IL

37 B8 **Regulation of caspase-3 mediated apoptosis and tumor angiogenesis**
Chandra S. Boosani, Dominic Cosgrove, Akulapalli Sudhakar, Cell Signaling and Tumor Angiogenesis Laboratory, Department of Genetics, Boys Town National Research Hospital, Omaha, NE

Control of Gene Expression

38 B9 **Ras up-regulates COL1A2 transcription through Smad2/3**
Silvia Smaldone, Magdalini Kypriotou, Francesco Ramirez, Department of Pharmacology, Mount Sinai School of Medicine, NY, NY

39 B10 **CDP/cux is capable of strong inhibition of the COL1A2 gene**
Maria Fragiadaki, Tetsuro Ikeda, David Abraham, George Bou-Gharios, Department of Medicine, Imperial College London, Hammersmith Campus, London; Centre for Rheumatology, University College,

Royal free campus, London; The Kennedy Institute of Rheumatology, Imperial College London, London, UK

40 B11 **Key Cis-acting sequences that regulate the Human Aggrecan Gene**
Andrea N. Layyous, Jerry Saklatvala, George Bou Gharios, Kennedy Institute of Rheumatology, Imperial College London, London, UK

41 B12 **Articular cartilage gene expression during MSC differentiation**
Deepa Edwin, Li Liang, Masahiro Iwamoto, Audrey McAlinden, Washington University, St Louis, MO; Thomas Jefferson University, Philadelphia, PA

42 B13 **The role of fibronectin synergy site in regulating alveolar EMT.**
Ashley E. Carson, Thomas H. Barker, Department of Biomedical Engineering at Georgia Tech and Emory University, Atlanta, GA

43 B14 **Changes in ECM in High/Low/Normal Oxygen in 3D Cultures**
Emmett Pinney, Frank Zeigler, Gail Naughton, Amy D. Bradshaw, Robert Kellar, Histogen Inc., San Diego, CA; Dept. of Med., MUSC, Charleston, SC

44 B15 **Global TNF-alpha regulated gene expression in chondrocytes**
Jason S. Rockel, Suzanne M. Bernier, Andrew Leask, CIHR group in Skeletal Development and Remodeling, Uni. of Western Ontario, London, ON, CA

45 B16 **The Protein Arginine Methyltransferase (PRMT5) Associates with Class II Transactivator (CIITA) to Repress Collagen Transcription**
Larry Luchsinger, B.D. Smith, Department of Biochemistry, Boston University School of Medicine, Boston, MA

Development

46 B17 **Modulation of Cell Adhesion and Migration by Bcl-2**
Christine M. Sorenson, Nader Sheibani, University of Wisconsin School of Medicine and Public Health, Madison, WI

47 B18 **Integrin-laminin interaction in Kidney Papillae Development**
Tetyana L. Vasylyeva, Qin Shan, Yingjie Liu, Charles Szekeres, Mary Taglienti, Jeffrey H. Miner, Jordan A. Kreidberg, Department of Medicine, Division of Nephrology, Children's Hospital Boston, Harvard Medical School, Boston, MA; Renal Division, Washington University School of Medicine, St. Louis, MO

48 B19 **Matrilin-1 in the vertebral column of salmon with deformities**
Mona E. Pedersen, Eva Veiseth, Harald Takle, Elisabeth Ytteborg, Grete Baeverfjord, Grethe Enersen, Kirsten O. Hannesson, Nofima Food Matforsk AS, Norway; Nofima Marine, Norway; Nofima Marine, Sunndalsøra, Norway

49 B20 **Temporal and spatial expression of collagens in heart valve ECM.**
Jacqueline D. Peacock, Joy Lincoln, Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL

50 B21 **Col IV NC1-induced disruption of ECM Alters Zebrafish Development**
Robert V. Intine, Billy G. Hudson, Michael P. Sarras Jr, Scholl College of Podiatric Med., Rosalind Franklin Univ., N. Chicago, IL; Dept of Med., Vanderbilt Univ., Nashville, TN; Dept of Cell Biol. and Anat.; Rosalind Franklin Univ., N. Chicago, IL

51 B22 **Evidences of osteoarthritis of the TMJ in Dmm and sedc mice**
Melissa Ricks, William Hale, Daniel Faulk, David Robinson, David W. Holt, Robert E. Seegmiller, Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT

52 B23 **Sedc Mutant Mice as a Model for Osteoarthritis (OA)**
S. A. Avery, M Baker, D. W. Holt, D Robinson, R. E. Seegmiller, Department of Physiology and Developmental Bio, Brigham Young Uni., Provo, UT

53 B24 **High Carrier Frequency for Recessive OI in West Africans**
Wayne A. Cabral, Aileen M. Barnes, Charles N. Rotimi, Lawrence Brody, Joan Bailey-Wilson, Susan R. Panny, David Chitayat, Forbes D. Porter, Joan C. Marini, BEMB, NICHD, NIH, Bethesda, MD; NHGRI, NIH, Bethesda, MD; Office of Genetics, Maryland DHMH, Baltimore, MD; Hosp for Sick Children, Toronto, Ontario, CA; HDB, NICHD, NIH, Bethesda, MD

54 B25 **Oxytalan fibers in the teleostean tooth and pedestal bone**
Keitaro Isokawa, Maki Yuguchi, Hideo Nagai, Department of Anatomy, Nihon Uni. School of Dentistry, Tokyo, Japan

55 B26 **Elastin Haploinsufficiency Results in Latent Aortic Valve Disease**
Robert B. Hinton, Jennifer Adelman-Brown, Varun Krishnamurthy, Hanna Osinska, Robert P. Mecham, Daria Narmoneva, D. Woodrow Benson, Division of Cardiology, Cincinnati Children's Hospital; Department of Biomedical Engineering, University of Cincinnati; Department of Cell Biology, Washington University, Cincinnati, OH

56 B27 **The role of Has2 on long bone development**
Peter J. Roughley, Judy Grover, Eunice R. Lee, Yu Yamaguchi, Shriners Hospital for Children, Montreal, QC; The Burnham Institute, La Jolla, CA

57 B28 **The role of NFATc1 in epicardial derived cells (EPDCs)**
Michelle D. Combs, Caitlin M. Braitsch, Katherine E. Yutzey, Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

58 B29 **Dose-Range Developmental Toxicity of rHuPH20 in Mice**
Sinisa Nadjsombati, Walter H. Bee, Deanna Newcomb, Monica L. Zepeda, Allan Hoberman, Gregory I. Frost, Halozyme Therapeutics, Inc, San Diego, CA; Charles River Laboratories, Horsham, PA

59 B30 **Genetic and functional studies of CTGF in bone development**
Bau-Lin Huang, Karen M. Lyons, Department of Oral Biology; Department of Molecular, Cell and Developmental Biology; Department of Orthopaedic Surgery, University of California, Los Angeles, CA

60 B31 **Characterization of Drole, Drosophila type XV/XVIII collagen**
Ryusuke Momota, Ichiro Naito, Yoshifumi Ninomiya, Aiji Ohtsuka, Department of Human Morphology; Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

61 B32 **Abnormal Lipid Homeostasis in Chondrocytes of S1P^{cko} Mice**
Debabrata Patra, Xiaoyun Xing, Jennifer Bryan, Guosheng Liang, Linda Sandell, Washington University, St. Louis, MO; UT Southwestern Medical Center, Dallas, TX

62 B33 **Impact of Exercise on Skeletal Muscle and Bone in *oim* Mice**
S Carleton, B Weber, A McCambridge, J Ferriera, MB Brown, C Phillips, Dept of Biochemistry/Child Health; Veterinary Pathobiology, Univ of Missouri, Columbia, MO

63 B34 **Insights into the function of SOX9 and campomelic dysplasia**
Kathryn S. Cheah, Department of Biochemistry, University of Hong Kong, Hong Kong

64 B35 **Expression of ADAMTS-9 in mouse growth plate cartilage**
Kanae Kumagishi, Keiichiro Nishida, Ryusuke Momota, Yuichiro Yamaai, Satoshi Hirohata, Kadir Demircan, Ichiro Naito, Yoshifumi Ninomiya, Aiji Ohtsuka, Department of Human Morphology; Department of Oral Function and Anatomy; Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

65 B36 **ADAMTSL-4 improves microfibril of Marfan syndrome derived cells.**
Masahiro Saito, Tomoko Wada, Ko Tsutsui, Naoto Suda, Ganburged Ganjargal, Kiyotoshi Sekiguchi, Toshiyuki Yoneda, Dept Mol Cell Biochem, Osaka Univ Grad Sch Dent, Osaka,Japan; Inst for Protein Res, Osaka Univ, Osaka,Japan; Maxillo. Ortho,Tokyo Medi and Dent Univ, Tokyo, Japan

66 B37 **TRIP11 is essential for skeletal development**
Patrick Smits, Andrew Bolton, Minh Hong, Lei Lu, Andrea Superti-Furga, Shiro Ikegawa, Daniel Cohn, Tom Kirchhausen, Matthew Warman, Dave Beier, Dept. Orthopaedic Surgery, Children's Hospital, Boston; Dept. Genetics, Brigham and Women's Hospital, Boston; Dept. Cell Biology, Harvard Medical Institute, Boston; Cedars-Sinai Medical Center, Los Angeles; RIKEN, Tokyo, Japan; Dept. Pediatrics, University of Freiburg, Germany

67 B38 **Abnormal differentiation of cardiac valves in Ltbp-1L null mice**
Vesna Todorovic, Erin Finnegan, Daniel Rifkin, Cell Biology Department, NYU Medical Center, New York, NY

68 B39 **ADAMTS proteases regulate BMP-mediated cell death**
Daniel R. McCulloch, Laura Collins, Courtney M. Nelson, Takako Sasaki, Marion A. Cooley, W. Scott Argraves, Suneel S. Apte, Department of Biomedical Engineering, Cleveland Clinic, Cleveland, OH; Shriners Hospital for Children, Portland, OR; Medical University of South Carolina, Charleston, SC

69 B40 **Autofluorescence in Multiple Tissues During Mouse Embryogenesis**
Xuehong Xu, Bruce E. Vogel, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, MD

ECM-Cytokines Interactions

70 B41 **LTBP-4 Function as a Modulator of TGF-β and Elastogenesis in Lung**
Branka Dabovic, Yan Chen, Jiwon Choi, Elaine Davis, Harry Dietz, Francesco Ramirez, Harold von Melchner, Daniel Rifkin, NYU School of Medicine, New York, NY; McGill University, Montreal, Canada; Johns Hopkins University , Baltimore, MD; Mount Sinai School of Medicine, New York, NY; University of Frankfurt Medical School, Frankfurt, Germany

71 B42 **Regulation of BMP Signaling By Fibrillin Microfibrils**
Gerhard Sengle, Valerie M. Carlberg, Noe L. Charbonneau, Sara F. Tufa, Douglas R. Keene, Francesco Ramirez, Lynn Y. Sakai, Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, OR; Shriners Hospital for Children, Portland, OR; Department of Pharmacology and Systems Therapeutics, Mt. Sinai School of Medicine, NY, NY

72 B43 **The Secretome of Keratocytes is Growth Factor Dependent**
LaTia Etheredge, Bradley Kane, John R. Hassell, Departments of Pathology & Cell Biology; Molecular Medicine, University of South Florida, Tampa, FL

73 B44 **LOX-PP inhibits FGF-2 signaling and DNA synthesis in osteoblasts**
Siddharth R. Vora, Philip C. Trackman, Boston University School of Dental Medicine, Boston, MA

74 B45 **Cochlea hypoxia in COL4a3 mice involves cytokine upregulation**
Lara Dunn, Marisa Zallocchi, Dominic Cosgrove, Michael Anne Gratton, University of Pennsylvania; BoysTown National Research Hospital, Philadelphia, PA

ECM Turnover

75 B46 **Fibrous tissues remodel to attain a preferred mechanical state**
Jasper Foolen, Corrinus C. van Donkelaar, Rik Huiskes, Keita Ito, Department of Biomedical Engineering, Eindhoven University of Technology, Netherlands

76 B47 **Does TIMP-3 protect articular cartilage in OA model**
Hiroyuki Nakamura, Phuong Vo, Ngee Han Lim, Ke Liu, Hideaki Nagase, George Bou-Gharios, The Kennedy Institute of Rheumatology, Imperial College of London, London, UK

77 B48 **A Type I Collagen Matrikine Can Drive Breast Cancer Osteolysis**
William Wu, Anna Drachuck, Izabela Podgorski, Bone and Joint Center, Henry Ford Hosptial, Detroit, MI; Department of Pharmacology, Wayne State University, School of Medicine, Detroit, MI

78 B49 **Matrix Remodeling in Fibroblast-Seeded Fibrin Gels**
Edward A. Sander, Sandy L. Johnson, Victor H. Barocas, Robert T. Tranquillo, Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

79 B50 **Calreticulin plays a role in collagen regulation**
Lauren B. Van Duyn, Mariya T. Sweetwyne, Joanne E. Murphy-Ullrich, Departments of Pathology and Cell Biology, University of Alabama at Birmingham, Birmingham, AL

80 B51 **Antibodies for targeting of collagenolytic processes**
Lars H. Engelholm, Daniel H. Madsen, Signe Ingvarsen, Henrik J. Jürgensen, Kenn Holmbeck, Niels Behrendt, Finsen Laboratory, Rigshospitalet, Copenhagen Biocenter, Copenhagen, Denmark; Craniofacial and Skeletal Diseases Branch, NIDCR, NIH, DHHS, Bethesda, MD

81 B52 **Type I collagen homotrimers may alter tissue remodeling**
Sejin Han, Elena Makareeva, Daniel J. McBride, Charlotte L. Phillips, Ulrike Schwarze, James M. Pace, Peter H. Byers, Robert Visse, Hideaki Nagase, Sergey Leikin, NICHD, National Institutes of Health, Bethesda, MD; University of Maryland School of Medicine; University of Missouri; University of Washington; Kennedy Institute of Rheumatology, Imperial College London, UK

82 B53 **Monitoring matrix turnover in fibrin-based tissue constructs**
Justin S. Weinbaum, Sandra L. Johnson, Katherine A. Ahmann, Robert T. Tranquillo, Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

83 B54 **Collagen Glomerulopathy and MMP Expression in oim Mouse Kidney**
Anna M. Roberts, Matthew H. Myles, Andrew Walker, Craig Franklin, Charlotte L. Phillips, Dept. of Biochemistry/Child Health, University

of Missouri-Columbia; Dept. of Veterinary Pathobiology, University of Missouri-Columbia, Columbia, MO

84 B55 **TAK1 regulates extracellular matrix remodelling by fibroblasts**
Xu Shiwen, Sunil Parapuram, Daphne Pala, David E. Carter, Chris P. Denton, David J. Abraham, Andrew Leask, Centre for Rheumatology, University College London (Royal Free Campus), London, UK; CIHR Group in Skeletal Development and Remodeling, Dental Sciences Building, University of Western Ontario, London ON, Canada; London Regional Genomics Centre, London, ON, Canada

85 B56 **Quantitative Proteomic Analysis of Hypertrophied Rat Myocardium.**
Amy D. Bradshaw, Catalin F. Baicu, John H. Schwacke, Kentaro Yamane, Tyler J. Rentz, John M. Lacy, Thomas N. Gallien, Kevin L. Schey, Michael R. Zile, Depts. of Medicine; Biostatistics, Bioinformatics & Epidemiology; Pharmacology, Medical University of South Carolina, Ralph H. Johnson V.A. Medical Center, Charleston, SC

86 B57 **p190RhoGEF (Rgnef) and FAK Promote Colorectal Cancer Invasiveness**
Hong-Gang Yu, Ju-Ock Nam, Lei Shi, Xiao Lei Chen, Alok Tomar, Ssang-Taek Lim, David D. Schlaepfer, Department of Reproductive Medicine, UCSD Moores Cancer Center, La Jolla, CA

87 B58 **Evidence for articular cartilage regeneration in MRL/MpJ mice**
Jamie Fitzgerald, Cathleen Rich, Dan Burkhardt, Justin Allen, Andrea S. Herzka, Christopher B. Little, Oregon Health and Science University, Portland, Oregon; Raymond Purves Bone and Joint Research Laboratories, Kolling Institute of Medical Research, University of Sydney at The Royal North Shore Hospital, Sydney, Australia; University of Melbourne and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia

88 B59 **Dissecting a fibronectin matrix assembly domain using FRET**
Jean E. Schwarzbauer, Nancy W. Karuri, Zong Lin, Hays S. Rye, Dept. of Molecular Biology, Princeton University, Princeton, NJ

89 B60 **Woundermatology: Analyzing ECM fragments in pressure ulcer wound**
Zenzo Isogai, Yusuke Murasawa, Koji Mizuno, Ken Watanabe, Masahiko Yoneda, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan; Hoshi University School of Pharmacy, Tokyo, Japan; Aichi Prefectural College of Nursing and Health, Nagoya, Aichi, Japan

Invasion/Migration

90 B61 **MIG-17/ADAMTS controls cell migration by recruiting nidogen**
Yukihiko Kubota, Kiyotaka Ohkura, Katsuyuki K. Tamai, Kayo Nagata, Kiyoji Nishiwaki, RIKEN CDB, Kobe, Japan; Department of Bioscience, Kwansei-Gakuin University, Sanda, Japan

91 B62 **TGF-β3 induction of BMSC induces bone formation *in vivo***
Jason T. Rich, Jennifer Bryan, Audrey McAlinden, Washington University, St. Louis, MO

92 B63 **A composite role of vitronectin and uPAR in cell morphology**
Thore Hillig, Signe Ingvarsen, Daniel H. Madsen, Henrik Gaardsvoll, Michael Ploug, Keld Dano, Lars Kjoller, Niels Behrendt, Lars H. Engelholm, The Finsen Lab, Copenhagen, Denmark; The Bartholin Institute, Copenhagen, Denmark

93 B64 **Novel role of Cdk4 in leukocyte adhesion and trafficking**
Lynn M. Schnapp, Li Liu, Barbara Schwartz, Yu-Hua Chow, Yoshiaki Tsubota, Elaine W. Raines, John M. Harlan, Dept of Med.; Dept of Pathology, Univ of Washington, Seattle, WA

94 B65 **Tumor Typing by Proteomic Analysis of Formalin Fixed Sample ECM**
Timothy M. Ritty, Elizabeth Frauenhoffer, Edward J. Fox, Bruce Stanley, Orthopaedics, PSU Cancer Institute; Pathology; Proteomics Facility; Penn State College of Medicine, Hershey, PA

Vascular Biology

95 B66 **Thrombospondin-1 Regulates Blood Pressure and Cardiac Response**
Jeff S. Isenberg, Yan Qin, Daryl Despres, Russell W. Bandle, Jurgen Schnermann, William A. Frazier, David D. Roberts, Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, MD; Kidney Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD; Mouse Imaging Facility, National Institutes of Health, Bethesda, MD; Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO

96 B67 **Cardiovascular mechanics in newborn ELN+/+, +/- and -/- mice**
Jessica E. Wagenseil, Attila Kovacs, Robert P. Mecham, Department of Cell Biology and Physiology, Washington University, St. Louis, MO; Department of Internal Medicine, Washington University, St. Louis, MO

97 B68 **HtrA1 - a Serine Protease that Regulates Vascular Calcification**
Colette A. Inkson, Kristen D. Hadfield, Fiona L. Wilkinson, Gillian A. Wallis, Ann E. Canfield, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

98 B69 **Vascular specific gene expression using the PDGFR-beta Promoter**
Renee J. LeClair, Qiaozeng Wang, Lucy Liaw, Volkhard Lindner, Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME

99 B70 **Fbln-2 and fbln-5 cooperate to assemble and maintain the IEL**
Shelby L. Chapman, F-X Sicot, Elaine C. Davis, Takako Sasaki, Mon-Li Chu, Hiromi Yanagisawa, Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX; Department of Dermatology and Cutaneous Biology, Thomas Jefferson University,Philadelphia, PA; Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada; Shriners Hospital for Children Research Center, Portland, OR

100 B71 **TG2, the missing link in the development of vascular stiffness ?**
Simon Moreau, Nicolas Chabot, Jeffrey Wayne Keillor, Pierre Moreau, University of Montreal, Faculty of Pharmacy; University of Montreal, Department of Chemistry, Montreal, QC, Canada

101 B72 **ECMPs act as centers of MMP activation and matrix remodeling.**
Thomas P. Lozito, Cassie M. White, Catherine K. Kuo, Juan M. Taboas, CBOB, NIAMS, NIH; Clemson University, Bethesda, MD

102 B73 **MSCs create a TIMP-rich, matrix-protective local environment.**
Thomas P. Lozito, Cassie M. White, Catherine K. Kuo, Juan M. Taboas, Rocky S. Tuan, CBOB, NIAMS, NIH; Clemson University, Bethesda, MD

103 B74 **MAPK/SMAD signaling in fibrin-based constructs grown in vitro**
Justin S. Weinbaum, Zeeshan H. Syedain, Robert T. Tranquillo, Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

104 B75 **GGCX and ABCC6 Gene Mutations in a Family with PXE-like Phenotype**
Qiaoli Li, Dorothy K. Grange, Nicole L. Armstrong, M. Yadira Hurley, Mark Rishavy, Kevin Hallgren, Kathleen L. Berkner, Leon J. Schurgers, Qiujie Jiang, Jouni Uitto, Jefferson Medical College, Philadelphia, PA; Washington University, St. Louis, MO; St. Louis University, St. Louis,MO; Case Western Reserve University, Cleveland, OH; Cardiovascular Research Institute and VitaK BV, University of Maastricht, Maastricht, The Netherlands

Poster Session II
Tuesday, December 9th
5:00-7:00pm

Glycosaminoglycans

129 B1 **Hyaluronan Role During Muscular and Craniofacial Development**
Paola Casini, Roberto Perris, Irma Nardi, Michela Ori, Department of Biology, University of Pisa, Pisa, Italy; Department of Genetics, Microbiology and Anthropology, Univerisity of Parma, Parma, Italy

130 B2 **Intrinsic aging/photoaging-dependent changes of GAG in human skin**
Jang-Hee Oh, Yeon Kyung Kim, Jeong-Eun Shin, Mi Hee Shin, Kyu Han Kim, Kwang Hyun Cho, Hee Chul Eun, Jin Ho Chung, Department of Dermatology, Seoul National University College of Medicine, Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Institute of Dermatological Science, Seoul National University, Seoul, Korea

131 B3 **Impact of modified hyaluronan on binding of biological mediators**
Vera Hintze, Ute Hempel, Matthias Schnabelrauch, Manuela Rösler, Hartmut Worch, Dieter Scharnweber, Max-Bergmann-Center of Biomaterials, TU Dresden, Germany; Institute of Physiological Chemistry, TU Dresden, Germany; Innovent e.V., Jena, Germany

132 B4 **Structure Function Analysis of the Human Hyaluronidase Enzymes**
Ge Wei, Panneer Selvam, Lou H. Bookbinder, Gregory I. Frost, Frost Halozyme Therapeutic Inc, San Diego, CA

133 B5 **Sulfated glycosaminoglycans regulate matrilysin cleavage activity and specificity**
Susanna Harju-Baker, Hyun-Jeong Ra, Bill C. Parks, John K. McGuire, Center for Lung Biology, University of Washington School of Medicine, Seattle, WA

Inflammation

134 B6 **ECM changes after spinal cord hemisection in Tenascin C-knockout**
Dietrich E. Lorke, Jenny Schreiber, Udo Schumacher, Department of Anatomy, FMHS, UAE University, Al Ain, UAE; Institute of Anatomy II, Hamburg University, Germany

135 B7 **Differential MMP Response to PGE2 During Flexor Tendon Healing**
Alayna E. Loiselle, Regis J. O'Keefe, Center for Musculoskeletal Research, University of Rochester, Rochester, NY

136 B8 **GULP Is Required for Stabilin-2-mediated Cell Corpse Engulfment**
Dong-Jun Bae, Seung-Yoon Park, Kae-Bok Kang, Narendra Tapha, Sang-Yeob Kim, Sung-Jin Lee, In-San Kim, Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University; Department of Biochemistry, School of Med., Dongguk Uni., Daegu, Korea

137 B9 **Cell Corpse Removal by Stabilin-2, a Phosphatidylserine Receptor**
Seung-Yoon Park, Mi-Yeon Jung, Ha-Jeong Kim, Sung-Jin Lee, Sang-Yeob Kim, Byung-Heon Lee, Tae-Hwan Kwon, Rang-Woon Park, In-San Kim, Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University; Department of Biochemistry, School of Medicine, Dongguk Uni., Daegu, Korea

138 B10 **Target-tropic accumulation of SLX-liposome in arthritis mouse**
Jun Minaguchi, Hideki Minematsu, Toshitaka Oohashi, Takayuki Otani, Kiichi Inagawa, Kazunori Oie, Keiichiro Nishida, Aiji Ohtsuka, Yoshifumi Ninomiya, Koichi Igarashi, Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine, Density, and Pharmaceutical Sciences; Department of Human Morphology, Okayama University Graduate School of Medicine, Density, and Pharmaceutical Sciences; Katayama Chemical Industries CO., LTD, Okayama, Japan

139 B11 **Ultrastructural Inflammatory Changes of Oculomotor Neurons**
Melani Rajendran, Arumugam Krishnamurti, Department of Anatomy,Sri Ramachandra University,Chennai, India; Department of Anatomy, Annamalai University, Annamalai Nagar, India

140 B12 **Syndecan-1 shedding by MMP-7 controls neutrophil activation**
Qinglang Li, Sean E. Gill, Ying Wang, Charles W. Frevert, Pyong W. Park, Bill C. Parks, Center for Lung Biology, University of Washington, Seattle, WA; Department of Pediatrics, Harvard University, Boston, MA

141 B13 **Proteomics of mouse cartilage degradation in vitro**
Richard Wilson, Belluoccio Daniele, Little Chrisopher, Fosang Amanda, Bateman John, Murdoch Childrens Research Institute; University of Melbourne, Australia; Kolling Institute of Medical Research, University of Sydney, Sydney, Australia

142 B14 **MMP-10 Controls Macrophage Activation in Response to Infection.**
Timothy P. Birkland, Ying Wang, Shawn Skerrett, Bill C. Parks, Center for Lung Biology, University of Washington, Seattle, WA; Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA

143 B15 **TGFBIp promotes monocytes adhesion, migration and chemotaxis.**
Ha-Jeong Kim, In-San Kim, Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu, South Korea

144 B16 **Epilysin plays a protective role in a model of atopic dermatitis**
Jane Yoo, Anne M. Manicone, Bill C. Parks, Dermatology, University of Washington, Seattle, WA; Center for Lung Biology, University of Washington, Seattle, WA

145 B17 **TGFBIp activates platelets and promotes thrombogenesis.**
Ha-Jeong Kim, Pan-Kyung Kim, Sang-Mun Bae, Hey-Nam Son, Thoudam D. Singh, Jung-Eun Kim, In-San Kim, Department of Biochemistry and Cell Biology, Cell and Matrix Research

Institute, School of Medicine, Kyungpook National University, Daegu, Korea; Department of Molecular Medicine, Kyungpook National University School of Medicine, Daegu, Korea

146 B18 **Angiogenic-immune regulation by thrombospondin 1**
Catherine Simone, Samantha Zak, Brandi Rasinger, Linda Gutierrez, Department of Biology, Wilkes University, Wilkes-Barre, PA

147 B19 **A Protective Role for TIMP3 in Acute Lung Injury**
Sean E. Gill, Isham Huizar, Eli M. Bench, Negin Nazarian, Kevin J. Leco, Rama Khokha, Bill C. Parks, Center for Lung Biology, University of Washington, Seattle, WA; University of Western Ontario, London, Ontario; University of Toronto, Toronto, Ontario

148 B20 **iNOS Deficiency Inhibits MMP-9 activation in Hepatic I/R Injury**
Takashi Hamada, Sei-ichiro Tsuchihashi, Ana J. Coito, The Dumont-UCLA Transplant Center, Los Angeles, CA

Integrins

149 B21 **Collagen and its receptors on articular cartilage vesicles**
Ann K. Rosenthal, Emily Muth, Claudia M. Gohr, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

150 B22 **Cellular Fibronectin Enhances Hepatic Stellate Cell Migration**
Abby L. Olsen, Rebecca G. Wells, University of Pennsylvania School of Medicine, Cellular and Molecular Biology Graduate Program; Department of Medicine, Division of Gastroenterology, Philadelphia, PA

151 B23 **FAK Activity is Essential for Vascular Development**
Ssang-Taek Lim, Xiao Lei Chen, Alok Tomar, Nichol L. Miller, David D. Schlaepfer, UCSD Moores Cancer Center, La Jolla, CA

152 B24 **Extracellular Ligands Involved in Learning and Memory**
Richard G. LeBaron, Amber M. Asher, David A. Medina, Mary M. Navarro, Maria V. Tejada-Simon, Department of Biology, University of Texas, San Antonio, TX; Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX

153 B25 **Sarcoma MMP Expression is Influenced by Integrin-ECM Interactions**
Adriana Roopnariane, Rajeev Boregowda, Brooke Krovic, Timothy M. Ritty, Penn State Cancer Institute; Orthopaedics, Penn State College of Medicine, Hershey, PA

154 B26 **VLA1 on RPE cells is required for retinal function.**
You-Wei Peng, Marisa Zallocchi, Daniel T. Meehan, Duane Delimont, Bo Chang, Norman Hawes, Weimin Wang, Dominic Cosgrove, Boys Town National Research Hospital, Omaha, NE; The Jackson Laboratories, Bar Harbor, ME

Matricellular Proteins

155 B27 **CCN2 is essential for early chondrogenesis**
Andrew Leask, Mohit Kapoor, Anita Woods, Karen Lyons, Frank Beier, David Carter, Daphne Pala, University of Western Ontario, London, ON; UCLA, Los Angeles, CA

156 B28 **Cell-specific inhibition of TSP-1 translation by glucose**
Sanghamitra Bhattacharyya, Tina E. Marinic, Irene Krukovets, Olga I. Stenina, Cleveland Clinic, Lerner Research Institute, Cleveland, OH

157 B29 **Mutant COMP causes intracellular retention in a mouse PSACH model**
Karen L. Posey, Alka C. Veerisetty, Huiqiu H. Wang, Peiman Liu, Roger Bick, Brian J. Poindexter, Joseph L. Alcorn, Jacqueline T. Hecht, Departmentsof Pediatrics, University of Texas Medical School at Houston, TX; Department of Pathology, University of Texas Medical School at Houston,TX; Shriners Hospital for Children, Houston, TX

158 B30 **SPARC regulates inhibition of pericyte migration by TGF-beta**
Lee B. Rivera, Shanna A. Arnold, Rolf A. Brekken, Hamon Center for Therapeutic Oncology Research, UT-Southwestern, Dallas, TX

159 B31 **The role of MAGP1 in bone homeostasis**
C.S. Craft, J. Weinbaum, T.J Broekelmann, K.L. Lee, M.J. Silva, R.P. Mecham, Cell Biology & Physiology, Washinton University in St. Louis; Orthopaedic Surgery, Washinton University in St. Louis; Biomedical Engineering, University of Minnesota-Twin Cities, Minneapolis, MN

160 B32 **Collagen Fibril Formation: A New Target to Limit Fibrosis**
Hye Jin Chung, Andrzej Steplewski, Kee Yang Chung, Jouni Uitto, Andrzej Fertala, Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA; Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, South Korea

161 B33 **Collagen Type XI in Zebrafish Axial Skeletal Development**
Jason S. Adams, Brian L. McMahan, Linda M. Mercer, Raquel J. Brown, Julia T. Oxford, Department of Biology, Boise State University, Boise, ID; Physiology and Developmental Biology, Brigham Young University, Provo, UT; School of Medicine, University of Washington, Seattle, WA

162 B34 **Collagen II R992C Mutation Leads to Unfolded Protein Response**
Hye Jin Chung, Andrzej Steplewski, Deborah Jensen, Katarzyna Gawron, Andrzej Fertala, Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA

163 B35 **P16 and P19; mineralization-related Echinoderm phosphoproteins.**
Keith Alvares, Saryu Dixit, Elizabeth Lux, Arthur Veis, Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University. Chicago, IL

164 B36 **Distinct OI Phenotype Caused by COL1 C-proteinase Site Mutations**
A. M. Barnes, K. Lindahl, M. Whyte, T. Hefferan, C-J. Rubin, A. Kindmark, W. McAlister, S. Mumm, Ö. Ljunggren, J. C. Marini, BEMB, NICHD/NIH, Bethesda, MD; Dept of Endo, Med Sci, Uppsala Univ, Sweden; Shriner's Hosp for Children, St. Louis, MO; Dept of Orthop, Mayo Clinic, Rochester, MN

165 B37 **Molecular Packing Structure of Native Type II Collagen**
Olga Antipova[1,2], Raul Barrea[1,2], Joseph P. Orgel[1,2]
[1]Illinois Institute of Technology, Chicago IL, [2]BioCAT, APS, Argonne National Lab, IL

166 B38 **Stabilin-2 Mediates Homophilic Interactions via Its FAS1 Domains**
Young Eun Yun, Seung-Yoon Park, Mi-Yeon Jung, In-San Kim, Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University; Department of Biochemistry, School of Medicine, Dongguk University; Daegu, Korea

167 B39 **DDR2 affects the Persistence length of Collagen type I Fibers**
Lalitha Sivakumar, Gunjan Agarwal, Department of Biomedical Engineering, Ohio State University, Columbus, OH

168 B40 **Thrombospondin-1 in an Early Uterine Fibroid Development Model**
Liping Feng, Friederike Jayes, Tamara Greene, Phyllis Leppert, Dept of Ob-Gyn, Duke Univ. Medical Center, Durham, NC

169 B41 **N-glycosylation of lysyl hydroxylases is linked to LH activity**
Linda C. Walker, Mayra A. Overstreet, Heather N. Yeowell, Division of Dermatology, Duke University Medical Center, Durham, NC

170 B42 **SPARC Effects Collagen Deposition in Myocardial Hypertrophy**
Amy D. Bradshaw, Catalin F. Baicu, Tyler J. Rentz, Christina C. Derienzo, Janet Boggs, An O. Van Laer, Michael R. Zile, Division of Cardiology, Department of Medicine, Medical University of South Carolina, Ralph H. Johnson V.A. Medical Center, Charleston, SC

171 B43 **SPARC Influences Collagen Interaction with the Cell Surface**
Yuhua Zhang, Lee B. Rivera, Rolf A. Brekken, Amy D. Bradshaw, Dept. of Medicine, MUSC, Charleston, SC; Hamon Center for Therapeutic Oncology Res. UT-Southwestern, Dallas, TX

172 B44 **The CRT-binding sequence of TSP1 alters the foreign body response**
M Sweetwyne, L Van Duyn, B Sun, M Pallero, J Murphy-Ullrich, Departments of Cell Biology & Pathology, University of Alabama at Birmingham, Birmingham, AL

173 B45 **Analysis of the new collagen VI chain: a4(VI), a5(VI) and a6(VI)**
Jamie Fitzgerald, Cathleen Rich, Uwe Hansen, Department of Orthopaedics and Rehabilitation, Oregon Health and Science University, Portland, OR; Institute for Physiological Chemistry and Pathobiochemistry, University Hospital of Muenster, Muenster, Germany

174 B46 **TGFBIp C-terminal fragment induces osteosarcoma cell apoptosis**
Richard G. LeBaron, Maria Somaraki-Cormier, Clyde F. Phelix, Rajesha Rupaimoole, Christine L. Miller, Rogelio Zamilpa, Department of Biology, University of Texas at San Antonio, San Antonio, TX

175 B47 **TGFBIp interacts with type I collagen in vitro**
Maria Somaraki-Cormier, Rogelio Zamilpa, Richard G. LeBaron, Department of Biology, University of Texas at San Antonio, San Antonio, TX

176 B48 **Protein consequences of a missense mutation in mouse Col2a1**
Lammy S. Kim, Sarah E. Funk, MaryAnn Weiss, Robert E. Seegmiller, David E. Eyre, Russell J. Fernandes, Department of Orthopaedics, Univ. of Washington, Seattle, WA; Department of Physiology, Brigham Young Univ., Provo, UT

177 B49 **Role of UPR in the retention of mutant COMP in PSACH chondrocytes**
Francoise Coustry, Karen L. Posey, Huiqiu R. Wang, Jing-Fang Lu, Sankar N. Maity, Jacqueline T. Hecht, Department of Pediatrics, University of Texas Medical School at Houston, TX; Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, TX; Shriners Hospital for Children, Houston,TX

Matrix Protein Biology

178 B50 **Fibrin Knob Binding Kinetics on Defined Surface Chemistries**
Sarah E. Stabenfeldt, Thomas H. Barker, Department of Biomedical Engineering, Georgia Institute of Technology / Emory University School of Medicine, Atlanta, GA

179 B51 **Engineering fibrin ECM using fibrin knob-pocket interactions**
Allyson Soon, Sarah Stabenfeldt, Cedric Paulou, Thomas Barker, Wallace H. Coulter Department of Biomedical Engineering, Georgia Tech & Emory University, Atlanta, GA

180 B52 **High expression and physical properties of bacterial collagen**
Ayumi Yoshizumi, Zhuoxin Yu, Geetha Thiagarajan, Teresita Silva, Angela Mohs, Takeshi Yoshida, Masayori Inouye, Barbara Brodsky, Department of Biochemistry,Robert Wood Johnson Medical School, Piscataway, NJ

181 B53 **Tissue-specific negative effects in a cutis laxa mouse model**
Hideki Sugitani, Adrian Shifren, Russell H. Knutsen, Christopher H. Ciliberto, Robert P. Mecham, Department of Cell Biology and Physiology, Washington University, St. Louis, MO

182 B54 **Collagen VI mutations: A review of 54 patients**
Naomi L. Baker, Rishika A. Pace, Leona Tooley, Laura Zamurs, Naomi E. Adams, Shireen R. Lamande, Murdoch Childrens Research Institute, and Dept of Paediatrics, University of Melbourne, Parkville, Australia

183 B55 **An a2(VI) C1 mutation prevents collagen VI microfibril formation**
Naomi L. Baker, Laura Zamurs, Leona Tooley, Rachel A. Peat, Kathryn N. North, Shireen R. Lamande, Murdoch Childrens Research Institute, and Dept of Paediatrics, University of Melbourne, Australia; Neurogenetics Research Unit, Children's Hospital at Westmead, Sydney, Australia

184 B56 **Collagen VI A-domain changes: mutations or polymorphisms?**
Leona Tooley, Naomi L. Baker, Rishika A. Pace, Laura Zamurs, Shireen R. Lamande, Murdoch Childrens Research Institute and Dept of Paediatrics, University of Melbourne, Australia

185 B57 **Fibrillin-1 Assembly is Dependent on Fibronectin**
Daliang Chen, Laetitia Sabatier, Christine Fagotto, Dirk Hubmacher, Douglas S. Annis, Deane F. Mosher, Dieter P. Reinhardt, Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada; Faculty of Dentistry, McGill University, Montreal, QC, Canada; Department of Medicine, University of Wisconsin, Madison, WI

186 B58 **Characterization of Fibrillin Interactions with Fibronectin**
Laetitia Sabatier, Christine Fagotto, Dirk Hubmacher, Daliang Chen, Douglas S. Annis, Deane F. Mosher, Dieter P. Reinhardt, Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada; Faculty of Dentistry, McGill University, Montreal, QC, Canada; Department of Medicine, University of Wisconsin, Madison, WI

Proteoglycans

187 B59 **Characterization of Osteoarthritic Articular Cartilage Perlecan**
Arivalagan Muthusamy, Charles M. Davis, III, Carlton R. Cooper, Ronald R. Gomes, Jr., Department of Orthopedics, The Pennsylvania State University College of Medicine, Hershey, PA.; Department of Biological Sciences, University of Delaware, Newark, DE

188 B60 **Podocytes use Syndecan-4 (Syn4) to adhere to basement membranes.**
Kevin McCarthy, Shoujun Chen, Deborah McCarthy, Anne Woods, Yu Yamaguchi, Toin van Kuppevelt, LSU Health Sciences Center-Shreveport; University of Alabama at Birmingham; Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; Burnham Institute, La Jolla, CA

189 B61 **AGEs stimulate transglutaminase activity in tenocytes.**
Ann K. Rosenthal, Emily Muth, Claudia M. Gohr, Department of Medicine, Medical College of Wisconsin, Madison, WI

190 B62 **Regulation of ECM genes during heart valve remodeling**
Santanu Chakraborty, Jonathan Cheek, Bhuvaneswari Sakthivel, Bruce J. Aronow, Katherine E. Yutzey, Division of Molecular Cardiovascular Biology, Cincinnati Children's Medical Center, Cincinnati, OH; Division of Biomedical Informatics, Cincinnati Children's Medical Center, Cincinnati, OH

191 B63 **HS/syndecan modulate proteolytic balance in airway inflammation**
Chi Hang Chan, Valeria On Yue Leung, Mary Sau Man Ip, Daisy Kwok Yan Shum, Department of Biochemistry, University of Hong Kong; Department of Medicine, the University of Hong Kong, Hong Kong

192 B64 **Role of Serglycin in Acute Lung Injury and Repair**
Michelle Lin, Peter Chen, Magnus Åbrink, Qinglang Li, Ying Wang, Bill C. Parks, Center for Lung Biology, University of Washington , Seattle, WA; Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

193 B65 **Confocal Microscopy Study of Decorin Binding to Collagen Fibrils**
Elena Makareeva, Mary B. Sutter, Angela M. DeRidder, Antonella Forlino, Antonio Rossi, Ruggero Tenni, Sergey Leikin, Section on Physical Biochemistry, NICHD, National Institutes of Health, Bethesda, MD; University of Pavia, Italy

194 B66 **Estrogen and Progesterone Modulate Versican in the Mouse Uterus**
Renato M. Salgado, Fernanda F. Fernandes, Rodolfo R. Favaro, Jocelyn D. Glazier, John D. Aplin, Telma M. Zorn, Department of Cell and Developmental Biology, University of Sao Paulo, Brazil; Maternal and Fetal Health Research Group, University of Manchester, UK

195 B67 **Diabetes Alters Deposition of Endometrial Extracellular Molecules**
Rodolfo R. Favaro, Priscila R. Raspantini, Munick Fulquim, Renato M. Salgado, Zuleica B. Fortes, Telma M. Zorn, Department of Cell and Developmental Biology, University of São Paulo, Brazil; Department of Pharmacology, Uni. of São Paulo, Brazil

196 B68 **Decorin, biglycan and human airway smooth muscle cell apoptosis**
Michelle L. D'Antoni, Marie-Claire Michoud, Pasquale Ferraro, Bruce Mazer, James G. Martin, Mara S. Ludwig, Meakins Christie Laboratories, McGill University, Montreal, Quebec, Canada; Uni. of Montreal Hospital Centre, Montreal, Quebec, Canada

197 B69 **G1 domain of versican recruits HA to microfibrils**
Yusuke Murasawa, Chika Orii, Masahiko Yoneda, Zenzo Isogai, Department of Advanced Medicine, National Center for Geriatrics and Gerontology,Obu, Aichi, Japan; Aichi Prefectural College of Nursing and Health, Nagoya, Aichi, Japan

198 B70 **Versican Induces Fibroblast Contraction of Collagen Gels**
Jon Carthy, Maziar Rahmani, Seti Boroomand, Darryl Knight, Bruce McManus, The James Hogg iCAPTURE Centre, Vancouver, BC, Canada

Proteolytic Pathways

199 B71 **Mutual Stabilization of P3H1 and CRTAP in ER Modification Complex**
W. Chang, A.M. Barnes, W.A. Cabral, J.C. Marini, BMB, NICHD, NIH, Bethesda, MD

200 B72 **Mitochondrion, a target for novel antitumor agent hydroxykauracid**
George G. Chen, Chun S. Lo, Jackie Leung, Billy C. Leung, Ursula F. Chan, Michael K. Hsin, Malcolm J. Underwood, Alexandrer C. Vlantis, Andrew C van Hasselt, Paul B. Lai, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

201 B73 **Dimerization of MT1-MMP regulates the activation of MMP-2**
Signe Ingvarsen, Daniel H. Madsen, Leif R. Lund, Kenn Holmbeck, Niels Behrendt, Lars H. Engelholm, The Finsen Laboratory, Rigshospitalet, Denmark; Matrix Metalloproteinase Unit, NIDCR, NIH, Bethesda, MD

202 B74 **The Role of Multimerisation in the Regulation of BMP-1 and mTLD**
Richard Berry, Thomas A. Jowitt, Manfred Roessle, Guenter Grossman, Elizabeth G. Canty, Richard A. Kammerer, Karl E. Kadler, Clair Baldock, Wellcome Trust Centre for Cell Matrix Research, University of Manchester, Hamburg, Germany; Synchrotron Radiation Department, Council for Central Laboratory of the Research Councils, Daresbury, Warrington, UK

ABSTRACTS
Biennial Meeting of the
American Society for Matrix Biology

1

Biglycan as a ligand to TLR-4 and –2 aggravates inflammation

Liliana Schaefer.
Dept. of Pharmacology, University of Frankfurt, Germany, 60590

During inflammation the ECM turns into a dynamic microenvironment, generating a host of regulatory molecules, which interact with resident and infiltrating cells. There is growing evidence that ECM molecules may convey proinflammatory signals. Recently, we showed that biglycan, a small leucine-rich proteoglycan, acts as an endogenous ligand of the innate immunity receptors Toll-like receptor-4 (TLR4) and -2 (TLR2) in macrophages, leading to rapid activation of p38, Erk and NF-κB, thereby stimulating the expression of TNF-α and macrophage inflammatory protein-2 (MIP-2). In agreement with these findings, the stimulatory effects of biglycan are significantly reduced in TLR4-mutant (TLR4-M), TLR2-/- and myeloid differentiation factor 88-/- (MyD88-/-) macrophages and completely abolished in TLR2-/-/TLR4-M macrophages. Biglycan-null mice have a considerable survival benefit in LPS- or zymosan-induced sepsis due to lower levels of circulating TNF-α and reduced infiltration of mononuclear cells in the lung, causing less end-organ damage. Importantly, macrophages, normally not expressing biglycan, start to synthesize biglycan when stimulated by proinflammatory factors. Thus, biglycan, upon release from the ECM or from macrophages, is capable to boost inflammatory reactions by signaling through TLR4 and TLR2, thereby enhancing the synthesis of TNF-α and MIP-2. This novel concept, that under certain conditions matrix components may act as ligands to distinct immunity receptors and thereby regulate inflammatory reactions, might be of considerable significance for the pathogenesis of and the therapeutic approach to inflammatory disorders.

2

Lumican regulates integrin-mediated migration of neutrophils

Seakwoo Lee[1], Shukti Chakravarti[1-3].
[1]Departments of Medicine, [2]Ophthalmology, [3]Cell Biology. The Johns Hopkins School of Medicine, Baltimore, Maryland 21205

Purpose: Lumican (*Lum*) is a leucine-rich repeat keratan sulfate proteoglycan of the extracellular matrix (ECM). We have shown that 1) lumican regulates toll-like receptor 4-mediated innate immune response and 2) influx of polymorphonuclear neutrophils (PMN) to the injured cornea is reduced and healing delayed in lumican-deficient mice. We investigated the role of lumican-integrin interactions in promoting PMN migration.
Methods: $Lum^{+/+}$ and $Lum^{-/-}$ peritoneal lavage PMN were tested for migration towards the pro-inflammatory chemokine CXCL1/KC (bottom chamber) in transwells, with or without recombinant lumican and function-blocking antibodies against specific integrins (upper chamber). Lumican and integrin interactions were investigated by co-immunoprecipitation and immunofluorescent confocal microscopy. Cell surface integrin contents were compared by flow cytometry.
Results and Discussion: There was no difference between lumican-deficient and wild type PMN in surface integrins. However, in transwell chemotaxis assays $Lum^{-/-}$ PMN showed reduced migration that could be increased to wild type levels by the addition of recombinant lumican. Furthermore, this lumican-aided PMN migration could be inhibited by anti-integrin antibodies. Confocal microscopy indicated lumican and integrin proximity on PMN cell surfaces. The co-immunoprecipitation experiments indicated prominent interaction between lumican and specific integrins. Taken together the results

suggest the presence of lumican on the surface of PMN where it interacts with integrins and promotes chemotactic migration.

3

Role of gamma1-Laminins in Renal Collecting Ducts.

Dong-Hua Yang[1], Zulin Chen[2], Roy Zent[3], Sidney Strickland[2], Carlton Bates[4], Karen K. McKee[1], Peter D. Yurchenco[1].
[1]Robert Wood Johnson Medical School, Piscataway, NJ 08854, [2]Rockefeller University, New York, NY 10021, [3]Vanderbilt University, Nashville, TN 37232, [4]Ohio State University, Columbus, OH 43210

The laminin-gamma1 gene was deleted in the ureteric bud (UB). At birth, laminin-deficient pups exhibited bilateral renal-ureteric agenesis or renal hypoplasia, the latter followed by emergence of diabetes insipidus and hydronephrosis. Laminin-deficient UBs were absent or under-branched within the metanephric mesenchyme at E10.5 to 11.5. By E12.5 to E13.5 there was reduced epithelial/mesenchymal proliferation, delayed mesenchymal condensation, and flattened and disorganized epithelia. UB basement membranes were initially severely attenuated to absent with marked reductions of laminin-gamma1, nidogen-1, collagen-IV and heparan sulfate (HS) proteoglycans. FGF-2, a HS-binding growth factor normally located within basement membranes, was largely absent. By E14.5, corresponding to the delayed appearance of renal vesicles and their basement membranes within the defective kidneys, increased deposition of the laminin gamma3 and gamma1 subunits, collagen-IV, nidogen and proteoglycans was detected. These results reveal the importance of gamma1-laminins for renal collecting duct basement membranes, maintenance of epithelial polarity, and growth.

4

The role of patient advocacy organizations in genetic research

Sharon F. Terry[1], Patrick F. Terry[1], Lionel G. Bercovitch[1], Jouni Uitto[2].
[1]Genetic Alliance, PXE International and Genetic Alliance BioBank, Washington, DC, [2]Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, PA

Advocacy organizations for genetic diseases are increasingly becoming involved in biomedical research, in order to meet the needs of the individuals that they serve. PXE International, an advocacy organization for the disease pseudoxanthoma elasticum, provides an example of how research can be accelerated by these groups. It has adopted methods that were pioneered by other advocacy organizations, and has integrated these along with new approaches into franchizable elements. The model has been followed for other conditions and has led to the establishment of a common infrastructure to enable advocacy groups to initiate, conduct and accelerate research. An example of the activities sponsored by PXE International is the Special Interest Group Symposium, "Heritable Diseases of the Elastic Structures – The Paradigms of Pseudoxanthoma Elasticum and Cutis Laxa", to be held at the Biennial ASBM Meeting in San Diego in December 2008. This Symposium highlights the progress made in understanding the molecular genetics and proposes putative pathomechanism underlying these complex heritable disorders affecting the extracellular matrix, primarily the elastic structures. The progress in this area of research forms the basis of translational applications in terms of improved diagnostics with refined classification, prerequisites for development of molecular therapies for these, currently intractable, diseases. Reference: *Nature Rev. Genet.* 8:157-164, 2007

5

Molecular genetics of pseudoxanthoma elasticum

Jouni Uitto[1], Qiaoli Li[1], Qiujie Jiang[1], András Váradi[2].
[1]Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, PA 19107, [2]Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary

Pseudoxanthoma elasticum (PXE), a heritable multi-system disorders, is characterized by pathologic mineralization of connective tissues. The causative gene was initially identified as *ABCC6* which encodes a transmembrane ABC transporter protein (ABCC6) expressed

primarily in the liver and the kidneys. The critical role of *ABCC6* in ectopic mineralization has been confirmed by the development of *Abcc6*$^{-/-}$ knock-out mice which recapitulate the features of PXE. Over 200 distinct loss-of-function mutations representative of over 1000 mutant alleles in *ABCC6* have been identified in patients with PXE. More recently, missense mutations in the *GGCX* gene, either in compound heterozygous state or digenic with a recurrent ABCC6 nonsense mutation (p.R1141X) *in trans*, have been identified in patients with PXE-like cutaneous findings and coagulation factor deficiency. *GGCX* encodes a carboxylase which catalyzes γ-glutamyl carboxylation of coagulation factors as well as of matrix gla protein (MGP); the latter in fully carboxylated form serves as a systemic inhibitor of pathologic mineralization. Collectively, these observations suggest the hypothesis that a consequence of loss-of-function mutations in the *ABCC6* gene is reduced γ-glutamyl carboxylation of MGP, with subsequent connective tissue mineralization. Further progress in understanding the detailed pathomechanisms of PXE should provide novel strategies to counteract, and perhaps cure, this complex heritable disorder at the genome-environment interface.

6

Extracellular Matrix Control of Stem Cell Niches

Marian F. Young, Tina M. Kilts, Yanming Bi.
Craniofacial and Skeletal Diseases Branch, NIDCR, NIH Bethesda, MD 20892

Extracellular matrix glycoproteins and proteoglycans bind a variety of growth factors and cytokines thereby regulating matrix assembly as well as musculoskeletal organization. However, little is known about the mechanisms by which extracellular matrix molecules modulate stem/progenitor cells involved in skeletal formation. Using mice deficient in members of the small leucine-rich proteoglycans (SLRPs) including biglycan (bgn), decorin (dcn) and fibromodulin (fmod), we uncovered a role for these extracellular matrix proteoglycans in modulating stem/progenitor cell function. Our studies showed bgn, dcn and fmod have critical roles in binding and sequestering TGF-b and BMP-2 within the ECM of skeletal tissues. The excess TGF-b and BMP-2 is then available to directly bind to their receptors on progenitor cells and over-activate their signaling transduction pathways. The predominant effect of the increased signaling is a "switch in cell fate". Specifically, in the case of bone, over-activation of TGF-b leads to increased proliferation and subsequent apoptosis of osteoprogenitor cells in the marrow of bgn/dcn deficient mice. In fmod/bgn deficient mice, ectopic bones form in the tendon through the activation of tendon stem/progenitor cells by BMP-2 thus directing them towards osteogenesis rather than tendon formation. These findings underscore the importance of the extracellular matrix micro-environment in controlling the fate of adult stem cells and reveal a novel cellular and molecular basis for the physiological and pathological control of skeletal function. Support: DIR-NIDCR, IRP-NIH, DHHS

7

Glycomics of Proteoglycan Biosynthesis in ESC Differentiation

Alison V. Nairn[1], Stephen Dalton[1], Toshihiko Toida[2], Michael Pierce[1], Kelley W. Moremen[1], Robert J. Linhardt[3].
[1]Complex Carbohydrate Research Center and the University of Georgia, 315 Riverbend Road, Athens, GA 30602, [2]Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, 260-8675 Japan, [3]Departments of Chemistry and Chemical Biology, Chemical and Biological Engineering, and Biology, Rensselaer Polytechnic Institute, Troy, NY 12180

Glycosaminoglycans (GAGs) play a critical role in binding and activation of growth factors involved in cell signaling critical for developmental biology. The biosynthetic pathways for GAGs have been elucidated over the past decade and now analytical methodology makes it possible to determine GAG composition in as few as 10 million cells. A glycomics approach was used to examine GAG content, composition and the level of transcripts encoding for GAG biosynthetic enzymes as murine embryonic stem cells (mESCs) differentiate to embryoid bodies (EBs) and to

extraembryonic endodermal cells (ExE) to better understand the role of GAGs in stem cell differentiation. Hyaluronan, chondroitin sulfate/dermatan sulfate, and heparan sulfate synthesis was enhanced following the transition from mESC to EB and ExE. Transcripts associated with the synthesis of the early precursors were largely unaltered, suggesting other factors account for enhanced GAG synthesis. The composition of GAGs also changed upon differentiation. Knowing the changes in GAG fine structure should improve our understanding the biological properties of differentiated stem cells.

8

Induction of Physiologic and Pathologic Angiogenesis by TIMP-free *pro*MMP-9 Derived Uniquely from Inflammatory Neutrophils

James P. Quigley.
Department of Cell Biology, The Scripps Research Institute, La Jolla, CA

Angiogenesis, the process by which normal or tumor-infiltrated tissues become newly vascularized, is generally believed to be induced by a number of well-established growth factors such as VEGF. Molecules that might inductively operate directly upstream of the angiogenic growth factors are not so well-established. Our laboratory, using both chick embryo and mouse models, has demonstrated that *pro*MMP-9 is a potent angiogenesis inducer at sub-nanomolar levels. In order to function as an inducer of angiogenesis, the *pro*MMP-9 must be completely free of its normally tightly-associated TIMP-1 molecule. A purified preparation of the *pro*MMP-9-TIMP-1 complex, which is the natural form of MMP-9 produced by most cell types, is not angiogenic even when added at 20 fold molar excess over the TIMP-1-free *pro*MMP-9. Inflammatory neutrophils appear to be one of the only cell types that produce *pro*MMP-9 in the TIMP-1-free form. These results are consistent with a large amount of data from a number of laboratories demonstrating a strong link between rapidly infiltrating inflammatory neutrophils and subsequent angiogenesis in those infiltrated tissues.

We recently explored mechanistically the biochemical features and reasons for the angiogenic potency of neutrophil TIMP-free *pro*MMP-9. Employing domain variants and mutants of human MMP-9, it appears that an intact catalytic site and the presence of the hemopexin domain are required for MMP-9's angiogenic ability. The *pro*MMP-9 zymogen must be activated and manifest its catalytic activity within the first 24 hours of a 70-80 hour *in vivo* angiogenesis assay. The *pro*MMP-9 in the natural stoichiometric complex with TIMP-1 fails to undergo either proteolytic or chemical activation, and this we suggest is the mechanistic reason for TIMP-1's angiogenic dampening abililty. Interestingly, the catalytic activity of activated TIMP-1-free MMP-9 induces a significant release of basic fibroblast growth factor (FGF-2) over that of VEGF. Furthermore, MMP-9's pronounced angiogenic capabilities are blocked by specific antibodies directed to FGF-2 and the FGF receptor, $FGFR_2$. Finally, neutrophil TIMP-1-free *pro*MMP-9 stimulates angiogenesis into a non-aggressive, non-angiogenic tumor developing on the chick embryo and the induced angiogenesis is blocked by anti-FGF-2 antibodies.

In summary, inflammatory neutrophils rapidly deliver a potent angiogenic inducer to tissues that are to undergo neovascularization. The inducer, TIMP-1-free *pro*MMP-9, is rapidly activated and proteolytically enhances the influx and generation of a number of protein factors, one of which, FGF-2, appears to be, within our *in vivo* models, the major downstream inducer of new blood vessel formation.

9

Fibulin-5 Inhibits Integrin-Induced ROS Production

Marie K. Schluterman[1], Shelby L. Chapman[2], Grzegorz Korpanty[1], Hiromi Yanagisawa[2], Rolf A. Brekken[1].
[1]Hamon Center for Therapeutic Oncology Research and Department of Surgery, UT-Southwestern, Dallas, TX 75390, [2]Department of Molecular Biology, UT-Southwestern, Dallas, TX 75390

Fibulin-5 (Fbln5), an integrin-binding matricellular protein, is expressed by vascular cells and is critical for the appropriate deposition and function of the vascular extracellular matrix. Here, we demonstrate that Fbln5 inhibits the production of reactive oxygen species (ROS) in

an integrin-dependent manner and through this pathway exerts control over the angiogenic process. We found that cells lacking endogenous Fbln5 produced higher levels of ROS in a β1 integrin and fibronectin-dependent manner in vitro. Additionally, we identified that elevated ROS in Fbln5-deficient (Fbln5-/-) animals is critical for vascular invasion of matrigel implants but detrimental to angiogenesis and growth of syngeneic tumors. Importantly, both effects were reverted with anti-oxidant treatment. We conclude that Fbln5 is a central and critical control factor in the regulation of the level of ROS in the vascular microenvironment, alteration of which can promote or suppress angiogenesis. These results suggest that Fbln5 may provide a unique target for therapy of angiogenesis-dependent disease.

10

Angiogenesis and Intravasation of PC-3 Dissemination Variants

Erin M. Conn[1,2], Elena I. Deryugina[1], James P. Quigley[1].
[1]Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037,
[2]University of California-San Diego, Molecular Pathology Graduate Program, La Jolla, CA 92037

Intravasation, i.e. the entry of aggressive tumor cells into the vasculature, is one early and potentially rate-limiting step in the metastatic cascade. To analyze this process, our lab has selected in vivo high and low disseminating cell variants derived from the human PC-3 prostate carcinoma cell line, i.e., PC-hi/diss and PC-lo/diss, using the human tumor-chick embryo spontaneous metastasis model. In this model, human tumor cells are grafted onto the chorioallantoic membrane (CAM) where they rapidly form tumors and intravasate into the vasculature. Cells disseminated to distant sites can be quantified by Alu-qPCR analysis. Using both in vitro and in vivo assays to recapitulate processes involved in intravasation, we have dissected out individual rate-limiting steps that are more successfully completed by PC-hi/diss. Notably, coinciding with enhanced intravasation potential, only PC-hi/diss induces a high level of tumor angiogenesis in chick and murine models, mediated by VEGF and IL-8 pathways. The new angiogenic vessels recruited by PC-hi/diss may serve as conduits for tumor cell intravasation and dissemination. The contributions of neoangiogenesis to PC-hi/diss dissemination can be determined by analyzing the functional relationships between these processes. In addition, PC-hi/diss is more migratory and invasive in vitro, suggesting enhanced ability to escape from the primary tumor and invade local stroma in vivo. Analyses of levels and activation of specific matrix proteases, performed to examine the mechanism of this differential, have revealed a functionally important increase in uPA expression in PC-hi/diss. Overall, the in vivo selection and comparative analysis of PC-3 intravasation variants is useful for identification of individual rate-limiting steps and key molecular changes ultimately leading to tumor cell intravasation.

11

Endorepellin evokes SHP1 activity in endothelial cells

Alexander Nystrom[1], Zabeena P. Shaik[1], Ambra Pozzi[2], Thomas Krieg[3], Beate Eckes[3], Renato V. Iozzo[1].
[1]Thomas Jefferson University, Philadelphia, PA,
[2]Vanderbilt University, Nashville, TN,
[3]University of Cologne, Cologne, Germany

Endorepellin, the C-terminal domain of perlecan, is a powerful inhibitor of angiogenesis. Here we aimed to further dissect the mechanisms of endorepellin-mediated endothelial silencing. Antibody arrays against tyrosine kinase receptors showed that endothelial cells exposed to endorepellin exhibited widespread reduction in overall receptor phosphorylation, especially affecting key receptors involved in regulating angiogenesis. Function-blocking antibodies against the integrin α2β1 abrogated the global dephosphorylation evoked by endorepellin suggesting that endorepellin activated tyrosine phosphatases via α2β1 integrin outside-in signaling. Tyrosine phosphatase assays showed an increase in general phosphatase activity after endorepellin treatment. Strong genetic evidence was provided by the lack of response in microvascular endothelial cells derived from α2β1 integrin null mice. SHP1, a tyrosine phosphatase, co-precipitated with the integrin α2

subunit and was phosphorylated in a dynamic fashion after endorepellin stimulation. Immunoblot of organs from integrin α2β1-deficient mice revealed a significant decrease in SHP1 content as compared to wild-type organs. Conversely, siRNA-mediated knockdown of the integrin α2 subunit caused a dose-dependent reduction of SHP1. Hence, both biochemical and genetic evidence strongly suggest a direct interaction of integrin α2β1 and SHP1, and indicate that SHP1 is an essential mediator of endorepellin antiangiogenic activity.

12

Hypertriglyceridemia caused by mutation of the basement membrane proteoglycan Type XVIII collagen

Jeffrey D. Esko[1], Joseph R. Bishop[1], Maria Rita Passos-Bueno[2], Kristin I. Stanford[1], Erika Yeh[2], Joseph L. Witztum[3], Andre Bensadoun[4], Karen S. Moulton[5].
Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, California 92093

The heparan sulfate proteoglycan Type XVIII collagen (Col18) is found ubiquitously in the basements membranes of the vasculature in vertebrates. Mice deficient in Col18 *(Col18a1*$^{-/-}$) are viable and fertile, but have broadened basement membranes, abnormal retinal vessel development and poor anchoring of vitreal collagen fibrils to the inner lining membrane of the retina. Similar retinal defects occur in subjects with Knobloch syndrome, a rare disorder characterized by myopia, vitreoretinal degeneration and retinal detachment attributed to mutations in Col18. Examination of *Col18a1*$^{-/-}$ mice demonstrates fasting and post-prandial hypertriglyceridemia characterized by large triglyceride-rich lipoproteins. These lipoproteins likely derive from chylomicrons based on size and apolipoprotein composition. *Col18a1*$^{-/-}$ mice exhibit delayed hydrolysis of triglycerides in the peripheral circulation due to inadequate access of particles to lipoprotein lipase, which is normally bound to receptors on the luminal side of the endothelium but deficient in mutant mice. Furthermore, patients with Knobloch Syndrome also have hypertriglyceridemia and accumulate triglyceride-rich lipoproteins under fasting conditions, a previously unrecognized consequence of the loss of Col18 in humans. These findings suggest that Col18 is required for transport or presentation of lipoprotein lipase in the vasculature and that alterations in its expression can lead to hypertriglyceridemia in mice and humans.

13

Syndecan-1 Shedding Facilitates Airway Re-epithelialization.

Peter Chen, Laura E. Abacherli, Ying Wang, Qinglang Li, Bill C. Parks.
Center for Lung Biology, University of Washington; Seattle, WA 98109

Introduction: Matrilysin (MMP-7) is essential for airway re-epithelialization. Matrilysin also functions to regulate lung inflammatory cell recruitment through the cleavage of syndecan-1 (SDC1). This study was to determine if matrilysin sheds SDC1 to facilitate re-epithelialization. **Methods:** Primary mouse tracheal epithelial cells were cultured at an air-liquid interface and injured as an *in vitro* model of re-epithelialization. Mice were also injured with naphthalene to study re-epithelialization *in vivo*. Cell lines were also retroviral transduced to stably express either a) wild-type or an uncleavable SDC1 or b) SDC1 shRNA. **Results:** Wounded wild-type epithelium shed SDC1 resulting in diminished SDC1 immunofluorescent signal at the wound front *in vitro* and *in vivo*. In contrast, SDC1 shedding was minimal, and SDC1 signal persisted in wounded matrilysin-deficient epithelium. Wounded COS-7 cells that stably co-express matrilysin and SDC1 repaired faster than cells co-expressing matrilysin and a cleavage-resistant SDC1. Consistent with these findings, injured SDC1-deficient airway epithelium repaired faster than wild-type epithelium *in vitro* and *in vivo*. Additionally, SDC1 knockdown increased migration and decreased adhesion of BEAS-2B cells compared to control cells. Moreover, β1 integrin activity was diminished in SDC1-deficient conditions. **Conclusion:** These data show that matrilysin sheds SDC1 during airway re-epithelialization. Moreover, SDC1 shedding removes restrictions to migration by reducing integrin-mediated adhesion. Because

wound edge epithelium can control acute inflammation and re-epithelialization, a dual role for matrilysin shedding of SDC1 may reflect co-opting of one process into others.

14

Synstatin, a Sdc1 peptide, blocks αvβ3-dependent angiogenesis

DeannaLee M. Beauvais, Brian J. Ell, Andrea R. McWhorter, Alan C. Rapraeger.
Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI 53706

Syndecan-1 (Sdc1), via an interaction site in its ectodomain, forms a ternary complex with the αvβ3 integrin and the insulin-like growth factor-1 receptor (IGF1R). Formation of this complex is required for αvβ3-dependent cell adhesion and invasion in human mammary carcinoma and endothelial cells. Thus, Sdc1's regulation of αvβ3 integrin activity likely affects tumor metastasis and tumor-induced angiogenesis. To study this, we derive a novel peptide called "synstatin", which contains the integrin regulatory site identified in the Sdc1 ectodomain. We show that synstatin (SSTN; IC50 ≅ 0.3 μM) competitively dissociates the Sdc1-integrin-IGF1R complex, thus blocking integrin activation, as evidenced by a loss in WOW1 and/or soluble fibrinogen binding (αvβ3 integrin activation sensors) and a block in cell adhesion, spreading and migration on vitronectin, an αvβ3-specific ligand. SSTN effectively blocks angiogenesis *in vitro* (i.e., 3D aortic ring assays) and *in vivo* when delivered systemically in mouse corneal-pocket angiogenesis assays. Moreover, using human mammary carcinoma cells subcutaneously implanted in nude mice, SSTN also inhibits tumor-induced angiogenesis - a prerequisite step for tumor growth and metastatic dispersion. Thus, Sdc1 plays a critical regulatory role during angiogenesis that can be potently targeted by the SSTN peptide. Disruption of this regulatory mechanism holds promise as an anti-cancer therapy.

15

Decorin binds to and downregulates the MET receptor

Silvia Goldoni[1], Ashley Humphries[1], Rick T. Owens[2], David J. McQuillan[2], Renato V. Iozzo[1].
[1]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA, [2]LifeCell Corporation, Branchburg, NJ

Decorin, a member of the small leucine-rich proteoglycan gene family, regulates tumor cell growth primarily by downregulating epidermal growth factor receptor (EGFR) activity and by interacting with other members of the ErbB family of receptor tyrosine kinase. Due to the complex binding repertoire of decorin towards multiple targets, we predicted a role for decorin in modulating the bioactivity of other tyrosine kinase receptors. We discovered that decorin binds to Met, the receptor for hepatocyte growth factor/scatter factor, leading to transient activation at the catalytic domain, followed by a rapid (T1/2 ~ 6 min) downregulation of total Met. This activity was independent of EGFR. Decorin induced Met suppression by a dual action of recruiting the ubiquitin ligase c-Cbl followed by enhanced proteasome degradation and by evoking shedding of the receptor ectodomain. In addition to physical downregulation, Met tyrosine kinase activity was also suppressed by decorin with a concurrent suppression of the cellular levels of β-catenin, a known downstream Met effector. Finally, decorin inhibited cell migration and growth via Met receptor-mediated events. Collectively these observations provide a novel mechanism through which decorin may exert its cytostatic activity, that is, by targeting multiple tyrosine kinase receptors implicated in cancer progression. This bioactivity would contribute to reducing in vivo primary tumor growth as well as inhibiting metastatic spread in situations where multiple tyrosine kinase receptors are coactivated.

16

Biological Functions of Membrane-Type Matrix Metalloproteinases

Kenn Holmbeck.
Matrix Metalloproteinase Unit, Craniofacial and Skeletal Diseases Branch, NIDCR, NIH, Bethesda, MD 20892.

Peri-cellular remodeling of the extracellular

matrix is considered essential for basic cellular functions such as proliferation and migration, and is necessary in development of all organisms. Membrane-type matrix metalloproteinases (MT-MMPs) display specificity for several of the more abundant substrates in the extracellular matrix, and have therefore received considerable attention in the context of both development and progression of diseases characterized by extensive matrix remodeling and tissue destruction. Through targeted deletion of some MT-MMP family members in mice, several aspects of their biological functions have now been elucidated. The results derived from these mouse models have provided insight into numerous aspects of peri-cellular proteolysis, which are vital to development, growth, and homeostasis of the connective tissues. Among the more notable findings is a separate remodeling paradigm for specific cartilage tissues in the body. The identification of this process not only highlights an important pathway in matrix remodeling, but also enables characterization of a specific cell population for which there may be potentially important therapeutic applications in cartilage reconstruction. The advent of MT-MMP animal models further allows direct testing of some of the most widely accepted hypotheses in those diseases characterized by tissue destruction, such as cancer and arthritis.

17

Extra- and intracellular collagen degradation linked by uPARAP

Daniel H. Madsen[1], Signe Ingvarsen[1], Lars H. Engelholm[1], Thomas H. Bugge[2], Niels Behrendt[1].
[1]Finsen Laboratory, Rigshospitalet, Denmark, [2]NIDCR, NIH, Bethesda, MD 20892

Degradation of collagens is a crucial process in physiological and pathological processes such as bone development and cancer growth and invasion. A recently discovered turnover route with importance for tumor growth involves intracellular collagen degradation and is governed by the collagen receptor uPARAP. uPARAP deficient cells are hence incapable of internalizing collagen for lysosomal degradation and in a mouse model of breast cancer the tumor growth in uPARAP deficient mice is delayed and accompanied by large accumulations of collagen in the tumor area. We here demonstrate that fibroblast-mediated collagen degradation proceeds as a sequential mechanism in which extracellular collagen degradation is followed by uPARAP-mediated endocytosis of collagen fragments. The first observation to support this is that collagen that has been pre-cleaved by a mammalian collagenase is internalized much more efficiently than intact, native collagen by uPARAP-positive cells. This preference is mainly governed by the acquisition of a gelatin-like structure by the collagen, occurring upon collagenase-mediated cleavage. The second observation is that the growth of uPARAP-deficient fibroblasts on a native collagen matrix leads to substantial extracellular accumulation of well defined collagen fragments, whereas, WT fibroblasts possess the ability to direct an organized and complete degradation sequence comprising both the initial cleavage, the endocytic uptake, and the intracellular breakdown of collagen. This study establishes uPARAP as a critical link between extra- and intracellular collagen degradation.

18

Joint Degeneration in Mice Lacking Adequate Perlecan Levels

Kathryn D. Rodgers, Ayla Turnquist, Olena Jacenko.
University of Pennsylvania

Perlecan is a conserved extracellular matrix (ECM) proteoglycan and is a core component of all basement membranes. Surprisingly, the absence of perlecan is primarily noted in the skeletal elements, where perlecan is a prominent member of cartilage ECM. In fact, complete absence of perlecan in man and mouse results in perinatal lethality due to respiratory distress. We recently reported the development and characterization of a novel mouse strain genetically engineered to contain a missense mutation in the perlecan gene found in a family with Schwartz-Jampel syndrome, a non-lethal progressive skeletal disease, and a neomycin selection cassette. These mice (C1532Yneo), and patients, have about 30% of normal perlecan expression and deposition and suffer from a wide array of skeletal dysplasias. Here, we have utilized these hypomorphic perlecan C1532Yneo

mice to investigate matrix turnover and non-inflammatory degenerative joint disease. Our results show decreased perlecan levels result in aberrant MMP activity in joint epiphyses, indicating disturbed tissue remodeling, a finding that is supported by altered number and activity of osteoclasts in long bones. This aberrant remodeling, likely combined with mechanical stresses due to abnormal bone shape and size, results in early-onset degeneration of all joints examined. These changes include chondrocyte clustering, loss of articular proteoglycan content, tissue shearing, osteophyte formation, and altered gait; all hallmarks of osteoarthritis in humans. Thus, our data indicate that C1532Yneo mice model early-onset non-inflammatory joint degeneration. Supported by NRSA F32 AR052246 to KDR and R01-DK-57904 to OJ.

19

The role of caveolin-1 in pulmonary matrix remodeling

Olivier Le Saux[1], Kelsa Teeters[1], Ji Won Choi[2], Barry Starcher[3], Elaine C. Davis[2], Claude Jourdan Le Saux[1].
[1]Department of Cell and Molecular Biology, University of Hawaii School of Medicine, Honolulu, HI 96813, [2]Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada, [3]Department of Biochemistry, University of Texas Health Center, Tyler, TX

Caveolin-1 (cav1) is a 22kDa membrane protein essential to the formation of small invaginations in the plasma membrane, called caveolae. Cav1 down-regulates transforming growth factor (TGF)-β signal transduction. In pulmonary pathologies such as interstitial fibrosis or emphysema, altered mechanical properties of the lungs are often associated with abnormal ECM deposition. In this study, we examined at various ages the deposition of selected ECM proteins in cav1-deficient mice (cav1-/-). We found that cav1-/- mice developed pulmonary edema, which was associated with progressive increase in deposition of collagen in airways and parenchyma. We also found abundant elastic fiber deposition primarily around airways. The higher deposition of collagen and elastic fibers was associated with increased tropoelastin and col1α2 and col3α1 gene expression in lung tissues, which correlated tightly with increased TGF-β/pSmad signal transduction. This remodeling of pulmonary ECM in cav1-/- mice most likely contributed to the reduced lung compliance, increased elastance and airway resistance we observed in these animals. Our study illustrates the important role played by cav1, as part of TGF-β signaling pathway, in the regulation of the pulmonary extracellular matrix and suggests that perturbation of cav1 function could also underlie cases of several pulmonary pathologies.

20

Retention of Thrombospondins in ECM, and views of TSP Evolution

Josephine C. Adams, Dae Joong Kim, Amber A. Bentley, Stephanie Haase.
Dept. of Cell Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195

Thrombospondins are extracellular, multimeric, calcium-binding glycoproteins. The five thrombospondins of humans have roles in wound repair and tumour biology (TSP-1, TSP-2), vascular pathologies and premature heart disease (TSP-1, TSP-2, TSP-4), and pseudoachondroplastic dysplasia (TSP-5). These activities depend on interactions of TSPs with cell surfaces, extracellular growth factors, proteases, and extracellular matrix (ECM) components. ECM deposition of TSP-1 occurs during development and in numerous pathophysiological contexts, however the molecular cellular mechanisms by which TSPs are retained in 3-dimensional ECM have been little studied. Through use of a panel of TSP-1 domain deletion mutants and a cell culture assay for 3-dimensional ECM, we identified that ECM retention of TSP-1 is mediated by its highly conserved C- terminal region in trimeric form, and not by C-terminal monomer, or trimers of the amino-terminal domain or type 1 repeats. ECM retention of C-terminal trimers involves a novel site in the L-lectin domain. Beta1 integrins are dispensible for ECM retention, but enhance activity. The status of actin cytoskeletal organisation also impacts on the ECM incorporation of TSP-1. We also identified that ECM retention is a evolutionarily-conserved property of TSPs from vertebrates, Ciona intestinalis and Drosophila melanogaster. To

understand TSP-ECM interactions with reference to a systems biology context, we have analysed multiple metazoan genome sequences for encoding of TSPs. These studies have established that TSPs are of early evolutionary origin within the metazoa and the gene family has expanded in the deuterostome and vertebrate lineages. This expansion was accompanied by functional diversification, in parallel with conservation of the ECM retention activity of the TSP C-terminal region.

21

COMP-specific Ribozyme: A New Strategy for Gene Therapy

Joseph L. Alcorn[1], Thomas M. Merritt[1], Mary C. Farach-Carson[2], Huiqui H. Wang[1], Jacqueline T. Hecht[1,3].
[1]University of Texas Medical School, Houston, TX, [2]University of Delaware, Newark, DE, [3]Shriners Hospital for Children, Houston, TX

Dominant-negative mutations in the homopentameric extracellular matrix glycoprotein cartilage oligomeric matrix protein (COMP) result in inappropriate intracellular retention of misfolded COMP in the rough endoplasmic reticulum of chondrocytes, causing chondrocyte cell death which leads to two skeletal dysplasias: pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (EMD1). However, COMP null mice show no adverse effects on normal bone development and growth, suggesting a strategy for therapy involving removal of COMP from the secretory pathway. The goal of this study was to assess the ability of a unique hammerhead ribozyme (Ribo56) designed against a specific COMP mutation (D469del) to reduce or eliminate COMP mRNA expression in chondrocytes. In transfected COS7 cells, Ribo56 significantly reduced overexpressed normal and mutant COMP mRNA in a dose-dependent manner and reduced mutant COMP protein expression by 45%. Using recombinant adenoviruses as a delivery system, Ribo56 significantly reduced expression of COMP mRNA in a dose-dependent manner by up to 50% in normal human costochondral cells (hCCC). In hCCCs containing COMP mutations known to cause PSACH (D469del, G427E, and D511Y) Ribo56 was significantly more effective at reducing mutant COMP mRNA (up to 70%) than normal COMP mRNA when assessed at several dosages. These results indicate that the Ribo56 ribozyme is effective at reducing mutant COMP levels in cells and suggests a possible mode of therapy to reduce the mutant protein load.

22

Regulation of fibronectin matrix assembly by tenascin-C

Wing S. To, Hideaki Nagase, Kim S. Midwood.
Kennedy Institute of Rheumatology, Imperial College, London, UK, W6 8LH

Fibronectin is a ubiquitous component of the extracellular matrix. Its assembly into 3D fibrillar matrices is essential during development and tissue repair to maintain tissue architecture and provide environmental signals to cells. Fibronectin deposition must be tightly regulated as excessive assembly is a major hallmark of fibrotic diseases and cancer. Tenascin-C is a large, multi-domain glycoprotein that is specifically expressed at sites of tissue remodelling. It is transiently induced during development and tissue repair, where it co-localizes with newly synthesized fibronectin fibrils, but is persistently expressed in fibrotic disease and cancer. We thus investigated the interactions between tenascin-C and fibronectin and the effect of tenascin-C on fibronectin assembly. We found that specific tenascin-C fibronectin type III repeats 1-8 (TNfn(1-8)), TNfn(1-3), TNfn(6-8) and the C-terminal fibrinogen globe (FBG) inhibited fibronectin matrix assembly, whilst full-length tenascin-C did not. We also found that TNfn(1-3) was essential for binding to fibrillar but not soluble fibronectin. Recombinant tenascin-C domains containing this region interfere with intermolecular fibronectin interactions during fibrillogenesis. Tenascin-C domains without these regions (i.e., TNfn(6-8) and FBG) inhibit fibrillogenesis without binding to fibronectin, possibly via interactions with cell surface receptors. These data suggest that proteolysis of tenascin-C during tissue remodelling limits fibronectin assembly. Persistent tenascin-C expression observed during conditions such as fibrosis and tumor growth may contribute to uncontrolled fibronectin deposition.

23

DMP1 Isoforms Promote Differential Cell Attachment and Migration

Zofia von Marschall, Larry W. Fisher.
CSDB, NIDCR, NIH, DHHS, Bethesda, MD

Dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), and osteopontin (OPN), are small integrin-binding glycophosphoproteins (SIBLINGs) co-expressed/secreted by skeletal and active ductal epithelial cells. Although the mechanisms remain unclear, DMP1 is the only one of these three SIBLINGs known to have mutations resulting in human disease and yet it remains the least studied. All three contain the conserved integrin-binding tripeptide RGD, and experiments comparing the cell attachment and haptotactic migration-enhancing properties of DMP1 to BSP and OPN were performed using human skeletal (MG63 and primary dental pulp cells) and salivary gland (HSG) cells. Mutation of any SIBLING's RGD destroyed all attachment and migration activity. Using its αVβ5 integrin, HSG cells attached to BSP but not to DMP1 or OPN. However, HSG cells could not migrate onto BSP. Expression of αVβ3 integrin enhanced HSG attachment to DMP1 and OPN and promoted haptotactic migration on all three. Interchanging the first four coding exons or the conserved amino acids adjacent to the RGD of DMP1 with corresponding sequences of BSP did not enhance DMP1's ability to bind to αVβ5. For αVβ3-expressing cells, intact DMP1, its BMP1-cleaved C-terminal fragment, and exon six lacking all post-translational modifications worked equally well but the proteoglycan isoform of DMP1 had greatly reduced ability for cell attachment and migration. The sequence specificity of DMP1's proposed BMP1-cleavage site was verified by mutation analysis. The differences among the SIBLINGs may lead to explanations as to why neither OPN nor BSP is able to compensate for the loss of DMP1 activity in the disorders.

24

Modeling single and collective cell movements in 3D matrices

Muhammad H. Zaman.
Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX 78712

Directed and stochastic cell migration models have focused nearly exclusively on cell movements on artificial 2D substrates. These substrates are not only far from in vivo and introduce artificial polarities, but also make it impossible to quantify the cooperative roles of matrix structure, mechanics and proteolysis in invasion. In order to address these critical deficiencies in our understanding of in vivo cell motility, we have developed a series of multi-scale models to probe various modes that exist in single and collective cell migration. Our models incorporate the mechanical and biochemical properties of the matrix, the dynamics of adhesion receptors on the cell surface, matrix-based inside out signaling and the effects of matrix degradation and remodeling. Our models allow us to study migration as a function of cell and matrix density, thereby enabling us to make a series of predictions in a variety of cancer systems. Results from our studies indicate individual and collective cell migration respond differently to matrix stiffness and matrix pore sizes. We also observe that the extent of migration of an individual cell in a high-density environment varies non-linearly with cell number. Interestingly, matrix mechanical properties play a more significant role in migration of individual cells than in collective cell migration. However, matrix mechanical properties are critical in regulating cell speed and persistence in both single and collective cell movements. Results from our show good agreement with existing experimental results, while at the same time, make a series of novel testable predictions for future experiments.

25

A Discrete Mathematical Model Simulates In Vitro Angiogenesis

Scott C. Sibole, Clayton J. Underwood, James E. Guilkey, Jeff A. Weiss.
University of Utah

Experimental investigation of the effects of mechanical factors on growth of microvascular networks is hindered by a lack of knowledge of the local mechanics in the vicinity of the vessel sprout. Computational models can potentially provide additional insight. The objective of this

research was to develop a discrete model to describe angiogenesis in vitro and to study the effects of extracellular matrix (ECM) fiber orientation and initial vessel density. Experimental growth data were gathered from 3D vessel cultures (N=5) imaged on culture days 1-7. Numerical simulations were conducted on a 2D grid, and ECM fiber orientations and local vessel densities were stored at grid nodes. Vessels, represented as discrete segments, were distributed randomly in the domain. Growth occurred via addition of segments to existing vessels, the length of each determined by a growth rate curve fit to experimental data. Growth direction was governed by local ECM orientation and density. Branching occurred stochastically in space, based on a constant that was optimized to match experimental data. Validation was performed through morphometric comparisons of predicted vessel length and branching with experimental data. The discrete angiogenesis model provided a good description of experimentally measured branching and vessel length, with RMS errors of 13% and 1%, respectively. Future work will couple this discrete model to an existing continuum mechanics model employing the Material Point Method, allowing the replacement of stochastic components of the angiogenesis model by mechanical stimuli. This will provide a framework for predicting the synergistic effects of mechanical factors with angiogenesis.

26

The glycosaminoglycan-binding domain of CXC-chemokines controls neutrophil migration into the lungs

Yoshi Tanino[1], Deirdre R. Coombe[2], Warren C. Kett[2], Sean E. Gill[1], Thomas R. Martin[1], Charles W. Frevert[1].
[1]Center for Lung Biology, University of Washington Seattle, WA, [2]Curtin University, Perth, Australia

Chemokine-glycosaminoglycan (GAG) interactions are thought to result in the formation of tissue-bound chemokine gradients. We hypothesized that the stable binding of chemokines to GAGs would increase neutrophil migration towards CXC-chemokines instilled into lungs of mice. To test this hypothesis we compared neutrophil migration towards recombinant human CXCL8 (rhCXCL8) and two forms of CXCL8, which show loss of GAG binding due to point mutations in the GAG-binding domain. Unexpectedly, mutant-CXCL8 recruited more neutrophils into the lungs and appeared in plasma at significantly higher concentrations. A comparison of the murine CXC-chemokines KC and MIP-2 revealed that KC was more effective in recruiting neutrophils into lungs and KC appeared in plasma at significantly higher concentrations. To model the diffusion of CXC-chemokines in lungs, the diffusion of CXC-chemokines was measured across an extracellular matrix *in vitro*. These studies show that mutant forms of CXCL8 and KC diffused more rapidly across an extracellular matrix *in vitro* when compared to rhCXCL8 or MIP-2. Next, kinetic binding studies were performed using heparin to model the molecular interactions between chemokines and GAGs in tissue. These studies show differential binding of KC, MIP-2, and rhCXCL8, with KC associating and dissociating more rapidly from immobilized heparin. In contrast, CXCL8 and MIP-2 have slower kinetics of binding to heparin. This suggests a new model whereby chemokine-GAG interactions control the spatial-temporal formation of chemokine-gradients in tissue, which results in different types of chemokine-gradients forming during an inflammatory response.

27

EGF-like domains of stabilin-2 recognizes PS during phagocytosis

Soyoun Kim[1], Seung-Yoon Park[2], Mi-Yeon Jung[1], Dong-Jun Bae[1], In-San Kim[1].
[1]Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu, 700-422, Korea, [2]Department of Biochemistry, School of Medicine, Dongguk University, Kyungju, 780-714, Korea

Exposure of phosphatidylserine (PS) on the cell surface occurs early during apoptosis and serves as a recognition signal for phagocytes. Clearance of apoptotic cells by a membrane PS-receptor is one of the critical anti-inflammatory functions of macrophages. However, the PS binding receptors and their recognition mechanisms have

not been fully investigated. Recently, we reported that stabilin-2 is a PS-receptor that mediates the clearance of apoptotic cells, thus releasing the anti-inflammatory cytokine, TGF-α. In this study, we showed that EGF-like domain repeats (EGFrp) in stabilin-2 can directly and specifically recognize PS using cell competition assay and fluorescence quenching assay. The EGFrps also competitively impaired apoptotic cell uptake by macrophages in in vivo models. We also showed that calcium ions are required for stabilin-2 to mediate phagocytosis via EGFrp. Interestingly, at least four tandem repeats of EGF-like domains were required to recognize PS and the second atypical EGF-like domain in EGFrp was critical for calcium-dependent PS-recognition. Considering that PS itself is an important target molecule for both apoptotic cells and nonapoptotic cells during various cellular processes, our results should help elucidate the molecular mechanism by which apoptotic cell clearance in the human body occurs and also have implications for targeting PS externalization of nonapoptotic cells.

28

Intact hyaluronan promotes maintenance of immune tolerance.

Paul L. Bollyky, Benjamin Falk, Buckner H. Jane, Evanko P. Stephen, Thomas T. Wight, Gerald T. Nepom.
Benaroya Research Institute, Seattle, WA 98103

The inflammatory milieu is thought to play a dominant role in the generation and regulation of adaptive immune responses. However the regulatory contributions of the extracellular matrix (ECM) components responsible for much of the physiology of inflammation are poorly understood. We have recently demonstrated (J Immunol. 2007 179(2):744-7) that the concentration and size of hyaluronan (HA), a prominent ECM component, substantially influences human TR. We observed that intact high-molecular weight HA (HMW-HA) promotes TR mediated suppression while low-molecular weight HA (LMW-HA), generated during injury and infection, does not. Herein we explore the mechanistic basis of these observations. We find that HMW-HA treatment promotes expression of FoxP3, a critical transcriptional regulator associated with TR. HMW-HA also promotes TR viability in settings of low IL-2. These effects are mediated by cross-linking of CD44. Consistent with this, TR from CD44 knock-out mice demonstrate functional impairment ex vivo. In explanation of these findings we find that CD44 cross-linking is a major co-stimulatory signal for IL-2 production by CD4+ T-cells, particularly at low-levels of TCR stimulation. CD44 cross-linking also promotes T-reg function via production of IL-10 and cell-surface TGF-β. Consistent with these findings T-reg from CD44 knock-out mice demonstrate impaired regulatory function ex vivo. These data reveal a novel role for CD44 cross-linking in the production of regulatory cytokines. We propose that intact ECM promotes immunologic tolerance in uninjured or healing tissue.

29

FN-α4β1 Interactions Regulate MMP-9 Expression in Liver IR Injury

Sergio M. Duarte, Takashi Hamada, Aline Mouradian, Ana J. Coito.
DUMONT-UCLA Transplant Center

Expression of fibronectin (FN) by sinusoidal endothelium is an early event after liver ischemia/reperfusion (I/R) injury. We test the effect of CS1 peptide mediated blockade of FN-α4β1 interactions in liver I/R Injury. **Methods and Results:** CS1 peptides were administrated through the portal vein of SD rat livers before and after 24h of cold storage (500 & μg/rat). Recipients of orthotopic liver transplants (OLTs) received an additional dose of peptides 1h post-OLT. CS1 therapy increased the OLT survival as compared to controls ($p<0.005$). CS1 peptides improved liver function as evidenced by the reduced sGOT levels (U/L) (1413±420 vs. 2866±864 $p<0.008$) at 6h. CS1 treated grafts showed a profound decrease in T (30±3 vs. 83±15, $p<0.0002$), NK (16±3 vs. 30±8, $p<0.001$), and ED1 (35±7 vs. 57±16, $p<0.0003$) cell infiltration. Leukocyte migration is dependent upon adhesive and focal matrix degradation mechanisms. Metalloproteinase-9 (MMP-9), expressed by infiltrating leukocytes, was depressed in CS1 treated OLTs at mRNA (0.33±0.19 vs. 1.13±0.22, $p<0.005$) and protein (0.15±0.07 vs. 0.7±0.14, $p<0.03$) levels. MMP-9

activity was reduced by 3-fold in the CS-1 grafts. Interestingly, phosphorylation of p38 MAPK (0.06±0.01 vs. 0.27±0.12, $p<0.02$) was reduced in the CS-1 treated livers. Moreover, treatment of macrophages plated on FN with a p38 MAPK inhibitor, decreased MMP-9 activity by ~70%. **In conclusion**, our work supports a regulatory role for FN-α4β1 interactions on MMP-9 expression associated with elevated leukocyte infiltration in I/R injury

30

Influences on the Angiogenic Response in ECM-Based Biomaterials

Amy M. Overby, Chad E. Johnson.
Research Department, Cook Biotech, Inc., West Lafayette, IN 47906

Porcine-derived small intestinal submucosa (SIS), an ECM-based biomaterial, has been used for surgical repair in humans for over 150,000 implants world wide. Angiogenesis is important for the long term success of extracellular matrix based biomaterials. SIS has been thoroughly studied for its composition, structure, and ability to remodel *in vivo*. Consisting primarily of fibrillar collagen, SIS contains other structural proteins, growth factors, glycosaminoglycans, and proteoglycans. Specifically, SIS contains collagens I, III, IV, and VI, fibronectin, hyaluronic acid, various sulfated GAGs, FGF-2, CTGF, and TGF-β. SIS is made into a clinical grade biomaterial through secondary drying processes that can leave the material open and porous or compressed and dense. The present study investigated the effect of composition and structure on angiogenesis in an *in vivo* murine implant model. The role of composition was investigated by comparing intact SIS to chemically stripped SIS. Fluorescent microangiography showed reduced angiogenic response in the chemically stripped SIS indicating decreased vessel ingrowth (<1mm for stripped vs. ~3mm for intact). The role of structure was investigated by comparing an open, porous SIS to compressed SIS. Fluorescent microangiography showed reduced vascular penetration in the compressed SIS (~1/3mm for compressed vs. ~3mm for open, porous SIS). Understanding the factors that promote angiogenesis into extracellular matrix based biomaterials will be beneficial in the development and design of the materials. This information will have significant influence on this emerging area of biomaterials.

31

TGFBIp/ βig-h3, an Endogenous Anti-angiogenic Molecule

Hye-Nam Son, Ju-Ock Nam, In-San Kim.
Cell and Matrix Research Institute, Department of Biochemistry and Cell Biology, Kyungpook National University School of Medicine, Daegu, Korea

TGFBIp/ βig-h3 is an extracellular matrix protein is highly induced by TGF-β has several motifs, which can interact with different sets of integrins such as α3β1, αvβ3 and αMβ2. We previously identified a motif containing FAS1 domain that can interact with endothelial αvβ3 integrin and have anti-angiogenic activity*in vitro* and *in vivo*. In this study, we showed that βig-h3 containing one EMI domain, 4 FAS1 domains and one RGD motif, had about 170-fold higher binding affinity ($Kd = 9.78 \times 10^{-8}$ M) for αvβ3 integrin than the one FAS1 domain (fastatin). The high binding affinity of βig-h3 was possibly due to the 4 FAS1 domains present in them.Furthermore, βig-h3 was able to elucidate high anti-angiogenic and anti-tumorigenic properties at concnetraion 100-fold less concentration than the effective dose of fastatin. In *In vivo* studies to confirm the function of βig-h3 in relation to tumor growth was carried out using transgenic mice that can constitutively over express βig-h3. Increase in the blood level of βig-h3 by 2.7-fold subsequently lead to 3-fold decrease in tumor growth in transgenic mice when compared to normal mice. The systemic treatment of transgenic mice with human βig-h3 antibody reversed the condition with elevation of tumor growth, possibly due to the neutralization of βig-h3 by the antibody. Taken together, our study proved that βig-h3 acts a potent endogenous anti-angiogenic and anti-tumorigenic molecule through avβ3 integrin interaction and thereby emphasize the importance of physiologic level of βig-h3 in inhibiting tumor growth.

32

Histidine-rich Glycoprotein Modulation of Vasculostatin

Philip A. Klenotic[1], Maria Febbraio[1], Roy L. Silverstein[1], Erwin G. VanMeir[2].
[1]Department of Cell Biology, Cleveland Clinic, Cleveland Ohio, [2]Emory University, Atlanta, Georgia

The human vasculature is normally kept in a quiescent state through a precise maintenance of balance between angiogenic inducers and inhibitors. During conditions of tumor development, however, this balance is disrupted and abnormal cellular growth is observed. Brain angiogenesis inhibitor 1 (BAI1) is a membrane protein expressed on glial cells within the brain. It has been shown to function in vivo to inhibit angiogenesis and tumor growth. It contains a large extracellular domain (termed vasculostatin) that can be cleaved from the cellular membrane and potentially bind to endothelial cells, however, a receptor for this process has not been identified. Our lab, in collaboration with Bouck and colleagues, identified CD36, a transmembrane glycoprotein on capillary endothelial cells, as a receptor that mediates antiangiogenic activity. Our laboratory has also identified a circulating protein, histidine-rich glycoprotein (HRGP) that shares significant homology with CD36. Through in vitro binding, in vivo cellular migration and tube formation assays, we show that CD36 is indeed the endothelial cell receptor for vasculostatin and that HRGP serves as a soluble "decoy" receptor and thereby inhibits the antiangiogenic activity of BAI1. Tumor growth in mice was dramatically increased when HRGP was introduced into tumor cells that contain vasculostatin when compared to tumors with vasculostatin alone. This result shows that HRGP is an important modulator of BAI1 within the tumor environment and the BAI1-CD36-HRGP interactions potentially can be exploited in future tumor therapies.

33

Chondrostatin Inhibits Angiogenesis

Zhepeng Wang[1], Jennifer Bryan[1], Brian J. Ell[2], Allan C. Rapraeger[2], Linda Sandell[1].
[1]Washington University, [2]University of Wisconsin

Cartilage is antiangiogenic and resistant to tumor invasion. There has been a good deal of research aimed at isolating inhibitors from cartilage , but the mechanism for the lack of vascularity of cartilage is still unknown. Chondrostatin (chon) is a fragment from type II procollagen. It is removed from procollagen prior to formation of collagen fiber in ECM. Chon contains an RGD sequence that is conserved across species, indicating potential biological function. We showed previously that the integrins αvβ3 and αvβ5 mediate cell adhesion to chon. Three different assays were performed to determine effects of chon on angiogenesis. In the tube formation assay, recombinant chon, but not the mutated chon, inhibited HUVEC cell tube formation in a dose-dependent manner: 0.1 μM of chon suppress tube formation by 50% of the control. In the aortic ring assay, chon, but not the mutated chon, inhibited FGF-stimulated microvessel outgrowth and the inhibition is dose-dependent. In the mouse corneal assay, chon, but not the mutated chon, inhibited vascularization as seen in the fluorescence-labeled mouse cornea. These results suggested that chon is an angiogenesis inhibitor that is dependent on the RGD motif. Since microvascular endothelial cells, which are recruited by tumors, have become an important target in tumor therapy, chon may be a potential antitumor agent. In addition, chon is liberated from the collagen molecule during biosynthesis, reaching the highest level during cartilage formation. As we show it inhibits angiogenesis, chon is an excellent candidate for the molecular mechanism by which cartilage is avascular.

34

Small molecule inhibitors of collagen-induced angiogenesis

Kari Habursky[1], Renato V. Iozzo[2], Kevin Turner[1], Michelle Burrows[2], Sung-wook Choi[3], Soni Basra[3], Joel S. Bennett[4], William F. DeGrado[3], James D. San Antonio[1].
[1]Dept Med, Thom Jeff U, Phil, PA, [2]Dept Path, Anat, Cell Biol, Thom Jeff U, Phil, PA, [3]Depts Chem, Biochem & Biophys, U PA, Phil, PA, [4]Dept Med, U PA, Phil, PA

Type I collagen induced angiogenesis requires ligation of collagens GFPGER sequence to the endothelial cell α2β1 integrin. We investigated

the effects of anti-α2β 1 integrin small molecule inhibitors (SMI) designed to disrupt integrin α2I- or β1I-domain function on angiogenesis. In control endothelial cells, fibrillar collagen induced widespread capillary tube formation by 12 h. In contrast, tube formation was reduced by ~70% only SMI 496, α β1 integrin A- domain inhibitor, and by function blocking anti-α2β1, but not α1β1 integrin antibodies. By flow cytometry, endothelial cells bound fluorescein-type I collagen, an interaction specifically inhibited (~65%) by SMI 496. Moreover, endothelial cells cultured on collagen and phalloidin-stained for filamentous actin were well-spread with abundant stress fibers, but SMI 496 caused cytoskeletal collapse, and delayed endothelial monolayer wound healing in vitro. SMI activities were examined on zebrafish (Danio rerio) embryos expressing green fluorescent protein under control of the vascular endothelial growth factor receptor 2 promoter. SMI 496, but not a control SMI interfered with angiogenesis by reversibly inhibiting sprouting from the axial vessels. Thus, an inhibitor of the integrin β1I-domain disrupts endothelial cell-collagen interactions and collagen-induced capillary morphogenesis and may represent a novel therapy to combat pathological angiogenesis in vivo.

35

MMP14 and TIMP regulation of angiogenesis in aortic ring cultures

Alfred C. Aplin[1], Eric Fogel[2], Roberto F. Nicosia[1,2].
[1]Department of Pathology, University of Washington, Seattle, WA, [2]VA Medical Center, Seattle, WA

The purpose of this study was to define the role of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in vascular regression following angiogenesis. Formation of new blood vessels in collagen-gel cultures of rat and mouse aortic rings is associated with collagen lysis and production of MMPs including membrane type 1-MMP (MMP14). By immunohistochemistry, high levels of MMP14 are detected at the tips of sprouting neovessels. Inhibition of MMP14 with specific antibodies blocks angiogenic sprouting in the aortic ring assay. Antiangiogenic effects are also obtained with recombinant TIMP -2, -3, and -4 which block the activity of MMP14 but not with TIMP-1, a poor inhibitor of MMP14. All four TIMPs are expressed in collagen gel cultures during angiogenesis, with increasing levels during the vascular regression phase. Interestingly, TIMPs and anti-MMP14 antibodies stabilize the neovasculature and allow vessels to survive longer than untreated controls if administered after the angiogenic growth phase. Conversely, vascular regression is accelerated in aortic cultures from TIMP-1 and TIMP-2 deficient mice. TIMPs with anti-MMP14 activity are more potent than TIMP-1 in preventing vascular regression. The vascular survival effect of these TIMPs is associated with complete inhibition of collagen lysis whereas TIMP-1 has no anti-collagenolytic effect. These results indicate that MMP14 plays a critical role in both angiogenesis and vascular regression and demonstrate that TIMPs with anti-MMP14 activity have opposite effects on angiogenesis depending on the stage of the angiogenic process.

36

ECM-ligand functionalized fibrillar peptides

Jangwook P. Jung, Joel H. Collier.
Department of Surgery, University of Chicago, Chicago, IL 60626

Regeneration of epithelial and endothelial cell monolayers after wounding is a complex sequence of events regulated by cell-matrix interactions, soluble growth factors, and the mechanics of the ECM. A major challenge for studying the biology of these processes is creating experimentally controllable microenvironments where a multitude of these factors may be independently and precisely adjusted. Towards this end, self-assembling multi-component biomaterials are increasingly seen as an attractive route. Here we report co-assembling fibril-forming peptides capable of forming hydrogels displaying fibronectin- and laminin-derived sequences. We investigated the secondary structure, morphology, and ligand presentation of the hydrogels' constituent nanofibers with circular dichroism, TEM, and nanogold labeling, respectively, and found that

both RGD and IKVAV were displayed on the surface of the self-assembled fibrils. The attachment and proliferation of human umbilical vein endothelial cells were significantly enhanced by incorporation of the RGD functionalized peptide, a response that was abolished with scrambled peptide controls. In addition, the stiffness and cytotoxicity of the scaffolds did not change with co-assembly of the ligand-presenting peptides, indicating that ligand density could be independently adjusted through simple mixing and co-assembly. Currently, we are systematically adjusting the concentration of each ligand to identify formulations that most effectively lead to rapid monolayer formation. This modular approach provides a route for producing precisely defined synthetic ECMs where the density of multiple ligands may be independently adjusted.

37

Regulation of caspase-3 mediated apoptosis and tumor angiogenesis

Chandra S. Boosani, Dominic Cosgrove, Akulapalli Sudhakar.
Cell Signaling and Tumor Angiogenesis Laboratory, Department of Genetics, Boys Town National Research Hospital, Omaha, NE 68131.

We recently reported α3β1 integrin dependent regulation of cyclooxygenase-2 signaling is specific to α3(IV)NC1 (tumstatin), in exerting antitumorogenic activities, as a potent inhibitor of angiogenesis. Here we show the binding of α3(IV)NC1 to α3β1/αVβ3 integrins and initiating cellular apoptosis by activating caspase-3, cleavage of poly (ADP-ribose) polymerase and eukaryotic translation initiation factor-2. Upon treatment with caspase-3 inhibitor, α3(IV)NC1 induced cellular apoptosis was remarkably inhibited. Interestingly α3 or β3 integrin null endothelial cells showed sustained caspase-3 activation upon treatment with α3(IV)NC1. Further, the flow cytometric analysis using integrin proteins α3β1/αVβ3 and α3(IV)NC1 in competition experiments with α3 and β3 integrin null endothelial cells showed abrogation of caspase-3 activation. In vitro studies using anti-α3β1/αVβ3 integrin antibodies showed increased apoptosis in endothelial cells, interestingly addition of α3(IV)NC1 further enhanced these antibodies induced apoptosis. Teratocarcinoma induced tumors in 129Sv mice showed increased apoptotic TUNEL positive microvasculature in tumors upon α3(IV)NC1 treatment, indicating inhibition of tumor angiogenesis and tumor growth. Further, the antitumor activity of α3(IV)NC1 was abrogated when caspase-3 inhibitor was used, protecting the tumor vasculature during apoptosis. These results unearth additional properties of α3(IV)NC1 as an endogenous angiogenesis inhibitor, inducing apoptosis in vitro and in vivo by activating integrins mediated apoptosis signaling.

38

Ras up-regulates COL1A2 transcription through Smad2/3

Silvia Smaldone, Magdalini Kypriotou, Francesco Ramirez.
Department of Pharmacology, Mount Sinai School of Medicine, NY, NY 10029

It has been reported that auto-antibodies against PDGFR stabilize Ras through ROS stimulation and ultimately promote tissue fibrosis in Scleroderma. This study explored the molecular connection between Ras stabilization and collagen I production using NIH-3T3 fibroblasts and primary human dermal fibroblasts (HDF) as experimental models and the proximal promoter of COL1A2 as the transcriptional read-out. We found that transient over-expression of constitutively active Ras (ca-Ras) increases COL1A2 promoter activity, and that neither TGFβ antagonism or ALK5 inhibition can reverse this transcriptional stimulation. By contrast, over-expression of dominant negative Smad3 or mutations in the Smad3-binding site of the COL1A2 promoter abrogated Ras-induced stimulation of COL1A2 transcription. These results strongly suggested a causal relationship between Ras stabilization, Smad2/3 activation and COL1A2 up-regulation in Scleroderma. To further support this notion we created NIH-3T3 cell clones that were stably transfected with a Tet-inducible Ras-expressing plasmid, and which therefore mimic Ras stabilization in Scleroderma fibroblasts. Consistent with the above observations, Tet-induced expression of Ras resulted in a rapid phosphorylation of

Smad2/3 and in a significant accumulation of COL1A2 transcripts. Interestingly, pre-treatment of the Ras inducible cell clones with an ALK5 inhibitor decreased but did not prevent Smad2/3phosphorylation and COL1A2 mRNA accumulation suggesting that Ras stabilization increases Smad2/3 signaling in part through an ALK5 independent mechanism.

39

CDP/cux is capable of strong inhibition of the COL1A2 gene

Maria Fragiadaki[1], Tetsuro Ikeda[1], David Abraham[2], George Bou-Gharios[1].
[1]Department of Medicine, Imperial College London, Hammersmith Campus, London W12 0NN, [2]Centre for Rheumatology, University College, Royal free campus, London NW3, [3]The Kennedy Institute of Rheumatology, Imperial College London, London W6, UK

Fibrosis is characterized by excessive accumulation of extracellular matrix (ECM). Type I collagen, a major ECM component, is in large part responsible for the functional alterations of organs in affected individuals. We have previously identified a cis-acting sequence that COL1A2 enhancer expression in fibroblasts. In this study, we characterised a novel repressor element within this promoter/enhancer and demonstrate its role in switching off type I collagen. In silico analyses identified CDP/cux as a potential negative regulator of the COL1A2 gene. Over expression of CDP was accompanied by a 2 fold decrease of COL1A2 mRNA levels, suggesting a significant role of CDP in switching off type I collagen. We performed DNA-protein binding assays (EMSA) and we showed that the CDP has the ability to bind to the COL1A2 promoter and thus silence COL1A2 expression. Type I collagen, which is the most abundant extra cellular matrix protein of the human body, is regulated tightly by a number or nuclear factors. This is the first report to link type I collagen regulation with CDP expression. CDP is a transcription factor that has been shown to be upregulated in response to TGF-β treatment (Mitchl P et al, cancer cell, 2006). This study is of particular interest since it suggests that TGF-β signaling may have compensatory effect which could be used for therapeutic purposes and as a method to switch off type I collagen. This work has been funded by Raynauds and Scleroderma Association and Kidney Research Campaign.

40

Key Cis-acting sequences that regulate the Human Aggrecan Gene

Andrea N. Layyous, Jerry Saklatvala, George Bou Gharios.
Kennedy Institute of Rheumatology, Imperial College London, London W6 8LH, UK

Aggrecan, a major structural component of cartilage, is a large aggregating chondroitin sulphate proteoglycan. Our understanding of the transcriptional regulatory mechanisms that govern the chondrocyte-specific expression of this gene is limited. In this study, we used the recombineering method to clone < 35kb of non-coding human genomic DNA into expression vectors with beta-galactosidase reporter transgenes. Transgenic embryos at E14.5 were stained to visualise expression of the transgene in chondrocytes. A ~20kb segment, including the first untranslated exon of the gene, was able to drive cartilage specific expression of the LacZ reporter gene in vivo. The expression was located exclusively in the developing cartilage of the long bones (hind and forelimbs), the ribs, digits as well as some craniofacial cartilages. We have further minimised this sequence to highlight the essential sequences within the promoter and the first intron of the gene that govern aggrecan expression during development. Further studies are underway to characterise the critical trans acting proteins that are necessary for cartilage-specific expression of the aggrecan gene.

41

Articular cartilage gene expression during MSC differentiation

Deepa Edwin[1], Li Liang[1], Masahiro Iwamoto[2], Audrey McAlinden[1].
[1]Washington University, St Louis, MO, [2]Thomas Jefferson University, Philadelphia, PA

Introduction: Current strategies to induce chondrogenesis of mesenchymal stem/stromal cells (MSCs) in vitro results in a phenotype resembling growth plate chondrocytes rather than articular chondrocytes. Methods to induce

articular chondrocyte differentiation will be vital for the field of cartilage tissue engineering. Methods: Human bone marrow-derived MSCs (BMSCs) were cultured in high density pellets with TGF-b3 for periods up to 28 days. Semi-quantitative or real-time PCR was used to analyze cartilage gene expression, including genes specific to articular cartilage: PRG-4/lubricin, cartilage intermediate layer protein (CILP), Tenascin-C (TN-C) and transcription factor (TF) ERG. Cartilage gene expression in human chondrocytes (TC28 cells) was examined after transient transfection of ERG expression constructs. Results: Expression levels of COL2A1, aggrecan and COL10A1 increased during the 28-day induction period while levels of the osteoblast TFs, Runx2 and Osterix, were also detected. TN-C expression gradually decreased over time. PRG-4 and CILP expression was only detected at early time points of differentiation. Real-time PCR showed high ERG expression at day 11 and expression decreased thereafter. Three isoforms of ERG were detected in human MSCs and preliminary results showed that ERG (full length and -81bp splice forms) increased expression of TN-C in human chondrocytes. Conclusion: TGF-b3 induction of human BMSCs in high density pellet cultures does not promote an articular chondrocyte phenotype. ERG may be a promising candidate to induce articular chondrocyte differentiation of BMSCs.

42

The role of fibronectin synergy site in regulating alveolar EMT.

Ashley E. Carson, Thomas H. Barker.
Department of Biomedical Engineering at Georgia Tech and Emory University, Atlanta, GA

Fibrosis, a potentially deadly pathology, is characterized by excessive deposition of extracellular matrix leading to the loss of tissue structure and function. In the lung, epithelial-to-mesenchymal transition (EMT) has been implicated in the progression of fibrosis. Recent evidence suggests that during fibrotic progression Type II alveolar epithelial (ATII) cells are driven toward a fibroblastic phenotype, theoretically increasing the number of synthetic mesenchymal cells depositing ECM. We hypothesize that the fibronectin (Fn) synergy site is critical to the regulation of EMT by directing integrin specificity. We have created recombinant fragments of Fn which display "stabilized" synergy and RGD (Fn9*10) or RGD alone (Fn10). Primary ATII cells were isolated and cultured on the fragments and cell phenotype analyzed by PCR and western blotting. Several genes associated with EMT are up-regulated in cells cultured in the absence of the synergy site including periostin, TGFβ, vitronectin, and several procollagens and MMPs. Early (48 hour) expression levels of E-cadherin (epithelial marker) as well as alpha-smooth muscle actin and vimentin (mesenchymal markers) indicate a shift toward a mesenchymal phenotype on Fn10 compared to Fn9*10. Attachment assays with function-blocking antibodies indicated that α3-integrin appears critical for ATII cell attachment to Fn9*10 but not Fn10, suggesting that integrin α3β1 binding to Fn may have a protective effect in the early onset of EMT. These studies indicate that, contrary to reports that Fn constitutively induces EMT, Fn-induced EMT may result from a decoupling of the synergy site from RGD.

43

Changes in ECM in High/Low/Normal Oxygen in 3D Cultures

Emmett Pinney[1], Frank Zeigler[1], Gail Naughton[1], Amy D. Bradshaw[2], Robert Kellar[1].
[1]Histogen Inc., San Diego, CA, [2]Dept. of Medicine, MUSC, Charleston, SC

We have seen significant changes in extracellular matrix (ECM) protein production by neonatal dermal fibroblasts in response to variations in oxygen tension. RNA gene chip analysis of cells grown in low, normal, and high oxygen demonstrated that transcriptional regulation of many ECM proteins produced by fibroblasts in a 3D nylon mesh were sensitive to oxygen tension. In low oxygen, elevated levels of collagens I, III, IV, V, VII, and XIVa were observed as well as increases in SPARC, thrombospondin, fibronectin and tenascin. Western blot analysis confirmed increases in protein levels of ECM proteins in low oxygen. Collagen quantification indicated an increase in protein deposition in low oxygen, however to a lesser extent than that demonstrated by gene expression analysis. Proteins indicative of

procollagen processing of propeptides were also increased in low oxygen. The differences between normal and high (28-30%) oxygen were not as significant as those seen in low oxygen. These data are consistent with changes observed between embryonic and adult ECM, suggesting that this culture system might be replicating "embryonic-like" conditions, similar to that seen during fetal development. These data support a manufacturing system designed to deliver low oxygen environments to improve output of these valuable ECM materials for a number of biomedical uses. Specifically, this ECM material has been successfully used as a raw material to surface coat commonly used biomaterials. The ability to surface-coat currently used biomaterials or medical devices, may help to improve clinical performance and patient outcomes.

44

Global TNF-alpha regulated gene expression in chondrocytes

Jason S. Rockel, Suzanne M. Bernier, Andrew Leask.
CIHR group in Skeletal Development and Remodeling, University of Western Ontario, London, ON, CA, N6A 5C1

Compared to normal joints, TNF-α levels are increased in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis. TNF-α regulates the expression of selective cartilage matrix genes including Col2a1, Agc1 and Hapln1 through the activation of downstream the ERK/MAPK pathway. However, the contribution of the ERK/MAPK signaling pathway to the global changes in gene expression induced by TNF-α has not been described. We identified that the TNF-α-activated ERK/MAPK pathway is primarily responsible for regulating the shift in chondrocyte gene expression profile to a catabolic phenotype by increasing transcripts of specific matrix metalloproteinases (MMP), including MMP-9 and MMP-12 and decreasing transcripts of selective cartilage matrix genes, including Col2a1, Hapln1 and Agc1. We determined that the activity of downstream transcription factor targets of TNF-α signaling, including NF-κB and Sox9, were independent of MEK1/2 activity. Promoter analysis of the rat Col2a1 and Agc1 genes identified overlapping Sp1/Egr-1 binding sites. Egr-1 DNA binding, but not nuclear localization was increased by TNF-α in a MEK1/2-dependent manner. Inhibition of TNF-α -induced Egr-1 genomic DNA binding by oligonucleotide decoy determined that Egr-1 is a primary regulator of Col2a1, Hapln1 and Agc1. Thus, Egr-1 activation is required to initiate at least part of the changes in chondrocyte gene expression in response to TNF-α-activated MEK/ERK. Breakdown of TNF-α signaling into its component pathways may uncover new therapeutic targets, such as Egr-1, for treatment of destructive joint disorders.

45

The Protein Arginine Methyltransferase (PRMT5) Associates with Class II Transactivator (CIITA) to Repress Collagen Transcription

Larry Luchsinger, B.D. Smith.
Department of Biochemistry, Boston University School of Medicine, Boston, MA, US, 02118

Wound healing proceeds through a series of overlapping, highly regulated stages with complex interplay of cells, extracellular matrix and soluble mediators to regenerate and remodel tissue. Investigation of collagen synthesis in wound healing has centered on repair, rather than inflammation. We have are characterizing the transcription factors responsible for up-regulation of immunity, which act by inducing major histocompatibility class II (MHC II) while simultaneously down-regulating collagen synthesis. Our published studies on the inflammatory cytokine interferon gamma (IFN) demonstrate that IFN increases expression of regulatory factor for X box 5 (RFX5) and class II transactivator (CIITA) to assemble a co-repressor complex at the collagen promoter. Our studies indicate that CIITA interacts with previously identified co-repressors (Sin3B and HDAC2) in a phosphorylation dependent manner through its proline/serine/threonine (PST) domain. However, the complete repertoire of molecules that aid CIITA in silencing collagen transcription remains unknown. MALDI-TOF mass spectrometry identified a novel protein, protein arginine methyltransferase

(PRMT5), that associates with the PST-domain in co-immunoprecipitation experiments. Interaction of PRMT5 with CIITA was confirmed by reciprocal co-immunoprecipitation of CIITA with FLAG-PRMT5. Methylation assays show that the immunoprecipitates contain methyl transferase activity. PRMT5 has been shown to preferentially symmetrically dimethylates histone arginine residues, H4R3 as well as H3R8, which places it in the rapidly expanding class of chromatin modifying proteins. The role of histone methylation by PRMT5 in gene expression remains elusive. Transfection of PRMT5 with collagen promoters indicates that PRMT5 represses collagen transcription; particularly in the presence of CIITA. Examination of the function of PRMT5 methylation on the collagen locus may lead to further insight into chromatin modification and its contribution in collagen repression.

46

Modulation of Cell Adhesion and Migration by Bcl-2

Christine M. Sorenson, Nader Sheibani.
University of Wisconsin School of Medicine and Public Health

Bcl-2 is the founding member of a family of proteins that regulate apoptosis. During kidney development bcl-2 not only acts as a survival factor but also impacts cell adhesive mechanisms, perhaps through modulation of the composition of the extracellular matrix (ECM) milieu. Our hypothesis is that bcl-2 regulates renal development through modulation of the composition of the extracellular microenvironment impacting cell adhesion, migration, and apoptosis processes. Here we examined the effect lack of bcl-2 has on adhesion, migration, and apoptosis of ureteric bud (UB) and their matured progeny collecting duct (CD)cells, and whether alterations in production of ECM proteins occurs in the absence of bcl-2. In the normal kidney, production of ECM proteins is developmentally regulated. Significant expression of thromobospondin-1 (TSP1) and osteopontin is observed in bcl-2 +/+ UB cells, and later in postnatal day 10 (P10) CD cells. Following renal maturation, the expression of these proteins is significantly down-regulated. Fibronectin expression remains at a modest level throughout kidney development in bcl-2 +/+ cells. In contrast, bcl-2 -/- UB cells and P10 CD cells demonstrated precocious down-regulation of TSP1 and osteopontin. The bcl-2 -/- cells also exhibited increased fibronectin expression, increased cell migration, and decreased adhesion to vitronectin and fibronectin compared to bcl-2 +/+ cells. Bcl-2 +/+ UB and collecting duct cells readily branched in Matrigel and collagen gel, while bcl-2 -/- cells did not undergo significant branching in either matrix protein. Taken together, these data suggest that bcl-2 expression significantly impacts cell adhesive and migratory mechanisms through modulation of ECM composition such that in its absence, branching morphogenesis is compromised both in vitro and in vivo.

47

Integrin-laminin interaction in Kidney Papillae Development

Tetyana L. Vasylyeva[1], Qin Shan[1], Yingjie Liu[1], Charles Szekeres[1], Mary Taglienti[1], Jeffrey H. Miner[2], Jordan A. Kreidberg[1].
[1]Department of Medicine, Division of Nephrology, Children's Hospital Boston, Harvard Medical School, Boston, MA, [2]Renal Division, Washington University School of Medicine, St. Louis, MO

Integrins, as receptors for the extracellular matrix, play a significant role in kidney development. Mice deficient in α 3β1 integrin have abnormal glomeruli due to podocyte damage, and do not survive beyond the birth. The focus of this study was to investigate the role of α3 β 1 integrin in the development of the kidney collecting system. Branching and elongation events were assessed in early kidney morphogenesis in α3 integrin null (KO) and wild type (WT) embryos. An α3 integrin conditional allele was crossed with HoxB7-cre transgenic mice to study postnatal kidney development. To study a role of integrin-laminin(L) interaction, Lama5 transgenic mice were created by knocking out the α subunit in L10, using Pax2Cre in Lama5- flox/null animals. Studies included morphometric analysis, immunohistochemistry for Ki67, TUNEL assay, immunofluorescent staining and measurement of proteinurea. Both tubular

elongation and branching were affected by a loss of α3 β 1 integrin or laminin. The difference in elongation became obvious in experimental embryonic kidneys by E18.5. Ki67 staining showed that the cKO had fewer proliferating cells in the tubular structures than did WT. cKO also had a higher number of apoptotic figures. We conclude that mice with deformed papillae can survive until adulthood under non-stress conditions. α 3β 1 integrin and L10 are important for normal development of collecting tubules, and play a significant role in tubular elongation and branching.

48

Matrilin-1 in the vertebral column of salmon with deformities

Mona E. Pedersen[1], Eva Veiseth[1], Harald Takle[2], Elisabeth Ytteborg[2], Grete Baeverfjord[3], Grethe Enersen[1], Kirsten O. Hannesson[1].
[1]Nofima Food Matforsk AS, N1430 Ås, Norway, [2]Nofima Marine, N1432 Ås, Norway, [3]Nofima Marine, N6600 Sunndalsøra, Norway

Skeletal formation and growth in fish species as Atlantic salmon include intramembranous bone formation and perichondral/periostal ossification in a similar way as in mammals. This involves cartilage and bone cellular activities dependent on interaction with extracellular matrix (ECM) components. In aquaculture farming, development of vertebral deformations occurs. Knowledge of the ECM composition of bone fish as salmon is limited, and a prerequisite to understand mechanisms involved in development of deformities. In this study the composition of proteins, extracted from normal and deform vertebrae, was compared by proteomics, using 2D-gels and Maldi-tof analysis. Deformities were induced by increasing water temperature during early development. A major difference between normal and deform samples was seen in the expression of matrilin-1. Furthermore, mRNA expression studies of matrilin-1 by real-time PCR verified a significant higher expression in the deform samples. In contrast, the mRNA expression of biglycan, another ECM component known to play a major role in bone development of mammals and interacting with matrilin-1, was lower. Immunohistochemistry at light microscopy level, using polyclonal antibody against matrilin-1, demonstrated a strong staining in the chondrocyte area in cryosections of both normal and deform vertebrae. Further studies will be performed in order to elucidate a possible functional role of matrilin-1, being highly upregulated in vertebral column with skeletal deformations.

49

Temporal and spatial expression of collagens in heart valve ECM.

Jacqueline D. Peacock, Joy Lincoln.
Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami FL 33136

Collagens are a major component of heart valve extracellular matrix, distinctly stratified to provide the valve leaflets with the necessary strength and flexibility to provide unidirectional blood flow through the heart. Although collagens have been shown to be important structural building blocks in heart valves, their patterning during valvulogenesis and valve maintenance has not been well defined. We examined the temporal and spatial expression of select collagen genes, including fibril-forming collagen genes *col1a1, col1a2, col2a1, col3a1, col5a1, col5a2, col11a1, col11a2,* and *col27a1*, FACIT collagens *col9a1, col9a2, col12a1* and *col14a1,* and collagen genes *col4a1, col4a2, col8a1, col8a2,* and *col10a1*in the AV valve regions in embryonic and adult mice. Gene transcript levels were examined in the endocardial cushions (E12.5), remodeling valves (E17.5), neonatal valves, and adult valves using TLDA. Of the genes examined, *col1a1, col2a1* and *col3a1* are the most highly expressed in endocardial cushions. Expression of *col1a1, col1a2, col2a1* and *col3a1, and col12a1* are the most highly expressed at E17.5. Maturing valves predominantly express *col1a1, col1a2, col3a1, col5a2, col11a1* and *col12a1*. Localization of highly expressed collagens was verified using immunofluorescence and in situ hybridization. Together, these expression data identify the differential spatial and temporal expression of collagens during heart valve development and maintenance, which likely relate to valve structure, integrity, and function.

50

Col IV NC1-induced disruption of ECM Alters Zebrafish Development

Robert V. Intine[1,3], Billy G. Hudson[2], Michael P. Sarras Jr[3].
[1]Scholl College of Podiatric Med., Rosalind Franklin Univ., N. Chicago, IL, [2]Dept of Med., Vanderbilt Univ., Nashville, TN, [3]Dept of Cell Biol. and Anat.; Rosalind Franklin Univ., N. Chicago, IL

Basement membranes (BM) are sheet-like networks of ECM that provide structural integrity to tissues and organs, but also function in the differentiation & regulation of cells. Their main collagenous components are col. IV protomers, which are triple-helical molecules made from three of the six col. IV chains (α 1-α 6). Proper col. IV expression is critical for development as improper expression leads to embryonic lethality in mice, flies, and worms. Here we report an initial characterization of col. IV in zebrafish and its role in the developing embryo with an emphasis on the NC1 domain. Sequence comparison with human counterparts revealed up to 99% similarity. In situ hybridization and RT-PCR results document that the col. IV α 1 and α 2 chains are present in the embryo from the mid-blastula stage onward; whereas the α 5 chain appears after 24 hours of development. Exogenous expression of the dominant negative NC1 domains from the ubiquitously distributed collagen IV α 1 or α 2 chains caused developmental abnormalities illustrating their requirement in embryogenesis. Furthermore, these abnormalities were accompanied by a perturbation of normal BM structure. In addition, the zebrafish collagen IV α 5 chain NC1 domain could not elicit this effect, whereas the human col. IV α 2 NC1 domain could. These results demonstrate both the selectivity of protomer formation and the sequence conservation of the zebrafish NC1 domains.

51

Evidences of osteoarthritis of the TMJ in Dmm and sedc mice

Melissa Ricks, William Hale, Daniel Faulk, David Robinson, David W. Holt, Robert E. Seegmiller.
Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT

Mutations of the Col2a1 gene, which encodes for type II and type XI procollagens, cause premature degeneration of articular cartilage in both disproportionate micromelia (Dmm) and spondyloepiphysial dysplasia congenita (sedc) mice. In the present study we analyzed the temporomandibular joint (TMJ) of both strains of mice to provide an animal model of TMJ arthritis in humans. Dmm/+ and sedc/sedc mice, along with age matched controls, were compared histologically at 2, 6, 9, and 12 months of age. The craniums were fixed in 4% paraformaldehyde, processed for paraffin sectioning, stained with H&E and analyzed under the light microscope. Evidence of OA was provided by recording number of fissures, grouping of cells and an estimation of cartilage thickness. Dmm mice showed fissuring of the articular cartilage at 6 months of age, whereas controls showed intact cartilage. Rather than aligning in columns as observed in the wild type, mutant chondrocytes were organized into clusters leaving numerous spaces devoid of cells within the matrix. Compared with Dmm, articular cartilage from sedc mice showed similar cellular disorganization accompanied by acellular regions. However, the extent of acellular regions appeared to be greater in sedc. These results suggest that the TMJ of both Dmm and sedc mice is subject to early onset OA. The disorganization of the chondrocytes in the articular cartilage appears to alter the integrity of the tissue thereby leading to degenerative changes. This study shows the importance of the collagen genes in establishing the structure of TMJ cartilage.

52

Sedc Mutant Mice as a Model for Osteoarthritis (OA)

S. A. Avery, M Baker, D. W. Holt, D Robinson, R. E. Seegmiller.
Department of Physiology and Developmental Biology, Brigham Young Uni., Provo, UT 84602

The Col2a1 gene encodes for types II and XI procollagen. Mutations in this gene have been shown to cause premature degeneration of articular cartilage in the disproportionate micromelia (Dmm) mouse. Recently, a naturally

occurring missesnse mutation known as spondyloepiphyseal dysplasia congenita (sedc) has been identified in the mouse Col2a1 gene. It is similar to human SEDC that leads to mild dwarfism and premature OA. The objective of our study is to establish the homozygous (sedc/sedc) mouse as a new murine model for investigating the mechanisms that lead to OA. We compared homozygous sedc mice with age matched wild type (+/+) controls by analyzing the histological and ultrastructural changes that occur in articular cartilage of the knee joint. Joints from both homozygous and wild type mice were taken at 2, 6, 9, and 12 months of age, and histological sections were analyzed for cartilage thickness and characteristic signs of OA. At 2 months, homozygotes had noticeably thicker cartilage than the control, and disorganized arrays of chondocytes were observed within the sedc cartilage matrix. In addition, we observed enlarged pericellular spaces surrounding the chondrocytes in the mutant, whereas wild type mice exhibited normal cell development. Ultrastructurally these enlarged spaces contained non-fibrillar material, which indicated a lack of fibril formation, thereby decreasing the integrity of extracellular matrix and causing early degeneration of articular cartilage. These histological and ultrastructural observations present the sedc mouse as a valuable research model for studying the mechanisms of OA.

53

High Carrier Frequency for Recessive OI in West Africans

Wayne A. Cabral[1], Aileen M. Barnes[1], Charles N. Rotimi[2], Lawrence Brody[2], Joan Bailey-Wilson[2], Susan R. Panny[3], David Chitayat[4], Forbes D. Porter[5], Joan C. Marini[1].
[1]BEMB, NICHD, NIH, Bethesda, MD, [2]NHGRI, NIH, Bethesda, MD, [3]Office of Genetics, Maryland DHMH, Baltimore, MD, [4]Hosp for Sick Children, Toronto, Ontario, CA, [5]HDB, NICHD, NIH, Bethesda, MD

Type VIII OI (OMIM #610915) is a lethal or severe recessive form of OI caused by mutations in *LEPRE1*, which encodes prolyl 3-hydroxylase 1. We have identified a recurring mutation, IVS5+1G>T, in 6 infants born to carrier parents of West African or African-American descent, suggesting it is an African founder mutation which was transported to the Americas. We investigated the carrier frequency for this mutation in African-American and contemporary West African populations and the molecular anthropology of the mutation. We screened gDNA from several populations using PCR and RE digestion, or a custom SNP assay. The mutation was identified in 5 of 995 Washington DC, 5 of 1429 Pennsylvania and 2 of 631 Maryland samples. Thus, Mid-Atlantic African-Americans have a carrier rate of 1/200-300 and a predicted incidence of homozygosity for this mutation of 1/160,000-380,000 births. Fifteen of 1097 unrelated individuals (1.37%) from Nigeria and Ghana were heterozygous for *LEPRE1* IVS5+1G>T, predicting that this mutation alone would cause recessive OI in 1/21,000 births in West Africa, which is equal to the incidence of *de novo* dominant OI, and an order of magnitude greater than the 5-7% proportion of recessive OI in North America. Haplotype analysis of the type VIII OI African-American and West African pedigrees revealed a conserved region of less than 450Kb, consistent with a single mutation that arose over 300 years ago.

54

Oxytalan fibers in the teleostean tooth and pedestal bone

Keitaro Isokawa, Maki Yuguchi, Hideo Nagai.
Department of Anatomy, Nihon University School of Dentistry, Tokyo 101-8310, Japan

Fibrillin microfibrils are integral components of elastic fibers and serve as a scaffold for elastin deposition. However, microfibrillar fibers (MFs) are not necessarily committed to develop into elastic fibers. Oxytalan fibers in the mammalian periodontal ligament are those of such MFs without elastin accumulation, but its architectural and functional significance remains unknown. Expecting extrinsic clues, we have examined the teleostean tooth with a hinged attachment consisting from a fibrous ligament and pedestal bone, a peculiar bony part on the dentary. 3D images generated through micro CT indicated that the ligament covering latero-lingual base of the tooth suffers a compressive but not extensible force. This is endorsed by ultrastructural observations and histochemistry;

i.e., the ligament contains tightly packed collagen fibrils and lacks positive staining for elastin or oxytalan fibers. In contrast, fibers positive for resorcin fuchsin only after oxone oxidation were demonstrated in dentin and pedestal bone at the latero-labial side of tooth base and pedestal bone. The latter fibers were distinct MFs at the ultrastructural level and a large number of disulfide bonds were detected by oxone-high iron diamine method. This newly found MFs devoid of elastin are unique in that they are embedded in calcified tissues but are disrupted in the tissue between tooth and pedestal bone. These findings could provide intriguing clues as to how the topological organization of oxytalan fibers has evolved in the periodontal tissue and how their functional significance has been changed during evolution.

55

Elastin Haploinsufficiency Results in Latent Aortic Valve Disease

Robert B. Hinton[1,2], Jennifer Adelman-Brown[1], Varun Krishnamurthy[2], Hanna Osinska[1], Robert P. Mecham[3], Daria Narmoneva[2], D. Woodrow Benson[1].
[1]Division of Cardiology, Cincinnati Children's Hospital, [2]Department of Biomedical Engineering, University of Cincinnati, [3]Department of Cell Biology, Washington University

Elastin is an extracellular matrix (ECM) protein component of heart valves. Based on findings in a patient with valve disease and elastin haploinsufficiency, we hypothesized that elastin deficient mice (ELN+/-) would manifest viable heart valve disease. Mice were analyzed at neonatal, juvenile, adult and aged adult stages. Histochemical and morphometric analyses demonstrated normal valve morphology at birth, but progressive cusp thinning and elongation with proteoglycan overgrowth of the annulus. In 1mo ELN+/- mice, ultrastructure analysis demonstrated loss of ECM stratification, separated collagen bundles and increased proteoglycans, mechanical testing showed increased aortic stiffness which was incrementally increased at 4mo, and gene expression profile analysis (Affymetrix Mouse Genome 430 2.0 Array) identified specific developmental, signaling and ECM protein abnormalities in aortas and aortic valves. Neonatal ELN+/- mice demonstrated increased valve interstitial cell proliferation. Valve structure and function was evaluated in vivo using echocardiography. ELN+/- mice demonstrated normal valve function at 1mo, and aortic valve disease in 1/6 (17%) at 2mo and 7/10 (70%) at 16mo; the annulus dimension was increased and the aortic valve cusps prolapsed. These findings establish a role for elastin in the pathogenesis of latent aortic valve disease, and identify the ELN+/- mouse as a model of viable progressive valve disease.

56

The role of Has2 on long bone development

Peter J. Roughley[1], Judy Grover[1], Eunice R. Lee[1], Yu Yamaguchi[2].
[1]Shriners Hospital for Children, Montreal, QC H3G1A6, [2]The Burnham Institute, La Jolla, CA 92037

A transgenic mouse line possessing a floxed Has2 gene was bred with a line processing Cre recombinase under control of a type II collagen promoter to allow knockout of Has2 expression and abolition of hyaluronan production by this enzyme specifically in cartilages. Homozygous knockout mice were disproportionate dwarfs, showing profound abnormalities throughout their skeletal system, and died just prior to or upon birth. In the knee joint, the interzone between the distal femur and proximal tibia was poorly differentiated with the menisci being poorly defined. The long bones were short and wide, and possessed a wide diaphyseal cortical bone collar that exhibited little evidence of bone modeling. There was also little evidence for the formation of an organized growth plate between the diaphysis and epiphysis, though adjacent regions expressing Indian hedgehog and type X collagen could be identified. There was more extensive cell proliferation in the epiphyseal cartilage of the knockout mice, resulting in a higher cell density, but there was no loss of aggrecan gene expression by the chondrocytes. The extracellular matrix of the epiphyseal cartilage exhibited Safranin O staining, suggesting the presence of aggrecan, which was confirmed by immunohistochemical localization. Retention of aggrecan is surprising as hyaluronan is thought to be necessary to

anchor aggrecan within the tissue. Thus, hyaluronan production by hyaluronan synthase-2 is essential for skeletogenesis and subsequent long bone development, and its absence severely impairs both cellular and extracellular matrix organization.

57

The role of NFATc1 in epicardial derived cells (EPDCs)

Michelle D. Combs, Caitlin M. Braitsch, Katherine E. Yutzey.
Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

During embryonic heart development, epithelial cells delaminate from the proepicardial organ (PEO) and migrate over the myocardium to form the epicardium. A subset of epicardial cells, EPDCs, will undergo a subsequent epithelial to mesenchymal transformation, invade the subepicardial extracellular matrix (ECM) and myocardium, and contribute to the fibrous skeleton and coronary vasculature of the heart. NFATc1 is a transcription factor necessary for cardiac valve growth and ECM remodeling. During valve growth, NFATc1 responds to VEGF signaling to increase valve cell proliferation. As the valves remodel, RANKL signaling activates NFATc1 signaling to induce expression of Cathepsin K (CtsK) a matrix proteinase that potentiates cell invasion and remodeling of ECM. The hypothesis is that VEGF/ NFATc1 signaling in epicardial cells increases cell proliferation, while RANKL/ NFATc1 signaling in cardiac epicardium and EPDCs induces CtsK expression to potentiate invasion. NFATc1 is expressed in PEO and epicardium and, mice lacking NFATc1 have a significant decrease in proliferating epicardial cells at embryonic day (E)10.5. This data supports the hypothesis that NFATc1 is necessary for normal epicardial cell cycle. In chicken, RANKL, NFATc1 and CtsK are expressed by the epicardium and a subset of EPDCs overlapping with expression of EPDC markers Tbx18 and Wt1. Strikingly, CtsK is expressed in EPDCs adjacent to myocardium, consistent with a role in invasion. Current studies are focused on determining the role of NFATc1 in epicardial proliferation, as well as EPDC invasion and differentiation.

58

Dose-Range Developmental Toxicity of rHuPH20 in Mice

Sinisa Nadjsombati[1], Walter H. Bee[1], Deanna Newcomb[2], Monica L. Zepeda[1], Allan Hoberman[2], Gregory I. Frost[1].
[1]Halozyme Therapeutics, Inc, San Diego, CA, 92121, [2]Charles River Laboratories, Horsham, PA, 19044

rHuPH20 is a highly purified recombinant form of the naturally occurring human hyaluronidase enzyme. It depolymerizes hyaluronan (HA), a major glycosaminoglycan and component of the cardiac jelly in the embryo. HAS2 nullizygous mice and embryo explant studies demonstrate the importance of HA in cardiac development. In this study, pregnant mice were injected with rHuPH20 once daily on days 6 through 15 of presumed gestation at doses of 0, 1, 3, 10 or 30 mg/kg/day. Viability, clinical observations, body weights, feed consumption, necropsy observations, Caesarean-sectioning and litter observations, fetal body weights, fetal sex and fetal gross external alterations were evaluated. All mice were sacrificed on DG 18. All mice survived to scheduled sacrifice. No maternal toxicity with the exception of large spleens in one 10 mg/kg and three 30 mg/kg dose group mice occurred. Four dams in the 30 mg/kg dose group had litters consisting of only resorbed conceptuses. The litter size (live fetuses) of the remaining dams in the 30 mg/kg dose group was reduced compared to controls. There were no test article related fetal gross alterations. Based on the results it can be speculated that the total litter losses observed at doses of 30 mg/kg were the result of the disruption or degradation of HA by rHuPH20, and that this dose produced the pharmacological equivalent of a knockout mouse. Lower doses of the test article did not appear to affect embryo development, indicating that the test article can be administered at safe levels that will not harm the developing conceptus.

59

Genetic and functional studies of CTGF in bone development

Bau-Lin Huang[1], Karen M. Lyons[2,3].
[1]Department of Oral Biology, [2]Department of Molecular, Cell and Developmental Biology, [3]Department of Orthopaedic Surgery, University of California, Los Angeles, CA 90095

It has been shown that CTGF deletion causes defects in osteoblast differentiation *in vitro*; however, the function of CTGF *in vivo* is not yet clear. We created Col I-2.3 Cre;CTGF $^{flox/flox}$ mice to generate conditional knockout mice lacking CTGF only in bone. We found 81% of mutant mice have warped ribs, and in some cases the size of mutant mice is smaller than wild-type littermates. We performed histomorphometric analysis and found that the volume and thickness of trabecular bones are less in mutant mice than in wild type littermates. In real-time PCR experiments, we found that mRNA level of CTGF is dependent on the stages of cell differentiation. The expression level of CTGF in mesenchymal stromal cells is low in the undifferentiated state, but is enhanced 14-fold after cells committed to osteogenesis. In addition, the expression level of CTGF in fresh osteoblasts isolated from calvarial bone is high, but it drops after cells become mature osteocytes. We suspected that CTGF may have a function in differentiation of committed osteo-progenitor cells to mature osteocytes. Furthermore, CTGF is a pro-angiogenesis factor, and others have shown that osteogenesis is coupled to angiogenesis. We measured the expression level of VEGF in CTGF mutant osteoblasts and found that the level of VEGF is markedly decreased in CTGF mutant cells compared to wild-type cells. Taken together, these findings suggest that CTGF may have direct and indirect functions in bone through regulating VEGF in bone development.

60

Characterization of Drole, Drosophila type XV/XVIII collagen

Ryusuke Momota[1], Ichiro Naito[1], Yoshifumi Ninomiya[2], Aiji Ohtsuka[1].
[1]Department of Human Morphology, [2]Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

Type XV and type XVIII collagens, homotrimeric basement membrane molecules, form a distinct subgroup called Multiplexin among the collagen family, characterized by multiple glycosaminoglycan attachment sites and by the central triple helical region with multiple interruptions flanked by N-terminal thrombospondin type I repeat and C-terminal endostatin domain. Type XV/XVIII and type IV collagens are the only collagens that have been conserved from nematode to human, implicating the important functions throughout the metazoan evolution.
Through the database search, we have identified a gene encoding type XV/XVIII collagen homologue, designated drole (**DRO**sophila co**L**lagen with **E**ndostatin). During cloning of drole cDNA, we found that two kinds of transcripts were generated from distantly located two promoters, resulting two kinds of proteins. By *in situ* hybridization on whole embryos, we observed similar expression pattern of both transcripts, ubiquitously distributed in basement membrane, prominently in the central nervous system. Immunostaining of whole embryos with antiserum raised against drole-GST fusion protein in mice also showed that Drole protein was accumulated in the central nervous system during embryogenesis. Moreover, loss of function mutants exhibited low surviving ratio during development and mildly abnormal larval locomotion.
Thus, drole is widely expressed in basement membrane and may play some important roles in the formation of the central nervous system.

61

Abnormal Lipid Homeostasis in Chondrocytes of S1P^{cko} Mice

Debabrata Patra[1], Xiaoyun Xing[1], Jennifer Bryan[1], Guosheng Liang[2], Linda Sandell[1].
[1]Washington University, St. Louis, MO 63110, [2]UT Southwestern Medical Center, Dallas, TX

Site-1 protease (S1P) plays an essential role in the regulated processing of endoplasmic

reticulum (ER) membrane-bound transcription factors SREBPs and ATF6. SREBPs are involved in cholesterol and fatty acid homeostasis; ATF6 is involved in ER stress signaling (ERSS). Cartilage-specific knockout of S1P in mice ($S1P^{cko}$) results in poor cartilage with a drastic decrease of collagen type IIB (Col IIB) in the matrix and consequent lack of endochondral bone formation. Most of the Col IIB appears trapped inside the cell. Ultrastructural analysis of the cartilage shows engorged and fragmented ER characteristic of ER stress. The ER is important for protein synthesis and folding and is the site of fatty acid and cholesterol synthesis. The ER is the essential secretory apparatus of the chondrocyte and therefore maintaining ER lipid composition would be critical for matrix synthesis. In $S1P^{cko}$ mice, ER stress signaling appears to function normally. However, genome-wide expression profiling followed by real-time PCR analysis demonstrated that fatty acid and cholesterol synthesis is down-regulated in $S1P^{cko}$ cartilage. This down-regulation may lead to poor ER membrane integrity and therefore ER fragmentation and engorgement presumably due to an inability to secrete Col IIB and therefore ER stress. These data suggest that in embryonic cartilage S1P is involved in maintaining ER membrane integrity by upholding lipid homeostasis and preventing ER stress. This study provides a link to the importance of lipids in cartilage and bone development and may be useful in cartilage diseases and tissue engineering.

62

Impact of Exercise on Skeletal Muscle and Bone in *oim* Mice

S Carleton[1], B Weber[2], A McCambridge[1], J Ferriera[2], MB Brown[2], C Phillips[1].
[1]Dept of Biochemistry/Child Health, [2]Veterinary Pathobiology, Univ of Missouri

Osteogenesis imperfecta (OI), a heritable connective tissue disorder commonly due to type I procollagen gene defects, is characterized by bone deformity and fragility. The homozygote *oim* mouse models moderately severe OI type III (*oim/oim*; homozygous for a functional null COL1A2 gene defect) with reduced bone mineral density and strength. Heterozygous mice (*oim*/+) model mild OI type I and have an intermediate phenotype between *oim/oim* and wildtype (Wt) mice. Normally, physical activity increases exercise tolerance, muscle strength and bone biomechanical integrity. We investigated whether treadmill (resistance) exercise will impact skeletal muscle and improve bone biomechanical integrity in Wt, *oim*/+ and *oim/oim* mice. We first characterized the hind limb skeletal muscles of 4 month old Wt, *oim*/+, and *oim/oim* mice with normal cage activity and determined that there was no evidence of muscle weakness or pathology. Mice exercised on a treadmill 30 min/day, 5 days/week at 10 m/min for 8 weeks starting at 8 weeks of age. Treadmill exercise resulted in decreased whole body and skeletal muscle mass for all muscles and genotypes studied. In addition, after exercise *oim/oim* mice had altered femoral bone geometry (μCT) and improved biomechanical strength (not yet significant), as seen by increased torsional ultimate strength, tensile strength and energy to failure as compared to non-exercise *oim/oim* mice. These results suggest that moderate weight-bearing physical activity may improve the amount and strength of bone in patients with OI.

63

Insights into the function of SOX9 and campomelic dysplasia

Kathryn S. Cheah.
Department of Biochemistry, University of Hong Kong, Hong Kong

Human SOX9 mutations cause the skeletal malformation syndrome campomelic dysplasia (CD). Complete inactivation of the Sox9 gene in mice results in failure of cartilage formation. Studies in zebrafish and Xenopus suggest that Sox9 may be crucial for specification of the otic placode. In mice, loss of Sox9 results in failure of otic placode invagination. Heterozygous mutations in human SOX9 result in conductive and sensorineural deafness in some CD patients, implying a later morphogenetic role but phenotypic details are limited. Sox9-/- null mice die before morphogenesis of the inner ear is complete, precluding investigation of the role of Sox9 later in ear development. Because all the SOX9 mutations are heterozygous and appear to cause loss of function, the CD phenotype has been attributed to haploinsufficency of SOX9.

However SOX9 proteins containing an intact HMG box and a truncated activation domain may act dominant negatively by competition with the wild-type for binding to target genes and interfere with interaction with partner factors via the transactivation domain. To assess whether such mutations in SOX9 may act via a dominant interference mechanism we generated transgenic and conditional knock'in mice expressing a mouse equivalent of a CD mutation, a Y440X nonsense mutation causing premature termination within the trans-activation domain of SOX9 (Sox9Y440X). Our data reveal an essential role for Sox9 for sensory and non-sensory morphogenesis of the inner ear. We compared the phenotypic impact of the Sox9 Y440X mutation with a Sox9 null mutation. These studies point to context dependent mechanisms for the Y440X nonsense mutation.

64

Expression of ADAMTS-9 in mouse growth plate cartilage

Kanae Kumagishi[1], Keiichiro Nishida[1], Ryusuke Momota[1], Yuichiro Yamaai[2], Satoshi Hirohata[3], Kadir Demircan[3], Ichiro Naito[1], Yoshifumi Ninomiya[3], Aiji Ohtsuka[1].
[1]Department of Human Morphology, [2]Department of Oral Function and Anatomy, [3]Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

Expression of ADAMTS-9 during the course of chondrocyte differentiation was examined by RT-PCR using ATDC-5 cells. Knee joints from 15 male ICR mice were obtained at 0, 7 and 14 weeks after birth (n=5 each). They were fixed, decalcified and embedded in paraffin. The expression of ADAMTS-9 in growth plate cartilage of proximal tibia were examined by *in situ* hybridization (ISH) and immunohistochemistry (IHC). Proliferative and hypertrophic chondrocytes were labeled in adjacent sections by IHC for PCNA and type X collagen, respectively.
ADAMTS-9 expression was noted at day 8 and 14 of ATDC5 cell culture, temporally decreased at day 21, and then increased up-to day 35. In the mouse growth plate cartilage, proliferative and hypertrophic chondrocytes showed strong expression for ADAMTS-9, but it was weaker in mature chondrocytes. There was no expression of ADAMTS-9 in undifferentiated mesenchymal cells. The strength of the signal in ISH and IHC decreased with age.
ADAMTS aggrecanases are known to degrade aggrecan, one of the major extracellular matrix components in cartilage. ADAMTS-9 is reportedly highly expressed throughout mouse development including skeletogenesis. The results of the current study suggested that ADAMTS-9 might have a role in the matrix degradation during the course of chondrocyte proliferation and hypertrophic differentiation in the growth plate.

65

ADAMTSL-4 improves microfibril of Marfan syndrome derived cells.

Masahiro Saito[1], Tomoko Wada[1], Ko Tsutsui[2], Naoto Suda[3], Ganburged Ganjargal[3], Kiyotoshi Sekiguchi[4], Toshiyuki Yoneda[1].
[1]Dept Mol Cell Biochem, Osaka Univ Grad Sch Dent, Osaka,Japan, [2]Inst for Protein Res, Osaka Univ, Osaka,Japan, [3]Maxillo. Ortho,Tokyo Medi and Dent Univ, Tokyo, Japan

Marfan syndrome (MFS) is a systemic disorder affecting connective tissues that is caused by mutations of the FBN1 gene encoding fibrillin-1, a major microfibril component. Prior observation suggested that MFS is associated with increasing susceptibility to severe periodontitis which associated with irreversible damage of periodontal ligament (PDL). However, the molecular mechanisms of microfibrils assembly in PDL formation remain largely unknown. Here, we report that ADAMTSL-4β, a novel microfibril binding protein, not only promotes fibrillin-1 microfibril assembly in PDL but also improves microfibril disorganization in cultured PDL cells obtained from MFS patient (M-HPDL). Expression patterning analysis revealed that *adamtsl4β* mRNA is strongly expressed in the dental follicle, the origin of the PDL, and ADAMTSL-4β protein is colocalized with the fibrillin-1 microfibril in the course of microfibril maturation during PDL development. In contrast, mice homozygous for a targeted hypomorphic allele (mgR/mgR) of *Fbn1*, which

served as a mice model of MFS, showed disorganization of PDL in association with progressive fragmentation of ADAMTSL-4β microfibrils. M-HPDL able to form insufficient fibrillin-1 microfibril, nevertheless overexpression of ADAMTSL-4β in M-HPDL markedly improved fibrillin-1 microfibril assembly. Our results suggest that ADAMTSL-4β regulates microfibril assembly of fibrillin-1 during PDL development, and could be a novel therapeutic target for the damaged PDL tissue in patients with MFS.

66

TRIP11 is essential for skeletal development

Patrick Smits[1], Andrew Bolton[2], Minh Hong[1], Lei Lu[3], Andrea Superti-Furga[6], Shiro Ikegawa[5], Daniel Cohn[4], Tom Kirchhausen[3], Matthew Warman[1], Dave Beier[2].
[1]Dept. Orthopaedic Surgery, Children's Hospital, Boston, [2]Dept. Genetics, Brigham and Women's Hospital, Boston, [3]Dept. Cell Biology, Harvard Medical Institute, Boston, [4]Cedars-Sinai Medical Center, Los Angeles, [5]RIKEN, Tokyo, Japan, [6]Dept. Pediatrics, University of Freiburg, Germany

Trip11 (Thyroid hormone interacting protein 11) null mice display an autosomal recessive, perinatal lethal, skeletal dysplasia characterized by short limbs, small thoraces and a failure to ossify vertebral bodies and skull bones, indicating impaired endochondral and intra-membranous ossification. Growth plates showed abnormalities in chondrocyte proliferation and maturation; mutant chondrocytes also had increased apoptosis rates. A reduction in Cbfa1 expression in the presumptive skull bones was consistent with impaired intra-membranous bone formation. All Trip11 null cells displayed an absence of Golgi stacks, however only chondrocytes and osteoblasts contained swollen endoplasmic reticulum (ER). Although mutant fibroblasts lack Golgi stacks, an oriented cis- and trans-Golgi network was present. However, this network was less efficient at protein trafficking, indicating that the phenotype is likely caused by defects in protein secretion. The human disorder Achondrogenesis type 1A (ACG1A, Houston-Harris type) is characterized by neonatal lethality due to thoracic insufficiency, short-limbed dwarfism and delayed vertebral body ossification. ACG1A chondrocytes also display ER swelling. We screened TRIP11 in ACG1A patients and identified nonsense, frameshift, and splice-site mutations in the majority of probands.

67

Abnormal differentiation of cardiac valves in Ltbp-1L null mice

Vesna Todorovic, Erin Finnegan, Daniel Rifkin. Cell Biology Department, NYU Medical Center, New York, NY 10016

LTBP-1 belongs to the LTBP/fibrillin family of extracellular proteins. Due to usage of different promoters, LTBP-1 exists in two major forms: long (L) and short (S), each expressed in a temporally and spatially unique fashion. Both LTBP-1 molecules covalently interact with latent TGF-ß and regulate its function. Lack of Ltbp-1L results in perinatal death due to abnormal heart development, with improper septation of the cardiac outflow tract and remodeling of the associated vessels. These cardiac anomalies present as persistent truncus arteriosus and interrupted aortic arch, which are associated with faulty cardiac neural crest function (Todorovic et al., Development 2007,134:3723-32). However, 40% of Ltbp-1L-null embryos die during fetal development due to abnormal valve differentiation. Cardiac valves derive from endocardial cushion tissue and Ltbp-1L is expressed in the myocardium underlying the regions where endocardial-mesenchymal transformation (EMT), which produces the cushions, takes place. During endocardial cushion differentiation and development, Ltbp-1L expression widens to the whole cushion. While ex vitro results suggested a crucial role for Ltbp-1 in cardiac EMT, our results show that lack of Ltbp-1L does not affect cardiac EMT but rather endocardial cushion fusion and remodeling. The malformed valve leaflets are misshaped, hyperplastic and thick, causing improper heart function and heart failure. Absence of Ltbp-1L during valve development causes decreased TGF-ß activity in the remodeling mesenchyme, revealing a critical role for Ltbp-1L as an extracellular regulator of TGF-ß activity during valve formation.

68

ADAMTS proteases regulate BMP-mediated cell death

Daniel R. McCulloch[1], Laura Collins[1], Courtney M. Nelson[1], Takako Sasaki[2], Marion A. Cooley[3], W. Scott Argraves[3], Suneel S. Apte[1].
[1]Department of Biomedical Engineering, Cleveland Clinic, Cleveland, OH USA, [2]Shriners Hospital for Children, Portland, OR, USA, [3]Medical University of South Carolina, Charleston, SC, USA

BMP-mediated apoptosis of interdigital mesenchyme regulates web regression in mammals. Failure of resorption leads to interdigital webbing, termed soft-tissue syndactyly (STS). We show that combinatorial ADAMTS protease deficient mice (*Adamts5 + Adamts20* (*bt*) and *Adamts5 + Adamts9*) have STS with greater penetrance and severity than deletion of these genes singly. Apoptosis is reduced in the affected webs. *Bmp2* and *Bmp4* mRNA expression is unaffected in mutant limbs, but application of BMP-4 beads rescues apoptosis, suggesting that these ADAMTS proteases act upstream of BMP signaling. *Adamts5*, *Adamts9* and *Adamts20* expression overlaps with the distribution of the proteoglycan versican, which they all cleave, and with fibulin-1, a co-factor for ADAMTS1 proteolysis of aggrecan. Versican cleavage is markedly reduced in the interdigital mesenchyme of *Adamts5-/-, Adamts20-/-* mice. Versican cleavage by ADAMTS5 is enhanced four-fold by fibulin-1, and *Adamts20-/-* mice haploinsufficient for either versican or fibulin-1 recapitulate the frequency and severity of STS seen in *Adamts5-/-, Adamts20-/-* mice. These genetic and biochemical data suggest the existence of a regulatory extracellular matrix network comprising ADAMTS proteases, versican, and fibulin-1, and support a new mechanism in which ADAMTS proteases act co- operatively to maintain critical thresholds of proteolytic activity.

69

Autofluorescence in Multiple Tissues During Mouse Embryogenesis

Xuehong Xu, Bruce E. Vogel.
Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, MD 21201

Antibodies with fluorescent tags and cloned fluorescent proteins are commonly used as reporters to monitor gene expression, protein distribution and the fate of injected cells in tissue slices and model organisms. However, interpretation of fluorescence data can be complicated by cell and tissue samples that either quench exogenous fluorescent labels or exhibit autofluorescence. Recently, autofluorescence made extensive attention in basic and clinic research. In cardiac stem-cell therapy, several clinical trials promoted by false information on myocardium regeneration in mouse have been terminated by failure of trans-differentiation from haematopoetic stem-cells in infarcted-heart tissue. In a survey of hemicentin distribution we systematically inspected autofluorescence within mouse embryo morphogenesis. We found that several tissues were intensely autofluorescent while most tissues exhibited little or no autofluorescence. In heart, strongest autofluorescence localizes to the internal elastic lamina of vein and aorta, and it also displays in myocardium, and the leaflet of aortic valves. Autofluorescence originated from extracellular matrix structures, including elastic fibers can be also detected in skin, spleen, bone and cartilage. Our data suggest that secreted proteins (e.g. members of the fibulin family) are particularly susceptible to this type of false positive data. In this study, we also identified that the autofluorescent tissues can be color specific. These results regarding tissue autofluorescence at selective wavelengths may be of value to reduce the chance of false positive while investigators study these tissues.

70

LTBP-4 Function as a Modulator of TGF-β and Elastogenesis in Lung

Branka Dabovic[1], Yan Chen[1], Jiwon Choi[2], Elaine Davis[2], Harry Dietz[3], Francesco Ramirez[4], Harold von Melchner[5], Daniel Rifkin[1].
[1]NYU School of Medicine, New York, NY, [2]McGill University, Montreal, Canada, [3]Johns Hopkins University , Baltimore, MD, [4]Mount Sinai School of Medicine, New York, NY, [5]University of Frankfurt Medical School, 60590 Frankfurt am Main, Germany

The latent transforming growth factor-β (TGF-β) binding proteins (LTBs) 1-4 are extracellular matrix proteins similar to the fibrillins. LTBPs interact with several matrix molecules, including fibronectin and fibrillin. LTBP-1, 3, and 4 form covalent bonds with latent TGF-β. Ltbp4 hypomorphic (Ltbp-4h/h) mice have developmental pulmonary emphysema with a defective elastic fiber structure and enlarged terminal air-sacs. The defect in the elastin structure at the tips of the growing septae may account for defective Ltbp-4h/h terminal lung septation. However, as TGF-β is a regulator of matrix molecule expression, the elastin anomaly may be TGF-β-dependent. In addition, it has been reported that LTBP4 binds only TGF-β1, but Tgf-β 1-/- mice have no obvious lung abnormalities, which raises an apparent contradiction in interpreting the Ltbp-4 h/h lung defects as a consequence of decreased TGF-β. To clarify the cause of the defects in Ltbp-4 h/h lungs, we examined TGF-β signaling and elastogenesis from E14.5 to P7. Elastin structure in the Ltbp-4 h/h lung was defective as early as E14.5-16.5. Contrary to what we expected, we found increased TGF-β signaling in Ltbp-4h/h lungs. Decreasing TGF-β signaling improved air-sac septation but did not reverse the defects in elastogenesis suggesting that regulation of TGF-β levels and elastogenesis represent two separable functions of LTBP-4.

71

Regulation of BMP Signaling By Fibrillin Microfibrils

Gerhard Sengle[1,2], Valerie M. Carlberg[1], Noe L. Charbonneau[2], Sara F. Tufa[2], Douglas R. Keene[2], Francesco Ramirez[3], Lynn Y. Sakai[1,2].
[1]Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, OR 97239, [2]Shriners Hospital for Children, Portland, OR 97239, [3]Department of Pharmacology and Systems Therapeutics, Mt. Sinai School of Medicine, New York, New York 10029

Fibrillin-1 and fibrillin-2 bind to the prodomains of BMPs with high affinity and participate in the extracellular targeting of BMP complexes to specific locations in the connective tissue space. Fibrillins may regulate BMP signaling by sequestering BMP complexes and/or facilitating the presentation of other factors required for appropriate BMP signaling. We present in vivo evidence of dysregulation of BMP signaling as a result of loss of fibrillin-2. Fbn2 null mice (E18.5 – P4) demonstrate abnormal activation of BMP signaling (pSmad 1/5/8) and loss of BMP-5 immunolocalization in skeletal muscle. BMP-5 immunolocalization appears normal at P4. During this time, skeletal muscle histology is abnormal (centrally located nuclei and evidence of degenerating muscle cells). Histological features normalize by P8. However, the skeletal muscle in Fbn2 null mice also exhibits an atrophic phenotype which does not recover by P8. Fbn2 null mice are born with joint contractures that resolve around P6 and were proposed to model Congenital Contractural Arachnodactyly, caused in humans by dominant mutations in FBN2. The skeletal muscle phenotype we observe may be related to joint contractures found in the mice. Biochemical analyses of muscle proteins suggest a delay in muscle differentiation, most notably in the expression of myosin heavy chain 8, a marker for perinatal muscle differentiation. Mutations in MYH8 cause a dominant disorder in humans called Trismus-Pseudocamptodactyly syndrome which is characterized by shortened flexor muscles causing distal arthrogryposis. The relationship between contractures in the Fbn2 null mouse and these two dominant human disorders is now open for fruitful speculation.

72

The Secretome of Keratocytes is Growth Factor Dependent

LaTia Etheredge[1], Bradley Kane[2], John R. Hassell[1,2].
[1]Departments of Pathology & Cell Biology, [2]Molecular Medicine, University of South Florida, Tampa, FL

Lumican, keratocan, and collagen type I are the major components of the extracellular matrix (ECM) of the corneal stroma and must be replaced by keratocytes during wound healing to ensure corneal strength and transparency. We studied the effect of IGF-I (IG), TGF-ß (TG), FGF-2 (FG), and PDGF (PD) on proliferation

and extracellular matrix synthesis by primary cultured bovine keratocytes. Keratocytes were collagenase-isolated from bovine corneas and plated at 20,000 cells/cm2 with DMEM/F12, or DMEM/F12 supplemented with 10ng IG, 2ng TG, 10ng FG, or 10ng PD/ml for 13 days. Cell growth was determined by measuring ^{3}H thymidine incorporation and DNA content (cyquant assay). ECM components synthesized by keratocytes and secreted into the media was determined by ^{3}H glycine incorporation into papain and collagenase sensitive protein and western blots with antibodies to procollagen I, fibronectin (FN), lumican (L), and keratocan (K). We found that FG caused the most proliferation, produced the lowest levels of total protein, and inhibited the synthesis of collagen. Keratocytes cultured in IG proliferated the least but synthesized the highest levels of total protein, collagen, and proteoglycans. Keratocytes treated with PD and TG had intermediate levels of these ECM components. The results of this study show that all of the tested growth factors (GFs) enhanced production of L, K, and FN, but that FG inhibited synthesis of total protein and collagen. This suggests that the levels of proliferation and ECM synthesis by keratocytes are GF dependent and GF specific.

73

LOX-PP inhibits FGF-2 signaling and DNA synthesis in osteoblasts

Siddharth R. Vora, Philip C. Trackman.
Boston University School of Dental Medicine, Boston, MA

Lysyl oxidase is secreted as a 50 kDa pro-enzyme and cleaved into a 30 kDa mature enzyme and a 18 kDa pro-peptide (LOX-PP). The importance of the mature enzyme in cross-linking collagen and elastin molecules is well established. Here we generate and purify recombinant LOX-PP in order to investigate a possible role in regulating osteoblast proliferation and differentiation. FGF-2 is an important mitogen for osteoblasts. Data show that LOX-PP inhibits FGF-2 induced [^{3}H]thymidine incorporation into MC3T3 osteoblasts in a dose dependent manner (0-8 μg/ml) by a maximum of 60%. The Ras-Raf-MEK-Erk pathway partly mediates the FGF-2 proliferative response. ELISA and Western blot analyses show that LOX-PP (0-8 μg/ml) inhibits FGF-2 induced Erk activation by a maximum of 65%. FRS2 (Fibroblast Growth Factor Substrate) an adaptor molecule downstream of the FGF receptors, mediates signals to the Ras-Raf-Erk pathway. LOX-PP significantly inhibits FGF-2 stimulated phosphorylation of FRS2 at 5 mins, by atleast 45%, indicating an effect at, or upstream of the receptors. Competitive ligand binding assays with [^{125}I]FGF-2, demonstrates a concentration-dependent inhibition of FGF-2 binding to MC3T3 cell layers by LOX-PP (0-8 μg/ml), similar to the LOX-PP concentration-dependence observed in DNA synthesis assays. Additionally, our study suggests that LOX-PP may inhibit the interaction of FGF-2 to its high affinity receptors. These data indicate that LOX-PP may play a role in regulating the FGF-2 induced proliferation of osteoblasts, perhaps allowing cells to exit from the cell-cycle and progress to the next stage of development.
Supported by NIH DE 14406

74

Cochlea hypoxia in COL4a3 mice involves cytokine upregulation

Lara Dunn[1], Marisa Zallocchi[2], Dominic Cosgrove[2], Michael Anne Gratton[1].
[1]University of Pennsylvania, [2]BoysTown National Research Hospital

Cochleae of 9 week old COL4α3 mutant mice have thickened basement membranes surrounding stria vascularis capillaries (SCBM). The strial tissue shows HIF-1α upregulation. Hypoxic environments upregulate cytokines, such as TNF α and IL-1β. These cytokines contribute to the amplification of matrix metalloproteinases (MMPs) and therefore, disrupt SCBM homeostasis. Specifically, TNFα induces MMP-2 and MMP-9 expression, while IL-1β enhances MMP-2 and MMP-3 expression. These MMPs have all been shown to be upregulated in the stria vascularis of COL4α3 mice compared to wild-type (WT) littermates. We hypothesized that COL4a3 mice have higher TNFα and IL-1β expression compared to WT mice especially after noise exposure. The noise increases the metabolic demand on the stria which increases hypoxia and subsequently cytokine expression. Expression of TNFα and IL-1β was evaluated in subsets of 3 and 9 week

old mice: WT, COL4α3, WT & noise exposure, COL4α3 & noise exposure. Results showed enhanced expression of TNFα and IL-1β in the COL4 α3 mice. The differential between WT and COL4α3 mice is maintained following noise exposure. In addition, cytokine expression in the WT mouse exposed to hypoxia mimicked the expression observed in the COL4α3 mouse. We conclude that the thickened SCBM creates a hypoxic environment that leads to a cascade of cytokine and MMP production that disrupts homeostasis within the cochlea.

75

Fibrous tissues remodel to attain a preferred mechanical state

Jasper Foolen, Corrinus C. van Donkelaar, Rik Huiskes, Keita Ito.
Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands

INTRODUCTION: Fibrous tissue remodeling follows the direction and magnitude of imposed load. However, the underlying mechanism is not fully understood. Based on our former work, we hypothesize that collagenous tissues remodel towards a mechanical equilibrium, which is at the end of the toe-region of the force-strain curve, i.e. at the transition in the tensile properties from a pliant tissue with curled collagen, to a much stiffer tissue with aligned collagen. METHOD: Periosteum of e15 chick embryos was mounted in a tensile tester and cultured for three days at 90%, 95% 100% or 105% of the in vivo length. Afterwards, force strain curves were obtained and material parameters were assessed. RESULTS: Independent of loading history, the toe-region always shifted to and approximated the strain applied. All tissues stiffened during culture, but the increase in stiffness proportionally increased with the initial strain applied. DISCUSSION: The results concur with the hypothesis that fibrous tissues strive to recover a preferred mechanical environment. This study shows that this mechanical equilibrium coincides with the transition from pliant to stiff properties. This corresponds to a condition where the majority of fibrils are marginally stretched in the aligned state. Presumably, this is in agreement with strain at which it has been shown that collagen is least susceptible to enzymatic degradation. When kept at the desired mechanical conditions the tissue strengthens, likely by synthesizing collagen and cross-links.

76

Does TIMP-3 protect articular cartilage in OA model

Hiroyuki Nakamura, Phuong Vo, Ngee Han Lim, Ke Liu, Hideaki Nagase, George Bou-Gharios.
The Kennedy Institute of Rheumatology, Imperial College of London, London W6, UK

The aggrecan proteoglycan of articular cartilage is primary target of osteoarthritic cartilage degradation. Recent studies using gene deletion mouse models have suggested that ADAMTS -5 might be a key aggrecanase involved in cartilage destruction. We previously reported that TIMP-3 inhibits ADAMTS-4 and -5 and also blocks aggrecan degradation in cultured articular cartilage stimulated with interleukin 1. We have characterized that TIMP-3 variant that has an extra Ala at the N-terminus ([-1A] TIMP-3) inhibits aggrecanases, but not MMPs. To investigate whether TIMP-3 or [-1A]TIMP-3 effectively block cartilage breakdown in osteoarthritis (OA), we have generated transgenic mice that overexpress TIMP-3 or [-1A]TIMP-3 together with β-galactosidase which are driven by type II collagen promoter. These mice of 10 weeks old were then challenged for OA by transecting meniscotibial ligament and the histological changes of their joints 8 weeks after surgery were analyzed. The expression of a surrogate marker for TIMP-3 expression, β-galactosidase, was detected at the side of cartilage injured 2 weeks after surgery. OA lesions were observed in the knee cartilage of the wild-type animals 8 weeks after surgery. The knee cartilage of [-1A] TIMP-3 transgenic mice was protected from destruction whereas the knee cartilage from TIMP-3 transgenic mice were only weakly protected. Transgenic mice expression higher levels of TIMP-3 failed to protect cartilage. These results suggest that selective inhibition of aggrecanase is crucial as a

therapeutic intervention to protect cartilage from degradation. This work is supported by Arthritis Research Campaign and National Institute of Health grants.

77

A Type I Collagen Matrikine Can Drive Breast Cancer Osteolysis

William Wu[1,2], Anna Drachuck[1], Izabela Podgorski[2].
[1]Bone and Joint Center, Henry Ford Hosptial, Detroit, MI 48202, [2]Department of Pharmacology, Wayne State University, School of Medicine, Detroit, MI, 48201

The major site of metastasis of breast cancer cells is bone. Bone metastases occur in 80% of patients with advanced disease and causes significant morbidity. This relationship was first described by Paget in 1889 and the term "seed and soil" was coined to explain this preferential metastasis of breast tumors to bone. Recently, studies of breakdown fragments of extracellular matrix (ECM) proteins have shown novel biological activity of the fragments. We have identified a type I collagen degradation fragment that can be generated either by MMPs or Cathepsin K cleavage of type I collagen and can act as a matrikine on tumor and bone cells. Real time PCR analysis of MDA-MB-231 cells stimulated with 25 ug/ml CB4II, showed a significantly upregulation of MMP-1, 2, 9, 13, 14, PTHrP and SPARC. Gelatin zymography of MMP-2 and MMP-9 of MDA-MB-231 condition media showed that CB4II stimulated both MMP-2 and MMP-9 activity. Osteoclast formation was measured by using purified rat monocyte/macrophages. Monocyte/macrophage cultures formed large multi-nucleated TRAP positive osteoclasts only in CB4II stimulated conditioned media suggesting CB4II stimulated osteoclast forming cytokines in MDA-MB-231 cells. We also measured the in vivo increase in production of this type I collagen matrikine fragment CB4II in mice tibiae injected with MDA-MB-231 from Rag -/- mice as compared to Rag -/-/CatK -/- mice by western blotting. This study proposes a new mechanism of breast tumor metastasis and osteolysis in bone.

78

Matrix Remodeling in Fibroblast-Seeded Fibrin Gels

Edward A. Sander, Sandy L. Johnson, Victor H. Barocas, Robert T. Tranquillo.
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455

Several tissue-engineering strategies have been employed to produce functional replacement tissues for diseased and damaged native tissues that cannot heal properly on their own. Our approach to tissue- engineered arteries and heart valves involves cell entrapment within a provisional fibrin matrix that is constrained by specially designed molds to produce fibril alignments similar to the native tissues. In the molds, the cells compact and remodel the fibrin through a number of complex and poorly understood interactions involving fibrinolysis and ECM deposition. The ECM composition and organization that results governs the construct's functionality and must be better understood and controlled so that functional tissues can be engineered. To study fibrin remodeling, we used histology and SEM to identify changes in the ECM of a simple test system, an adherent cell-seeded fibrin gel hemisphere (HMS). Neonatal human dermal fibroblasts were combined with fibrin forming solution to form HMS 1 cm in diameter. HMS were cultured up to 5 weeks with supplemented DMEM. Each week, HMS were prepared for histology and SEM imaging. Histology included Masson's trichrome stain and picrosirius red was used to visualize gross changes. Microstructural imaging was conducted with a Hitachi S-900 FESEM. High pressure freezing and cryo-SEM allowed examination of the hydrated microstructure, while conventional SEM facilitated immunogold labeling of specific ECM components. The HMS primarily compacted during the first week of culture, after which ECM synthesis accompanied fibrin degradation resulting in interpenetrating fibril networks.

79

Calreticulin plays a role in collagen regulation

Lauren B. Van Duyn, Mariya T. Sweetwyne, Joanne E. Murphy-Ullrich.
Departments of Pathology and Cell Biology, University of Alabama at Birmingham

Chronic wounds and delayed wound healing are a major problem in the elderly, diabetics, and patients with cardiovascular disease. There is emerging evidence that soluble calreticulin (CRT) plays a role in wound healing. Gold et al. showed that topical application of soluble CRT in a porcine model enhances dermal wound healing (J Invest Derm Symp Proc. 2006). Our lab and others have shown that CRT on the cell surface mediates cellular functions important to wound healing including signaling of apoptotic cell clearance, cell motility, and cell survival. This led us to hypothesize that CRT plays a role in ECM remodeling during tissue repair. In initial studies, we examined CRT localization by immunohistochemistry in tissues undergoing remodeling. We observed staining for CRT in granulation tissue of mouse excisional wounds and in rabbit atherosclerotic arteries. To examine the role of CRT in ECM production, we assayed wild type and CRT null fibroblasts for soluble and ECM-bound collagen. The CRT null fibroblasts have reduced secretion and deposition of type I collagen in the detergent-insoluble ECM as compared to wild type fibroblasts, measured by immunoblot analysis. In addition, mouse L fibroblasts (gift, Dr. Michal Opas) engineered to express 2-fold higher levels of CRT as compared to parental cells have a ~2-3 fold increase in collagen I gene expression, soluble collagen in the conditioned medium, and ECM deposition of collagen I. Together, these findings show that CRT is upregulated in areas of injury and that CRT expression plays a role in extracellular matrix remodeling through regulation of collagen (NIH HL079644).

80

Antibodies for targeting of collagenolytic processes

Lars H. Engelholm[1], Daniel H. Madsen[1], Signe Ingvarsen[1], Henrik J. Jürgensen[1], Kenn Holmbeck[2], Niels Behrendt[1].
[1]Finsen Laboratory, Rigshospitalet, Copenhagen Biocenter, Ole Maaløes Vej 5, DK-2200 Copenhagen N, Denmark, [2]Craniofacial and Skeletal Diseases Branch, NIDCR, NIH, DHHS, Maryland 20892, USA

The directed turnover of collagens in various ECM compartments is a crucial process in normal development as well as in several pathological conditions. Degradation of collagens is undertaken by soluble and membrane-bound proteases, mainly belonging to the MMP family, and by specific endocytic receptors that direct collagen to lysosomal degradation. Studies on combined gene deficiency in mice have identified various key components in collagenolysis that cannot be inactivated simultaneously without fatally affecting early development. Thus, combined deficiency for the endocytic collagen receptor, uPARAP/Endo180, and the membrane-associated collagenase, MT1-MMP, is incompatible with postnatal survival due to severe defects in collagen turnover (Wagenaar-Miller R. et al. (2007), Mol.Cell.Biol. 27, 6309–6322). With the aim to target these components at the protein level in adult animals, we are currently developing function-blocking monoclonal antibodies against uPARAP/Endo180 and MT1-MMP. To allow administration in vivo, antibodies against the murine components are generated in knock-out mice that lack the antigen in question. This work has led to an antibody against uPARAP/Endo180 which, when added to cultured fibroblasts, leads to a complete blocking of collagen uptake. The current strategy should prove useful for therapy experiments in mice, specifically targeting single steps in ECM turnover in cancer models.

81

Type I collagen homotrimers may alter tissue remodeling

Sejin Han[1], Elena Makareeva[1], Daniel J. McBride[2], Charlotte L. Phillips[3], Ulrike Schwarze[4], James M. Pace[4], Peter H. Byers[4], Robert Visse[5], Hideaki Nagase[5], Sergey Leikin[1].
[1]NICHD, National Institutes of Health, Bethesda, MD 20892, [2]University of Maryland

School of Medicine, [3]University of Missouri, [4]University of Washington, [5]Kennedy Institute of Rheumatology, Imperial College London, UK

Insufficient or abnormal synthesis of the α2(I) chain results in replacement of normal type I collagen by α1(I) homotrimers in various pathological conditions. For instance, synthesis of α1(I) homotrimers in individuals with a polymorphism in the SP1 binding region of COLIA1 may contribute to osteoporosis. Homotrimer synthesis is also observed, e.g., in cancer, glomerular sclerosis, and several forms of Osteogenesis Imperfecta and Ehlers-Danlos Syndrome. To understand how α1(I) homotrimers may contribute to pathology, we investigated murine and human homotrimers and their mixtures with the corresponding heterotrimers. The most significant findings were: (a) the segregation of the homo- and heterotrimers at a subfibrillar level and (b) the significantly reduced susceptibility of the homotrimers to cleavage by tissue collagenases. A more detailed study with rhMMP-1 revealed that the lack of the α2(I) chain does not alter the enzyme binding but hinders opening of the triple helix necessary for presenting the unwound chains to the catalytic site. In mixtures, rhMMP-1 degraded the normal type I heterotrimers before noticeable cleavage of the homotrimers. We hypothesize that subfibrillar segregation of the homotrimers and selective proteolytic degradation of the heterotrimers may alter tissue remodeling, resulting in accumulation of MMP-resistant fibers.

82

Monitoring matrix turnover in fibrin-based tissue constructs

Justin S. Weinbaum, Sandra L. Johnson, Katherine A. Ahmann, Robert T. Tranquillo.
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455

A significant hurdle in engineering completely biological artificial vessels has been achieving enough mechanical strength to withstand hemodynamics. We fabricated constructs from vascular smooth muscle cells (vSMC) embedded in a tubular fibrin gel. vSMC-mediated turnover of the fibrin gel into an aligned collagen- and elastin-rich matrix is needed for a hardy vascular equivalent. Until now, a non-destructive means to monitor this turnover in real time has been unavailable. Here we present three new non-invasive methods to quantify fibrinolysis and to monitor collagen production in real time during construct maturation. Fibrinolysis is performed by plasmin and MMPs, leading to the release of fibrin degradation products (FDPs). We developed an ELISA for measuring FDPs in the construct medium. We also devised a zymographic technique to monitor fibrinolytic enzyme activity in the same medium. Significant fibrinolysis occurred very early in the maturation process and was delayed by plasmin inhibition. Increased early fibrinolysis also correlated with increased collagen deposition and a stronger tissue construct, highlighting the importance of turnover. In addition, we have established stably transfected vSMC cell lines that report type I collagen transcription using a luciferase reporter, this allows viable luminescence monitoring of our constructs. Collagen expression peaks two-fold after a week of incubation before returning to basal levels. Since these new methods preserve the maturing construct they will allow real-time optimization en route to a functional tissue-engineered blood vessel.

83

Collagen Glomerulopathy and MMP Expression in oim Mouse Kidney

Anna M. Roberts[1], Matthew H. Myles[2], Andrew Walker[1], Craig Franklin[2], Charlotte L. Phillips[1].
[1]Dept. of Biochemistry/Child Health, University of Missouri-Columbia, [2]Dept. of Veterinary Pathobiology, University of Missouri-Columbia

Though *oim* (COL1A2-deficient) mice are known for their bone fragility, they also develop progressive glomerulosclerosis characterized by accumulation of homotrimeric type I collagen. The glomerulosclerosis exhibits a gene dose effect, initiates postnatally within 2 weeks of age, and leads to proteinuria. To investigate whether deficiencies in matrix metalloproteinases are associated with disease progression, we extracted glomeruli from 1 and 3 month (mo) old wildtype (Wt) and homozygous *oim* mice and examined MMPs-2, -

3 and -9 expression. MMP-2 steady-state mRNA levels were similar between *oim* and Wt glomeruli at 1 mo of age; whereas, at 3 mo of age mRNA levels had decreased by 57% in Wt mice, but remained elevated in the *oim* mice. MMP-2 protein (pg/glomerulus) was significantly elevated in *oim* glomeruli by 3-and 2-fold as compared to Wt at 1 and 3 mo, respectively. *Oim* MMP-3 mRNA levels were slightly elevated at 1 mo (not significant), but had increased 126% by 3 mo as compared to Wt. *Oim* MMP-3 protein was elevated 9-fold in 1 and 3 mo glomeruli as compared to age-matched Wt glomeruli; for both, glomerular MMP-3 increased with age. Transcription of MMP-9 was equivalent in Wt and *oim* glomeruli at 1 mo, but increased 2- and 5-fold by 3 mo, respectively. MMP-9 protein was not elevated in *oim* glomeruli at 1 or 3 mo as compared to Wt, but appeared to decrease between 1 and 3 mo of age. In summary elevation in MMP expression in *oim* glomeruli does not appear effective at controlling accumulation of homotrimeric type I collagen

84

TAK1 regulates extracellular matrix remodelling by fibroblasts

Xu Shiwen[1], Sunil Parapuram[2], Daphne Pala[2], David E. Carter[3], Chris P. Denton[1], David J. Abraham[1], Andrew Leask[2].
[1]Centre for Rheumatology, University College London (Royal Free Campus), London, UK NW3 2PF, [2]CIHR Group in Skeletal Development and Remodeling, Dental Sciences Building, University of Western Ontario, London ON, Canada, N6A 5C1, [3]London Regional Genomics Centre, London, ON, Canada N6A 5K8

TGFβ -activated kinase 1 (TAK1) is a key TGFβ signaling pathway component. We use control cells and TAK1-deficient fibroblasts, and fibrotic cells from scleroderma (SSc; n=6) to probe the contribution of TAK1 in basal and TGFβ stimulated transcriptional and functional programs of fibroblasts. Gene expression profiling was assessed using MOE430 Affymetrix gene arrays, followed by confirmation and functional studies, including RT-PCR, western blot, matrix remodeling, myofibroblast differentiation and extracellular matrix production. Cluster analysis revealed that expression of TGFβ induced transcripts were reduced in TAK1-deficient cells, including thrombospondin 1, TIMP3, vinculin, and several collagen genes ($p<0.05$). Results were verified by alternative RNA and protein analysis. TAK-/- cells alone exhibited reduced matrix contraction, and TGFβ did not induce matrix contraction or a cohort of fibrotic genes, including alpha smooth muscle actin (α -SMA) in TAK1-deficient cells ($p<0.05$). The ability of TGFβ to induce JNK phosphorylation was impaired in the absence of TAK1, and phosphorylation of TAK1 was reduced by the FAK/src inhibitor PP2. Activated TAK-1 was found in the majority of SSc fibroblasts but not in normal fibroblasts. Our results uncover new insights into the contribution of TAK1 to tissue repair, remodeling and fibrotic responses of fibroblasts.

85

Quantitative Proteomic Analysis of Hypertrophied Rat Myocardium.

Amy D. Bradshaw[1], Catalin F. Baicu[1], John H. Schwacke[2], Kentaro Yamane[1], Tyler J. Rentz[1], John M. Lacy[1], Thomas N. Gallien[1], Kevin L. Schey[3], Michael R. Zile[1].
[1]Depts. of Medicine, [2]Biostatistics, Bioinformatics & Epidemiology, [3]Pharmacology, Medical University of South Carolina, Ralph H. Johnson V.A. Medical Center, Charleston, SC

Chronic pressure-overload (PO) causes myocardial hypertrophy and increased extracellular matrix (ECM) collagen concentration. Fibrillar collagen concentrations are known to increase in response to hypertrophy, however the full complement of ECM proteins that make up the hypertrophic cardiac interstitium is not fully characterized. Therefore, a quantitative analysis of PO-induced changes in protein expression in the right ventricle (RV) was performed with particular emphasis placed on identification of ECM proteins. Six 3-month old rats were subjected to pulmonary artery band (PAB) for 4 weeks to generate PO-induced hypertrophic RVs. PAB rats had an increase in RV mass (increased RV mass to body weight ratio [RV/BW]) that confirmed a hypertrophic response to banding. iTRAQ-facilitated quantitative proteomic

analyses of RV proteins from age-matched normal (n=6, pooled samples) against each PO-induced hypertrophic heart were performed. Complimentary biochemical assays were done to confirm results from proteomic analyses. Total, soluble (1M NaCl extraction), and insoluble (mature cross-linked) RV collagen content (hydroxyproline assay) and specific ECM protein expression (immunoblot) were determined. Proteomic analysis using iTRAQ generated a robust list of differentially expressed proteins in the RV of PO rats versus normal including identification of several ECM-associated and integral ECM components.

86

p190RhoGEF (Rgnef) and FAK Promote Colorectal Cancer Invasiveness

Hong-Gang Yu, Ju-Ock Nam, Lei Shi, Xiao Lei Chen, Alok Tomar, Ssang-Taek Lim, David D. Schlaepfer.
Department of Reproductive Medicine, UCSD Moores Cancer Center,3855 Health Sciences Dr., La Jolla, CA 92093

Colorectal tumor metastasis is a leading cause of cancer-related death. The peptide hormone gastrin enhances colorectal carcinoma spread via activation of the G-protein-coupled cholecystokinin 2 receptor (CCK2R) and Rho GTPase-dependent cytoskeletal changes. Here, we show that gastrin promotes CCK2R-dependent human DLD1 colorectal cell E-cadherin internalization, cell scattering-motility, and matrix-degrading invadopodia formation. Gastrin triggers cytoplasmic tyrosine kinase FAK and c-Src activation, FAK recruitment to newly-formed focal adhesions, and the formation of a complex between FAK, c Src, paxillin, and the Rho guanine nucleotide exchange factor p190RhoGEF (Rgnef). Pharmacological inhibitors to Src or FAK suppress gastrin-stimulated DLD1 cell scattering and inhibit paxillin (Y31) tyrosine phosphorylation. Knockdown of FAK or Rgnef in DLD1 cells inhibit cell scattering and invadopodia formation whereas c-Src knockdown was without effect due to compensatory Src-family kinase up-regulation. Importantly, gastrin-stimulated paxillin tyrosine phosphorylation was inhibited after Rgnef knockdown, supporting the notion that Rgnef functions upstream of FAK-Src-paxillin. As Rgnef over-expression enhanced both spontaneous and gastrin-stimulated DLD1 scattering and invadopodia formation, and elevated Rgnef mRNA levels were selectively detected in Stage III-IV colorectal cancer tissues, our results identify Rgnef as a new regulator of colorectal cancer cell motility associated with an invasive tumor phenotype.

87

Evidence for articular cartilage regeneration in MRL/MpJ mice

Jamie Fitzgerald[1,3], Cathleen Rich[1], Dan Burkhardt[2], Justin Allen[3], Andrea S. Herzka[1], Christopher B. Little[2].
[1]Oregon Health and Science University, Portland, Oregon, USA, [2]Raymond Purves Bone and Joint Research Laboratories, Kolling Institute of Medical Research, University of Sydney at The Royal North Shore Hospital, Sydney, Australia, [3]University of Melbourne and Murdoch Childrens Research Institute, Royal Childrens Hospital, Melbourne, Australia

A major clinical problem in Orthopaedics is the repair of traumatic articular cartilage lesions. The MRL/MpJ strain of mice have the remarkable ability to regenerate ear hole punch wounds seamlessly including the scarless replacement of multiple tissues. We assessed whether articular cartilage defects repair or regenerate in the MRL/MpJ 'healer' strain of mice. Full thickness and partial thickness lesions were introduced into trochlear groove articular cartilage of MRL and C57/bl6 mice, which do not undergo ear hole regeneration. The wound sites were assessed 12-weeks post-surgery. The partial thickness lesions did not repair in either strain. However, with the full thickness lesions the MRL/MpJ mice had a superior healing response with abundant chondrocytes and an extracellular matrix rich in proteoglycan and collagens II and VI at the wound site. The enhanced cartilage healing was restricted to male MRL/MpJ mice. In contrast, the C57/bl6 control strain produced an extracellular matrix at the wound site that completely lacked proteoglycan and collagens II and VI. We conclude that male MRL/MpJ mice possess an

intrinsic ability to regenerate articular cartilage. Understanding, in detail, the biochemical and genetic basis for articular cartilage regeneration may open up new treatment options for traumatic articular cartilage defects.

88

Dissecting a fibronectin matrix assembly domain using FRET

Jean E. Schwarzbauer, Nancy W. Karuri, Zong Lin, Hays S. Rye.
Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544

As a major fibrillar component of the extracellular matrix, fibronectin (FN) interacts with cell surface integrin receptors to influence cell functions. Binding to integrins is also required to promote interactions between FN molecules and initiate matrix assembly. The FN type III modules III1 and III2 each contain a FN binding site and together they play a regulatory role in FN assembly. A seventeen amino acid linker between these modules may allow intermodular interactions that sequester the FN binding sites, which could then be exposed by cell tension during matrix assembly. To test this hypothesis, we probed the conformation of the III1-2 domain using CIIIY, a conformational sensor consisting of the fluorescence resonance energy transfer (FRET) pair cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) fused to the amino- and carboxy-termini of the III1-2 domain, respectively. Spectroscopic and donor lifetime studies showed significant FRET in CIIIY indicating that opposite ends of III1-2 are in close proximity. FRET was due to intramolecular interactions between modules as shown by proteolytic cleavage or treatment with mild denaturant. Mutagenesis at predicted sites of interaction between III1 and III2 revealed several charged residues required to stabilize a compact conformation of III1-2. A mutant form of CIIIY showed increased binding to the 70 kDa amino-terminal fragment of FN supporting the hypothesis that the FN binding sites can be exposed by conformational changes in III1-2. We propose a model in which a compact conformation of the III1-2 domain regulates FN-FN interactions during matrix assembly.

89

Woundermatology: Analyzing ECM fragments in pressure ulcer wound

Zenzo Isogai[1], Yusuke Murasawa[1], Koji Mizuno[2], Ken Watanabe[1], Masahiko Yoneda[3].
[1]National Center for Geriatrics and Gerontology, Obu, Aichi, Japan, [2]Hoshi University School of Pharmacy, Tokyo, Japan, [3]Aichi Prefectural College of Nursing and Health, Nagoya, Aichi, Japan

Clinical findings and wound healing process of pressure ulcer are heterogeneous. Since wound tissue lacks epithelial tissue, ECM in the wound surface can be sampled non-invasively. Therefore, we attempted to analyze the ECM fragments in pressure ulcer wound. We focused on microfibril components including fibrillin-1, versican, latent TGF-beta binding protein-1 (LTBP-1) and fibronectin. The ECM fragments detected from wound surface were different from monomeric molecules produced from dermal fibroblast. For instance, the amino-terminus of versican in the wound surface lost its hyaluronan binding capacity and was separated from the carboxyl-terminus of versican. In another example, LTBP-1 fragment detected in wound surfaces was similar to the fragment that was generated by plasmin treatment from normal skin. Analyses of wound surface ECM provided information not only for turnover (degradation and assembly) of each ECM molecule during wound healing process, but also for the pathogenesis of pressure ulcer. We further developed a novel method that we call WOUND BLOTTING. By this method, the proteins in wound surface were transferred onto nitro-cellulose membranes by site and stage specific manner. Then the blots were used for immunostainings. Using this method, the ECM fragments of wound can be superimposed on clinical findings. We are proposing WOUNDERMATOLOGY that is consisted of clinical description and wound surface ECM analyses.

90

MIG-17/ADAMTS controls cell migration by recruiting nidogen

Yukihiko Kubota[1,2], Kiyotaka Ohkura[1], Katsuyuki K. Tamai[1], Kayo Nagata[1], Kiyoji

Nishiwaki[1,2].
[1]RIKEN CDB, Kobe, Hyogo 650-0047, Japan, [2]Department of Bioscience, Kwansei-Gakuin University, Sanda, Hyogo 669-1337, Japan

Remodeling of the extracellular matrix is an important process for organ morphogenesis in animal development. In *C. elegans*, the U-shape of the gonad is determined by the migration path of the gonadal leader cells. We previously demonstrated that a secreted metalloprotease of the ADAMTS family, MIG-17, regulates the directional migration of the leader cells {Nishiwaki et al. (2000)}. We also found that gain-of-function (*gf*) mutations in the gene *fibulin-1 (fbl-1)* can suppress the leader cell migration defects of *mig-17* mutants {Kubota et al. (2004)}, suggesting that the FBL-1 protein interacts with MIG-17 in the gonadal basement membrane to control leader cell migration. In this study, we characterized a second suppressor locus, *let-2*, which encodes the α 2 chain of type IV collagen. Amino acid substitutions in the C terminus of LET-2 result in *gf* mutations that strongly suppress the leader cell migration defects of *mig-17* mutants. Mammalian Type IV collagen and fibulin-1 are reported to bind nidogen in vitro. Interestingly, we found that the suppression by *fbl-1(gf)* depends on NID-1/nidogen, whereas that by *let-2(gf)* does not. The basement membrane localization of NID-1 was reduced in the loss-of-function mutants *mig-17, let-2*, and *fbl-1* but was recovered to wild-type levels in *mig-17; fbl-1(gf)* and *mig-17; let-2(gf)* double mutants. Furthermore, overexpression of NID-1 in *mig-17* mutants substantially rescued the leader cell migration phenotypes. We will discuss the functional interaction of FBL-1, LET-2 and NID-1 in the downstream of MIG-17-dependent proteolysis to control leader cell migration.

91
TGF-B3 induction of BMSC induces bone formation *in vivo*
Jason T. Rich, Jennifer Bryan, Audrey McAlinden. Washington University, St. Louis, MO

Introduction: TGF-β3 is commonly used to induce cartilage formation from adipose- and bone marrow-derived stromal cells (AdSC, BMSC). However, there is limited data on the phenotypic stability of these tissues following *in vivo* transplantation. **Methods:** AdSC and BMSC were induced with TGF-β3 for 28 days to form cartilage pellets (AdCP, BMCP), which were then harvested for histological and RT-PCR analysis or placed subcutaneously in SCID mice. At 6 weeks the pellets were harvested and evaluated. **Results:** Following *in vitro* culture with TGF-β3, AdCP and BMCP expressed Sox9, Osterix, and Runx2. BMCP expressed much more COL10A1 than AdCP, and only BMCP expressed COL2A1. Von Kossa staining was negative in both groups. After *in vivo* transplantation, AdCP architecture was no longer identifiable. In BMCP, von Kossa staining revealed significant bone growth at the periphery of each pellet, with osteocytes embedded within the bone and abundant osteoblasts lining the outer edges of the bone. Within BMCP there was abundant cellularity, vascular channels, adipocytes, and bony trabeculae, consistent with bone marrow formation. No chondrocytes were present. **Conclusion:** Induction of BMSC with TGF-β3 results in a hypertrophic chondrocyte phenotype *in vitro*, and bone and bone marrow formation after *in vivo* transplantation, consistent with endochondral ossification. AdCP demonstrated minimal chondrocyte formation *in vitro* and did not endure *in vivo* transplantation. These findings suggest that induction of BMSC with TGF-β3 results in excellent bone formation following *in vivo* transplantation and that alternative induction methods for engineering stable hyaline cartilage from BMSC must be explored.

92
A composite role of vitronectin and uPAR in cell morphology
Thore Hillig[1], Signe Ingvarsen[1], Daniel H. Madsen[1], Henrik Gaardsvoll[1], Michael Ploug[1], Keld Dano[1], Lars Kjoller[1], Niels Behrendt[1], Lars H. Engelholm[1,2].
[1]The Finsen Lab. section 3735, Ole Maaloes Vej 5, 2200 Copenhagen N, Denmark, [2]The Bartholin Institute section 3731, Ole Maaloes Vej 5, 2200 Copenhagen N, Denmark

The urokinase receptor, uPAR, is a GPI-anchored membrane protein engaged in pericellular proteolysis and cellular adhesion, migration and modulation of cell morphology. A direct matrix adhesion is mediated through the binding of uPAR to vitronectin and this event is followed by downstream effects including changes in the cytoskeletal organization. However, it remains unclear if the adhesion through uPAR-vitronectin is the only event capable of initiating these morphological rearrangements, or if lateral interactions between uPAR and other membrane proteins can induce the same response. Here we show that both of these triggering mechanisms can be operative and that uPAR dependent modulation of cell morphology can indeed occur independently of vitronectin binding. Expression of wildtype uPAR on HEK-293 cells led to pronounced vitronectin adhesion and cytoskeletal rearrangements whereas a mutant uPAR, $uPAR_{W32A}$ with defective vitronectin binding, failed to induce both phenomena. However, upon saturation of $uPAR_{W32A}$ with the protease ligand, pro-uPA or its receptor-binding domain, the ability to induce cytoskeletal rearrangements was restored even though this did not rescue the vitronectin binding and adhesion capability. On the other hand, using other uPAR variants, we could show that uPAR-vitronectin adhesion is indeed capable and sufficient to induce the same morphological rearrangements.

93

Novel role of Cdk4 in leukocyte adhesion and trafficking

Lynn M. Schnapp[1], Li Liu[1], Barbara Schwartz[1], Yu-Hua Chow[1], Yoshiaki Tsubota[2], Elaine W. Raines[2], John M. Harlan[1].
[1]Dept of Medicine, [2]Dept of Pathology, Univ of Washington, Seattle, WA

Leukocyte emigration is a tightly regulated process essential for an appropriate inflammatory response. The current paradigm describes discrete steps in the process: rolling, activation, adhesion, and migration through subendothelial extracellular matrix (ECM). We report a new adhesion pathway that may be important in post-trafficking, i.e. mediating leukocyte interactions to and migration through subendothelial ECM. We found several novel features of this pathway that distinguish it from "conventional" stimulated adhesion: it is dependent on Cdk4 activity; it requires microtubules; it does not require exogenous leukocyte activation; and it does not require Rap-1 activity. Because this novel pathway allows leukocyte adhesion to physiological relevant substrates such as exposed endothelial matrix in the absence of exogenous stimulation, we have termed it "Ligand-induced Adhesion". Monocytes and lymphocytes, but not neutrophils, are capable of LIA using several ligands, including purified FN and endothelial cell-derived ECM. The known substrates of Cdk4, Rb and Smad, are not required for ligand-induced adhesion suggesting the involvement of a novel Cdk4 substrate. Furthermore, we show that mice lacking Cdk4 have impaired recruitment of lymphocytes in bronchoalveolarlavage fluid following bleomycin induced lung injury. Thus, Cdk4 may be a novel therapeutic target for regulating lymphocyte recruitment during lung injury and inflammation. Further characterization of this novel pathway and its role during injury will be critical to our understanding of leukocyte trafficking.

94

Tumor Typing by Proteomic Analysis of Formalin Fixed Sample ECM

Timothy M. Ritty[1,4], Elizabeth Frauenhoffer[2,4], Edward J. Fox[1,4], Bruce Stanley[3,4].
[1]Orthopaedics, PSU Cancer Inst., [2]Path., [3]Proteomics Facility, [4]Penn St. Coll. of Medicine, Hershey, PA

A vast and mostly unused resource of tumors and normal stroma is harvested daily by surgical resection, and is formalin fixed/paraffin embedded (FFPE) for pathology. Molecular analysis of FFPE tumors is difficult due to covalent cross-linking induced by formalin. Consequently, these samples are an untapped fund of molecular information. New techniques allow the extraction of peptides from FFPE samples for proteomic analysis. We used these methods to test the hypothesis that tumors arising from different tissues can be distinguished by the ECM proteins they make. We chose to study osteosarcoma and chondrosarcoma, both neoplasms of

mesenchymal origin, but believed to arise from osteoblasts and chondrocytes, respectively. Importantly, it is often difficult to differentiate chondrosarcoma from osteosarcoma with standard histopathology. Making an accurate diagnosis between the two malignancies is clinically critical because these sarcomas respond differently to chemotherapy. Laser capture microscopy was used to isolate only ECM from precise regions for each tumor type (N=3). This strategy results in samples of lower complexity, and increases the capacity to detect differences. Samples were treated with proprietary solutions (Expression Pathology, Inc.), trypsinized, 2D liquid chromatographic separation linked to tandem MS/MS. Distinct ECM protein signatures were identified for the two tumor types. This demonstrates that proteomic analysis of FFPE tumor ECM is possible, and has potential for basic and translational research, and for improving clinical diagnostic accuracy.

95

Thrombospondin-1 Regulates Blood Pressure and Cardiac Response

Jeff S. Isenberg[1], Yan Qin[2], Daryl Despres[3], Russell W. Bandle[1], Jurgen Schnermann[2], William A. Frazier[4], David D. Roberts[1].
[1]Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, MD 20892, [2]Kidney Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, [3] Mouse Imaging Facility, National Institutes of Health, Bethsda, MD, [4]Dept of Biochemistry and Molecular Biophysics, Washington Uni. School of Med., St. Louis, MO

Aims - Nitric oxide (NO) regulates regional vascular resistance and systemic blood pressure by modulating blood vessel tone. The ability of thrombospondin-1 via its receptor CD47 to locally limit NO-mediated vasodilation and alter regional blood flow suggested that it may also regulate systemic cardiovascular physiology. Methods – Wild type, thrombospondin-1 and CD47-null mice underwent analysis of several cardiovascular endpoints including cardiac output, ejection fraction and telemetric blood pressure before and after vasoactive challenge. Analysis of skin blood flow and core temperature change following vasoactive challenge was also performed. Results - Mice lacking thrombospondin-1 exhibit an activity-associated increase in diastolic blood pressure and have decreased pulse pressure. CD47-deficient mice have elevated resting blood pressure. Both null mice show exaggerated decreases in MAP and pulse pressure and increased cardiac output and ejection fraction in response to NO. They are also resistance to the hypertensive effects of epinephrine. Conversely, autonomic blockade induce exaggerated hypotensive responses in thrombospondin-1 null mice. In combination, these agents cause premature cardiovascular collapse and death of thrombospondin-1 null mice. Conclusions - Thrombospondin-1 signaling via CD47 is an acute physiological regulator of blood pressure and maintains blood pressure through resistance of nitric oxide stimulated vasodilation.

96

Cardiovascular mechanics in newborn ELN+/+, +/- and -/- mice

Jessica E. Wagenseil[1], Attila Kovacs[2], Robert P. Mecham[1].
[1]Department of Cell Biology and Physiology, Washington University, St. Louis, MO, [2]Department of Internal Medicine, Washington University, St. Louis, MO

ELN+/- mice have increased blood pressure, smaller vessels and increased elastic lamellae. ELN-/- mice die soon after birth due to occlusive proliferation of smooth muscle cells (SMCs). In both cases, decreased elastin levels and altered mechanical signals may influence SMC phenotype. We have measured blood pressure, aortic wall structure and aortic mechanical properties of ELN+/+, +/- and -/- mice on postnatal day 0 (P0). Blood pressure in P0 ELN-/- mice is higher than ELN+/+ and +/-. Aortic inner diameter is significantly different between all genotypes, being smallest in ELN-/-, then ELN+/- and largest in ELN+/+. P0 ELN-/- aorta show disorganized, proliferating SMCs at the intimal surface, instead of the circumferentially organized layers seen in ELN+/+. ELN+/- aorta show elastin producing SMCs in the adventitia, instead of collagen producing fibroblasts as in ELN+/+. Adventitial progenitor cells can be stimulated to differentiate into elastin producing myofibroblasts after stretch induced injury.

ELN-/- aorta have lower circumferential stresses at similar pressures compared to ELN+/+ and +/-, but almost identical stresses at physiologic pressure. ELN+/- aorta have increased circumferential stretch ratios at similar pressures compared to ELN+/+ and -/-. At P0, the characteristics of ELN-/- and +/- aorta suggest that circumferential stress induces SMCs to proliferate, while circumferential stretch induces progenitor cells to differentiate. This hypothesis will be investigated through similar studies at additional developmental time points.

97

HtrA1 - a Serine Protease that Regulates Vascular Calcification

Colette A. Inkson, Kristen D. Hadfield, Fiona L. Wilkinson, Gillian A. Wallis, Ann E. Canfield.
Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

Calcification of blood vessels is highly correlated with increased morbidity and mortality in patients with atherosclerosis, diabetes and chronic kidney disease. To identify factors involved in vascular calcification we performed differential analysis of mineralizing pericytes and identified HtrA1 (High Temperature Requirement protein A) a secreted protein with serine protease activity. Recent data from our lab and others suggests that HtrA1 is involved in the development, pathogenesis and mineralization of skeletal tissues. Therefore, this study tested the hypothesis that HtrA1 also regulates mineralization in the vasculature. Analysis of pericyte and vascular smooth muscle cell (VSMC) cultures revealed that HtrA1 is expressed by these cells and is down-regulated when mineralization occurs. Immunolocalization demonstrated that HtrA1 is expressed at sites of mineralization in human atherosclerotic arteries, co-localizing with matrix Gla protein (MGP) and osteopontin. To determine if HtrA1 regulates mineral deposition, VMSC were treated with recombinant HtrA1 (rHtrA1); these cells exhibited significantly less matrix mineralization compared to controls. To analyse the effect of HtrA1 protease activity on VSMC a Ser to Ala mutation of the active site triad was introduced; this protease inactive HtrA1 does not inhibit mineralization. Finally, we have shown that rHtrA1 cleaves elastin, decorin and matrix Gla protein. Together these results suggest that HtrA1 regulates mineral deposition by VSMC and experiments are currently in progress to determine the mechanism by which this occurs.

98

Vascular specific gene expression using the PDGFR-beta Promoter

Renee J. LeClair, Qiaozeng Wang, Lucy Liaw, Volkhard Lindner.
Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME 04074

The platelet-derived growth factor (PDGF) signaling pathway is of particular relevance to vascular biology as it regulates mural cell recruitment and smooth muscle cell migration. Currently many promoters used to drive expression in vascular smooth muscle in vivo have decreased activity in dedifferentiated SMC of the neointima. To overcome these limitations we generated transgenic mice with Cre recombinase under the control of the PDGF receptor-β(PDGFR-β) promoter, which displays increased activity following vascular injury. 19 transgenic founder lines were generated in the ROSA26 background for characterization of transgene expression by LacZ staining in embryonic and postnatal tissues as well as after vascular injury. Remarkable differences between transgenic lines with respect to transgene activity in different tissues were observed. Strong expression was commonly observed in the cartilage, sub epidermal mesenchyme, vascular smooth muscle in several organ beds, kidney mesenchymal cells and in the brain. Unique lines were identified showing high expression only in the smooth muscle cells, vascular endothelium and inner and outer granular layers of the brain. Another line had strict expression in the vascular smooth muscle, collecting ducts and mesenchymal cells of the kidney. After vascular injury, promoter activity was either unchanged or enhanced relative to basal levels of activity in large vessels.Generation of these transgenic animals is an important tool for vascular bed specific targeting of gene deletion and over expression.

These lines will be especially useful for directing gene expression or deletion during vascular remodeling by overcoming the limitations of current Cre driven vascular promoters.

99

Fbln-2 and fbln-5 cooperate to assemble and maintain the IEL

Shelby L. Chapman[1], F-X Sicot[2], Elaine C. Davis[3], Takako Sasaki[4], Mon-Li Chu[2], Hiromi Yanagisawa[1].
[1]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA, [2]Department of Dermatology and Cutaneous Biology, Thomas Jefferson University,Philadelphia, PA, USA, [3]Dept of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada, [4]Shriners Hospital for Children Research Center, Portland, Oregon

Recent findings on the role of fibulin-5 have provided substantial progress in understanding the molecular mechanism of elastic fiber assembly in vitro. However, little is known about the differential roles of fibulin proteins in the formation of elastic fibers in blood vessels. Here, we test the involvement of fibulin-2 and fibulin-5 in the formation of the internal elastic lamina (IEL) in vivo. Fibulin-2 is distinctly expressed in the IEL, whereas fibulin-5 is observed throughout the vessel wall. Although fibulin-2 and fibulin-5 exhibited strong binding to tropoelastin, no interaction was observed between fibulin-2 and fibulin-5. We generated double knockout mice for the fibulin-2 and fibulin-5 genes (termed DKO) and found that the IEL was severely disorganized in DKO mice. Furthermore, DKO vessels displayed abnormal remodeling, thrombus formation and aneurysmal change after carotid artery ligation-injury, underscoring the importance of the IEL in maintenance of the integrity of the vessel wall. In conclusion, fibulin-2 and fibulin-5 cooperatively function to form the IEL during postnatal development by directing assembly of elastic fibers, and are responsible for maintenance of adult vessel walls after injury. In additon, the DKO mouse will serve as a unique animal model to test the effect of vessel integrity during various pathological insults.

100

TG2, the missing link in the development of vascular stiffness ?

Simon Moreau[1], Nicolas Chabot[2], Jeffrey Wayne Keillor[2], Pierre Moreau.
[1]University of Montreal, Faculty of Pharmacy, [2]University of Montreal, Department of Chemistry

Transglutaminase 2 (TG2) catalyzes the formation of crosslinks of extracellular matrix proteins, like collagen and fibronectin (FN). These links promote the accumulation of matrix proteins, leading to fibrosis. Large artery stiffness is charactarized by an increased calcium and collagen deposition (fibrosis) in the extracellular matrix, with hemodynamic consequences. We sought to determine if TG2 could play a role in the development of vascular stiffness. Rats were treated with Warfarine (20mg/kg/d) and Vitamin K (15mg/kg/d) for 1, 3, 5, 7, 14, 21 and 28 days to induce calcification and vascular stiffness. Hemodynamic parameters and calcium amounts were evaluated. We measured the expression of TG2, FN and CTGF by western blot, ET-1 by RIA and binding of TG2 to FN by immunoprecipitation. TG2 activity was determined by probing aortic cryosections with a TG2 irreversible inhibitor coupled to rhodamine for fluorescence detection. Collagen was detected by histology. TG2 activity was augmented after 7 and 14 days of treatment. Collagen increased progressively from day 14 to day 28. We noted an increased expression of FN and ET-1 at 21 days of treatment, while TG2 and CTGF remained unchanged. Calcium increased significantly at 21 and 28 days, while stiffness reached significance at 28 days. We previously observed the early implication of MMP-9 and TGF-ß in our model, followed later by Cbfa-1, calcification, fibrosis and stiffness. Herein, we show that TG2 is activated early. Knowing that TG2 was recently implicated in vascular calcification, it could be the link between early and late events in vascular stiffness.

101
ECMPs act as centers of MMP activation and matrix remodeling.
Thomas P. Lozito[1], Cassie M. White[1,2], Catherine K. Kuo[1], Juan M. Taboas[1].
[1]CBOB, NIAMS, NIH, [2]Clemson University

Endothelial cell microparticles (ECMPs) are small membrane vesicles that are released from endothelial cells (ECs) during pathological conditions. Here we investigated the interactions between ECMPs and vascular matrix and the role of ECMPs in matrix remodeling. Our results showed that, first, ECMPs bound surfaces coated with vascular matrix molecules. Next, ECMPs exhibited significantly higher MMP-2 and MMP-9 activities than the surrounding EC conditioned medium (EC CM) via fluorescence-based enzyme assays. ECMPs also lacked the TIMP-1 found in EC CM, and, while EC CM strongly inhibited MMP-2 and MMP-9, ECMPs did not. Furthermore, not only did ECMPs display endogenous MMP-2 and MMP-9 activities, but ECMPs were also shown to activate exogenous MMP-2. Our results showed that this was mediated by MMP-14, which was detected solely in its active form in ECMPs. ECMPs also bound to vascular matrix activated exogenous MMP-2 despite high levels of TIMPs in the surrounding environment. Finally, MMP-2 activated by matrix-bound ECMPs was able to modify that matrix, again despite the presence of EC-secreted TIMPs. Thus, amidst the TIMP-rich local environment created by ECs, ECMPs were shown to bind to vascular matrix and create a local zone where MMP-2, either provided by ECs or other cells of the vascular environment, was activated and able to remodel the surrounding matrix. These findings may have applications in therapies targeting the pathological consequences of ECMP-mediated disruption of the vascular matrix. (Supported by NIAMS IRP Z01AR4 131)

102
MSCs create a TIMP-rich, matrix-protective local environment.
Thomas P. Lozito[1], Cassie M. White[1,2], Catherine K. Kuo[1], Juan M. Taboas[1], Rocky S. Tuan[1].
[1]CBOB, NIAMS, NIH, [2]Clemson University

There is evidence to suggest mesenchymal stem cells (MSCs) are perivascular, occupying a prime location for regulating vessel stability. For example, MSCs are known to secrete potentially vessel-disrupting proteases, including MMPs, as well as TIMPs. Here we investigated MSC secretion of MMP-9 and MMP-2 (MMP-9/2), two proteases strongly implicated in disrupting vascular matrix. We also considered MSC secretion of TIMPs and whether their interactions with MMP-9/2 contributed to matrix remodeling or stabilization. First, despite MMP-9/2-secretion by MSCs, MMP-9/2 activity was not detected in MSC conditioned medium (MSC CM) by fluorescence-based assays. Reverse zymography showed that MSCs secreted functional TIMPs. MSC CM also strongly inhibited exogenous MMP-9/2, comparable to known MMP inhibitors. In addition, we showed that MSC CM protected vessels from disruption by exogenous MMP-2 in vitro. Lastly, we investigated the effects of pro-inflammatory cytokines and hypoxia on MSC-secretion of MMP-9/2 and TIMPs and found that, even under conditions traditionally considered conducive to MMP-activation, MSCs remained matrix-protective. While several of these conditions increased MMP secretion, this was matched with corresponding increases in TIMP secretion. In fact, the endogenous MMP-9/2 activities of MSC CM remained undetectable, and the MMP inhibitory properties of MSC CM were maintained. These results show that MSCs, residing in the perivascular niche, serve as robust sources of TIMP-mediated MMP-9/2 inhibition, capable of protecting the vascular environment from high levels of MMP-9/2 despite pathological conditions. (Supported by NIAMS IRP Z01AR4 131)

103
MAPK/SMAD signaling in fibrin-based constructs grown in vitro
Justin S. Weinbaum, Zeeshan H. Syedain, Robert T. Tranquillo.
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455

Replacement of diseased vessels by a fully biological tissue-engineered blood vessel (TEBV) would be an ideal solution to a major

clinical need. Our strategy uses vascular smooth muscle cells to remodel a fibrin tube, resulting in a collagen-rich media-equivalent (ME) layer for the TEBV. Our goal is that such an ME will have sufficient mechanical strength for vascular replacement. In order to understand and optimize ME maturation during static incubation or in response to mechanical stimulation, we investigated two signaling cascades. Mitogen-activated protein kinases (MAPKs) were investigated since cyclic distension (CD) is known to activate MAPK pathways. Both p38 and p42/44 MAPKs, but not JNK, were activated during the initial two weeks of static culture but were attenuated during the next two weeks. Fibroblast-based constructs were used to look at the p42/44 response to CD; constant CD leads to increased collagen deposition and mechanical strength that is further improved by incremental CD. Three weeks of constant CD failed to activate p42/44 MAPK but incremental CD led to a twofold increase over the static control. This suggests adaptation of the p42/44 signal to constant mechanical stimulation which was overcome by incremental stimulation. TGF-β1 promoted collagen deposition in the MEs; therefore, SMAD signaling was investigated in static culture. SMAD2 activity remained constant but SMAD7 protein level decreased over time indicating enhanced signaling overall. We believe that by targeting pathways activated during ME maturation that we will be able to strengthen our TEBV to withstand human hemodynamics.

104

GGCX and ABCC6 Gene Mutations in a Family with PXE-like Phenotype

Qiaoli Li[1], Dorothy K. Grange[2], Nicole L. Armstrong[2], M. Yadira Hurley[3], Mark Rishavy[4], Kevin Hallgren[4], Kathleen L. Berkner[4], Leon J. Schurgers[5], Qiujie Jiang[1], Jouni Uitto[1].
[1]Jefferson Medical College, Philadelphia, PA, [2]Washington University, St. Louis, MO, [3]St. Louis University, St. Louis,MO, [4]Case Western Reserve University, Cleveland, OH, [5]Cardiovascular Research Institute and VitaK BV, University of Maastricht, Maastricht, The Netherlands

A characteristic feature of classic PXE, an autosomal recessive disorder caused by mutations in the *ABCC6* gene, is aberrant mineralization of connective tissues. Here, we report a family with PXE-like cutaneous features in association with multiple coagulation factor deficiency, an autosomal recessive disorder associated with *GGCX* mutations. The proband and her sister, both with severe skin findings including mineralization, were compound heterozygotes for missense mutations in *GGCX*, which were shown to result in reduced g-glutamyl carboxylase activity and in under-carboxylation of matrix gla protein. The proband's mother and aunt, also manifesting with PXE-like skin changes, were heterozygous carriers of a missense mutation (p.V255M) in *GGCX* and a null mutation (p.R1141X) in the *ABCC6* gene, suggesting digenic nature of their skin findings. Thus, reduced g-glutamyl carboxylase activity in individuals either compound heterozygous for a missense mutation in *GGCX* or with haploinsufficiency in *GGCX* in combination with heterozygosity for *ABCC6* gene expression results in aberrant mineralization of skin leading to PXE-like phenotype. These findings expand the molecular basis of PXE-like phenotypes, and suggest a role for multiple genetic factors.

105

The Bone Marrow Microenvironment in Neuroblastoma Progression

YVES A. DE CLERCK.
The Saban Research Institute of Childrens Hospital Los Angeles, USC Keck School of Medicine

It has become increasingly evident that the microenvironment contributes to tumor progression. In this aspect, the bone marrow and the bone are particularly fertile environments. In our laboratory, we have studied the contribution of the bone marrow to the progression of human neuroblastoma, the second most common solid tumor in children and a cancer that frequently metastasizes to the bone and the bone marrow. In the presence of bone marrow stromal cells (BMSC), human neuroblastoma cells stimulate the production of interleukin-6 (IL-6) by these cells. This stimulatory effect does not require cell-cell contact but is mediated by soluble factors like prostaglandin-E2 and galectin-3 binding protein, a glycosylated protein that binds to galectin-3, present at the surface of BMSC. The production of IL-6 in the bone marrow

microenvironment has multiple effects that promote tumor progression. It stimulates osteoclast maturation and activity and the formation of osteolytic lesions. It also has a paracrine effect on neuroblastoma cells that express a functional IL-receptor (IL-6R). As a result, neuroblastoma cells proliferate more rapidly and become resistant to drug-induced apoptosis. Consistent with IL-6 being a positive contributor to neuroblastoma progression, we found elevated levels of IL-6 in the serum and the bone marrow of patients with neuroblastoma bone metastasis and an increased expression of IL-6 in BMSC obtained from these patients. Thus IL-6 is a critical cytokine produced by BMSC in the bone marrow microenvironment that promotes the proliferation and survival of neuroblastoma cells.

106

The proteoglycan metastatic signature of a cancer cell

Silvia Rossi[1], Katia Lacrima[1], Pier Andrea Nicolosi[2], Sabrina Cattaruzza[2], Elena Garusi[1], Jorge Filmus[3], Guido David[4], Roberto Perris[1,2]. [1]Dep. of Genetics, University of Parma, Italy, [2]National Cancer Institute Aviano, Italy, [3]Sunnybrook and Women's College of Health Sciences, Toronto, Canada, [4]Dep. of Mol. and Devel. Genetics, University of Leuven, Belgium

Cell surface proteoglycans PGs are known key regulators of tumor progression and several of them are now emerging as clinically relevant targets. The tumorigenic function of the 11 primary PGs, including syndecans-1-4, glypicans-1-6 and NG2, is only in part resolved for some of these PGs individually, but not cumulatively. We have approached this problem by creating sarcoma cell lines with diverse PGs surface profiles and have comparatively assayed these engineered cells in vitro and for their migratory abilities and tumour growth/metastasis formation capacities in vivo. To this end we have developed a unique system for isolating native matrices from cultured cells; a system for the accurate monitoring and quantification of tumour cell-endothelial interactions (under flow); and a novel system for examining the tumour cells' interactions with normal human tissues in vitro. Misexpression of glypican-4, -5 and –6 on diverse constitutive PG backgrounds modulates the in vitro and in vivo behaviour of the cells. To further define how given PG surface profiles may modify the behavioural traits of the cells, we combined the ectopic/overexpression of the above glypicans with RNAi knockdown of endogenous glypican-1, syndecan-1 and syndecan-4. The outcome of these investigations provide the first evidence that defined surface PGs pattern differentially control tumorigenesis.

107

A central role for decorin during vertebrate convergent extension

Jason J. Zoeller[1], Wittaya Pimtong[1], Helen Corby[1], Angela McQuillan[1], Silvia Goldoni[1], Alex E. Iozzo[1], Rick T. Owens[2], David J. McQuillan[2], Shiu-Ying Ho[1], Renato V. Iozzo[1]. [1]Thomas Jefferson University, Philadelphia, PA 19107, [2]LifeCell Corporation, Branchburg, NJ 08876

Decorin, an archetypal member of the small leucine-rich proteoglycan gene family, regulates collagen fibrillogenesis and cell growth. To further explore its biological function, we examined the role of decorin during zebrafish development. Zebrafish decorin is a chondroitin sulfate proteoglycan which exhibits a high degree of conservation with its mammalian counterparts and displays a unique spatiotemporal expression pattern. Morpholino-mediated knockdown of zebrafish decorin identifies a developmental role for decorin during medial-lateral convergence and anterior-posterior extension of the body plan, as well as in craniofacial cartilage formation. Decorin morphants display a pronounced shortening of the head to tail axis as well as compression, flattening and extension of the jaw cartilages. The morphant phenotype could be rescued by zebrafish decorin mRNA. Unexpectedly, microinjection of excess zebrafish decorin mRNA or proteoglycan/protein core into one-cell stage embryos caused a defect in convergent extension associated with cyclopia. Overall, the results of our investigation indicate a central function for decorin during key early events of body plan development and cartilage formation, and further suggest that decorin may play a role in the Wnt and/or Hedgehog signaling pathways.

108

Prolyl 3-hydroxylase 1 null mice have abnormal bones and tendons

Janice A. Vranka[1], Elena N. Pokidysheva[1], Kerry Maddox[1], Lauren K. Hayashi[1], Sara F. Tufa[1], Doug R. Keene[1], Emily Larson[3], Robert Klein[3], Hans Peter Bächinger[1,2].
[1]Research Dept, Shriners Hospitals for Children, Portland, OR 97239, [2]Dept of Biochem and Molec Biology, Oregon Health and Sci Uni., Portland, OR 97239, [3]Bone and Mineral Unit, Oregon Health and Science Uni., Portland, OR 97239

Osteogenesis imperfecta (OI) is a skeletal disorder primarily caused by mutations in collagen I genes. However, recent investigations have revealed that mutations in cartilage associated protein (CRTAP) or prolyl 3-hydroxylase 1 (P3H1) can cause a severe, recessive form of OI. These reports show minimal 3-hydroxylation of key residues in types I and II collagens as a result of CRTAP or P3H1 deficiency and demonstrate the importance of P3H1 and CRTAP to bone structure and development. P3H1 and CRTAP have previously been shown to form a stable complex with cyclophilin B (CYPB), and P3H1 was shown to catalyze the 3-hydroxylation of specific proline residues in procollagen I in vitro. Here we describe a mouse model in which the P3H1 gene has been inactivated. Our data demonstrate abnormalities in collagen fibril ultrastructure in tendons from P3H1 null mice by EM. Differences are also seen in skin architecture, as well as in developing limbs by histology. Additionally bone density was measured by microCT and determined to be significantly lower in the P3H1 mice as compared with wild type littermates. Altogether these investigations demonstrate disturbances of collagen fiber architecture in tissues rich in fibrillar collagen including bone, tendon and skin. This model system presents a good opportunity to study underlying mechanisms of recessive OI and understand its effects in humans.

109

MT1-MMP, vascular guidance tunnels, and EC-pericyte tube assembly

George E. Davis, Amber N. Stratman.
Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, MO 65212

Recent work in our laboratory reveals that endothelial cell (EC) tube morphogenesis is controlled by the cell surface proteinase, MT1-MMP, through its ability to create proteolyzed spaces, termed vascular guidance tunnels, within collagenous extracellular matrices (ECM). These EC generated spaces represent a physical imprint in the matrix allowing for EC motility and tube remodeling. In EC-pericyte cocultures, there is marked pericyte recruitment into these vascular guidance tunnel spaces along the ablumenal surface of EC tubes resulting in polarized and dynamic EC-pericyte interactions. These interactions result in extracellular deposition of the basement membrane matrix components, collagen type IV, laminin, nidogens 1/2, perlecan and fibronectin. This ECM deposition does not occur in EC only cultures. Both cell types produce each basement membrane matrix component (as detected by mRNA and protein analyses) while EC-pericyte interactions selectively induce the production and deposition of three basement membrane components: fibronectin, nidogen-1 and TIMP-3 which possess ECM bridging and stabilization functions. Furthermore, EC integrin $\alpha5\beta1$ blocking antibodies or addition of a 70kDa fragment of fibronectin (which blocks fibronectin assembly) cause marked morphologic changes of EC tubes in EC-pericyte cocultures but not in EC only cultures, while anti-$\alpha2\beta1$ integrin antibodies cause tube collapse in EC only cultures but not in EC-pericyte cocultures. Also, we show that pericyte expression of TIMP-3 in cocultures is necessary to maintain collagen type IV integrity around developing tubes. Thus, MT1-MMP-generated vascular guidance tunnels provide a conduit for polarized EC-pericyte interactions leading to dynamic ECM remodeling events leading to basement membrane matrix assembly and tube stabilization.

110

Epilysin (MMP-28) functions in promoting epithelial cell survival

Anne M. Manicone[1], Victoria Mai[1], Sara Illman[2], Timothy P. Birkland[1], Bill C. Parks[1].
[1]Center for Lung Biology, Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA,

[2]Department of Pathology, University of Helsinki, Helsinki, Finland

Epilysin (MMP-28) is constitutively expressed in numerous tissues, and in adult mice, it has the highest expression in the lung, where it is produced by bronchial epithelial cells. The expression of epilysin in uninjured, non-inflamed tissues suggests that this proteinase functions in epithelial homeostasis. Indeed, compared to wildtype mice, we observed increased apoptosis in the airway epithelium of MMP-28-null mice following influenza infection and upregulation of stress-response genes in lungs of naïve knock-out mice. We also generated stable lines in human respiratory epithelial cells over-expressing catalytically-active (WT) or catalytically-inactive (Mu) human epilysin. The resultant cells proliferate normally with unaltered morphology, wound closure rates, and adherence and spreading on type I collagen, fibronectin, and laminin-111. Similar to our in vivo observations, we found that cells over-expressing WT epilysin were more resistant to apoptosis induced by serum-deprivation or staurosporine than were Mu cells. Together, these results suggest that epilysin plays a role in promoting epithelial cell survival.

111

TGFβ+EGF promotes PAI-1 sensitive collagen gel lysis and invasion.

Cynthia E. Wilkins, Qunhui Ye, Paul J. Higgins. Center for Cell Biology & Cancer Research, Albany Medical College, Albany, NY 12208

Epidermal growth factor receptor (EGFR) amplification and an altered cellular response to transforming growth factor-β (TGF- β),accompany the progression of epithelial tumor cells from a non-invasive phenotype to an aggressive, metastatic carcinoma. During this pathological epithelial-to-mesenchymal transition (EMT), despite increased autocrine/paracrine expression of TGF- β, cells become refractory to its growth-suppressive effects. TGF-β promotes tumor invasive properties through the induction of stromal remodeling genes which paradoxically support matrix disruptive as well as stabilizing processes. In response to TGF- β1+EGF stimulation, transformed human keratinocytes (HaCaT-II4)undergo phenotypic changes characteristic of EMT, become highly collagenolytic and invade typ-1 collagen gels. Enhanced collagenolysis was coupled to incresed expression of matrix metalloproteinase -10 (MMP-10) and involved a plasmin/MMP-10/MMP-1 proteolytic axis. Neutralization of any one component in this cascde blocked collagen degradation, as did addition of plasminogen activator inhibitor type-1 (PAI-1). PAI-1 is the most highly expressed member of the TGF-β1 response gene set in HaCaT-II4 cells and was synergistically up-regulated in response to TGF-β1+EGF. The present findings Provide a mechanism for TFF- β1+EGF initiated matrix remodeling in malignant human keratinocytes and propose a criticl role for PAI-1 in regulating stromal degradation/tumor invasion. (Supported by NIH grant:GM57242)

112

Scleraxis is required for normal heart valve formation in vivo.

Agata K. Levay[1], Jacqueline D. Peacock[1], Yinhui Lu[2], Manuel Koch[3], Robert B. Hinton, Jr.[4], Karl E. Kadler[2], Joy Lincoln[1].
[1]Department of Molecular and Cellular Pharmacology, Leonard M. Miller School of Medicine, University of Miami, FL, 33136, USA, [2]Wellcome Trust Center for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, M13 9PT, UK, [3]Division of Molecular and Cardiovascular Biology, Cincinnati Children's Hospital, Cincinnati, OH, 45229, [4]Dept of Cell and Developmental Bio, Oregon Health and Sci Uni, Portland, OR

Heart valve structures derived from mesenchyme precursor cells are composed of differentiated cell types and extracellular matrix arranged to facilitate valve function. However, the molecular mechanisms required to form complex valve structures from undifferentiated precursor cells are poorly understood. Scleraxis (scx) is a transcription factor expressed in tendons and required for tendon matrix formation. In this study, we observe additional scx expression in developing heart valve structures using scx-GFP mice. The in vivo function of scx during heart valve formation was determined using mice null for scx. Scx-/- mice

display significantly thickened heart valve structures from E17.5, and valves from mutant mice show alterations in valve precursor cell differentiation and matrix organization. This is indicated by decreased expression of the tendon-related collagen type XIV, increased expression of cartilage- associated genes including sox9, as well as persistent expression of mesenchyme cell markers including msx1. In addition, ultra-structure analysis reveals ECM disarray. Thickened valve structures in juvenile scx-/- mice show characteristics of human valve disease including increased expression of matrix remodeling genes and excessive collagen deposition. Collectively our studies have identified new roles for scx during valvulogenesis and demonstrate its requirement for cell lineage differentiation and matrix distribution during heart valve development in vivo.

113

Skeletal abnormalities in quad-KO mice (TSP1, 3, 5, and Col 9)

Karen L. Posey[1], Kurt Hankenson[2], Alka C. Veerisetty[1], Paul Bornstein[3], Jack Lawler[4], Jacqueline T. Hecht[1,5].
[1]Department of Pediatrics, University of Texas Medical School at Houston, TX, [2]University of Pennsylvania School of Veterinary Medicine, PA, [3]Departments of Biochemistry and Medicine, University of Washington, Seattle, WA, [4]Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, [5]Shriners Hospital for Children, Houston, TX

Thrombospondin-5 (TSP5) is a large extracellular matrix glycoprotein found in musculoskeletal tissues. TSP5 mutations cause two skeletal dysplasias, pseudoachondroplasia and multiple epiphyseal dysplasia; both show a characteristic growth plate phenotype with retention of TSP5, as well as type IX collagen and matrilin-3, in the rough endoplasmic reticulum. While, most studies focus on defining the disease process; few functional studies have been performed. TSP5 knock-out mice have no obvious skeletal abnormalities, suggesting that TSP5 is not essential in the growth plate and/or other TSPs may compensate. In contrast, type IX collagen knock-out mice have diminished matrilin-3 in the extracellular matrix and early onset osteoarthritis. To define the roles of TSP1, TSP3, TSP5 and type IX collagen in the growth plate, each knock-out and combinatorial strains were analyzed using histomorphometric techniques. While significant alterations in growth plate organization were found in some of the single knock-out mouse strains, skeletal growth was only mildly disturbed. In contrast, dramatic changes in growth plate organization in TSP3/5/Col9 knock-out mice result in a 20% reduction in limb length corresponding to short stature in humans. These studies suggest that 1) type IX collagen may regulate growth plate width, 2) TSP3, TSP5 and type IX collagen contribute to growth plate organization, and 3) TSP1 may help define the timing of growth plate closure when other extracellular proteins are absent.

114

Dystroglycan is required for neural maintenance and guidance.

Robert P. Johnson, Seonghoon Kang, James M. Kramer.
Department of Cell and Molecular Biology and Center for Genetic Medicine, Northwestern University, Chicago, IL 60611

Mutants lacking *C. elegans* dystroglycan DGN-1 display a neural mispositioning phenotype in which cell bodies of posterior neurons are positioned normally in mid-stage embryos but become progressively displaced beginning in late embryogenesis. DGN-1 is thus required for positional maintenance of posterior neurons. We have identified potential *dgn-1* interacting genes through a screen for mutations producing the neural mispositioning phenotype. One of them, *dig-1*, encodes a novel extracellular matrix protein recently implicated in the positional maintenance of neural cell bodies and axons. Analysis of single and double mutants supports a role for a DIG-1/DGN-1 pathway in maintaining neural position. Another gene identified, *anc-1*, encodes an actin-binding protein that anchors nuclei in the syncitial

hypodermis. ANC-1 acts in a neural maintenance pathway parallel to DGN-1 and DIG-1 in late embryos. The dependence of specific neurons on these genes indicates that mechanisms controlling positional maintenance vary between neuron types. In addition, *dgn-1* mutants show an axon guidance defect in which posterior neuron axons migrating to the ventral nerve cord (VNC) fail to traverse the lumbar commissure, a phenotype also seen in *unc-6*/netrin mutants. UNC-6 is mainly required for guidance of the pioneering PVQ axons to the VNC, whereas DGN-1 functions in both PVQ-dependent and –independent guidance of follower axons. We are using cell type-specific rescue of DGN-1 to determine critical sites of DGN-1 function in neural maintenance and guidance.

115

Proteoglycan desulfation regulates endochondral ossification

Carmine Settembre[1,2], Emilio Arteaga-Solis[1], Marc D. McKee[3], Raquel de Pablo[2], Qais Al Awqati[1], Andrea Ballabio[2], Gerard Karsenty[1].
[1]Department of Genetics & Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA, [2]Telethon Institute of Genetic and Medicine (TIGEM), Via P. Castellino 111a, Naples, 80131,Italy., [3]Faculty of Dentistry, and Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada H3A 2B2, [4]Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy

Cartilage extracellular matrix (ECM) contains large amounts of proteoglycans that are made of a protein core decorated by highly sulfated sugar chains, the glycosaminoglycans (GAGs). GAGs desulfation, a necessary event, is exerted by sulfatases that are activated by another enzyme, sulfatase modifying factor 1 (Sumf1). In humans, Sumf1 inactivation leads to severe skeletal abnormalities. We show here that despite being equally expressed in osteoblasts and proliferating chondrocytes Sumf1 does not overtly affect osteoblast differentiation. Conversely, in chondrocytes it favors ECM production and autophagy and promotes proliferation by limiting FGF signaling. Thus, proteoglycan desulfation is a critical and specific regulator of several aspects of chondrogenesis.

116

Heparan sulfate function in limb synovial joint formation

Christina Matticola[1], Kevin Quann[1], Takanaga Ochiai[1], Yu Yamaguchi[2], Eiki Koyama[1], Maurizio Pacifici[1].
[1]Thomas Jefferson University, Philadelphia, PA 19107, [2]Burham Institute for Biomedical Research, La Jolla, CA 92037

Formation of synovial joints is a fundamental process in skeletogenesis, but relatively little is known about its underlying mechanisms of regulation. Since BMP, Wnt, Hedgehog and other heparin-binding signaling proteins are involved in joint formation, we asked whether their action is mediated by, and dependent on, local heparan sulfate (HS) synthesis. Thus, we mated floxed *Ext1* mice with transgenic mice expressing Cre-recombinase (*Gdf5-Cre*) in mesenchymal cells (collectively known as the interzone) present at prospective joint sites in early limbs, and examined the resulting *Ext1*-deficient mice at prenatal and postnatal time points. We found that joint formation was markedly deranged by local HS deficiency. At early stages, formation of a typical tripartite mesenchymal interzone was hampered in mutants. With further development, the joint defects became more severe. Mutant articular cartilage was disorganized, lacked a compact cell layer facing the synovial cavity, and expressed markedly lower levels of essential extracellular matrix components, including lubricin and collagen IIA. Interestingly, the defects were not limited to the joint area, but affected the entire epiphyseal region and the secondary ossification center in particular. The data reveal that local HS synthesis is essential for limb synovial joint formation, and abnormalities in this process have serious repercussions for overall long bone development. Ongoing experiments aim to clarify the signaling pathways involved in the joint and epiphyseal abnormalities.

117

Role of heparan sulfate in limb skeletal development

Kazu Matsumoto[1], Yoshihiro Matsumoto[1], Fumitoshi Irie[1], Jun-ichi Fukushi[2], William B.

Stallcup[2], Yu Yamaguchi[1].
[1]Sanford Children's Health Research Center, [2]Cancer Center, Burnham Institute for Medical Research, La Jolla, CA 92037

Heparan sulfate (HS) is an integral component of many morphogen signaling pathways. However, its mechanisms of action appear to be diverse, depending on the type of morphogen and the developmental context. Here we show that HS plays a critical role in regulating the range of BMP signaling during endochondral ossification. Limb mesenchyme-specific conditional mutants lacking Ext1, which encodes an essential glycosyltransferase for HS synthesis, display severe limb defects, including hypoplastic limb bones, oligodactily, and fusion of joints. Chondrogenic differentiation of mutant mesenchymal condensations is delayed and impaired, whereas the area of differentiation is expanded without clear boundaries. Mutant mesenchymal cells show delayed chondrocyte differentiation and poor response to exogenous BMPs in vitro. In micromass cultures, the tight spatial control of BMP signaling observed in wild-type chondrogenic nodules is disrupted in mutant nodules, suggesting aberrant distribution of BMP proteins in the absence of HS. Consistently, the distribution of BMP2 in the mutant limb mesenchyme is markedly broadened and diffuse. Our results indicate that HS regulates the spatial distribution of BMP proteins by restricting their diffusion. On the other hand, our data are not consistent with the notion that HS acts as an obligatory coreceptor for BMPs. The impaired BMP signaling in mutants is likely the consequence of the dilution of BMP proteins due to increased diffusion.

118

Integrin-mediated activation of TGFβ

Dean Sheppard.
Department of Medicine, University of California, San Francisco

TGFβ isoforms are secreted and stored in latent form cross-linked to the extracellular matrix of most tissues. Much of the in vivo regulation of TGFβ function occurs at the level of activation of these latent complexes. Recent evidence suggests that αv integrins play an important role in this activation process. We have utilized mice lacking the integrin β6 subunit to identify roles for αvβ6-mediated TGFβ activation in regulating pulmonary and renal fibrosis, acute lung injury and pulmonary emphysema. Ligation of a small group of G protein-coupled receptors triggers an inside-out signaling pathway that regulates αvβ6-mediated TGFβ activation through induction of actin-myosin contraction. Using mice generated by Louis Reichardt's laboratory expressing conditional null alleles of the integrin β8 subunit, we have identified important roles for αvβ8 mediated TGFβ activation by dendritic cells or T cells in both negatively and positively regulating immune responses in the colon, central nervous system and lung.

119

Merlin Blocks Tumorigenesis by Inhibiting a Nuclear E3 Ligase

Filippo G. Giancotti, Liru Liu, Wei Li, Gaia Schiavon, Angela Pepe.
Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065

Integrin-mediated activation of PAK disrupts an intramolecular interaction that maintains Merlin in a closed, growth inhibitory conformation, thereby removing a block to mitogenesis. However, the mechanism through which the de-phosphorylated, closed form of Merlin suppresses cell proliferation and tumorigenesis is currently unclear. By using TAP followed by mass spectrometry, we have found that the closed form of Merlin interacts selectively with the receptor component of a novel Cul4 E3 ubiquitin ligase. Biochemical and functional studies indicate that Merlin suppresses the enzymatic activity of the ligase, placing Merlin upstream of the ligase. Several tumor-derived missense mutants of Merlin display severely reduced binding to the ligase, suggesting that Merlin's interaction with the ligase is important for growth inhibition. Silencing of the ligase suppresses the ability of Merlin-deficient cells to exit from contact inhibition and hyperproliferate, suggesting that the ligase operates to remove a block to mitogenesis. In addition, depletion of

the ligase suppresses the ability of Merlin-null tumor cells to form soft agar colonies in vitro and subcutaneous tumors in nude mice. Biochemical fractionation and immunofluorescence studies indicate that Merlin interacts with the ligase in the nucleus. Furthermore, DNA microarray studies indicate that expression of Merlin or depletion of the ligase induces a virtually identical gene expression program. These results indicate that Merlin suppresses tumorigenesis by inhibiting a novel nuclear ligase.

120

Pyk2 FERM regulates p53 in the absence of FAK

Ssang-Taek Lim, Nichol L. Miller, Xiao Lei Chen, Yangmi Lim, David D. Schlaepfer.
UCSD Moores Cancer Center, La Jolla, CA 92093

Proline-rich kinase-2 (Pyk2) and focal adhesion kinase (FAK) are related cytoplasmic tyrosine kinases that share similar N-terminal 4.1, ezrin, radixin, moesin (FERM), central kinase, and C-terminal focal adhesion targeting (FAT) domains and function as integrin-activated tyrosine kinases. We recently showed that FAK inactivation during mouse development results in p53-dependent growth arrest and that nuclear FAK promotes cell survival through FAK FERM-enhanced p53 turnover (Lim et al, Mol. Cell 29: 9-22, 2008). As Pyk2 expression increases upon FAK inactivation in fibroblasts and endothelial cells, we tested whether Pyk2 may promote cell survival in the absence of FAK. In early passage FAK-/-p21-/- fibroblasts, Pyk2 levels increase with passage and this coincides with decreased p53 levels and enhanced cell growth rate. Pyk2 knockdown prevents changes in p53 and Pyk2 FERM re-expression maintains low p53 levels. Autophosphorylation (Y402F) or kinase-dead (K457A) Pyk2 mutants also reduce p53 levels, confirming that this connection is not through a canonical Pyk2 kinase signaling pathway. Mutation of the F2 lobe within the Pyk2 FERM domain (R184T/R185T, TT) prevents Pyk2 FERM nuclear localization, weakens Pyk2 binding to p53, and blocks the ability of the Pyk2 FERM domain to regulate p53 levels. In human fibroblasts, FAK knockdown plus cisplatin addition triggers p53-dependent apoptosis that can be rescued by Pyk2 FERM WT but not Pyk2 FERM-TT expression. Together, our results support the importance of compensatory Pyk2 expression in promoting cell survival in a kinase independent manner through FERM-dependent p53 regulation.

121

The alpha5beta1 integrin as mechanotransducer in chondrocytes

Linda M. Kock[1,2], Corrinus C. van Donkelaar[1], Ronny M. Schulz[2], Augustinus Bader[2], Keita Ito[1].
[1]Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands, [2]Department of Cell Techniques and Applied Stem Cell Biology, University of Leipzig, Germany

Mechanical loading has been proposed as a mechanism for improving the functionality of engineered cartilage tissue. However, the precise mechanotransduction pathways are unknown. Recent studies have shown that integrins can act as mechanoreceptors in articular cartilage. In this study, we examined the role of integrin $\alpha5\beta1$ in the mechanoregulation of both ECM gene expression and ECM protein synthesis in tissue-engineered chondrocyte/agarose constructs. Chondrocytes, isolated and seeded in 3% agarose constructs, were compressed at 0.33 and 1 Hz, in the presence or absence of GRGDSP (a blocker for integrin $\alpha5\beta1$). mRNA levels for aggrecan, collagen II and MMP-3 were determined at several time points up to 24 hours post-stimulation. Cell viability, DNA and sGAG content were determined at several time points up to 28 days post-stimulation. mRNA levels for all genes were upregulated upon loading, except for collagen II, loaded at 0.33 Hz. Incubation with GRGDSP counteracted upregulation. sGAG levels were significantly lower in constructs loaded at 0.33 Hz compared to the unstrained control at day 28. In contrast, loading at 1 Hz caused a significant increase in sGAG deposition at this timepoint, which was counteracted by blocking the $\alpha5\beta1$ integrin. We conclude that the $\alpha5\beta1$ integrin acts as a mechanotransducer in the regulation of both ECM gene expression and matrix biosynthesis

for chondrocytes seeded in agarose under the loading regimes applied. However, this regulation appears frequency dependent.

122

Mice deficient in the ECM protein WARP have nerve defects

Justin M. Allen[1,2], Bent Brachvogel[4], Ursula Schlötzer-Schrehardt[5], Jamie Fitzgerald[1,6], John F. Bateman[1,3].
[1]Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia, [2]Harvard Medical School, Children's Hospital Boston, Boston, MA, USA, [3]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia, [4]Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany, [5]Department of Ophthalmology, University Erlangen-Nurnberg, Erlangen, Germany, [6]Department of Orthopaedics and Rehabilitation, Oregon Health and Science Uni, Portland, OR

WARP is a recently identified extracellular matrix molecule with restricted expression in permanent cartilages and a distinct subset of basement membranes in peripheral nerves, muscle and the central nervous system vasculature. WARP interacts with perlecan and type VI collagen, suggesting a function in bridging connective tissue structures. To understand the in vivo function of WARP, we generated a WARP-deficient mouse strain. WARP-null mice were healthy, viable and fertile with no overt abnormalities. Motor function and behavioral testing demonstrated that while general motor function was unaffected, the WARP-null mice exhibited a delayed response to acute painful stimulus and impaired fine motor coordination suggesting compromised peripheral nerve function. Immunostaining of perlecan and laminin revealed an apparently normal endoneurial basement membrane, however the collagen VI microfibrillar matrix was severely reduced and mislocalized. In addition, ultrastructural analysis revealed reduced fibrillar collagen deposition and partial fusing of adjacent Schwann cell basement membranes. In contrast, other WARP-deficient tissues such as articular cartilage and intervertebral discs were indistinguishable from wild type littermates and displayed no predisposition to degenerative changes. Our data suggest a peripheral nerve-specific role for WARP in the interaction of basement membranes with the interstitial matrix which may be important for nerve function.

123

A Laminin-Nidogen Chimera Facilitates Basement Membrane Assembly

Stephanie Capizzi, Karen McKee, Peter D. Yurchenco.
Robert Wood Johnson Medical School. Piscataway, NJ 08854

Laminin self-assembly contributes to the formation of basement membranes, requiring α, β, and γ LN domains. Those laminins possessing only two of three LN domains cannot self-assemble. In laminin-α2 deficient congenital muscular dystrophy there is increased expression of the laminin-α4 subunit in muscle and nerve. However, it is ineffective at compensating for the α2 subunit, in part because it cannot polymerize. We asked whether laminin-411 and related two short-arm laminins could be converted to polymerizing laminins, increasing their ability to assemble a basement membrane. To address this issue, the nidogen-binding site of laminin-111 was used to attach a modified third short-arm onto laminin. This chimeric short-arm consists of laminin-α1 LN-LEa domains and nidogen-1 domains G2-rod-G3. The α1LN-LEa domains enable interactions with laminin short-arms and allow laminin polymerization, where domains G2-rod-G3 maintain type-IV collagen binding and attachment to laminin. The recombinantly expressed α1Lm-Nd chimeric protein, was evaluated for structure, binding and self-assembly, and its ability to promote basement membrane assembly on cultured Schwann cells. This 156 kDa protein had a triple dumb-bell shape by rotary shadowing electron microscopy, bound to laminin and type-IV collagen, and facilitated polymer formation of an alpha short-arm deleted laminin-111. Furthermore, it supported significant accumulation of laminin and type-IV collagen in

Schwann cell basement membranes. The ability to convert non-polymerizing laminins into laminins that can self-assemble, offers a new approach to treat laminin-α2 deficient congenital muscular dystrophy.

124

Mutant COL4A1 triggers oxidative stress in a genetic model of AMD

Yi-Chinn Weng[1], David J. Dilworth[1], Richard T. Libby[2], Simon W. M. John[3], Douglas B. Gould[1].
[1]Departments of Ophthalmology, University of California, San Francisco, San Francisco, CA 94143, [2]Departments of Ophthalmology, University of Rochester, Rochester, NY 14642, [3]Howard Hughes Medical Institute and The Jackson Laboratory, Bar Harbor, ME 04609

Age-related macular degeneration (AMD) is a complex disease and a leading cause of irreversible visual impairment in the elderly. Multiple genetic and environmental factors interact and contribute to the disease process. Genetic and biochemical approaches indicate a central role for de-repression of the alternative complement pathway of innate immunity in the late stages of disease. Although the triggers that underlie complement de-repression are unknown, several lines of evidence implicate oxidative damage. Oxidative stress and endoplasmic reticulum (ER) stress are intimately related cellular processes. Here, we test the hypothesis that misfolded-protein-induced ER stress contributes to oxidative stress in a mouse genetic model of AMD. Mice with a mutation in the type IV collagen alpha 1 gene (*Col4a1*) have several hallmark phenotypes of AMD. Misfolded mutant COL4A1 proteins are not secreted and cause ER stress in some tissues. Labeling eyes from mutant mice with markers of oxidative damage to proteins and nucleic acids revealed oxidative damage at sites of retinal lesions. Our data supports ER stress, from misfolded proteins or other sources, as a primary trigger that leads to oxidative damage and complement de-repression in AMD. We propose that therapeutics targeted for ER and oxidative stress pathways could prevent or delay vision loss in patients with AMD.

125

Molecular regulation of vascular calcification

Ann Canfield.
Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

Vascular calcification is a common complication of many diseases including atherosclerosis, diabetes, and end-stage kidney disease. Recent studies have shown that there is a strong association between the presence of vascular calcification and adverse clinical events such as myocardial infarction and stroke. We have shown that vascular pericytes and smooth muscle cells (VSMC) can differentiate into osteoblasts and chondrocytes *in vitro* and *in vivo* and deposit a calcified matrix resembling that found in calcified atherosclerotic plaques, suggesting that these cells may mediate, at least in part, vascular calcification. 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 vascular calcification. 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 these new data will be presented at this meeting.

126

MAPKp38 determines Smad2/3 signaling in the fbn1 null mouse aorta

Luca Carta[1], Silvia Smaldone[1], Lior Zilberberg[2], David Lock[3], Harry C. Dietz[3], Daniel B. Rifkin[2], Francesco Ramirez[1].
[1]Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY, [2]Department of Cell Biology, New York University School of Medicine, New York, NY, [3]Departments of Pediatrics, Medicine and Molecular Biology and Genetics, and the Howard Hughes Med Institute, Johns Hopkins Uni School of Med, Baltimore, MD

Aneurysm progression and rupture in mouse models of Marfan syndrome, a connective tissue disorder caused by mutations in extracellular fibrillin-1, has been associated with TGF-β -driven unproductive tissue repair. Here we report that constitutively active TGF-β signaling is the cell-autonomous phenotype of primary vascular smooth muscle cell (VSMC) cultures explanted from the thoracic aorta of Fbn1 mutant mice with neonatal onset or progressively severe aneurysm. Additional experiments revealed abnormal activation of stress-response pathways, including MAPKp38,in Fbn1 null (mgN/mgN) VSMC cultures. Furthermore, MAPKp38 activation was found to contribute to abnormal Smad2/3 signaling in response to stress response and non-canonical TGF-β signaling cascades, in addition to up-regulating expression of the latent TGF-β activator MMP-9. Importantly, in vivo analyses demonstrated that MAPKp38 stimulation in the aorta of mgN/mgN mice precedes Smad2 activation, and that MAPKp38 inhibition reduces Smad2 stimulation. These data provide the basis for a new model of chronic TGF-β signaling in the mgN/mgN aorta that extends knowledge of the molecular pathogenesis of aneurysm in Marfan syndrome.

127

C-terminal Domain Modification in Mature Elastin

Thomas J. Broekelmann, Christopher H. Ciliberto, Adrian Shifren, Robert P. Mecham.
Department of Cell Biology and Physiology, Washington University School of Medicine, Saint Louis, MO 63110

The carboxyl terminus of tropoelastin is a highly conserved, atypical region of the molecule with sequences that define both cell and matrix interactions. This domain also plays a critical but unknown role in the assembly and crosslinking of tropoelastin during elastic fiber maturation. Using a competitive ELISA with an antibody to an elastase- resistant epitope in the C-terminus of tropoelastin (domain 36), we quantified levels of the domain 36 sequence in elastase-derived peptides from mature, insoluble elastin. We found that the amount of C-terminal epitope is well below the expected value, assuming each tropoelastin monomer that is incorporated into the insoluble polymer has an intact C-terminus. Differential trypsin sensitivity suggests that the low values were due to potential lysine-derived cross-links in this region, which alter the extractability and antigenicity of the C-terminal epitope. These results indicate that there is little or no unmodified domain 36 in mature elastin, indicating that the cell and matrix binding activities associated with this region of tropoelastin are lost or modified as elastin matures. A crosslinking function for domain 36 may serve to help register the multiple cross-linking sites in elastin and explains why mutations that alter the domain 36 sequence have detrimental effects on elastic fiber assembly.

128

Granzyme B and Perforin in atherosclerosis and skin pathologies

Wendy A. Boivin, Rani P. Cruz, Hongyan Zhao, Tyler Varnals, David C. Walker, David J. Granville.
The James Hogg iCAPTURE Centre, Canada

Granzyme B (GrB) is a key player in immune cell-mediated apoptosis and exhibits an extracellular matrix (ECM) proteolytic capacity. Perforin (perf) is a pore forming protein that is required for GrB-dependent apoptosis but not for extracellar GrB activity. We have shown that when GrB is knocked out of apolipoprotein E knockout (apoE-KO) mice, the characteristic atherosclerosis and skin pathologies in these mice are abolished. We propose that this may be due to the extracellular activity of GrB. To identify novel ECM substrates for GrB, ECM cleavage assays were performed. ApoE-KO, GrB-KO, GrB/apoE-DKO, Perf-KO, and perf/apoE-DKO mice were fed a high fat diet for 30weeks and skin, aortic root, and aorta sections were examined by histology and electron microscopy for evidence of atherosclerosis, ECM degradation and other pathologies. ECM cleavage assays identified fibronectin, fibrillin-1, fibrillin-2 and fibulin-2 to be cleaved by GrB. Interestingly, perf/apoE-DKO aortic plaques are more extensive than those of apoE-KO mice, suggesting perf may be protective and that both the intracellular and extracellular roles of GrB

are involved. The skin of apoE-KO mice contains evidence of immune cell infiltration, lipid deposition, foam cell formation and ECM degradation that are absent from GrB/apoE-DKO and Perf/apoE-DKO skin. We conclude that GrB cleaves microfibril proteins in vitro and the absence of either perf or GrB in apoE-KO mice prevents ECM degradation and xanthomatosis. Conversely, the absence of GrB in apoE-KO mice prevents advanced atherosclerosis while the absence of perf exacerbates plaque formation.

129

Hyaluronan Role During Muscular and Craniofacial Development

Paola Casini[1], Roberto Perris[2], Irma Nardi[1], Michela Ori[1].
[1]Department of Biology, University of Pisa, 56010 Pisa, Italy, [2]Department of Genetics, Microbiology and Anthropology, Univerisity of Parma, 43100 Parma, Italy

Hyaluronan (HA) is a crucial glycosaminoglycan of vertebrate embryonic extracellular matrix. We are using Xenopus laevis to study the role of HA and CD44 in vivo during cell migration and differentiation processes. We have described the spatio-temporal gene expression pattern of the three known vertebrate hyaluronan synthases (XHas1, XHas2 and XHas3) showing a very close conservation of the spatio-temporal expression profile of Xenopus Has genes with that of mammals. Recently we demonstrated a critical role of XHas2 and XCD44 during muscle formation and precursor muscle cell migration. To further dissect the role of these molecules, on migration and differentiation processes, we are now focusing on cranial neural crest cells (NCC) development, knocking down the XHas1, XHas2 and XCD44 gene functions. We showed that the hyaluronan synthases and the hyaluronan receptor present a dynamic expression pattern during cranial NCC development suggesting multiple roles in the various steps of cranial NCC migration and differentiation. Nor the gene loss of function of XHas1 or XHas2 interferes with early events of the cranial NCC induction and specification but, in concert with XCD44, they are involved in the migration and differentiation processes, influencing the craniofacial skeleton morphology determination. We are now exploring the possibility of alternative molecular mechanism of action of HA during development studying possible functional interactions of HA with alternative receptors, such as RHAMM, and hyaluronan binding proteins partners such as the proteoglycan versican.

130

Intrinsic aging/photoaging-dependent changes of GAG in human skin

Jang-Hee Oh, Yeon Kyung Kim, Jeong-Eun Shin, Mi Hee Shin, Kyu Han Kim, Kwang Hyun Cho, Hee Chul Eun, Jin Ho Chung.
Department of Dermatology, Seoul National University College of Medicine, Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Institute of Dermatological Science, Seoul National University, Seoul, Korea

Glycosaminoglycans (GAGs) are ones of major components in the human skin tissue, however, their aging- or photoaging-related changes in skin are not well-defined and controversial. GAGs are thought to have structural functions, but their physiological functions are recently reported. To investigate intrinsic aging/photoaging-dependent changes of GAG in human skin, sun-protected buttock and sun-exposed forearm skins were biopsied from healthy young (20-39 year, n=16) and old (70-89 year, n=16) male and female volunteers (total n=32). In the dermis and epidermis, respectively, tissue water content, total uronic acid (UA) amount, total sulfated GAG (sGAG) amount, and hyaluronic acid (HA) amount were measured. Total UA amount, HA amount, and tissue water content of buttock dermis showed no change in old groups, but showed significant increase in the forearm dermis. However, total sGAG amount was decreased in both old buttock and forearm dermis. In epidermis, total UA amount, tissue water content, and total sGAG amount decreased in both buttock and forearm of old group, except in the buttock of old males. HA amount of the epidermis decreased in all the groups except in the forearm of old females. These series of investigations about GAG changes in skin aging process probably provide more fundamental understandings about the functions of GAGs in human skin.

131

Impact of modified hyaluronan on binding of biological mediators

Vera Hintze[1], Ute Hempel[2], Matthias Schnabelrauch[3], Manuela Rösler[2], Hartmut Worch[1], Dieter Scharnweber[1].
[1]Max-Bergmann-Center of Biomaterials, TU Dresden, Germany, [2]Institute of Physiological Chemistry, TU Dresden, Germany, [3]Innovent e.V., Jena, Germany

Titanium and its alloys exhibit sound biocompatibility and are widely used as implant materials. However there is still the need for improving the integration into the surrounding tissue. The cells at the interface of implant and tissue are mainly held responsible for integration. Besides direct interactions with the extracellular matrix (ECM) they are mainly influenced by biological mediators (growth factors, cytokines). In tissues the ECM serves as a scaffold for cells and as a reservoir for growth factors. Through targeted use of ECM components we aim on creating biomimetic implant coatings acting as a defined microenvironment to selectively influence in vivo mediators and thereby improving cell adhesion, proliferation and differentiation. This approach seems promising to enhance healing of bone prosthesis and stability of regenerated tissue. The artificial ECMs are mainly based on fibrillar collagen and modified hyaluronan (HAD). We have focused our investigation on HADs with different degrees of sulphate modification. Due to their involvement in healing and remodelling of bone we have emphasized on the mediators BMP-2 and -4, TGF-beta1, IL-1 and IL-6. Interaction studies (ELISA, surface plasmon resonance) with HAD and selected mediators have revealed differences in binding depending on which mediator and sugar derivative used. We will present the newest developments in characterizing the differently modified model substances on binding and activity of mediators and their impact on osteoblastic cells.

132

Structure Function Analysis of the Human Hyaluronidase Enzymes

Ge Wei, Panneer Selvam, Lou H. Bookbinder, Gregory I. Frost.
Frost Halozyme Therapeutic Inc, San Diego, CA

Mammalian hyaluronidases (Hyals) are a family of endo-beta-(1,4) N-acetyl-hexosaminidase enzymes (Hyal1-5 and PH20) that hydrolyze hyaluronan (HA). Hyaluronidases are processed through the ER and Golgi of cells, and are found natively modified with carbohydrate chains, and some may contain other post-translational modifications. Mammalian Hyal1 and bee venom hyaluronidase (bvHyal) have been crystallized and their structure contains a distorted (beta/alpha) 8 triose phosphate isomerase (TIM) barrel as their catalytic domain. In addition to the TIM catalytic domain, mammalian hyal1 has an additional EGF-like domain at their carboxyl termini. Mammalian hyaluronidases show low levels of homology in their carboxyl terminal sequences, and some contain a GPI-anchor after that to attach the hyaluronidase (PH20) to membranes. Because of the variability demonstrated among mammalian hyaluronidases carboxyl termini, it has been suggested that this region is not essential for enzymatic activity. To understand the function of the carboxyl terminal domain and the influence of N-glycosylation on hyaluronidase enzymatic activity, a panel of human PH20 hyaluronidase carboxyl terminal deletion mutants were generated. These mutants were transfected into CHO cells and the secreted proteins and cell pellets were analyzed for PH20 expression by western blot and for hyaluronidase enzymatic activity by digestion of Biotin-HA. Data demonstrate that the carboxyl-terminal domain is crucial for hyaluronidase enzymatic activity. Mutants with amino acid sequences shorter than C464 do not produce or secrete measurable hyaluronidase enzyme activity into the tissue culture media. The lack of hyaluronidase enzymatic activity is due to the poor expression of these truncated forms of PH20 (western blots of cell pellets and supernatants) by the CHO cells, compared to soluble active recombinant human PH20 hyaluronidase. Some mutants, which end with hydrophobic amino acids located within the GPI anchor sequences, also did not show any hyaluronidase expression or enzymatic activity. Complete deglycosylation of soluble active recombinant human PH20 hyaluronidase results in the total loss of hyaluronidase enzymatic activity. Partial deglycosylation does not

significantly alter the enzymatic activity. Recombinant human PH20 has six N-glycosylation sites, all of which are occupied with glycans. Amino acid sequence alignment of mammalian hyaluronidases indicate that only N333 of human PH20 is conserved among all human hyaluronidases. This site is located in the linker region between the N-terminal catalytic and C-terminal EGF-like domains of the hyaluronidases. These observations suggest that N333 might play a role in enzymatic function of mammalian hyaluronidases. Experiments are underway to further investigate the role the C-terminal domain and the N333 glycans mediate, on functional hyaluronidase specific enzymatic activity.

133

Sulfated glycosaminoglycans regulate matrilysin cleavage activity and specificity

Susanna Harju-Baker, Hyun-Jeong Ra, Bill C. Parks, John K. McGuire.
Center for Lung Biology, University of Washington School of Medicine, Seattle, WA

Matrilysin (matrix metalloproteinase-7) is highly expressed by injured epithelium of many tissues. Studies with knock-out mice demonstrated that matrilysin is required for wound closure and that E-cadherin is a physiological substrate during healing. Matrilysin has several other in vivo substrates, such as pro-defensins and syndecan-1, yet the mechanism controlling its cleavage specificity are not known. We sought to identify the matrilysin cleavage site in mouse E-cadherin and to determine whether glycosaminoglycans (GAGs) play a role in regulating the specificity of the cleavage. Tandem mass spectrometry was used to identify matrilysin cleavage sites in a E-cadherin/Fc fusion protein and in E-cadherin ectodomain shed from HEK-293 cells stably expressing mouse E-cadherin and matrilysin. Wound healing assays using transfected CHO-K1 cells impaired in GAG production suggested that cleavage of E-cadherin by matrilysin was reduced in the absence of GAGs. In vitro, addition of heparin or chondroitin sulfate GAGs altered the kinetics and/or cleavage products in reactions of matrilysin with Ecad/Fc or a fluorogenic OmniMMP substrate. Together these data suggest that GAGs function in regulating matrilysin cleavage of E-cadherin during wound healing.

134

ECM changes after spinal cord hemisection in Tenascin C-knockout

Dietrich E. Lorke[1,2], Jenny Schreiber[2], Udo Schumacher[2].
[1]Department of Anatomy, FMHS, UAE University, Al Ain, UAE, [2]Institute of Anatomy II, Hamburg University, Germany

The role of the ECM glycoprotein tenascin C (TNC) is somewhat paradoxical because in vitro it can be both neurite-outgrowth promoting and repellent. To assess its effects in vivo, lumbar spinal cord (SC) hemisection was studied both in TNC knockout (-/-) and wildtype (TNC +/+) mice (n=20 each). 1, 3, 7 and 14 days post surgery, SC sections were examined histologically for the expression of neurofilament (NF), GFAP (astrocytes), F4/80 (microglia/macrophages) and the ECM molecules collagen IV, laminin (LN) and fibronectin (FN). Staining intensity was quantified by densitometry. 1 day after lesion, both TNC -/- and control mice showed an early inflammatory response with neuronal damage, neutrophil infiltration and discrete FN deposits. After 3 days, the late inflammatory response consisted of lympho-monocyte infiltration, microglial activation, few reactive astrocytes and increased collagen IV, LN and FN deposits. Day 7 (proliferation phase) showed beginning astrogliosis, densely packed fibroblasts and large amounts of ECM molecules. 14 days post surgery, a scar was formed, consisting of collagen I and IV, LN, FN and densely packed astrocytes. Despite these similarities, TNC -/- mice showed distinct differences: lymphocyte infiltration started 2 days earlier and neutrophil infiltration was more prominent. FN was significantly decreased over the entire period, and collagen IV and LN were reduced on day 14. On day 14 thin NF-positive fibers were markedly increased in TNC -/- SC, indicating that TNC deficiency alters ECM composition so that axonal fibers more easily penetrate SC scar tissue.

135

Differential Mmp Response to PGE2 During Flexor Tendon Healing

Alayna E. Loiselle, Regis J. O'Keefe.
Center for Musculoskeletal Research, University of Rochester, NY 14642

Murine flexor tendons heal with increased *Mmp-9* during the inflammatory phase, and increased *Mmp-2* during the remodeling phase. To understand the cellular and molecular events involved in differential Mmp expression during flexor tendon healing, we tested the hypothesis that bone marrow cells produce *Mmp-9* in response to PGE_2, while tenocytes produce *Mmp-2*, with these processes mediated by EP4. *Mmp-9* expression was confined to a small population of cells that did not express *Scx* or Fibroblast Specific Protein-1, suggesting that these cells are not of tendon origin, nor are they fibroblasts. Expression of *Mmp-2* was confined to fibroblastic granulation tissue that bridges the repair site. To determine the identity of these cells, primary tenocytes and bone marrow cells were treated with PGE_2. Bone marrow cells, which can be recruited to healing tendons, responded to PGE_2 with a robust increase in *Mmp-9* expression and activity. In contrast, stimulation of tenocytes with PGE_2 resulted in increased *Mmp-2* expression and activity. There were no significant changes in *Mmp-2* and *Mmp-9* expression or activity in EP2-/- bone marrow or tenocytes, suggesting that Mmp induction is not mediated by EP2. Treatment of EP2-/- bone marrow with an EP4 agonist resulted in decreased *Mmp-9* activity, and decreased *Mmp-2* expression and activity in tenocytes. This study suggests that the unique expression profile of Mmp's during flexor tendon healing is mediated through the EP4 receptor in two distinct populations of cells. Understanding the complex cellular events may provide novel targets to mediate the tendon healing process and decrease adhesion formation.

136

GULP Is Required for Stabilin-2-mediated Cell Corpse Engulfment

Dong-Jun Bae[1], Seung-Yoon Park[2], Kae-Bok Kang[1], Narendra Tapha[1], Sang-Yeob Kim[1], Sung-Jin Lee[1], In-San Kim[1,3].
[1]Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, [2]Department of Biochemistry, School of Medicine, Dongguk University, [3]Corresponding author: iskim@knu.ac.kr

The prompt clearance of cells undergoing apoptosis is critical during embryonic development and normal tissue turnover, as well as during inflammation and autoimmune responses. We recently demonstrated that stabilin-2 is a phosphatidylserine receptor that mediates the clearance of apoptotic cells, thereby releasing the anti-inflammatory cytokine, TGF-β. However, the downstream signaling components of stabilin-2-mediated phagocytosis are not known. Here, we provide evidence that the adaptor protein, GULP, physically and functionally interacts with stabilin-2. Using FRET analysis and biochemical approaches, we show that GULP directly binds to the cytoplasmic tail of stabilin-2. Knockdown of endogenous GULP expression decreased stabilin-2-mediated phagocytosis. Conversely, overexpression of GULP caused an increase in aged cell engulfment. The phosphotyrosine-binding (PTB) domain of GULP was sufficient for the interaction with stabilin-2, therefore, transduction of TAT-fusion PTB domain acts as dominant negative, resulting in impaired engulfment of aged RBCs in stabilin-2 expressing cells. In addition, the PTB domain of GULP was able to specifically interact with the NPXY motif of the stabilin-2 cytoplasmic tail. Taken together, these results indicate that GULP is a likely downstream molecule in the stabilin-2-mediated signaling pathway and plays an important role in stabilin-2-mediated phagocytosis. Supported by the Brain Korea 21 Project.

137

Cell Corpse Removal by Stabilin-2, a Phosphatidylserine Receptor

Seung-Yoon Park[2], Mi-Yeon Jung[1], Ha-Jeong Kim[1], Sung-Jin Lee[1], Sang-Yeob Kim[1], Byung-Heon Lee[1], Tae-Hwan Kwon[1], Rang-Woon Park[1], In-San Kim[1,3].
[1]Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, [2]Department of Biochemistry, School of

Medicine, Dongguk University, [3]Corresponding author: iskim@knu.ac.kr

Rapid phagocytic clearance of apoptotic cells is crucial to prevent inflammation and autoimmune responses. Phosphatidylserine (PS) to the external surface of the plasma membrane has been suggested as a general “eat me” signal for apoptotic cells. Although several soluble bridging molecules have been proposed in the recognition of PS, the PS-specific membrane receptor that binds directly to the exposed PS and provides tickling signal has not been identified. Here we provide evidence that stabilin-2 is a novel PS receptor that plays a key role in rapid cell corpse clearance. It recognizes PS of aged red blood cells (RBCs) and apoptotic cells and mediates the engulfment of them. Downregulation of stabilin-2 expression in macrophages significantly inhibits phagocytosis, and anti-stabilin-2 mAb provokes the release of anti-inflammatory cytokine, TGF-β. Furthermore, time-lapse video analysis suggests that stabilin-2 plays a crucial role in rapid clearance of aged and apoptotic cells. These data suggest that stabilin-2 are the first membrane PS receptors providing tethering and tickling signals, and could be involved in the resolution of inflammation and the prevention of antoimmunity. Supported by the Brain Korea 21 Project.

138

Target-tropic accumulation of SLX-liposome in arthritis mouse

Jun Minaguchi[1,2], Hideki Minematsu[3], Toshitaka Oohashi[1], Takayuki Otani[3], Kiichi Inagawa[1], Kazunori Oie[3], Keiichiro Nishida[2], Aiji Ohtsuka[2], Yoshifumi Ninomiya[1], Koichi Igarashi[3]. [1]Dept of Molecular Bio and Biochemistry, Okayama Uni. Grad. School of Medicine, Density, and Pharmaceutical Sciences, [2]Department of Human Morphology, Okayama Uni. Grad. School of Med, Density, and Pharmaceutical Sciences, [3]Katayama Chemical Industries CO., LTD.

Objective: The aim of the current study was to investigate the specific accumulation of Sialyl Lewis X (SLX) liposome (SLX-Lipo) to inflammation in the collagen-antibody induced arthritis (CAIA) model mice. Methods: We prepared the SLX-Lipo encapsulating fluorescent substance for the study. The SLX-Lipo was administrated intravenously from mouse caudal vein. After one or 24 hours, the accumulation of SLX-Lipo was observed using in vivo fluorescent imaging equipment, or the joints were removed for histological analysis. Results: By the in vivo imaging, the detected signal was confined to the inflammatory site in the CAIA mice in the inflammatory-dependent manner. The signal intensity was stronger after 24 hours than after one hour. In the histological sections, the fluorescent signals were detected at the periarticular soft tissue, especially at hyperplastic synovium including pannus invasion with inflammatory cells in the CAIA. Intense signals were observed in vessel-like structures after one hour. Then diffused signals from the vessels were observed after 24 hours. Conclusion: SLX-Lipo accumulated specifically in the inflammatory joints of the CAIA mice. Our results demonstrate the feasibility and potential use of SLX-Lipo as a vehicle for active targeting drug delivery to inflammatory tissue.

139

Ultrastructural Inflammatory Changes of Oculomotor Neurons

Melani Rajendran[1], Arumugam Krishnamurti[2]. [1]Department of Anatomy ,Sri Ramachandra University,Chennai , India, [2]Dept of Anatomy , Annamalai University , Annamalai Nagar , India

The role of neuron cell body in maintaining the integrity of neuritis is perhaps best shown after interruption of the axon.A study was carried out to find out the ultrastructural cellular inflammatory changes of neurons of the oculomotor nucleus in Bonnet monkeys (Macaca Radiata)after axotomy of oculomotor nerve. A transtemporal axotomy of oculomotor nerve was done in bonnet monkeys. After four days of survival,the midbrain portion at the level of superior colliculus were removed.The position of the oculomotor nucleus was identified by staining with toluidine blue.Only left side oculomotor nucleus was cut into 1mm size and were processed in 3% buffered gluteraldehyde (pH 7.4)and postfixed in 1%osmic acid.Ultra thin sections were viewed under electron microscope. Following changes were observed.Piknosis and eccentric position of nuclei,swollen neurons with vacuolated

cytoplasm,condensation of neuro filaments, abundant mitochondria without cristae, distorted golgi apparatus , increased free ribosomes,clumps of endoplasmic reticulum and increased gliosis. The hypertrophy with vacuolation may be due to intense synthetic activity with neuronal disturbances.Condensation of neuronal filaments usually responsive to local compressive and expansive processes.Free ribosomes and clumps of endoplasmic reticulum are due to increased protein synthesis. Morphological changes of mitochondraia may be due to alterations in the oxidative enzyme activity. Increased gliosis are due to phagocytosis.

140

Syndecan-1 shedding by MMP-7 controls neutrophil activation

Qinglang Li[1], Sean E. Gill[1], Ying Wang[1], Charles W. Frevert[1], Pyong W. Park[2], Bill C. Parks[1].
[1]Center for Lung Biology, University of Washington, Seattle, WA, [2]Department of Pediatrics, Harvard University, Boston, MA

As neutrophils exit the vasculature, move through interstitial spaces and cross the epithelium, they go through progressive stages of activation. Thus, specific interactions in these compartments would either promote or constrain activation. We have determined that the transepithelial migration of neutrophils is controlled by matrilysin (MMP-7) shedding of syndecan-1 with the CXC chemokine KC bound to its glycosaminoglycan chains. In matrilysin-null (*Mmp7*$^{-/-}$) mice, neutrophils halt at the epithelium, and the null mice are resistant to injury-mediated lethality. We hypothesize that an interaction with cell- bound KC/syndecan-1 complexes not only constrains neutrophil movement but also activation, thereby preventing oxidative burst at the epithelial cell surface. To test this idea, we exposed neutrophils to injured or infected cultures of primary lung epithelial cells and assessed activation by release of myeloperoxidase. Whereas neutrophils activated in contact with wounded wildtype epithelial monolayers, only basal activation was detected when plated with cells from *Mmp7*$^{-/-}$ or syndecan-1-null (*Sdc1*$^{-/-}$) mice. The expression of KC did not differ among genotypes, but its release into the medium was dependent on matrilysin. In addition, although KC was released into the medium of *Sdc1*$^{-/-}$ cultures, it did not promote neutrophil activation, demonstrating the importance of being complexed to syndecan-1. These findings indicate that matrilysin, syndecan-1, and KC function together to control neutrophil activation at the epithelial- interstitial interface.

141

Proteomics of mouse cartilage degradation in vitro

Richard Wilson[1], Belluoccio Daniele[1], Little Chrisopher[3], Fosang Amanda[1,2], Bateman John[1,2].
[1]Murdoch Childrens Research Institute, [2]University of Melbourne, Australia, [3]Kolling Institute of Medical Research, University of Sydney, Australia.

To explore the molecular mechanisms of cartilage degradation in arthritis, we used proteomic techniques to analyze a mouse femoral head cartilage explant model system under basal and cytokine stimulated conditions. Cartilage extracts and conditioned media was analyzed by 2-D PAGE and tandem MS, we identified novel proteins and fragments released into the media of control, interleukin-1a (IL-1) and all-trans-retinoic acid (RetA)-treated explants. Fluorescent difference gel electrophoresis (2-D DIGE) was used to quantify protein expression changes. We also measured changes in mRNA expression to distinguish transcriptional and post-translational regulation of released proteins. We identified 20 differentially-abundant proteins in media from control and treated explants, including fragments of TSP-1 and CTGF. IL-1 stimulated release of the cartilage degeneration marker MMP-3, as well as proteins with uncharacterized roles in cartilage pathology, such as neutrophil gelatinase-associated lipocalin. RetA stimulated release of extracellular matrix proteins COMP, link protein and matrilin-3 into the media, accompanied by dramatic reduction in corresponding mRNA transcripts levels. Gelsolin, implicated in cytoskeletal reorganization in arthritic synovial fibroblasts but not previously associated with cartilage pathology, was regulated by IL-1 and RetA. This

first analysis of mouse cartilage degradation in vitro using proteomics has identified proteins and fragments, some of which represent candidate biomarkers for cartilage degradation.

142

MMP-10 Controls Macrophage Activation in Response to Infection.

Timothy P. Birkland[1], Ying Wang[1], Shawn Skerrett[2], Bill C. Parks[1].
[1]Center for Lung Biology, University of Washington, Seattle, WA 98109, [2]Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA 98104

Matrix metalloproteinases (MMP) control various processes of innate immunity, such as re-epithelialization and leukocyte influx. Because stromelysin-2 (MMP-10) is highly expressed in essentially all injured, inflamed, and diseased tissues, we explored its role in the host response to lung infection with Pseudomonas aeruginosa (PA). MMP-10 was not expressed in lungs of naïve mice but was detected by 4 h post- infection, peaked at 48 h, and waned thereafter. Cell culture models suggested that the early expression of MMP-10 was by epithelium and that the later, more potent production was by infiltrated macrophages. When MMP-10 expression peaked in wildtype (WT) mice, Mmp10-/- mice died or were moribund. The difference in mortality between WT and null mice was not associated with a difference in bacterial clearance. Rather, Mmp10-/- had more severe inflammation and significantly more ($p < 0.05$) CD45+ leukocytes in lung tissue than did WT mice. NOS2 levels, a marker of the M1 pathway of macrophage activation, were significantly reduced in Mmp10-/- mice, whereas IL- 10 and arginase-1 (M2 pathway) were elevated. Furthermore, peritoneal macrophages from Mmp10-/- mice expressed much higher levels of the chemokine MCP-1/CCL2 after exposure to PA than did WT cells suggesting that endogenous MMP-10 moderates the proinflammatory activity of macrophages. Overall, these data indicate that MMP-10 functions to moderate overall inflammation and the activation status of macrophages in response to infection.

143

TGFBIp promotes monocytes adhesion, migration and chemotaxis.

Ha-Jeong Kim, In-San Kim.
Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University

Monocyte recruitment from the blood in response to chemoattractant gradients is a key phenomenon in inflammation. Various extracellular matrix proteins, at the site of inflammation, have chemoattractant activity and mediate monocyte adhesion and migration as ligands of integrins. In this report, we demonstrate that TGFBIp/βig-h3, as an extracellular matrix protein, mediates monocytes adhesion under both static and flow conditions mainly through integrin αMβ2. FAS1 domains of TGFBIp/βig-h3 are responsible for the interaction with integrin αMβ2, not only enhances monocyte migration in both chemotactic and haptotactic manners but also mediates their transendothelial migration and subendothelial matrix invasion. These activities are also mediated through integrin αMβ2. Intraperitoneal injection of TGFBIp/βig-h3 promotes the recruitment of monocytes but not neutrophils. Our results demonstrate that TGFBIp/βig-h3 produced at inflammatory sites is a novel chemoattractant for monocytes and interacts with integrin αMβ2 to serve as a substrate for their migration, suggesting that TGFBIp/βig-h3 plays an important role in inflammation.

144

Epilysin plays a protective role in a model of atopic dermatitis

Jane Yoo[1,2], Anne M. Manicone[2], Bill C. Parks[2].
[1]Dermatology, University of Washington, Seattle, WA, [2]Center for Lung Biology, University of Washington, Seattle, WA

Epilysin (MMP-28) is expressed constitutively by keratinocytes and other epithelial cell types. Recent evidence in an asthma model has suggested a potential role in modulating Th2-type allergic inflammation. We hypothesized that epilysin would demonstrate a suppressive

role in a model of atopic dermatitis (AD). We used a mouse model based on intraperitoneal and epicutaneous sensitization with ovalbumin, which we have shown to recapitulate human AD. To evaluate temporal expression during inflammation, we followed cutaneous epilysin mRNA expression over time and correlated this with stage and degree of inflammation. We found that epilysin expression was decreased during acute stages of inflammation, while its expression increased over time as inflammation proceeded. This later increase in expression was consistent with previous findings that epilysin expression is upregulated by TNF-Î±. Next, we used epilysin-null mice to determine the effects of loss of epilysin on allergic inflammation in our AD model. Epilysin-null mice are otherwise healthy and phenotypically normal. Upon epicutaneous ovalbumin challenge, however, they showed more robust inflammation compared to challenged wild-type mice. Together, these data support our hypothesis that during the acute stage of Th2-type allergic inflammation, initiators of the inflammatory process cause downregulation of epilysin to allow inflammation to proceed, whereas later, the accumulation of proinflammatory cytokines such as TNF-Î± turns expression back on in order to counterbalance the inflammatory response.

145

TGFBIp activates platelets and promotes thrombogenesis.

Ha-Jeong Kim[1], Pan-Kyung Kim[1], Sang-Mun Bae[1], Hey-Nam Son[1], Thoudam D. Singh[1], Jung-Eun Kim[2], In-San Kim[1].
[1]Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu, Korea, [2]Department of Molecular Medicine, Kyungpook National University School of Medicine, Daegu, Korea

TGFBIp/βig-h3 is a 68 kDa extracellular matrix protein which is functionally associated with the cell-matrix adhesion, migration, proliferation and cell differentiation of various cells. The presence of TGFBIp in platelets led us to study the role of this protein in the regulation of platelet functions. Upon activation, platelet TGFBIp was released from the granules, which get associated with the external surface of platelets membrane. TGFBIp induces not only platelet adhesion and spreading but also activates phosphatidylserine exposure, α-granule secretion and integrin αIIbβ3 affinity. TGFBIp also promotes thrombi formation on type I fibrillar collagen under flow condition of whole blood in vitro. In vivo experiments, systemic injection of TGFBIp induces the pulmonary embolism in mice. Moreover, transgenic mice, which have about 2-fold higher blood TGFBIp concentration, are significantly more susceptible to collagen and epinephrine to produce the pulmonary embolism than the wild type mice. These results suggest that TGFBIp, one of human platelet proteins, play important roles in platelet activation and thrombus formation. Our finding will increase our understanding of the novel mechanism of platelet adhesion and aggregation, contributing to a better understanding of thrombotic pathways, and may subsequently show us the way for the development of new anti-bleeding and antithrombotic therapies.

146

Angiogenic-immune regulation by thrombospondin 1

Catherine Simone, Samantha Zak, Brandi Rasinger, Linda Gutierrez.
Department of Biology, Wilkes University, Wilkes-Barre, PA 18766

Transforming growth factor beta 1 (TGFß1) is one of the main regulators of the immune response. TGFß1 is activated by thrombospondin 1 (TSP1) which also induces cell death on leukocytes and endothelial cells (EC). EC secrete a variety of growth factors and cytokines, which initiate and modulate inflammation. We recently showed that TSP1-/- mice treated with dextran sulfate sodium (DSS) display a major mucosal damage, inflammation and angiogenesis in colitic lesions. Colons also displayed higher apoptotic indices in EC and secreted lower level of soluble fas ligand (FasL) compared with wild type (WT) mice. Our data indicate that TSP1 might decrease angiogenesis by reducing the levels of pro-angiogenic factors and inducing apoptosis in EC through the Fas/FasL pathway. We further investigated the effects of the axis TSP1 /TGFß1 on EC

apoptosis and secretion of mediators of the immune response. Our data show that TSP1-/- vascular cells are less susceptible to FasL mediated apoptosis. Increased angiogenesis in TSP1-/- intestines from mice under DSS induced colitis may be a consequence of a natural resistance of TSP1-/- endothelial cells to apoptotic mechanisms, including the Fas-FasL pathway. Furthermore, our results also indicate that TSP1 and TGFß1 activation regulate the vascular secretion of interleukin 6 (IL-6). These factors exert important functions during the inflammatory process and they have significant therapeutic potential.

147

A Protective Role for TIMP3 in Acute Lung Injury

Sean E. Gill[1], Isham Huizar[1], Eli M. Bench[1], Negin Nazarian[1], Kevin J. Leco[2], Rama Khokha[3], Bill C. Parks[1].
[1]Center for Lung Biology, University of Washington, Seattle, WA, 98109, [2]University of Western Ontario, London, Ontario, [3]University of Toronto, Toronto, Ontario

Tissue inhibitor of metalloproteinases 3 (TIMP3) is a unique member of the TIMP family as it inhibits not only matrix metalloproteinases (MMPs) but also ADAMs (a disintegrin and metalloproteinase domain). Mice lacking TIMP3 (*Timp3*$^{-/-}$) typically have exaggerated inflammatory responses, predominantly due to the unopposed ability of ADAM17 to activate latent TNFα. Because specific MMPs regulate inflammation and repair, we used *Timp3*$^{-/-}$ mice to assess the role of this inhibitor in a model of acute lung injury. Following bleomycin-induced injury, inflammation, as indicated by the presence of neutrophils in bronchoalveolar lavage (BAL) fluid, was more pronounced in *Timp3*$^{-/-}$ mice compared to wild type (WT) mice. Additionally, while the infiltration of macrophages and neutrophils peaked in the WT mice at 7 days post- injury and began to wane thereafter, inflammation persisted in *Timp3*$^{-/-}$ mice. Further evidence for augmented inflammation was provided by examination of cytokine profiles in the BAL fluid. In *Timp3*$^{-/-}$ mice, MCP1 (CCL2), interferon γ, and TNFα were significantly elevated in BAL fluid compared to their levels in WT mice suggesting disregulation of the mechanisms controlling leukocyte influx. Examination of total MMP activity demonstrated increased activity in the BAL fluid from *Timp3*$^{-/-}$ mice confirming previous reports. Thus, these data demonstrate that TIMP3 functions to moderate leukocyte recruitment into the lung following injury by controlling the levels of proinflammatory mediators.

148

iNOS Deficiency Inhibits MMP-9 activation in Hepatic I/R Injury

Takashi Hamada, Sei-ichiro Tsuchihashi, Ana J. Coito.
The Dumont-UCLA Transplant Center

Nitric oxide (NO), generated by the inducible NO synthase (iNOS), participates in extracellular matrix proteolysis. We have shown that MMP-9 is a critical mediator of leukocyte recruitment in damaged livers. We test the hypothesis that iNOS expression has a regulatory function upon MMP-9 activation in liver Ischemia/Reperfusion (I/R) injury.
Methods and Results: iNOS$^{-/-}$ mice (iNOS-KO), and wild-type (WT) controls were submitted to 90 min partial warm ischemia followed by reperfusion. Liver function and histology were improved in iNOS-KO mice [ALT(IU/l) 6h: 1,368 ± 1,239 vs. 18,810 ± 4,316, $p<0.001$; 24h: 241 ± 98 vs. 1485 ± 306, $p<0.001$] as compared to controls. Infiltration of Ly-6G+ neutrophils (2.3 ± 0.6 vs. 19.3 ± 1.5, $p<0.001$), CD3+ lymphocytes (4.0 ± 1.0 vs. 8.3 ± 0.6, $p<0.03$), and Mac-1+leukocytes (2.7 ± 0.6 vs. 21.7 ± 3.1, $p<0.001$) was depressed in iNOS-KO livers. MMP-9 activity was significantly reduced in iNOS-KO livers (0.04 ± 0.008 vs. 1.28 ± 0.09 ng/g of protein, $p<0.0001$) as compared to controls. Furthermore, LPS-activated macrophages, which produced high levels of NO, increased MMP-9 activity and neutrophil transmigration across fibronectin-coated transwell inserts; however, treatment with ONO-1714, a selective iNOS inhibitor, significantly depressed NO release (3.5 ± 3.1 vs. 33.8 ± 4.4, uM, $p<0.003$), MMP-9 activity (0.35 ± 0.05 vs. 1.27 ± 0.26, ng/ml, $p<0.01$), and neutrophil migration (37.22 ± 2.36 vs. 67.98 ±

11.04, %, p<0.02). **Conclusion:** Our results provide evidence for a novel mechanism by which MMP-9 can mediate iNOS induced liver I/R injury.

149

Collagen and its receptors on articular cartilage vesicles

Ann K. Rosenthal, Emily Muth, Claudia M. Gohr.
Department of Medicine, Medical College of Wisconsin

Objective: Pathologic mineralization is a common occurrence in osteoarthritic cartilage and results in the formation of calcium crystals such as calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals. These crystals further damage osteoarthritic cartilage. CPPD and BCP crystals are likely generated in cartilage matrix by extracellular organelles known as articular cartilage vesicles (ACVs). ACVs are present in both normal and osteoarthritic cartilage matrix. Paradoxically, ACVs isolated from human normal and osteoarthritic cartilage mineralize poorly compared to those from healthy porcine cartilage. We recently demonstrated that type II collagen dramatically suppressed ACV mineralization in vitro, and sought to determine the types of collagens and collagen receptors on human and porcine ACVs. Methods: ACVs were obtained from old and young human and porcine articular cartilage by sequential enzymatic digestion. Western blotting was used to determine the presence types I, II, VI and X collagen, discoidin domain receptors (DDRs), and integrins on ACVs. Quantities of collagen bound to ACVs were measured with the hydroxyproline assay. Results: DDR2 as well as α1, α2 and β1 integrins, and types VI and X collagen were present on all ACVs. No type I collagen was detected, and very little type II collagen was present on porcine ACVs. Human ACVs contained easily measurable quantities of type II collagen which was of higher molecular weight in old human ACVs. Conclusions: ACVs contain crucial collagen receptors. Increased quantities of type II collagen in human ACVs may explain the seemingly paradoxical mineralization pattern in isolated ACVs from human cartilage.

150

Cellular Fibronectin Enhances Hepatic Stellate Cell Migration

Abby L. Olsen[1,2], Rebecca G. Wells[1,3].
[1]University of Pennsylvania School of Medicine, Philadelphia, PA 19104, [2]Cellular and Molecular Biology Graduate Program, [3]Department of Medicine, Division of Gastroenterology

Fibronectins regulate diverse cell behaviors via integrin signaling. Cellular fibronectin (cFN) can contain two alternatively spliced domains, extra domain A (EDA) and B (EDB), which are excluded from plasma fibronectin (pFN). EDA- and EDB-containing isoforms of cFN are are upregulated during liver fibrosis, in which EDA has been reported to promote myofibroblastic differentiation of hepatic stellate cells (HSC). We hypothesized that EDA and EDB influence HSC behavior by altering integrin signaling. To model myofibroblastic differentiation, we cultured HSC on plastic or polyacrylamide supports coated with pFN or cFN. HSC grown on cFN had decreased organization of αSMA into stress fibers and increased lamellipodia, suggestive of enhanced cell motility. This motility difference was confirmed by transwell migration assays, in which HSC plated on cFN-coated inserts had increased baseline chemotaxis toward serum as well as a greater enhancement of migration in response to treatment with PDGF. HSC grown on cFN also demonstrated greater expression of the integrin signaling mediators phosphoFAK and phospho-paxillin, and smaller but more numerous focal adhesions. These effects were further augmented by treatment with PDGF. Collectively, these data demonstrate a clear role for cFN in promoting HSC motility and suggest that this effect may be due to changes in integrin signaling that occur via synergy with PDGF. Thus, in concert with PDGF, cFN may contribute to the pathology of liver fibrosis by promoting HSC migration to areas of injury within the liver.

151

FAK Activity is Essential for Vascular Development

Ssang-Taek Lim, Xiao Lei Chen, Alok Tomar, Nichol L. Miller, David D. Schlaepfer.
UCSD Moores Cancer Center, La Jolla, CA

Extracellular matrix regulates cellular architecture during development via integrin activation. Integrins transmit intracellular signals via activation of protein tyrosine kinases such as focal adhesion kinase (FAK), a key mediator of cell motility and survival. FAK deletion during development results in p53-dependent growth arrest, we found FAK promotes cell survival in a kinase-independent manner through FAK FERM-mediated p53 regulation. To test whether FAK activity is important during development, we created a kinase-dead (KD) FAK knockin mouse by homologous recombination. Homozygous KD FAK is lethal at E9.5 with irregular yolk sac and embryo vascular structures and associated hemorrhage. However, in contrast to FAK-null cells that exhibit p53-dependent growth arrest, primary KD FAK cells grow in culture. KD FAK mouse embryonic fibroblasts (MEFs) exhibit reduced FAK Y397 phosphorylation and an overall reduction in cellular phosphotyrosine levels consistent with the loss of FAK activity. FAK-related Pyk2 expression and phosphorylation is similar in KD FAK and WT MEFs. Time-lapse and indirect fluorescent microscopy revealed that KD FAK MEFs initially spread normally on fibronectin, but form an over-abundant focal adhesions and exhibit elevated numbers of membrane protrusions within 1-2 h. These morphological alterations of KD FAK MEFs are associated with severe motility defects. Together, our results support the notion that KD FAK embryo lethality is associated with defects in vascular cell morphogenesis and that FAK activity is not essential for cell survival. Our studies highlight a differential role for FAK in cell migration-survival.

152

Extracellular Ligands Involved in Learning and Memory

Richard G. LeBaron[1], Amber M. Asher[1], David A. Medina[1], Mary M. Navarro[1], Maria V. Tejada-Simon[2].
[1]Department of Biology, University of Texas, San Antonio, TX 78249, [2]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030

Models describing mechanisms of CNS synaptic plasticity increasingly include cell-surface adhesion receptors as important molecules in long-term potentiation (LTP). LTP is an activity-dependent prolonged increase in synaptic transmission that represents a cellular substrate for some forms of learning and memory. In rat CA1 excitatory synapses, integrin receptors are crucial for the maintenance of LTP, thereby implicating integrin ligands in important complement roles. However, integrin ligands that are crucial for hippocampal LTP have not been definitively identified. To test for ligands, substrata derived from preparations of mouse hippocampal neurosynaptosomes were probed with cells that express the integrins that play roles in hippocampal LTP. Proteins within the molecular mass ranges of 50-60 kDa and 70-80 kDa sustained cell attachment. Peptide mass fingerprints of the regions revealed candidate integrin ligands. In a complement approach, the chemical Bis(Sulfosuccinimidyl) suberate was used in rat hippocampal slices to crosslink integrin β1 subunits to their near-neighbor proteins. Peptide mass fingerprints of crosslinked β1 pull-downs identified proteins that were detected by cell probe analysis. Candidate ligands include molecules of the ECM and cell surface proteins that will be applied to rat hippocampal slices to determine whether field excitatory post- synaptic potentials are diminished, thereby showing functionally that the candidate ligands operate in slice preparations of CA1 LTP.

153

Sarcoma MMP Expression is Influenced by Integrin-ECM Interactions

Adriana Roopnariane[2], Rajeev Boregowda[2], Brooke Krovic[2], Timothy M. Ritty[1,2].
[1]Penn State Cancer Institute, [2]Orthopaedics, Penn State College of Medicine, Hershey, PA 17030

During metastasis, osteosarcoma cells encounter a variety of microenvironments. Matrix metalloproteinases (MMPs) are known to have multiple functions that include facilitation of cell migration. We hypothesize that metastasizing osteosarcoma cells alter MMP production in response to their integrin-perceived extracellular environment. To test this hypothesis, we have quantified the protein levels of seven secreted

MMPs produced after human MG63 osteosarcoma cells attach to specific ECM proteins. MG63 cells specifically down-regulate production of MMP1 and MMP3 after attachment to type I collagen and fibronectin. Further, we have selectively down-regulated expression of integrin subunits alpha 2, 5, V and beta 3 using siRNA produced by stably transfected plasmids we constructed. The loss of alpha 2 expression results in dramatic up-regulation of MMP1 and MMP3 protein levels when grown on type I collagen and fibronectin. Interestingly, loss of alpha 2 integrin expression is widely reported to be associated with increased metastatic potential in various carcinomas, though a role in sarcoma is not yet clear. Additionally, we have recently developed an MG63 line that over-expresses the alpha 2 integrin subunit and are evaluating its MMP production characteristics as compared to the parent line. Finally, the metastatic potential of these cell lines is currently being evaluated in an *in vivo* murine model.

154

VLA1 on RPE cells is required for retinal function.

You-Wei Peng[1], Marisa Zallocchi[1], Daniel T. Meehan[1], Duane Delimont[1], Bo Chang[2], Norman Hawes[2], Weimin Wang[1], Dominic Cosgrove[1].
[1]Boys Town National Research Hospital, Omaha, NE, [2]The Jackson Laboratories, Bar Harbor, ME

The role of integrin/cell matrix interactions between the RPE and the basement membrane in retinal maintenance and function is not well characterized. In this study the functional importance of α1β1 integrin (also called VLA1) for RPE cell homeostasis and retinal health was assessed by comparing α1 integrin knockout mice with strain/age matched wild type mice. We found that integrin α1β1 localizes to the basal aspect of retinal pigment epithelial cells co-localizing with the basal lamina of the RPE. Integrin α1 null mice have delayed onset progressive retinal degeneration associated with thickening of the basement membrane, dysmorphology of basal processes, synaptic malformations and funduscopic abnormalities. Integrin α1-null mice display marked delay in transducin translocation compared to wild type mice following exposure to light in dark-adapted animals. Collectively, these data suggest an essential role for α1β1 integrin/basement membrane interactions in the RPE in basement membrane metabolism and translocation of transducin in photoreceptors. This is the first report describing evidence supporting an essential role for integrin/basement membrane interaction in the RPE. Further, this report demonstrates a direct link between integrin α1β1 function in RPE and molecular defects in photoreceptor cell function before retinal pathology is apparent.

155

CCN2 is essential for early chondrogenesis

Andrew Leask[1], Mohit Kapoor[1], Anita Woods[1], Karen Lyons[2], Frank Beier[1], David Carter[1], Daphne Pala[1].
[1]University of Western Ontario, London, ON, N6A 5C1, [2]UCLA, Los Angeles, CA, 90095

Adhesive signaling plays a key role in cellular differentiation, including in chondrogenesis. Herein, we probe the contribution to early chondrogenesis of two key modulators of adhesion, namely FAK/src and CCN2 (Connective tissue growth factor, CTGF). We use the micromass model of chondrogenesis to show that FAK/src signaling, which mediates cell/matrix attachment, suppresses early chondrogenesis including the induction of Ccn2, Agc and Sox6. The FAK/src inhibitor PP2 elevates Ccn2, Agc and Sox6 expression in wild-type mesenchymal cells in micromass culture, but not in cells lacking CCN2. Our results suggest a critical feature permitting chondrogenic differentiation is a reduction in FAK/src signaling, and that CCN2 operates downstream of this loss to promote chondrogenesis.

156

Cell-specific inhibition of TSP-1 translation by glucose

Sanghamitra Bhattacharyya, Tina E. Marinic, Irene Krukovets, Olga I. Stenina.
Cleveland Clinic, Lerner Research Institute

Molecular mechanisms causing aberrant angiogenesis in diabetics are unknown. Pathological regulation of angiogenesis in diabetic patients occur in a tissue-specific

manner: angiogenesis is increased in some tissues and decreased in others. We have reported that high glucose regulates expression of TSP-1, a potent antiangiogenic agent associated with multiple diabetic complications, in a cell type specific manner. TSP-1 mRNA levels were upregulated in response to high glucose in all vascular cells. However, TSP-1 protein levels decreased dramatically in microvascular endothelial (MVEC) and retinal pigment epithelial cells (RPE). Increased levels of mRNA in MVEC suggested that inhibition of TSP-1 production was regulated at the post transcriptional level and was not due to decreased TSP-1 mRNA stability. Neither stability of the protein nor its secretion were affected. The regulation was at the level of TSP-1 mRNA translation and was controlled by the untranslated regions. Upon glucose stimulation the UTRs caused uncoupling of a reporter mRNA from polysomes, resulting in its translocation from a fraction of actively translated messages to nonpolysomal fraction. Bioinformatic analysis predicted 6 miRNA target sites on TSP1 3'UTR, 3 of which were upregulated by high glucose, 7 folds for hsa miR 467a, 1.5folds for hsa let7b and 1fold for hsa let7i . Transient transfection with specific antisense RNA oligonucleotides relieved the translational inhibition and increased reporter gene activity, thereby suggesting a possible role of these miRNAs in regulating TSP1 expression in response to high glucose. In conclusion, such glucose mediated cell specific post transcriptional regulation of TSP-1 a potent anti angiogenic protein, could provide a molecular basis for the aberrant angiogenesis in diabetic patients.

157

Mutant COMP causes intracellular retention in a mouse PSACH model

Karen L. Posey[1], Alka C. Veerisetty[1], Huiqiu H. Wang[1], Peiman Liu[1], Roger Bick[2], Brian J. Poindexter[2], Joseph L. Alcorn[1], Jacqueline T. Hecht[1,3].
[1]Departmentsof Pediatrics, University of Texas Medical School at Houston, TX, [2]Department of Pathology, University of Texas Medical School at Houston,TX, [3]Shriners Hospital for Children, Houston, TX

Cartilage oligomeric matrix protein (COMP) is a pentameric extracellular matrix protein expressed in cartilage and other musculoskeletal tissues. Mutations in the cartilage oligomeric matrix protein gene cause two skeletal dysplasias, pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia. These two skeletal dysplasias have characteristic growth plate chondrocyte pathology of intracellular retention of COMP and other extracellular matrix proteins in large rER cisternae. These extracellular matrix proteins form an ordered matrix inside the rER and are not exported or degraded by the cell. This accumulation is cytotoxic and causes premature chondrocyte cell death depleting the pool of chondrocytes needed to form the cartilage anlagen for linear bone growth. In this study, we used a tetracycline-inducible promoter to drive high expression of mutant (MT-) COMP in mice. Growth plates from 1 month-old mice were examined. The control mice show normal growth plate organization and distribution of extracellular matrix proteins. In contrast, the MT-COMP growth plate is disorganized and intracellular retention of COMP, type IX collagen and matrilin-3 was observed. Importantly, an order intracellular matrix previously described in human PSACH chondrocytes was found. This mouse model has many of the hallmarks of the PSACH cellular pathology: disorganized growth plate, enlarged rER cisternae with intracellular matrix composed of COMP, types II and IX collagens and matrilin-3 and increased chondrocyte apoptosis. The disease manifestations are observed with heterozygote MT-COMP expression and thus exactly models the human PSACH condition.

158

SPARC regulates inhibition of pericyte migration by TGF-beta

Lee B. Rivera, Shanna A. Arnold, Rolf A. Brekken.
Hamon Center for Therapeutic Oncology Research, UT-Southwestern, Dallas, TX 75390

TGF-beta is a pleiotropic regulator of angiogenesis, influencing both endothelial cell and pericyte behavior. In pericytes, this cytokine signals through type I receptor ALK5 to inhibit migration. Pericytes also express the type-III TGF-beta receptor endoglin, which can control

cell migration and regulate activation of ALK5. Previously, we observed a significant reduction of pericyte-associated blood vessels and a significant increase in the level of active TGF-beta in pancreatic tumors grown in SPARC-null compared with wild-type animals. SPARC is a matricellular protein that has been shown to modulate cellular response to TGF-beta. Therefore, we set out to determine if SPARC regulates pericyte migration through a TGF-beta dependent pathway. We found that migration of primary SPARC-null pericytes was enhanced in the presence of a function blocking anti-TGF-beta antibody, in contrast to wild-type pericytes. Using 10T1/2 cells as a pericyte model, we found that inhibition in SPARC activity reduced pericyte migration towards fibronectin. Surprisingly, this effect was not observed in the presence of TGF-beta inhibition via a function-blocking antibody or a small molecular weight inhibitor. We next examined whether SPARC interacts with TGF-beta receptors. We immunoprecipitated ALK1, ALK5, and endoglin from pericyte lysates and found that SPARC co-immunoprecipitated with endoglin but not with ALK5 or ALK1. Furthermore, we found that SPARC binds recombinant endoglin by ELISA. These data suggest that SPARC by interacting with endoglin expressed on pericytes functions to limit TGF-beta-mediated inhibition of pericyte migration.

159

The role of MAGP1 in bone homeostasis

C.S. Craft[1], J. Weinbaum[3], T.J Broekelmann[1], K.L. Lee[2], M.J. Silva[2], R.P. Mecham[1].
[1]Cell Biology & Physiology, Washinton University in St. Louis, [2]Orthopaedic Surgery, Washington Uni in St. Louis, [3]Biomedical Engineering, University of Minnesota-Twin Cities

Functional microfibrils are pivotal to the homeostasis of multiple organ systems. The major structural components of microfibrils are fibrillins—large glycoproteins rich in calcium binding EGF-like domains, and microfibril-associated glycoproteins (MAGPs)—small, cysteine-rich proteins of unknown function. Gene targeted inactivation of MAGP1 reveals a complex phenotype that includes increased body weight and size due to excess body fat, an altered wound healing response in bone and skin, a bleeding diathesis, and bone abnormalities. To better characterize the bone phenotype in this mouse, we utilized micro CT, DEXA body composition analysis, and whole-bone mechanical testing. Micro CT and DEXA work demonstrated reduced bone mineral density (BMD) in the MAGP1 deficient animals. Reduced BMD was associated with reduced whole-bone strength, as determined by 3-point bending. Microfibrils are also important regulators of ligands of the TGFβ superfamily (TGFβ and BMP), and the TGFβ superfamily has a well-documented role in bone development. Consequently, we investigated the interaction between this family and MAGP1. We found recombinant MAGP1 is capable of binding the mature forms of some of these ligands with high affinity. We then utilized MAGP1-deficient RFL-6 cells to show that the addition of recombinant MAGP1 to cell media induces activation of the TGFβ superfamily signaling cascade. Together, our data provides evidence that MAGP1 has a functional role in bone homeostasis, and its mechanism may involve regulation of the TGFβ superfamily.

160

Collagen Fibril Formation: A New Target to Limit Fibrosis

Hye Jin Chung[1], Andrzej Steplewski[1], Kee Yang Chung[2], Jouni Uitto[1], Andrzej Fertala[1].
[1]Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA, [2]Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul 120-752, South Korea

Excessive accumulation of collagen is the hallmark of a number of localized fibrotic diseases, such as keloids and hypertrophic scars, and systemic fibrosis. At present, several biological processes critical for the development of fibrotic lesions are considered potential targets to serve as inhibitors of fibrosis. Because most of these potential targets are involved not only in pathological fibrosis but also in a number of physiological processes, their inhibition is frequently associated with significant adverse effects. Here, we tested a

new approach to reduce excessive scarring by specifically targeting the extracellular process of formation of collagen fibrils, a main component of fibrotic scars. By employing custom-designed antibodies that specifically bind to the C-terminal telopeptide of the α2-chain of collagen I we demonstrated that blocking telopeptide-mediated collagen/collagen interaction limits accumulation of collagen fibrils in vitro and in organotypic constructs formed by keloid-derived fibroblasts. Because excessive deposition of collagen fibrils is characteristic of all fibrotic processes, we predict that the basic design for the inhibitors of collagen fibril formation we tested in a skin-based keloid model will be applicable for reducing a number of localized and systemic fibrotic changes in other tissues and organs as well.

161

Collagen Type XI in Zebrafish Axial Skeletal Development

Jason S. Adams[1,2], Brian L. McMahan[3], Linda M. Mercer[1], Raquel J. Brown[1], Julia T. Oxford[1]. [1]Department of Biology, Boise State University, Boise, ID 83725, [2]Physiology and Developmental Biology, Brigham Young University, Provo, UT 84602, [3]School of Medicine, University of Washington, Seattle, WA 98195

Autosomal dominant chondrodystrophies, Stickler syndrome type 2 and Marshall syndrome, are characterized by facial abnormalities, eye defects, hearing loss, and joint problems caused by mutations in COL11A1. To study the functional role of COL11A1 in craniofacial and axial skeleton development, a knockdown of this gene was performed in zebrafish. The expression of col11a1 isoforms begins at ten and one-third hours post fertilization and continues to be expressed through adulthood. Although multiple splice forms were present, we observed that exons 6A and 8 were the predominant splice forms of col11a1 in zebrafish. A knockdown experiment of col11a1 revealed abnormalities of Meckel's cartilage, the otoliths of the inner ear, defects of the notochord, shortening of the total body length, and an increased mortality at 72 hours post fertilization. Knockdown of exon 6A produced the most severe effects in zebrafish. The results of these experiments provide evidence that col11a1 is essential for normal zebrafish development. Similarities exist between the findings reported here and the human birth defects such as Stickler syndrome type 2 and Marshall syndrome and in the experimental model, the chondrodystrophic mouse (cho/cho). Therefore, zebrafish provide an animal model with which to investigate the mechanisms of human chondrodystrophies and to establish potential treatments.

162

Collagen II R992C Mutation Leads to Unfolded Protein Response

Hye Jin Chung, Andrzej Steplewski, Deborah Jensen, Katarzyna Gawron, Andrzej Fertala. Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA

Single amino acid substitutions in collagen II are associated with spondyloepiphyseal dysplasia. These mutations cause alterations of the structure of individual collagen molecules, their fibrillar assembly and affect the ability of collagen molecules to properly interact with extracellular matrix components. Recently we demonstrated that certain mutations not only affect the structure of mutant molecules but also induce intracellular accumulation of mutant proteins which can lead to apoptosis. Here we analyzed the effects of R992C on collagen II thermostability, the presence of intramolecular disulfide bonds and cell response to the presence of the mutant molecules. We demonstrated that R992C alters the melting profile of the collagen triple helix, introduces atypical disulfide bonds, decreases the rate of secretion of mutant molecules and increases intracellular accumulation. Expression of mutant collagen II in a chondrocytic cell line demonstrated that these cells undergo apoptosis in response to the presence of mutant collagen II. Biochemical and microscopic assays have shown that apoptotic changes were associated with processes typical of the unfolded protein response in cells expressing R992C. Overall our study suggests that mutations in collagen II associated with atypical unfolding of the triple-helical domain of collagen may trigger a cascade of intracellular events cumulating in apoptosis. We postulate

that the described changes are part of the pathomechanism of heritable disorders of cartilage caused by mutations in collagen II.

163

P16 and P19; mineralization-related Echinoderm phosphoproteins.

Keith Alvares, Saryu Dixit, Elizabeth Lux, Arthur Veis.
Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University. Chicago, IL. 60611

Acidic proteins of similar character have been implicated in mineral deposition in vertebrate bones and teeth and invertebrate tissues such as shells, spicules and teeth. To explore the relationships between the vertebrate and invertebrate proteins, we have focused on the mineral-related proteins of the sea urchin teeth *L.Varigatus*, which have a rich cellular component, and a magnesium-rich calcite mineral phase. The mature mineralized portion of 100 teeth was solubilized in 0.6N HCl, after an initial 6.0 M Gdn-HCl extraction. The extracted proteins were separated by SDS polyacrylamide gel electrophoresis, and by passage over a CHT ceramic hydroxyapatite column, separating distinct apatite binding and non-binding fractions. The most prominent component in each fraction was purified, digested with trypsin, fractionated by HPLC and peptide peaks sequenced by automated Edman degradation. The peptides shared homology with predicted proteins P16 and P19, previously described from the sea urchin spicules by mRNA isolation. The full-length protein sequences were cloned from our *L.Varigatus* tooth cDNA library. P19, a Glu-rich intracellular protein, did not bind to ^{45}Ca or apatite while P16 bound to ^{45}Ca and hydroxyapatite and had a transmembrane sequence with a dentin phosphophoryn-like Ser-Asp-rich domain in the extracellular space. Both proteins were phosphorylated in vivo. Although both proteins were occluded in the mineral phase they appear to have distinct functions relative to the mineralization process.

164

Distinct OI Phenotype Caused by COL1 C-proteinase Site Mutations

A. M. Barnes[1], K. Lindahl[2], M. Whyte[3], T. Hefferan[4], C-J. Rubin[2], A. Kindmark[2], W. McAlister[3], S. Mumm[3], Ö. Ljunggren[2], J. C. Marini[1].
[1]BEMB, NICHD/NIH, Bethesda, MD, [2]Dept of Endo, Med Sci, Uppsala Univ, Sweden, [3]Shriner's Hosp for Children, St. Louis, MO, [4]Dept of Orthop, Mayo Clinic, Rochester, MN

Osteogenesis imperfecta (OI) is often caused by mutations in the type I collagen genes. Mutations in the type I procollagen C-propeptide cleavage site are of interest because they disrupt a processing step. We identified two children with mild OI who had cleavage site mutations in *COL1A1* (P1: α1(I)D1041N) or *COL1A2* (P2: α2(I)A1029T). P1 DEXA z-score and pQCT vBMD were +3, contrasting with radiographs demonstrating osteopenia and os-in-os vertebrae, and histomorphometry revealing increased bone remodeling, without a mineralization defect or signs of osteosclerosis. P2 had a DEXA z-score of 0, gracile long bones with radiographic osteopenia, and decreased BV/TV and increased BFR without a mineralization defect on histomorphometry. Steady-state collagen electrophoresis showed slight backstreaking of α1(I) and α2(I) in cell layers of both probands, with a slight baseline delay in P1. Chain incorporation was normal in P1 and slightly delayed in P2. Pericellular processing of P1 was delayed, with increases in both pCα1 and proα2, while P2 had increased pCα2 and proα2 and normal processing kinetics. Together with an adult with an α1(I)A1040T substitution (Int Conn Tis 82S1: CC01), our cases suggest that defects in proα1(I) processing lead to high childhood BMD, with signs of osteopetrosis occurring subsequently. Proα1(I) cleavage appears crucial to C-propeptide processing, while defective proα2(I) cleavage occurs after α1(I) processing.

165

Molecular Packing Structure of Native Type II Collagen

Olga Antipova[1,2], Raul Barrea[1,2], Joseph P. Orgel[1,2]
[1]Illinois Institute of Technology, Chicago IL, [2]BioCAT, APS, Argonne National Lab, IL

The naturally crystalline arrangement of collagen molecules in fibrils found in some tissues, allows the use of fiber diffraction methods for structural characterization. This alternative biophysical method has the potential to give structural information about collagen type II with minimum interference from sample preparation and may give the opportunity to produce relatively detailed three-dimensional visualization of the fibrils sub-structure. Towards this end, experiments with Multiple Isomorphous Replacement (MIR) were carried out to so that a one-dimensional electron density map of native collagen structure may be determined. Several experiments were performed at the BioCAT facility at Argonne National Laboratory with variations of: sample holder designs, sample preparation procedures, heavy atoms for MIR, temperatures and setups for small and medium angle diffraction. Some more optimum combinations of these produced data of resolution 15 Å or better in the axial direction. This study revealed that the parameters of collagen type II fibrils from lamprey notochord are very similar if not the same as collagen type II fibrils in mammalian tissues: 30 nm in diameter, axial periodicity of 67 nm, amino acid distribution is the same. Analysis of the one dimensional electron density map showed that the telopeptides, which are crucial for fibrillogenesis and organization of collagen type II tissues, have a very specific folded conformation, reminiscent of that seen in the C-telopeptide of type I collagen. This type of structural information is essential for understanding the mechanisms of development and disease pathologies.

166
Stabilin-2 Mediates Homophilic Interactions via Its FAS1 Domains
Young Eun Yun[1], Seung-Yoon Park[2], Mi-Yeon Jung[1], In-San Kim[1,3].
[1]Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, [2]Department of Biochemistry, School of Medicine, Dongguk University, [3]Corresponding author: iskim@knu.ac.kr

Stabilin-2 was recently shown to undergo a heterophilic interaction with the leukocyte beta2 integrin Mac-1, thereby mediating interactions between sinusoidal endothelium and lymphocytes in inflammatory cell recruitment. Because FAS1-containing proteins have been suggested to self-assembles to form a fibrillar structure, we hypothesized that stabilin-2 might also mediate homophilic cell-cell interactions. Here, we demonstrated that stabilin-2-expressing cells mediate a significant level of cell aggregation, and this aggregation is significantly inhibited by anti-stabilin-2 antibody. Stabilin-2-mediated aggregation is mediated by homophilic interactions and enhanced in the presence of Ca^{2+} and Mg^{2+}. Interestingly, exogenous addition of FAS1 domain enhanced stabilin-2-mediated cell aggregation, suggesting role of FAS1 domains in stabilin-2-mediated aggregation. To assess further the involvement of the FAS1 domains, a chimera replacing ectodomain of stabilin-2 with human βig-h3, which has four FAS1 domains, were expressed. This chimera also mediated cell aggregation, similarly to stabilin-2. Taken together, our results indicate that stabilin-2 mediates cell-cell interaction via homophilic interaction, and FAS1 domains are responsible for stabilin-2-mediated homophilic interaction. Supported by the Brain Korea 21 Project.

167
DDR2 affects the Persistence length of Collagen type I Fibers
LALITHA SIVAKUMAR, GUNJAN AGARWAL.
Department of Biomedical Engineering, Ohio State University, Columbus, OH 43210

Collagen fibers in the vertebrate tissue are responsible for its tensile strength. A disruption in the morphological or rheological properties of collagen fibers is bound to impact tensile strength and contractility of tissues and affect several cellular processes. We had recently established that interaction of discoidin domain receptor (DDR2) with collagen type I results in disruption of the native structure and morphology of collagen fibers. These results led us to investigate if DDR2 affects the mechanical properties of collagen fibers. Persistence length (P_L) is one way of quantifying the mechanical property of biopolymers. We present here, a simple and straightforward method for

determining P_L of collagen fibers using transmission electron microscope (TEM) images of immobilized collagen fibers. The fluctuations in the curvature of the collagen fibers formed in-vitro (with and without DDR2) were analyzed to ascertain the P_L of collagen fibers. Our results show that P_L increases as a function of collagen concentration even for native collagen fibers. In the presence of DDR2 extracellular domain, a significant reduction in P_L was observed, dependent upon concentration of DDR2. The P_L values and fiber-diameter measurements were utilized to ascertain the Young's Modulus of collagen fibers formed in the presence and absence of DDR2. Our results signify a physiological role of DDR2 in modulating matrix stiffness which may be especially important in developing tissues.

168

Thrombospondin-1 in an Early Uterine Fibroid Development Model

Liping Feng, Friederike Jayes, Tamara Greene, Phyllis Leppert.
Dept of Ob-Gyn, Duke Univ. Medical Center, Durham, NC 27710

The etiology of uterine fibroids is unclear and there is no universally accepted model for this common condition often causing pain, bleeding and infertility. Recent studies indicate uterine fibroids are formed by accumulation of ECM rather than by cellular proliferation and that altered wound healing leading to fibrosis may be the underlying cause. We aim to develop an invitro model of early fibroid development. Smooth muscle cells from fresh myometrium obtained at surgery from non-pregnant women were cultured in 10% serum until 70% confluent followed by 72 h serum-starvation (SS). Cells were evaluated for cell viability and examined by electron microscopy (EM) to determine the cell types present after SS. We measured mRNA and/or protein expression of thrombospondin-1 (THBS-1) at 0, 1, 2, 4, 24, 32, and 96 h after serum add-back. Cells were stained with anti-CD45, anti-CD42d or anti-α-SMA and analyzed by confocal microscopy. Cell viability after 72 h SS did not differ from controls. Under EM we noted 60-80% of SS cells had notched nuclei and a decreased cellular/nuclear ratio characteristic of myofibroblasts. THBS-1 mRNA and protein expression were increased within 2 h after serum add-back and remained elevated. Cells stained positive for human α-SMA, but negative for CD45 and CD42d, markers for leukocytes and platelets respectively, suggesting that myometrial derived cells were the source of THBS-1. We conclude that a majority of the SS cells appear to be myofibroblasts and that unlike many wounds where THBS-1 is derived from leukocytes or platelets, in this culture system it is produced by the myometrial derived cells themselves.

169

N-glycosylation of lysyl hydroxylases is linked to LH activity

Linda C. Walker, Mayra A. Overstreet, Heather N. Yeowell.
Division of Dermatology, Duke University Medical Center, Durham, NC 27710

The family of lysyl hydroxylases (LH1, the alternatively-spliced forms of LH2, LH2(long) and LH2(short) that differ by a single exon coding for 21aa, and LH3) are important posttranslational modifying enzymes that hydroxylate specific lysine residues during collagen biosynthesis. The hydroxylysine residues are precursors for the collagen cross-linking essential for the tensile strength of collagen. Although the sizes of the LH isoforms are similar, there are considerable differences between their electrophoretic mobilities, which may in part be linked to levels of N-linked glycosylation. As each isoform has a different number of potential glycosylation sites: LH1(4 sites), LH2(long) and LH2(short) (7 identical sites), and LH3 (2 sites), we undertook a study to determine the effect of N-deglycosylation on LH activity and electrophoretic mobility. Overnight incubation of the individual recombinant LHs with the deglycosylation enzyme PNGaseF at 30◦ resulted in the following loss of LH activity expressed as percent of each untreated control: LH1 (40%), LH2(long) (50%), LH2(short) (52%), and LH3 (10%). In addition the deglycosylated enzymes showed a predictable increase in electrophoretic mobility. Mutagenesis experiments using recombinant LH enzymes are underway to ascertain the effects of individually-mutated

glycosylation sites on LH activity. Structure-function analysis should identify those sites essential for enzyme activity and confirm our current findings that N-glycosylation of the LH family makes a significant contribution to enzyme activity.

170

SPARC Effects Collagen Deposition in Myocardial Hypertrophy

Amy D. Bradshaw, Catalin F. Baicu, Tyler J. Rentz, Christina C. Derienzo, Janet Boggs, An O. Van Laer, Michael R. Zile.
Division of Cardiology, Department of Medicine, Medical University of South Carolina, Ralph H. Johnson V.A. Medical Center, Charleston, SC

Chronic pressure-overload (PO) causes myocardial hypertrophy and increased extracellular matrix (ECM) collagen concentration. Increased myocardial collagen concentration is associated with increased stiffness and decreased diastolic function. We hypothesized that one determinant of these PO-induced changes is extracellular processing of procollagen mediated by SPARC. To determine whether PO-induced changes in collagen content and diastolic function are abrogated by the absence of SPARC, age-matched wild-type (WT) and SPARC null mice underwent transverse aortic constriction (TAC) for 4 weeks. In WT mice, TAC increased LV mass, SPARC expression, myocardial diastolic stiffness and total, salt-soluble, and insoluble collagen. In SPARC null mice, TAC increased LV mass and total collagen to an extent similar to WT mice. However, the proportion of LV collagen, which was insoluble, was less in SPARC Null TAC mice compared with WT TAC mice. In addition, the proportion of collagen, which was soluble, was greater in SPARC Null TAC mice compared with WT TAC mice. As a result, myocardial diastolic stiffness was lower in the SPARC Null TAC mice (0.075±0.005) then in WT TAC mice (0.045±0.005, $p < 0.05$). We conclude that the absence of SPARC reduced PO-induced alterations in ECM fibrillar collagen and diastolic function. These data support the hypothesis that SPARC plays a key role in procollagen processing and the development of mature cross-linked collagen fibrils in normal and pressure-overloaded myocardium.

171

SPARC Influences Collagen Interaction with the Cell Surface

Yuhua Zhang[1], Lee B. Rivera[2], Rolf A. Brekken[2], Amy D. Bradshaw[1].
[1]Dept. of Medicine, MUSC, Charleston, SC, [2]Hamon Center for Therapeutic Oncology Res. UT-Southwestern, Dallas, TX

Abnormalities in collagen assembly in the extracellular matrix (ECM) can disrupt cell behavior and tissue function. SPARC (Secreted Protein Acidic and Rich in Cysteine) is a collagen-binding protein with counter-adhesive activity. In SPARC-null mice, the level of collagen is reduced dramatically in skin and in response to fibrotic stimuli. To study the function of SPARC during collagen production and processing we stimulated fibroblasts isolated from wild-type (WT) or SPARC-null animals with ascorbic acid. Upon initial induction of collagen production, we found that higher levels of collagen I were associated with SPARC-null dermal fibroblast cell layers. We also found that processing of procollagen I to collagen I was enhanced by the absence of SPARC. Furthermore, addition of collagenase to fibroblast cultures followed by immunoblot analysis demonstrated that the higher levels of procollagen I and collagen I in SPARC-null cell layers is predominantly extracellular. We have gone on to show that levels of collagen IV in SPARC-null cell layers are also elevated in comparison to WT cells. Addition of rSPARC decreased collagen I and IV association with cell layers in a concentration-dependent manner. Preliminary experiments demonstrate a partial inhibition of collagen binding to SPARC-null cell layers mediated by function-blocking anti-integrin α1 antibodies. We hypothesize that the absence of SPARC increases collagen interaction with cell surface receptors and leads to decreases in collagen accumulation in SPARC-null mice.

172

The CRT-binding sequence of TSP1 alters the foreign body response

M Sweetwyne, L Van Duyn, B Sun, M Pallero, J Murphy-Ullrich.
Departments of Cell Biology & Pathology,

University of Alabama at Birmingham, Birmingham, AL

Thrombospondin-1 (TSP1) induces focal adhesion disassembly and increases cell survival and motility by signaling through cell surface calreticulin (CRT) and LDL receptor related protein1 (LRP1). These actions are mimicked by a TSP1 peptide of the CRT binding domain. To determine the role of the TSP1/CRT/LRP1 co-complex in tissue remodeling, we modeled the foreign body response to local expression of the TSP1 CRT-binding sequence by filling PVA sponges with collagen and a plasmid of the TSP1 signal peptide followed by the CRT-binding sequence and tagged with enhanced green fluorescent protein [HBD(1-35)-EGFP]. Sponges were implanted subcutaneously in wt mice. Cells invading the sponge became locally transfected. A plasmid expressing the CRT-binding sequence with two amino acid substitutions was used as an inactive control. Expression of the constructs from days 5-22 was confirmed by detection of EGFP. Sponges treated with HBD(1-35)-EGFP have increased collagen capsule organization and reduced foreign body giant cells at days 7 and 14. *In vitro*, fibroblasts treated with the TSP1-CRT binding domain or with the TSP1 N-terminal domain (NoC1) show increased collagen secretion and deposition into the ECM. NoC1 stimulation of collagen was prevented by a peptide which blocks TSP1/CRT interactions. Preliminary RT-PCR shows the TSP1 CRT-binding sequence increases collagen 1α2 mRNA over untreated or control peptide treated fibroblasts. These studies identify a novel function for the CRT-binding sequence of TSP1 and suggest that this signaling mediates collagen deposition during wound repair. (NIH HL079644 &T32 HL007918)

173

Analysis of the new collagen VI chain: a4(VI), a5(VI) and a6(VI)

Jamie Fitzgerald[1], Cathleen Rich[1], Uwe Hansen[2].
[1]Department of Orthopaedics and Rehabilitation, Oregon Health and Science University, Portland, OR 97239, USA, [2]Institute for Physiological Chemistry and Pathobiochemistry, University Hospital of Muenster, 48129, Muenster, Germany

Collagen VI is present in all musculoskeletal tissues in close association with basement membranes. It is composed of six genetically distinct polypeptide chains; α1(VI), α2(VI), α3(VI), α4(VI), α5(VI) and α6(VI). The α1(VI), α2(VI) and α3(VI) chains have been studied for the past 20 years revealing many important insights into collagen VI biology such as collagen VI assembly and its role in musculoskeletal disease. However, very little is known about the recently identified new chains, α4(VI), α5(VI) and α6(VI). While the majority of mammals contain an intact col6a4 gene the human COL6A4 gene has been disrupted by a chromosome break. Immunohistochemical analysis demonstrated that the new chains are expressed in skeletal tissues including cartilage, tendon and ligament. In addition, COL6A6 mRNA and α6(VI) protein was present in skeletal muscle making the COL6A6 gene a candidate gene for mutations in congenital muscular dystrophy. The three new chains superficially resemble the α3(VI) chain and there is evidence from SaOs-2 cell transfection experiments that the α4(VI) chain co-assembles with α1(VI) and α2(VI). However, the α5(VI) and α6(VI) chains were not competent to assemble with α1(VI) and α2(VI) in this system suggesting that alternative collagen VI assemblies are possible. In summary, the discovery of three additional collagen VI chains doubles the collagen VI family and adds a layer of complexity to collagen VI assembly and function in the extracellular matrix.

174

TGFBIp C-terminal fragment induces osteosarcoma cell apoptosis

Richard G. LeBaron, Maria Somaraki-Cormier, Clyde F. Phelix, Rajesha Rupaimoole, Christine L. Miller, Rogelio Zamilpa.
Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249

TGFBIp, transforming growth factor beta induced protein, is secreted into the extracellular space. When fragmentation of the C-terminus is blocked, apoptosis is low, even when the protein is overexpressed. If fragmentation occurs, apoptosis is observed. Whether full-length TGFBIp or an RGD fragment released from its

C-terminus is necessary for apoptosis remains equivocal. More importantly, the exact portion of the C-terminus that conveys the pro-apoptotic property of TGFBIp is uncertain. It is reportedly within the final 166 amino acids. We sought to determine if this property is dependent upon the final 69 amino acids containing the integrin-binding, EPDIM and RGD, sequences. With MG-63 osteosarcoma cells, TGFβ1 treatment increased expression of TGFBIp over 72 hours ($p<0.001$). At this time point, apoptosis was significantly increased ($p<0.001$) and was prevented by an anti-TGFBIp, antibody ($p<0.05$). Overexpression of recombinant TGFBIp produced a 2-fold increase in apoptosis ($p<0.01$). Exogenous TGFBIp at concentrations of 37 to 150 nM produced a dose dependent increase in apoptosis ($p<0.001$). Mass spectrometric analysis of TGFBIp isolated from conditioned medium of cells treated with TGFβ1 revealed that TGFBIp lacked integrin-binding sequences in the C-terminus. Recombinant TGFBIp truncated, similarly, at amino acid 614 failed to induce apoptosis. A fragment encoding the final 69 amino acids of the TGFBIp C-terminus produced significant apoptosis at a level comparable to that induced by TGFβ1 and dependent on the C-terminus most likely to interact with integrins.

175

TGFBIp interacts with type I collagen in vitro

Maria Somaraki-Cormier, Rogelio Zamilpa, Richard G. LeBaron.
Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249

Transforming Growth Factor-β Induced protein (TGFBIp) binds molecules of the ECM including collagens. Considerable interest in TGFBIp was spurred by the findings that mutations in the TGFBIp gene have been linked to various types of autosomal dominant corneal dystrophies (CD). Confocal imaging of non-CD donor human corneas localized TGFBIp alongside collagen fibrils in the corneal stroma, BowmanÕs and the epithelial layer. TGFBIp localization indicates TGFBIp binds type I collagen (col-I) in vitro, however, there is not much information available on the biological effects of TGFBIp association with col-I. In this study we investigate the role of TGFBIp in col-I fibrillogenesis and binding strengths between the two proteins. Human recombinant TGFBIp was utilized in turbidity and solid-phase binding assays with col-I. Binding is concentration-dependent and changes in the absorbance of collagen solutions recorded as a function of time showed typical sigmoidal curves composed of three phases. The time of the lag phase was longer in the collagen solution mixed with TGFBIp compared to the control of col-I alone. The increase rate of the absorbance in the growth phase decreased with increasing concentration of TGFBIp added in collagen solutions. Similarly, the absorbance in the plateau phase increased with increasing concentration of TGFBIp. We conclude that TGFBIp alters col-I fibrillogenesis by delaying fibril formation and increasing diameter of collagen fibrils leading to the assumption that TGFBIp hinders, to a degree, col-I fibrillogenesis and perhaps guides fibril organization in order to maintain equilibrium in the ECM.

176

Protein consequences of a missense mutation in mouse Col2a1

Lammy S. Kim[1], Sarah E. Funk[1], MaryAnn Weiss[1], Robert E. Seegmiller[2], David E. Eyre[1], Russell J. Fernandes[1].
[1]Department of Orthopaedics, Univ. of Washington, Seattle, WA 98195, [2]Department of Physiology, Brigham Young Univ., Provo, UT 84602

Type II collagen, the major structural protein of cartilage, is essential for normal embryonic skeletal development and the mechanical properties of cartilage. Mutations in the *Col2a1* gene cause chondrodysplasia and osteoarthritis in mice and humans. The type II collagen monomer, a homotrimer, is synthesized by chondrocytes as procollagen molecules with extension peptides at the N- and C-terminal ends. The process by which the newly synthesized chains associate and fold in the rough endoplasmic reticulum is not well characterized for type II procollagen although the last 78 amino acids at the C-terminus of the molecule have been found to be important for the nucleation of the triple helix. A new, naturally occurring, missense mutation in the

mouse *Col2a1* gene within exon 48 predicts a cysteine substitution for arginine at amino acid position 992 within the triple-helix. The resulting phenotype in affected homozygous (*sedc/sedc*) mice shows similarities to the human chondrodysplasia, spondyloepiphyseal dysplasia (SED) congenita. A cysteine at this position provides the opportunity for disulfide bonds to form, interfering with efficient folding of collagen molecules and assembly into fibrils. We have used the *sedc/sedc* mouse to investigate the consequences of this mutation on the assembly of stable triple-helical type II collagen protein. We also report on the formation of inter-molecular covalent cross-links within the collagen that frames the cartilage of homozygous and heterozygous mice.

177

Role of UPR in the retention of mutant COMP in PSACH chondrocytes

Francoise Coustry[1], Karen L. Posey[1], Huiqiu R. Wang[1], Jing-Fang Lu[2], Sankar N. Maity[2], Jacqueline T. Hecht[1,3].
[1]Department of Pediatrics, University of Texas Medical School at Houston, TX, [2]Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, TX, [3]Shriners Hospital for Children, Houston.TX

COMP is a pentameric glycoprotein found in matrices surrounding chondrocytes. COMP mutations cause two skeletal dysplasias, pseudoachondroplasia and multiple epiphyseal dysplasia. Mutations in COMP result in abnormal protein folding, aberrant protein trafficking, and accumulation in the endoplasmic reticulum (ER). These cellular processes trigger the unfolded protein response (UPR). Ultimately, COMP retention leads to premature chondrocyte death and dwarfism. This study examines UPR components in chondrocytes over expressing mutant-COMP. Human chondrocytes and rat chondrosarcoma cells were cultured in monolayer and infected with a TET-inducible wild-type or mutant-COMP adenovirus. RNA and proteins were analyzed by RT-PCR and Western blot. Preliminary results indicate that mutant-COMP expression in chondrocytes up regulates chaperone grp78, involved in protein folding, at the mRNA and protein level. The protein levels of the transcription factor Xbp-1, that regulates select UPR genes, and the proapoptotic factor, CHOP, were not changed by mutant-COMP expression. Mutant-COMP over expression did not repress general protein synthesis as measured by phosphorylated-eIF2α levels suggesting that relentless mutant protein synthesis may overwhelm the clearance mechanisms. Further studies will be conducted to analyze the ER associated degradation pathway in chondrocytes to define why mutant-COMP is not efficiently degraded.

178

Fibrin Knob Binding Kinetics on Defined Surface Chemistries

Sarah E. Stabenfeldt, Thomas H. Barker.
Department of Biomedical Engineering, Georgia Institute of Technology / Emory University School of Medicine, Atlanta, GA 30332

Conversion of fibrinogen to fibrin follows a complex, yet well established chain of enzymatic events. Ultimately, thrombin cleaves fibrinopeptides from fibrinogen exposing fibrin knob domains that in turn interact with the pocket domains on other fibrin(ogen) monomers. The interaction between exposed knob peptides and pocket domains has been well characterized with traditional binding assays. However, with the emergence of Surface Plasmon Resonance (SPR) and advances in surface chemistry technology, direct protein/peptide binding interactions can now be characterized in a well-defined and controlled environment. Coupling of these technologies (SPR and Surface Chemistry) enables us to more rapidly explore alterations in binding dynamics stemming from species-specific A-knob peptide modifications, peptide length, and amino acid point mutations. For this study, fibrin A-knob peptide variants will be synthesized and covalently immobilized to self-assembled monolayers (SAMs) of functionalized dialkyl disulfides on gold films allowing high levels of control of both the peptide orientation and surface density. Using SPR, binding affinity and dissociation constants will be determined by flowing the fibrinogen analyte over the immobilized peptides. The specificity of the SAMs surfaces have been validated with thiol

consumption assays and enzyme linked immunosorbent assays. The results from this study will further identify critical elements of the fibrin A-knob domains necessary for binding pocket domains, species differences, and potential links to hereditary clotting disorders.

179
Engineering fibrin ECM using fibrin knob-pocket interactions
Allyson Soon, Sarah Stabenfeldt, Cedric Paulou, Thomas Barker.
Wallace H. Coulter Department of Biomedical Engineering, Georgia Tech & Emory University, Atlanta, GA

Fibrin is a provisional matrix that forms in response to vascular injury, acting to stimulate and support cellular infiltration. The thrombin-catalyzed conversion of fibrinogen to fibrin involves the cleavage of fibrinopeptides, exposing N-terminal fibrin 'knobs' that bind to C-terminal 'pockets' on fibrin(ogen), resulting in the formation of a polymer matrix that is replaced as cells invade and the tissue heals. Fibrin knob-pocket interactions have been studied using classic binding assays and more recently, with laser tweezers and surface plasmon resonance. We are interested in exploiting these stable non-covalent interactions to modify the structure and/or function of fibrin matrices for enhanced wound repair. We have created a simple 'plug-and-play' vector system allowing the expression of recombinant proteins displaying fibrin knobs for delivery of therapeutic proteins in fibrin matrices. Using the 9th and 10th fibronectin Type III repeats (Fn9-10) as a model protein, we have shown that non-fibrin proteins displaying 'knobs' are capable of binding fibrinogen and being incorporated into fibrin polymers. Release assays indicate that the non-covalent incorporation of proteins is sufficient to enhance their retention in fibrin matrices. We are currently exploring the delivery of Fn9-10 for controlling integrin engagement during wound repair and tissue-type plasminogen activator to regulate fibrin degradation rate. We believe this biology-inspired technology will allow the development of ECM-based therapies targeting tissue regeneration and enable new investigations into fibrin polymer dynamics.

180
High expression and physical properties of bacterial collagen
Ayumi Yoshizumi, Zhuoxin Yu, Geetha Thiagarajan, Teresita Silva, Angela Mohs, Takeshi Yoshida, Masayori Inouye, Barbara Brodsky.
Department of Biochemistry,Robert Wood Johnson Medical School, Piscataway, NJ, USA

Collagens are the characteristic structural molecules of the extracellular matrix of multicellular animals. Numerous products based on collagen have been designed for biomaterial and biomedical uses. However, there are increasing concerns over the use of animal-derived collagen products, including the risk of contamination by infectious agents. Efficient production of recombinant mammalian collagen encounters issues of post-translational modification, suggesting that a high yield expression system for a stable collagen-like protein with no post-translational modifications such as found in bacteria could be useful . Even though it lacks hydroxyproline, the triple-helix module of *Streptococcus pyogenes* has a high stability because of its highly charged sequence.A single triple-helix module $(Gly\text{-}X\text{-}Y)_{79}$ and a construct composed of two triple-helix modules based on collagen-like sequences from *S. pyogenes* have been expressed in a high yield cold shock system in *E. coli* , and the collagenous domains were isolated by trypsin digestion. The thermal stability, calorimetric enthalpy, and hydrodynamic properties of the monomer and dimmer were compared. Solubilities of bacterial collagen constructs are compared with that of bovine skin collagen at different pH and temperature values. The efficient expression of stable bacterial bacterial collagen-like modules in *E.coli* and knowledge about their aggregation properties may provide a basis for useful design of biomaterial products .

181
Tissue-specific negative effects in a cutis laxa mouse model
Hideki Sugitani, Adrian Shifren, Russell H. Knutsen, Christopher H. Ciliberto, Robert P. Mecham.
Department of Cell Biology and Physiology,

Washington University, St. Louis, MO

Cutis laxa, an extremely rare disorder with lax, redundant, and inelastic skin, is caused by defective elastic fiber formation. The majority of autosomal dominant cutis laxa (ADCL) single base deletion mutations have been mapped in exon 30, 32, and 33 of tropoelastin. To fully understand the molecular mechanism whereby missense mutations in the carboxy-terminal end of elastin exert their effect on elastic fiber assembly or function, we generated transgenic mice using bacterial artificial chromosome with (hCL-BAC) or without (hWT-BAC) a single nucleotide deletion (2012G in exon 30). Studies of lung and arterial function showed that elastin from the hWT-BAC interacts with mouse elastin to form functional elastic fibers and, when expressed in the elastin haploinsufficient background, reverses the vascular and pulmonary changes associated with that phenotype. Protein from the hCL-BAC transgene, however, produced worse tissue function in the lung but had little, if any, effect on vascular tissue. Semi-quantitative RT-PCR, Western blot analysis, and immunofluorescence studies showed that the tissue-specificity of this negative function in hCL-BAC mice may depend on protein deposition or elastin maturation levels but not protein or RNA expression levels. These data suggest a possible strong tissue bias for effects of CL mutations affecting the C-terminal region of elastin.

182

Collagen VI mutations: A review of 54 patients

Naomi L. Baker, Rishika A. Pace, Leona Tooley, Laura Zamurs, Naomi E. Adams, Shireen R. Lamande.
Murdoch Childrens Research Inst, and Dept of Paediatrics, Uni. of Melbourne, Parkville, Australia

Mutations in the three collagen VI genes cause a spectrum of disorders ranging from relatively mild muscle weakness to profound disability. We have studied 54 patients with clinical features consistent with a collagen VI disorder and have identified amino acid substitutions, premature stop codon mutations, and in-frame deletions in the three collagen VI chains. We have also examined collagen VI biosynthesis, and intracellular and extracellular assembly in patient fibroblasts. The mutations are providing fundamental new information about the domains important for assembly of collagen VI monomers, dimers, tetramers and microfibrils and genotype/phenotype correlations are emerging. Twenty eight patients have dominant in-frame deletions at the N-terminal end of the triple helix and 9 have dominant glycine mutations in the same region. Distinct regions of the N-terminal end of the helix are critical for dimer and tetramer formation. Five patients have dominant, and 2 have recessive, A-domain mutations. Some A-domain mutations prevent chain association and triple helix formation, while other act later in the assembly pathway and interfere with tetramer or microfibril assembly, or with interactions in the extracellular matrix. Only five patients have recessive mutations. Thirteen patients (24%) do not have a collagen VI mutation; however, several of these patients have reduced collagen VI immunostaining in muscle biopsies suggesting that a collagen VI interacting protein may also be involved in this spectrum of diseases.

183

An a2(VI) C1 mutation prevents collagen VI microfibril formation

Naomi L. Baker[1], Laura Zamurs[1], Leona Tooley[1], Rachel A. Peat[2], Kathryn N. North[2], Shireen R. Lamande[1].
[1]Murdoch Childrens Research Institute, and Dept of Paediatrics, University of Melbourne, Australia, [2]Neurogenetics Research Unit, Children's Hospital at Westmead, Sydney, Australia

The collagen VI muscular dystrophies form a continuum of clinical phenotypes. Bethlem myopathy patients have dominant mutations, while both dominant and recessive mutations cause severe UCMD. The majority of recessive mutations lead to in-frame premature stop codons and nonsense-mediated mRNA decay. Collagen VI is not secreted by patient cells and there are no collagen VI microfibrils in the extracellular matrix. We have identified a patient with recessive UCMD who has unusual compound heterozygous mutations in COL6A2. The first mutation, inherited from her father, is a

splice site mutation that causes skipping of exon 24, introduces a premature stop codon, and leads to mRNA decay. The second mutation, inherited from her mother, is a two amino acid deletion in the C1 domain of the α 2(VI) chain. The deleted residues are adjacent to the DXSXS sequence that makes up part of the MIDAS motif of the A-domain. The α 2(VI) chain with the 2 amino acid deletion is stable and able to assemble with α 1(VI) and α 3(VI) inside the cell into monomers, dimers and tetramers which are secreted. Normal intracellular assembly was surprising because the C1 domains are thought to be critical for initial chain association. Despite secretion of collagen VI tetramers, patient fibroblasts do not deposit collagen VI microfibrils into the extracellular matrix, indicating that the α 2(VI) C1 domain contains structural information essential for end-to-end assembly of tetramers into microfibrils.

184

Collagen VI A-domain changes: mutations or polymorphisms?

Leona Tooley, Naomi L. Baker, Rishika A. Pace, Laura Zamurs, Shireen R. Lamande.
Murdoch Childrens Research Institute and Dept of Paediatrics, University of Melbourne, Australia

Most of the collagen VI structural mutations identified in muscular dystrophy patients are clustered at the N-terminal end of the triple helix of the three collagen VI chains. These mutations have a clear negative effect on collagen VI dimer, tetramer, or microfibril assembly. Amino acid substitutions in the A-domains have also been identified; 32 are in healthy controls, making it difficult to know if a change in a patient is a mutation or a polymorphism, particularly if parents are not available. We have identified changes in patients in the α2(VI) C1 and C2, and the α3(VI) N2 A-domains. Three changes in α2(VI) A- domains are recessive and we are sure they are mutations because they affect collagen VI assembly, either by disturbing folding and preventing association of the mutant chains, or by preventing microfibril formation. Four heterozygous A-domain aa substitutions are also likely to be mutations; two are de novo, one is found only in affected members of a 3 generation family, and one is present in an affected father and daughter. In one case we are unsure if an A-domain change is a mutation or a polymorphism because parents are not available. It is not known how the dominant A-domain mutations cause disease. The majority of the mutations are located in highly conserved regions, or β-sheets, or are adjacent to amino acids that make up the MIDAS motif of the A-domains. We are conducting detailed analyses of collagen VI assembly in these patients, and in SaOS-2 cells transfected with normal and mutant α3(VI) chains to determine the affect of A-domain mutations on collagen VI assembly.

185

Fibrillin-1 Assembly is Dependent on Fibronectin

Daliang Chen[1], Laetitia Sabatier[1], Christine Fagotto[1], Dirk Hubmacher[1], Douglas S. Annis[3], Deane F. Mosher[3], Dieter P. Reinhardt[1,2].
[1]Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada, [2]Faculty of Dentistry, McGill University, Montreal, QC, Canada, [3]Department of Medicine, University of Wisconsin, Madison, WI, USA

Fibrillin-1 constitutes the major backbone of extracellular microfibrils. Proper assembly mechanisms are crucial for the formation and function of microfibrils. We have used dermal fibroblasts to analyze assembly of fibrillin-1 in dependence of other matrix molecules. Inhibition studies by siRNA demonstrated that the fibrillin-1 assembly was dependent on the presence of extracellular fibronectin fibers. However, fibronectin assembly was not dependent on the presence of fibrillin-1. Fibrillin-1 assembly was not dependent on the presence of fibulin-2, a microfibril associated protein capable of interacting with fibrillin-1. Immunofluorescence experiments showed that fibrillin-1 colocalized with fibronectin fibers at early stages of the assembly of both proteins. Protein binding assays demonstrated that fibronectin interacted with the C-terminal region of fibrillin-1, a region that coincides with the self-assembly and the heparin/heparan sulfate interaction epitopes in fibrillin-1. However, heparin did not inhibit the fibronectin-fibrillin-1

interaction, indicating different binding sites. Further interaction mapping studies revealed that the fibrillin-1 binding region in fibronectin is localized to the collagen/gelatin interaction region in fibronectin. In summary, our results show that the initial fibrillin-1 network formation requires fibronectin. This mechanism is mediated through direct molecular interactions between fibronectin and fibrillin-1.

186

Characterization of Fibrillin Interactions with Fibronectin

Laetitia Sabatier[1], Christine Fagotto[1], Dirk Hubmacher[1], Daliang Chen[1], Douglas S. Annis[3], Deane F. Mosher[3], Dieter P. Reinhardt[1,2].
[1]Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada, [2]Faculty of Dentistry, McGill University, Montreal, QC, Canada, [3]Department of Medicine, Uni. of Wisconsin, Madison, WI

Fibrillins are members of a protein family that constitutes extracellular microfibrils. Assembly of fibrillins into microfibrils is not very well understood and is often compromised in connective tissue disorders caused by fibrillin mutations. Recently, we have found that fibronectin is required for the initial network formation of fibrillin-1. Here we have analyzed the molecular properties of fibronectin-fibrillin interactions. C-terminal halves of fibrillin-1, -2 and -3 interacted with fibronectin, indicating that the interaction epitopes are conserved among the fibrillins. These C-terminal halves partially multimerize in association with cells. After separation of monomeric, intermediate and multimeric fractions of all fibrillins, we found that the monomeric forms bound very little, whereas the multimeric forms bound fibronectin with high affinity. These data suggest that low affinity binding sites for fibronectin are transformed to high avidity binding sites by fibrillin multimerization. The interaction can be inhibited by anionic or amphoteric detergents while high salt concentrations had no inhibitory effects, indicating a hydrophobic nature of the interaction. Gelatin inhibits binding of fibrillins to fibronectin, positioning the fibrillin binding site between FN type I domains #6 and #9. This finding was further corroborated by mapping the binding site to the same region using recombinant and proteolytic fibronectin fragments.

187

Characterization of Osteoarthritic Articular Cartilage Perlecan

Arivalagan Muthusamy[1], Charles M. Davis, III[1], Carlton R. Cooper[2], Ronald R. Gomes, Jr.[1].
[1]Department of Orthopedics, The Pennsylvania State University College of Medicine, Hershey, PA., [2]Department of Biological Sciences, University of Delaware, Newark, DE

Deposition of perlecan is enhanced in osteoarthritic articular cartilage, and may contribute to disease progression. We hypothesize that glycosaminoglycan (GAG) chains of human perlecan (HnPln) derived from osteoarthritic articular cartilage are differentially sulfated relative to non-diseased cartilage. Alterations in sulfate composition may differentially modulate heparin-binding growth factor bio-availability. To test our hypothesis, HnPln was purified from disease-free/"normal" (N-HnPln) and osteoarthritic articular cartilage (OA-HnPln), biochemically characterized, then interactions with heparin-binding growth factors assessed. Perlecan, isolated by density gradient centrifugation, enriched and purified by anion exchange and gel filtration chromatography was monitored by protein, uronic acid, and dot blot analysis using anti-perlecan antibodies. Contrasting OA-HnPln, the majority of N-HnPln did not bind to the anion-exchange column. The bound portion of N-HnPln was eluted, and further characterized. Heparan sulfate disaccharide analysis revealed significant differences in Δdi-6S and Δdi-diS1 content for OA-HnPln. Solid phase binding assays suggest that OA-HnPln binds more FGF-2 and VEGF165 relative to N-HnPln, and such interactions were heparan sulfate dependent. Soluble forms of OA-HnPln inhibit FGF-2 and VEGF165 induced-capillary tube-like formation, in vitro. Thus, GAG chains attached to OA-HnPln are well suited to regulate heparin-binding growth factor activity and their impact on articular cartilage integrity, and function.
Support: NIH AR054915-01A1, and PSUCOM Dean's Feasibility Grant to RRG.

188

Podocytes use Syndecan-4 (Syn4) to adhere to basement membranes.

Kevin McCarthy[1], Shoujun Chen[1], Deborah McCarthy[1], Anne Woods[2], Yu Yamaguchi[4], Toin van Kuppevelt[3].
[1] LSU Health Sciences Center-Shreveport, [2] University of Alabama at Birmingham, [3]Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [4] Burnham Institute, La Jolla, CA

PEXTKO mouse (podocyte specific Ext1 knockout mouse) podocytes do not make heparan sulfate glycosaminoglycans (HSGAGs) and their foot process are effaced. This suggests that loss of cell surface HSGAGs has negative effects on cell-matrix adhesion. Syn4, a cell surface HSPG, modulates cell adhesion, but its role in podocyte adhesion was unknown. Staining tissue sections from control and PEXTKO animals with anti-Syn4 antibodies showed that podocytes make Syn4, but the pattern of Syn4 distribution in PEXTKO animals was altered vs controls, having a patchy rather than linear appearance. To examine Syn4-mediated podocyte-matrix interactions, adenoviral delivery of Cre- recombinase to immortalized $Ext1^{fl/fl}$ podocytes was used to make cell lines incapable of assembling HSGAGs(HSGAG-). Control cell lines were made using an empty adenoviral vector. In cell adhesion assays on fibronectin, fewer HSGAG- podocytes attached compared to controls and had smaller spread cell area compared to HS- GAG+ cells ($p<0.0001$). Vinculin staining, a marker of focal adhesions, showed that HSGAG-podocytes failed to form focal adhesions. Syn4 staining in HSGAG-podocytes was primarily diffuse whereas in HSGAG+ podocytes Syn4 was concentrated in clusters at the periphery of the cell. In HSGAG+ cells, α-actinin 4 was localized at Syn4+ clusters. Stress fiber formation was also enhanced in HSGAG+ compared to HSGAG- cells. The data indicate that HSGAG on Syn4 plays a role in mediating podocyte-matrix interactions.

189

AGEs stimulate transglutaminase activity in tenocytes.

Ann K. Rosenthal, Emily Muth, Claudia M. Gohr.
Department of Medicine, Medical College of Wisconsin

Aims: Tendon abnormalities, including excess stiffness, thickness, and calcification, occur commonly in diabetic patients and cause considerable disability. These changes are often attributed to excess cross-linking of collagen by advanced glycation end-products (AGEs). However, AGEs also alter extracellular matrix production by resident connective tissue cells, potentially affecting biomechanics and calcification. We determined the effect of AGEs on tenocyte extracellular matrix production and on the calcification-promoting transglutaminase (Tgase) enzymes. Methods: Primary adult porcine tenocytes were exposed to the AGE, Nε-carboxymethyl-lysine (CML)- type I collagen or control collagen. Proteoglycan production was measured by 35S uptake. Hydroxyproline levels were used to estimate collagen content. Tgase was measured by a radiometric activity assay and by protein and mRNA levels of the Tgase enzymes. We determined whether these effects were dependent on reactive oxygen species or associated with apoptosis. Results: CML-collagen (160-400 μg/ml) decreased tenocyte proteoglycan production by 60% compared to collagen controls. No changes in hydroxyproline content were seen. CML-collagen increased Tgase activity 2.3-5.6 fold over collagen controls without altering protein levels of either Tgase enzyme. Antioxidants reduced the effect of CML-collagen on Tgase activity. Markers of apoptosis were unchanged by CML-collagen. Conclusions: AGE-mediated suppression of proteoglycan production and increased activity of the protein cross-linking Tgase enzymes in tenocytes may contribute to the altered tissue biomechanics and pathologic calcification in diabetic tendons.

190

Regulation of ECM genes during heart valve remodeling

Santanu Chakraborty[1], Jonathan Cheek[1], Bhuvaneswari Sakthivel[2], Bruce J. Aronow[2], Katherine E. Yutzey[1].
[1]Division of Molecular Cardiovascular Biology, Cincinnati Children's Medical Center, Cincinnati, OH 45229, [2]Division of Biomedical Informatics, Cincinnati Children's Medical Center, Cincinnati, OH 45229

Atrioventricular (AV) valves of the heart develop from undifferentiated mesenchymal endocardial cushions (EC) that later remodel into stratified valves with diversified extracellular matrix (ECM). Although some ECM components and remodeling enzymes have been identified, a complete gene expression profile of heart valve maturation and ECM remodeling program has not been established. In order to identify gene expression profile of developmental valve remodeling, Affymetrix gene expression profiling analysis was performed on murine embryonic day (E)12.5 AV cushion compared with E17.5 AV valves. The most highly upregulated family of genes detected in the E17.5 and adult valves compared to E12.5 EC were members of the small leucine-rich proteoglycan (SLRP) family including Asporin and Osteoglycin. Interestingly, Asporin is a known negative regulator of matrix mineralization and could be important in valve disease mechanisms. Consistent with ECM maturation and remodeling, collagens associated with specific types of connective tissue, including Col14a1 and Col1a1 are increased in late E17.5 AV valves. In contrast, cartilage-associated type IX Col9a3 and Col2a1 are increased in early E12.5 EC. Several ECM remodeling enzymes and tissue inhibitor of matrix metalloproteinases (TIMPs) are also differentially expressed during mouse AV valve development. Adamts12 is identified as one of the most increased genes in E17.5 AV valves, whereas Adamts15, Mmp11 and Adamtsl1 are increased in early E12.5 EC. The differential regulation of multiple ECM proteins and remodeling enzymes is critical for normal valve structure and function with implications in valve degeneration and disease mechanisms.

191

HS/syndecan modulate proteolytic balance in airway inflammation

Chi Hang Chan[1,2], Valeria On Yue Leung[1], Mary Sau Man Ip[2], Daisy Kwok Yan Shum.
[1]Department of Biochemistry, the University of Hong Kong, [2]Department of Medicine, the University of Hong Kong

Persistent tissue injury in chronic airway inflammation is contributed by the unopposed neutrophil elastase (NE) activity. In sputum sols of patients with bronchiectasis, alpha-1-antitrypsin (α_1-AT) was found in excess of unopposed NE at molar ratios that averaged 16:1, suggesting the existence of imbalance between protease and anti-protease. Western blot analysis of sputum sol samples however found NE in a supramolecular complex with syndecan-1 and as such, inhibition of NE activity was incomplete even with addition of α_1-AT. To confirm that NE binding to heparan sulfate (HS) moieties of syndecan-1 limits the anti-elastase effect, recombinant syndecan-1 was recovered from stable syndecan-1 transfectants of ARH-77 cells. Western ligand blot confirmed that NE bound only to HS moieties of syndecan-1. Inhibition of NE activity by standard additions of α_1-AT was incomplete unless syndecan-1 had been deglycanated by heparitinase treatment. SPR analysis revealed that NE binding to HS could be competed out by heparin variants. To test if heparin fragments can displace NE from the complex, heparin oligosaccharides were incubated with sputum sol samples. Western blot analysis indicated that heparin oligosaccharides (dp $\geq$4) displaced NE from the complex and the displaced NE became accessible to inhibition by endogenous α_1-AT. MALDI TOF-MS analysis further revealed subunit composition of the heparin oligosaccharides. Our results therefore suggest the possible use of heparin oligosaccharides in restoring the balance between protease/anti-protease in chronic airway inflammation.

192

Role of Serglycin in Acute Lung Injury and Repair

Michelle Lin[1], Peter Chen[1], Magnus Åbrink[2], Qinglang Li[1], Ying Wang[1], Bill C. Parks[1].
[1]Center for Lung Biology, University of Washington , Seattle, WA, [2]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

Proteoglycans influence cell-cell and cell-matrix interactions, as well as the functions of chemokines, enzymes, and growth factors through sequestration, transport, and activation of substrates or target cells. Serglycin is a heparin/heparan- and chondroitin-sulfated proteoglycan involved in the retention and

maturation of several proteases inside storage granules and secretory vesicles of hematopoietic cells and endothelial cells. In addition, secreted serglycin can further modulate activity of proteins through their interactions. Using immunohistochemistry and qRT-PCR, we determined that serglycin is expressed by alveolar type II cells, and we hence assessed if this proteoglycan functions in the response to acute lung injury. We found an increase in the number of leukocytes, particularly macrophages, in the bronchoalveolar lavage (BAL) fluid of serglycin-null mice compared to wildtype animals after bleomycin-induced injury. Furthermore, serglycin expression was increased in response to mechanical injury in organotypic airway epithelial cell cultures. In the absence of serglycin, the epithelial cells had decreased wound closure and increased total MMP activity as measured with a fluorogenic substrate. These data suggest that serglycin is involved in modulating inflammation and injury repair.

193

Confocal Microscopy Study of Decorin Binding to Collagen Fibrils

Elena Makareeva[1], Mary B. Sutter[1], Angela M. DeRidder[1], Antonella Forlino[2], Antonio Rossi[2], Ruggero Tenni[2], Sergey Leikin[1].
[1]Section on Physical Biochemistry, NICHD, National Institutes of Health, Bethesda, MD 20892, [2]University of Pavia, Italy

Most studies of protein and proteoglycan binding to collagen are based on techniques that do not account for the supramolecular organization of collagen fibers and physiological environment. To overcome these limitations, we developed a confocal microscopy binding assay with differential fluorescent labeling of collagen and its ligands. For the present study, purified type I human fibroblast collagen was labeled with AlexaFluor488 (A488) and recombinant human decorin was labeled with AlexaFluor546 (A546). Decorin solution was added to reconstituted collagen fibrils at 37 °C. Three dimensional confocal microscopy imaging revealed a non-uniform binding pattern of decorin with more proteoglycan bound at fibril edges, kinks, junctions, and within poorly organized collagen aggregates. Such binding heterogeneity was caused by variation in the exposed collagen surface. The amount of decorin bound to isolated fibrils was directly proportional to the fibril surface area. The A546/A488 intensity ratio normalized to the surface area followed the classical binding isotherm with the dissociation constant Kd ~ 50 nM. From competitive binding experiments, we found the effect of decorin labeling to be minimal. The isotherms on fibrils reconstituted from collagen with intact and pepsin-cleaved telopeptides were indistinguishable, indicating that telopeptides do not play a significant role in decorin binding. We are currently investigating the role of GAG chains and different cofactors in decorin binding.

194

Estrogen and Progesterone Modulate Versican in the Mouse Uterus

Renato M. Salgado[1], Fernanda F. Fernandes[1], Rodolfo R. Favaro[1], Jocelyn D. Glazier[2], John D. Aplin[2], Telma M. Zorn[1].
[1]Department of Cell and Developmental Biology, University of Sao Paulo, Brazil, [2]Maternal and Fetal Health Research Group, University of Manchester, UK

Versican is a hyaluronan-binding proteoglycan found in various soft tissues. This molecule undergoes RNA alternative splicing, originating four distinct isoforms, which play important roles in physiological and pathological conditions. In this study, we analyze the synthesis and distribution of versican in mouse uterine tissues during the estrous cycle, in ovariectomized animals and after hormonal replacement. Uteri samples were fixed in methacarn and embedded in paraplast for immunoperoxidase, or immersed in RNAlater solution for quantitative Real Time PCR. In diestrus and proestrus, versican was exclusively expressed in the endometrial stroma, conspicuously stronger in the superficial stroma. In estrus and metaestrus, the whole stroma was immunoreactive, as well as the internal layer of the myometrium. In ovariectomized and control groups, versican was abolished in all uterine tissues. E2-treament recovered versican expression, forming a delicate network in the stroma, except in regions of edema. P4-

treatment induced similar immunoreaction observed in diestrus. In E2+P4-treated groups, versican immunoreaction was strong in the whole stroma and present in myometrium. Real Time PCR analysis showed that versican increases considerably in groups with high levels of estradiol. Estrogens appear to play a role in the organization of versican in the ECM. Furthermore, the control exerted by ovarian hormones on versican expression suggests important roles for this molecule in the uterine environment.

195

Diabetes Alters Deposition of Endometrial Extracellular Molecules

Rodolfo R. Favaro[1], Priscila R. Raspantini[1], Munick Fulquim[1], Renato M. Salgado[1], Zuleica B. Fortes[2], Telma M. Zorn[1].
[1]Department of Cell and Developmental Biology, University of São Paulo, Brazil, [2]Department of Pharmacology, University of São Paulo, Brazil

In rodents, decidua formation requires a notable reorganization of cellular and extracellular components of the endometrium, building a unique environment to embryo development. The impact of diabetes in early pregnant uterus is poorly understood. This work investigated the effects of diabetes on the decidual ECM composition in early pregnancy of mice. Deposition of the proteoglycans biglycan, decorin, fibromodulin and lumican were analyzed through immunohistochemistry and Western blotting techniques. Diabetes was induced in female Swiss mice by intravenous injection of alloxan (40mg/Kg). Diabetic (glycemia >400mg/dl) and normal females were mated with normal males 85-100 days after alloxan or vehicle administration and sacrificed within 168 hours of pregnancy. The diabetic females were characterized by: high glycemic levels, glycosuria, increased food and water consumption and decreased body mass weight. No cetonuria was detected. Diabetes promotes the reduction in the number and dimension of implantation sites and affects the deposition of biglycan and lumican. No reactivity for decorin and fibromodulin was detected. Our experimental model shows physiological parameters compatible with diabetes type I. In addition, negative impact of the diabetes on the cytoarchitecture of the uterine tissues and their ECM was detected. These alterations in the uterine environment may be partially responsible for the impaired embryo development observed in diabetic pregnancies.

196

Decorin, biglycan and human airway smooth muscle cell apoptosis

Michelle L. D'Antoni[1], Marie-Claire Michoud[1], Pasquale Ferraro[2], Bruce Mazer[1], James G. Martin[1], Mara S. Ludwig[1].
[1]Meakins Christie Laboratories, McGill University, Montreal, Quebec, Canada, [2]University of Montreal Hospital Centre, Montreal, Quebec, Canada

Changes in small leucine-rich proteoglycans (SLRP), such as biglycan (bgn) and decorin (dcn), are described in the airway wall in asthma. Since these SLRP are known to affect apoptosis, in other cell systems, we questioned whether they could affect airway smooth muscle (ASM) apoptosis and thereby, ASM mass. ASM cells, obtained from lung transplant recipients, were seeded on dcn or bgn (1, 3 and 10 μg/ml). After a 48 h growth arrest period (time 0), cells were stimulated with platelet-derived growth factor (PDGF) and cell number was assessed at 0, 48 and 96 h by cell counting. Control cells were seeded on plastic. ASM apoptosis was measured at 0 and 48 h by annexin V and propidium iodide (PI) staining, using flow cytometry. A significant decrease in cell number was observed with cells seeded on dcn (10μg/ml) at 0, 48 and 96 h ($p<0.01$) compared to control. Bgn initially decreased cell number at time 0 ($p<0.05$) compared to control, but this decrement was not sustained at 48 or 96 h. In cells seeded on dcn, the annexin V positive, PI negative, cell population was increased at 0 and 48 h ($p<0.01$) compared to control. This suggests an increase apoptosis, rather than simply cell necrosis. Bgn affected neither annexin V nor PI staining. These studies demonstrate that dcn and bgn have differential effects on human ASM cell number and apoptosis. Previous authors have described decreases in dcn in the asthmatic airway wall; this could permit a more exuberant ASM hyperplasia, due to the loss of the pro-apoptotic effect.

197

G1 domain of versican recruits HA to microfibrils

Yusuke Murasawa[1], Chika Orii[1], Masahiko Yoneda[2], Zenzo Isogai[1].
[1]Department of Advanced Medicine, National Center for Geriatrics and Gerontology,Obu, Aichi, Japan, [2]Aichi Prefectural College of Nursing and Health, Nagoya, Aichi, Japan

Versican connects elastic matrix and hydrated matrix through interaction with both fibrillin-microfibrils and hyarulonan (HA). However, hyaluronan binding region (HABR) of versican is extracted by 6 M guanidine hydrochloride buffer from skin and is separated from the carboxyl-terminus. Therefore, we investigate whether HABR of versican apart from the carboxyl terminus still associate microfibrils and impart hydrated nature to microfibrils. Sequential extraction by hyaluronidase and 6 M guanidine hydrochloride buffer suggested that versican was not simply trapped by interaction with HA in dermis. A blot overlay assay showed that a recombinant polypeptide for versican□$B!G□(Bs HABR, rVN specifically bound to versican core protein produced by dermal fibroblast. By solid phase binding assay, rVN bound to non-degraded microfibrils and native versican purified from conditioned medium, but not to fibrillin-1. Binding assay using newly expressed carboxyl-terminus of versican (rVC) demonstrated that rVN bound to both rVN and rVC. Overlay assay onto skin tissue using rVN as a soluble ligand showed that exogenous rVN deposited to microfibrils and recruits more HA to microfibrils. From our data, homotypical interaction of versican is a critical factor for tissue visco-elasticity. HABR of versican recruits HA to microfibrils by versican self-interactions.

198

Versican Induces Fibroblast Contraction of Collagen Gels

Jon Carthy, Maziar Rahmani, Seti Boroomand, Darryl Knight, Bruce McManus.
The James Hogg iCAPTURE Centre, Vancouver, BC, Canada

Versican, a chondroitin sulphate proteoglycan, is one of the main components of the extracellular matrix (ECM) where it provides hygroscopic properties to create a loose and hydrated matrix necessary to support key events during development and disease. The current study evaluated the hypothesis that versican can also modulate tissue remodeling by altering fibroblast-mediated contraction of the ECM. To accomplish this, we have cloned human versican and set up an inducible (doxycycline controlled) expression system in NIH3T3 fibroblasts. Cells were cultured in 3D native type I collagen gels in the presence or absence of versican overexpression, and contraction was followed for a period of 24 hours. Results suggest versican significantly increased fibroblast contraction of the collagen gels ($27.9 \pm 1.2\%$ vs $64.5 \pm 1.4\%$ of initial gel area, $p<0.01$). Immunohistochemistry and confocal imaging of these gels demonstrated a marked change in cell phenotype: versican expressing fibroblasts exhibited cellular protrusions that were associated with smooth muscle alpha-actin (SMA) positive stress fibres, while non-induced cells were rounded and negative for SMA. Further, versican appeared to form a pericellular coat around these cellular protrusions suggesting it may be influencing cell receptor-to-matrix interactions. In support of this idea, Western blotting demonstrated that versican overexpression dramatically increased the expression of beta 1 integrin. The ability of versican to modulate fibroblast function suggests that versican may be an important regulator of tissue remodeling in both normal wound healing and in the development of fibrosis.

199

Mutual Stabilization of P3H1 and CRTAP in ER Modification Complex

W. Chang, A.M. Barnes, W.A. Cabral, J.C. Marini.
BMB, NICHD, NIH, Bethesda, MD 20892

Null mutations in CRTAP and P3H1/LEPRE1 cause two novel recessive forms of OI, Types VII and VIII, respectively. CRTAP and P3H1 form a complex with cyclophilin B in the ER which 3-hydroxylates α1(I)Pro986 and α1(II)Pro986. We used cultured dermal fibroblasts from type VII and VIII OI probands to investigate the interaction of CRTAP and

P3H1. P3H1 is absent in CRTAP-null fibroblast lysates and, conversely, CRTAP is minimally detectable in P3H1-null lysates. Immunofluoresence microscopy confirmed reduced CRTAP levels in P3H1-null cells and ER localization of CRTAP. However, P3H1 or CRTAP mRNA levels were normal in cells in which null mutations in the other gene caused reduced transcript levels due to NMD. Moreover, CRTAP and P3H1 are stable in normal fibroblasts after translation inhibition with cyclohexmide. These data imply that P3H1 and CRTAP are mutually stabilizing. In contrast, cyclophilinB was as abundant in both sets of mutant cell lines as in control. Stable transfection of CRTAP expression plasmid into CRTAP null fibroblasts restored both CRTAP and P3H1, and reduced type I collagen overmodification. CRTAP was not restored in P3H1-null fibroblasts treated with the proteosomal inhibitor MG132; instead, the increased CRTAP in media may reflect absence of a CRTAP ER retention signal. P3H1 is detectable in lysates of CRTAP-null cells treated with MG132. However, some P3H1 apparently occurs in insoluble pellets from CRTAP-null cell lysates, suggesting P3H1 aggregates in the absence of the complex. The mutual stabilization of P3H1 and CRTAP in collagen prolyl 3-hydroxylation complex may be the underlying mechanism for the similar characteristics of types VII and type VIII OI.

200

Mitochondrion, a target for novel antitumor agent hydroxykauracid

George G. Chen, Chun S. Lo, Jackie Leung, Billy C. Leung, Ursula F. Chan, Michael K. Hsin, Malcolm J. Underwood, Alexandrer C. Vlantis, Andrew C van Hasselt, Paul B. Lai.
The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

Mitochondrion plays an important role in the maintenance of homeostasis and energy balance for cells. The dysfunction of mitochondrion has contributed to apoptosis in a number of pathological conditions. We isolated several compounds from Pteris semipinnata Linn (PsL) and found that one compound, ent-11¦Á-hydroxy-15-oxo-kaur-16-en-19-oic-acid (5F) significantly inhibited the growth of a number of human cancer cells including hepatocellular carcinoma, laryngeal cancer, lung cancer and thyroid cancer. In order to identify the possible target of 5F, we employed cDNA microarray to screen for molecules. The result showed that 5F affected several molecules related to the mitochondrion. We further examined these molecules at protein levels and confirm that 5F enhance the expression of Bax, Bak, cytochrome c, casepase 8, caspase-3 but decrease the level of Bcl-2 and Bcl-xl. Since most of these molecules reside in the mitochondrion, we isolated the mitochondria to confirm that the most molecules tested above are also altered in the mitochondrial protein. Furthermore, we employed flow cytometry to examine the mitochondrial membrane potential (MMP) and found that 5F markedly reduce the MMP, leading to apoptosis as measured by TUNEL assay. Interestingly, the apoptosis induced was found to be negatively associated with the reduced activity of nuclear factor kappaB, which is in line with the fact that nuclear factor kappaB is able to repress the alteration of MMP. Taken together, our study has demonstrated that 5F induces apoptosis in cancer cells via targeting the mitochondria.

201

Dimerization of MT1-MMP regulates the activation of MMP-2

Signe Ingvarsen[1], Daniel H. Madsen[1], Leif R. Lund[1], Kenn Holmbeck[2], Niels Behrendt[1], Lars H. Engelholm[1].
[1]The Finsen Laboratory, Rigshospitalet, Denmark, [2]Matrix Metalloproteinase Unit, NIDCR, NIH, Bethesda, MD 20892, USA

Matrix metalloproteases (MMPs) are centrally engaged in the processes of extracellular matrix turnover. An important MMP cascade reaction is initiated by the membrane-anchored matrix metalloprotease, MT1-MMP, which serves to activate the proenzyme form of the secreted gelatinase, MMP-2. This reaction occurs in an interplay with the MMP inhibitor, TIMP-2, and the proposed mechanism involves two molecules of MT1-MMP in complex with one TIMP-2 molecule. To study this, as well as other roles of MT1-MMP, we have now raised a panel of monoclonal antibodies against the protein. These antibodies have been raised in MT1-MMP knock-out mice and react against conserved

epitopes in murine and human MT1-MMP. Using one of these antibodies we provide positive evidence that proMMP-2 activation is governed by dimerization of MT1-MMP on the surface of fibroblasts and fibrosarcoma cells. The antibody in question is directed against the hemopexin domain of MT1-MMP and has no effect on the catalytic activity of the protease domain. The antibody induces dimerization of endogenous MT1-MMP on the cell surface. Through this reaction it stimulates the formation of active MMP-2 products. This effect is a consequence of MT1-MMP dimerization because it requires the divalent monoclonal antibody with no effect being obtained with monovalent Fab fragments. Since only a negligible level of MMP-2 activation is obtained with MT1-MMP expressing cells in the absence of dimerization, our results identify the dimerization event as a critical level of proteolytic cascade regulation.

202

The Role of Multimerisation in the Regulation of BMP-1 and mTLD

Richard Berry[1], Thomas A. Jowitt[1], Manfred Roessle[2], Guenter Grossman[3], Elizabeth G. Canty[1], Richard A. Kammerer[1], Karl E. Kadler[1], Clair Baldock[1].
[1]Wellcome Trust Centre for Cell Matrix Research, University of Manchester, M13 9PT, UK, [2]EMBL-Hamburg Outstation, c/o DESY, Notkestrasse, 22603 Hamburg, Germany, [3]Synchrotron Radiation Dept, Council for Central Laboratory of the Research Councils, Daresbury, UK.

The BMP-1/TLD family of metalloproteinases are a group of evolutionary conserved enzymes that are fundamental to dorsal-ventral patterning and tissue morphogenesis. Although the number of identified substrates is ever expanding, precisely how this group of proteins is regulated remains unknown. In particular, it is a conundrum why BMP-1, which lacks three of the seven non-catalytic domains present in all other tolloid members, is the most effective proteinase of the family. Using a variety of complimentary biophysical techniques we have investigated the structure of BMP-1 and mTLD. Herein we show that mTLD, but not BMP-1, forms a calcium-ion dependent antiparallel dimer under physiological conditions. We propose the activity of both proteinases are restricted by distinct substrate exclusion mechanisms. Whereas this is intra-molecular in the case of BMP-1, it is inter-molecular in mTLD, the latter a consequence of dimerisation. These results provide an explanation for the functional difference between BMP-1 and mTLD, and have broad implications for regulation of the activity of tolloid family and related proteinases during BMP signaling and tissue assembly.

203

Matrix elasticity directs differentiation & de-differentiation

Dennis E. Discher
University of Pennsylvania, Philadelphia, PA

Most tissue cells need to anchor to a 'solid' for viability, and over the last decade it has become increasingly clear that the physical 'elasticity' of that solid-like matrix is literally ‘felt’ by cells. In fact, matrix elasticity as an insoluble cue now appears to synergize with the effects of soluble factors [1]. We will show that Mesenchymal Stem Cells (MSCs) specify lineage and commit to phenotypes with extreme sensitivity to the elasticity typical of tissues [2]. In serum only media, soft matrices that mimic brain appear neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous ‘osteoid’ prove osteogenic. Inhibition of myosin II blocks all lineage specification, indicating that the cell pulls on matrix through adhesions. In addition to the work with stem cells, studies of isolated, beating cardiomyocytes on elastic matrices demonstrate the sensitivity of cell differentiation: cardiomyocytes stop beating most rapidly on matrices as rigid as an infarct scar – and this is shown to be about as hard as osteoid. The results predict that an infarct scar not only opposes cardiogenesis through de-differentiation but would tend to promote osteogenesis, which appears consistent with recent observations in mouse [3]. The results have also motivated development of a proteomic-scale method to identify mechano-responsive protein structures [4].
[1] Wells... Science Signaling (2008)

[2] Engler... Cell (2006).
[3] Breitbach... Blood (2007)
[4] Johnson... Science (2007)

204

GSK3b controls the intensity of tissue repair by regulating ET-1

Andrew Leask[1], Shangxi Liu[1], Christopher Denton[2], David Abraham[2], Mohit Kapoor[1].
[1]University of Western Ontario, London, ON N6A 5C1, [2]University College London, London, UK

Glycogen synthase kinase-3 (GSK-3) is a widely expressed and highly conserved serine/threonine protein kinase encoded by two genes (GSK3 α and GSK3 β), but the role of GSK in tissue repair and fibrogenesis is unknown. To investigate the function of GSK3 β in fibroblasts, we generated mice containing a fibroblast-specific deletion of GSK3 β . These mice show protracted enhanced wound closure, including increased inflammatory responses, angiogenesis, collagen production and myofibroblast formation. GSK3 β -deficient mice were unable to terminate tissue repair, resulting in excessive scarring. In cultured GSK3 β -deficient fibroblasts, adhesion, spreading, migration and wound closure were significantly enhanced. GSK3 β -deficient mice and fibroblasts showed increased endothelin-1 (ET-1) production. Antagonizing ET-1 reversed the phenotype of GSK3- β deficient fibroblast in vitro and GSK3- β *f*ndeficient animals in vivo. Thus GSK3-β controls the progression of wound healing and fibrosis, via modulating ET-1 levels. Our results are consistent with the notion that targeting the GSK3-β or ET-1 may be of benefit in controlling tissue repair and fibrogenic responses in vivo.

205

Cell polarity regulated by a FAK-p120RasGAP-p190RhoGAP complex

Alok Tomar[1], Ssang-Taek Lim[1], Yangmi Lim[2], David D. Schlaepfer[1].
[1]University of California San Diego, Moores Cancer Center, Dept of Reproductive Medicine, [2]Mogam Research Institute, Giheung-gu, Korea

Directional cell migration is important in development, angiogenesis, wound healing, and tumor spread. Cell polarization along the anterior-posterior axis facilitates directional movement. Focal adhesion kinase (FAK) promotes cell migration, but a role for FAK in cell polarity regulation is not defined. Here, using wound healing motility and Golgi reorientation analyses, we show that FAK expression is necessary for polarized motility of normal and carcinoma cells. Polarity defects of FAK -/- mouse embryo fibroblasts (MEFs) are rescued by re-expression of wild-type and phosphorylation site mutants (F861 and F925) of FAK, but not FAK that is mutated at Y397 nor FAK that is catalytically-inactive (R454). FAK activity is required for fibronectin-stimulated p190RhoGAP (p190A) tyrosine phosphorylation and p190A-p120RasGAP complex formation. Over-expression of GAP-inactive (R1283A) or double phosphorylation site mutated (Y1087F, Y1105F) p190A in FAK+/+ MEFs blocks cell polarity. Interestingly, knockdown of p120RasGAP prevents FAK-p190A complex formation, p190A tyrosine phosphorylation, and FAK+/+ MEF polarity. Further, although p190A is tyrosine phosphorylated in Src-transformed FAK-/- MEFs, FAK is required for leading edge p190A localization and cell polarity. Our studies show that FAK expression and activity facilitate the recruitment and formation of a p120RasGAP-p190A complex at leading edge focal contacts connected to the spatial and temporal regulation of directional cell movement.

206

SPARC is required for collective cell migration during oogenesis

Nathalie Martinek[1], Rebecca Parsons[1], David Bowtell[1,2], Helena Richardson[1].
[1]Cell Cycle and Development, Peter MacCallum Cancer Centre, East Melbourne, Australia, [2]Cancer Genomics and Biochemistry

SPARC is a 32-35 kD Ca2+-binding matricellular glycoprotein that modulates cell-extracellular matrix interactions and influences cell-cell adhesion, migration and invasion in vitro and in vivo. SPARC is expressed throughout oogenesis and is specifically expressed in a pair of cells found at the poles of each developing egg chamber. These polar cells are crucial for patterning the egg chamber and

for recruiting the adjacent border cells to delaminate from the epithelium to form the border cell cluster. Together, the polar cells, which are not migratory and form the core of the cluster, and the border cells will then invade the germ line and collectively migrate toward the oocyte. SPARC protein is only observed at high levels in the polar cells prior to border cell cluster formation and low levels are distributed throughout the cluster during migration. Knockdown of SPARC expression in the polar cells by RNAi delays border cell cluster migration, disrupts E-Cadherin distribution and alters border cell cluster morphology but does not affect levels of SPARC protein present within the cluster during migration. Generating mutant clones of one or both polar cells prevents border cell cluster migration but not invasion suggesting that the SPARC supplied by the polar cells may provide spatial information to the border cells to promote their collective migration. We are currently addressing the molecular mechanisms underlying these observations and live imaging of border cell cluster migration combined with our initial findings will be presented.

207

The response of articular chondrocyte microRNAs to stress

Maozhou Yang, Liang Zhang, William Gibson, Gary Gibson.
Bone and Joint Center, Henry Ford, Hospital, Detroit, MI, 48202

MicroRNAs (miRNAs) are ~22 nucleotide, non-coding RNAs that play critical roles in the regulation of gene expression. Many cellular functions are regulated by miRNAs and aberrant expression has been linked to several diseases particularly those associated with cellular stress. To characterize miRNA expressed by articular chondrocytes we have adapted the classical acid-phenol RNA extraction protocol for miRNA purification. The expression of miRNA in isolated chondrocytes was compared with that in intact cartilage as a model of the response of miRNA to stress. MiRNA array analysis employed two distinct platforms: a microfluidic array platform and LNA microarrays. Expressions of individual miRNAs were also confirmed using RT-PCR and quantitative real-time RT-PCR using specific primers. Approximately 100 miRNAs were shown to be robustly expressed by chondrocytes and could be detected in the tissue or isolated chondrocytes. Six (3 upregulated 3 down regulated) characterized miRNAs and five (all down regulated) currently uncharacterized miRNAs showed greater than 2 fold changes in response to chondrocyte isolation. From predicted target genes it appears that the upregulated miRNAs are associated with regulation cell division, those down-regulated have predicted gene targets associated with matrix, MMP and growth factor pathways. Our initial studies have shown that chondrocytes express a large number of miRNAs. Some of these appear responsive to the stress of chondrocyte isolation and are associated with regulation of cell division and matrix metabolism.

208

Factors affecting splicing of scleroderma-associated LH2 mRNA

P Seth[1], L C. Walker[1], D M. Mauger[2], M A. Garcia-Blanco[2], H N. Yeowell[1].
[1]Dermatology, [2]Mol.Gen. & Microbiology, Duke Univ. Med. Center, NC 27710

Lysyl hydroxylases (LH) catalyze hydroxylation of specific lysines in collagen. Hydroxylysines then act as precursor for formation of collagen crosslinks essential for maintaining collagen integrity. LH2 is the only member of LH family that is alternately spliced. Long form of its mRNA, LH2(long), includes an extra exon that is excluded in the short form. Since different reports suggest that the overexpression of LH2(long) in skin is related to fibrosis, our goal is to identify factors regulating the LH2 alternate splicing. Here we report 3 different RNA binding proteins:T-cell-restricted Intracellular Antigen 1 (TIA1), TIA1 Like protein 1 (TIAL1) and Polypyrimidine Tract Binding protein (PTB) play important roles in regulating the LH2 alternative splicing. While overexpression of TIA1 and TIAL1 led to an increase in the levels of LH2(long), an overexpression of PTB had the opposite effect. Similarly, knockdown of both TIA1 and TIAL1 decreased the levels of LH2(long), whereas a knockdown of PTB led to its increase. Knockdown of TIA proteins in fibroblasts derived from scleroderma patients by

specific siRNAs significantly reduced the levels of LH2(long). Real Time PCR of PTB in scleroderma fibroblasts also revealed a striking reduction in PTB mRNA in fibrotic cells vs normal controls. This could partly explain the increased expression of LH2(long) observed in scleroderma. Our results provide insights towards understanding the molecular basis behind an increase in LH2(long) mRNA observed in fibrosis. This could lead to potential therapy against fibrosis by reducing the overexpression of LH2(long) in scleroderma cells.

209

Postnatal Ablation of Sox9 in Mouse Cartilage

Stephen P. Henry, Chuan-Wei Jang, Zhaoping Zhang, Jenny Deng, Richard R. Behringer, Benoit de Crombrugghe.
Department of Genetics, University of Texas MD Anderson Cancer Center

Although the transcription factor SOX9 has been coined as a master control gene in chondrocyte differentiation and proliferation, most of the genetic analysis has focused on embryonic studies of mouse growth plate. Mice heterozygous for a null mutation of *Sox9* die at birth; therefore, to circumvent this perinatal lethality, we have established a genetic system in which genes can be inactivated specifically in postnatal cartilage by an inducible system. We have generated a "knockin" mouse at the aggrecan locus in which a construct creER^{T2} is targeted to the 3' UTR of mouse aggrecan. To demonstrate the efficacy of the inducible component of *Agc1creER*^{T2} mice, we have bred these mice to the ROSA26 reporter strain. Postnatal injections of the inducer, tamoxifen, in 2 week-, 2 mon-, and 6 mon *Agc1creER*^{T2}; ROSA mice resulted in high percentage of cre-mediated recombination in articular and growth plate chondrocytes. We have bred the *Agc1creER*^{T2} mice to the conditional *Sox9*$^{flox/flox}$ mice to inactivate Sox9 postnatally. Two-month-old *Agc1creER*^{T2}; *Sox9*$^{flox/flox}$ mice were injected with tamoxifen for five consecutive days. Within two weeks, several phenotypic changes occurred in articular, growth plate cartilages and underlying trabecular bone. The mice developed premature osteoarthritis with a total loss of proteoglycan staining in the articular chondrocytes located above the tidemark as judged by Safranin-O staining. Furthermore, the aggrecan mRNA and protein were not detected by RNA *in situ* hybridization and immunostaining respectively in the articular chondrocytes above the tidemark in mutant mice.

210

TGFβ1 Enhances Pancreatic Tumor Progression in SPARC-Null Mice

Shanna A. Arnold[1], Lee B. Rivera[1], Juliet G. Carbon[1], Sean P. Dineen[1], Amy D. Bradshaw[2], Rolf A. Brekken[1].
[1]Hamon Center for Therapeutic Oncology Research, UT-Southwestern, Dallas, TX 75390, [2]Department of Medicine, Medical University of South Carolina, Charleston, SC 29412

SPARC is a matricellular protein that governs ECM deposition and maturation during tissue remodeling, particularly, during wound healing and tumorigenesis. In the present study, we evaluate the function of endogenous host-derived SPARC in an orthotopic model of pancreatic cancer. Pancreatic tumors grown in *SPARC-null* mice are more invasive than tumors grown in *wild-type* counterparts. Surprisingly, we found active TGFβ1 to be increased significantly in tumors grown in *SPARC-null* mice. TGFβ1 is known to contribute to many aspects of tumor development including metastasis, endothelial cell permeability, inflammation and fibrosis, all of which are altered in the absence of endogenous SPARC. Given these results, we performed a survival study to assess the contribution of increased TGFβ1 activity to tumor progression in *SPARC-null* mice using losartan, an AT1 angiotensin II receptor antagonist that diminishes TGFβ1 production in vivo. Tumors grown in *SPARC-null* mice progressed more quickly than those grown in *wild-type* littermates with a median survival of 28d and 36d, respectively (p=0.018). However, losartan therapy extended median survival of *SPARC-null* animals to 40d, equivalent to losartan treated *wild-type* controls. This data supports the idea that aberrant TGFβ1 activation in the absence of host SPARC contributes significantly to tumor progression and suggests that SPARC, by controlling ECM deposition and maturation, can regulate cytokine availability and activation.

211

Extracellular regulators of TGF-β signaling in bone.

H Nistala, S Lee-Arteaga, S Smaldone, F Ramirez.
Mt Sinai School of Medicine, New York

Fibrillin-rich microfibrils impart structural integrity to connective tissues, and participate in extracellular (local) control of TGFβ signals. Given that reduced bone mass is a common finding in both Marfan and Beals syndrome (caused by Fbn1 and Fbn2 mutations respectively), and that osteoblasts are unique examples of cells secreting both small and large latent TGFβ complexes, we investigated the role of microfibrils in primary calvarial osteoblast (COb) cultures from Fbn1 or Fbn2 null mice. Loss of Fbn1 or Fbn2 was correlated with elevated TGFβ signaling and normative TGFβ gene expression. TGFβ activity was 2 fold higher in Fbn2 than Fbn1 null COb cultures, implying that the two proteins differentially regulate local TGFβ bioavailabilty in these cells. Consistent with this conclusion, Fbn2 null COb assembled seemingly normal Fbn1 and LTBP1, whereas LTBP1 was somewhat reduced in Fbn1 null cultures. Higher TGFβ activity translated into reduced matrix deposition and mineralization in Fbn2 null compared to Fbn1 null cultures. Impaired differentiation of Fbn2 null COb was rescued by TGFβ antagonism or BMP2 addition ruling out an earlier change in cell fate. In line with this observation, RNA interference in primary COb recapitulated the phenotypes of Fbn1 and Fbn2 null cultures. Finally, co-culture experiments revealed that both Fbn1 and Fbn2 null Cob stimulate osteoclastogenesis as the result of ALK5 independent increase in RANKL expression. Collectively, our findings demonstrate the differential roles of fibrillins in local control of matrix assembly, TGFβ bioavailability, and resident cell differentiation during bone remodeling.

212

Collagen X is required for proper hematopoietic development

Elizabeth Sweeney, Olena Jacenko.
University of Pennsylvania, Philadelphia, PA

During endochondral ossification (EO), the hypertrophic cartilage (HC)-containing growth plate is replaced by trabecular bone and a hematopoietic marrow. We propose that EO establishes the hematopoietic stem cell (HSC) niche. This is based on the skeleto-hematopoietic disease phenotypes in mice where the function of collagen X, a unique HC matrix protein, was disrupted either by transgenesis (Tg mice), or by gene inactivation (KO mice). Murine defects manifested as aberrant growth plates, reduced trabecular bone, marrow hypoplasia, altered lymphocyte profiles throughout life, and compromised immunity. A fraction of all mice succumbed to perinatal lethality at week-3 due to inability to suppress opportunistic infections, and displayed marrow aplasia and lymphatic organ atrophy. Serum cytokine arrays revealed mis-expression of inflammatory and hematopoietic cytokines in all collagen X mice. Of note, many of these cytokines bind heparan sulfate proteoglycans (HSPGs), which are reduced or lacking in the chondro-osseous junctions (coj) of the collagen X mice. Neonatal bone marrow transplantations confirmed that the hematopoietic defects result from an altered coj and marrow environment in the collagen X mice, rather than from defects in their HSCs. Finally, in vitro co-cultures with wild type HSCs and different cell types from the coj demonstrated an altered ability of osteoblasts and HC from collagen X mice to support B lymphopoiesis. We propose that the collagen X/HSPG network sequesters hematopoietic cytokines and growth factors at the coj, and that disruption of collagen X function causes an imbalance in cytokine metabolism, leading to impaired hematopoiesis and immunity.

213

Molecular based Structure and Ligation of the Collagen Fibril

Joseph P. Orgel[1,2], Olga Antipova[1,2], Aya Eid[1], Rong Wang[1], Dengli Qiu[1], Shawn Sweeney[3], John Scott[4], James San Antonio[3].
[1]Illinois Institute of Technology, Chicago IL, [2]BioCAT, APS, Argonne National lab, IL, [3]Thomas Jefferson University, Philadelphia, PA, [4]Manchester University, Manchester U.K.

The fibrous collagens are the fundamental constituents of the Extracellular Matrix (ECM)

of animals, forming the structural basis of all known mammalian connective tissues and organ systems. Yet, despite the fundamental biological importance of collagen, many of us are perplexed by the complexity of the assemblies that the collagens form. This is particularly true at what may be the most significant aspect of collagen structure from a cellular point of view, at the intermediate sub-fibrillar (and or surface) level where many important biological processes occur in growth, development and disease. These include but are not limited to: fibrillogenesis, tissue remolding and in forming the scaffolding upon which organ systems, bones, cartilage, etc., i.e. the animal body, are built upon. Clearly, obtaining an unambiguous and contextualized visualization of collagen molecules would be of significant value to the scientific community. We have recently determined the structure of the type I collagen microfibril and fibril at the molecular level from whole intact rat-tail tendons. Using this data it is possible to map the amino acid chemistry, ligand binding data and other observations onto the defining shape modality of the fibrillar collagen ECM. In so doing, we have been able to propose the first fibrillar based mechanism of collagenolysis and provide a number of illuminating observations regarding other collagen fibril - ligand interactions.

AUTHOR INDEX
Biennial Meeting of the
American Society for Matrix Biology

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z