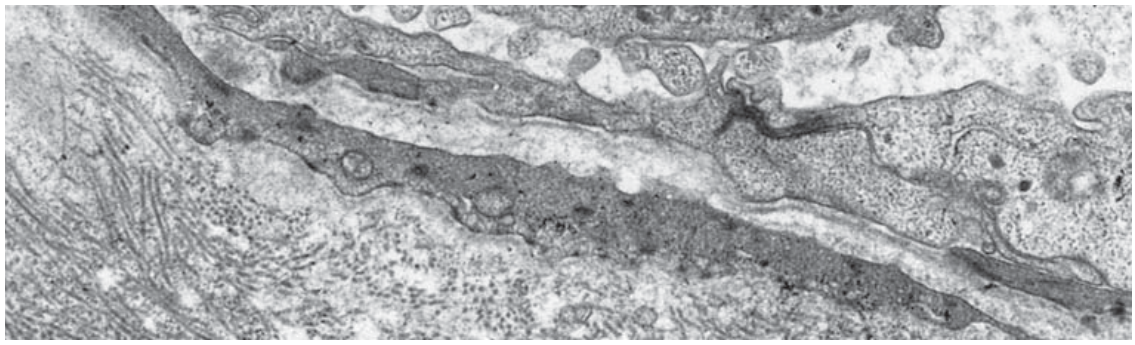


# ISMB NEWSLETTER

## August 2009

Dear Fellow Matrix Biologists,

As I mentioned to you in the January newsletter, the major goals of our society are to "communicate" with your fellow matrix biologists, establish an interactive dialogue and improve the international visibility of ISMB by fostering matrix biology research and by improving networking across nations and continents.

As you all know by now, in this part of the Atlantic (and also the Pacific) there is a new sheriff in town, by the name of Barack Obama. Immediate changes in the administration might have profound effects on our ability to sustain and improve our research capability locally and, in turn, on our ability to collaborate at an international and intercontinental level.

What about the stimulus package? Here, in the United States of America, we have received a stimulus package from the Obama administration under the so called American Recovery and Reinvestment Act. I am one of the investigators who did not submit any proposal directly, although I was associated with a couple of "crazy" investigators who hope to be funded with the so called "challenging" grants. These grants are just for two years, for a maximum of $500,000/year; so each grant would be ~ 1 million. Since the stimulus package allotted 200 million for these grants, only 200 grants will be funded. NIH has received an avalanche of challenge grant applications, >20,000! So, the probability of getting funded is ~2% or less. In addition, how could we sustain a program that has just started? After 2 years, where will the funds come from since this is a one time deal? It would have been savvier to invest these funds by supporting some of the fantastic grant applications that were borderline and could not be funded because of lack of funds diverted toward other issues by the previous administration.

Another thing I have noticed is that in the past 4-5 years there has been an apparent decline (again this is my personal view not scientifically proven) in papers in our field of research from NIH-funded investigators and USA based investigators. I performed a search for the past 10 years using key words related to matrix biology appearing in the title and abstract and using JBC as the standard journal. In several instances there was a downward trend reaching a significant decline up to 40%. Is this due to the catastrophic effects of the previous administration on NIH funding? Thus, any infusion of federal monies in the system is welcome. I just argue about the way it is spent. I hope that the stimulus package (which should be a continuous package) will have a positive impact not only on "retaining" the best talents but also on "attracting" new blood to our field of research. Those are some of my thoughts…….and welcome any comments.

I have asked Vince Hascall and Jody Buckwalter to write something in honor of Larry Rosenberg. They have assembled a very nice piece as well a beautiful collage of Larry's famous pictures and models of the cartilage aggregate.

This year is filled with outstanding meetings, both small and large, on matrix biology. I generally prefer the small meetings where I can interact with old friends and experts in the field. However, the larger meetings (I mean open meetings) are also important because I can meet young graduate students and postdoctoral fellows. As Greg Petsko (President of the ASBMB) wrote in a recent editorial: "Small meetings are about reinforcing your expertise, big meetings are about expanding your horizons". I truly like this view and I would like to add that large, open meeting would allow senior investigators to have a direct, personal contact with new potential researchers. One of the greatest pleasures I have at a meeting is to review posters and talk about details, bolts and nuts. I like the vibrant and inspired presentations of findings by a young researcher. It reminds me of when I started my career and of my first oral presentation in front of an audience. What a traumatic experience! But I survived.

Among the matrix biology meetings highlighted below are the following: September 13-17, 6th International Conference on Proteoglycans, Aix-les-Bains, France; October 4-6; Klenk Symposium on Extracellular Matrix in Health and Disease, Cologne, Germany (no registration fee!); October 11-14, Meeting of the Matrix Biology Society of Australia/New Zealand, Novotel Barossa Valley Resort, Australia.

Lastly, I wish to thank the ISMB Council members who are stepping down for their service to the society (Klaus von der Mark, Monique Aumailley and Leena Bruckner-Tuderman) and welcome on board four new Council members (Reinhard Fässler, David Birk, Karl Kadler and Shireen Lamandé).

So, fellow matrix biologist, pack lightly and travel around the world.
Warmest regards to all,

Renato V. Iozzo, M.D.
President ISMB

## From the ISMB Secretary

Dear ISMB-member,
The other day, I updated the ISMB membership files. I can gladly announce that we have been successful recently in recruiting new members, including several graduate students who have joined the ranks. This is a reliable indicator that matrix biology is a viable field of science attracting the attention of young people trying to orient themselves in the vast area of life sciences.

Having just returned home from the Collagen Gordon Conference with an exceptionally high level of participation by graduate students, I can assure you that there are many brilliant young people out there. Billy Hudson, the Chair of the next Collagen Gordon Conference, is determined to take advantage of a new initiative by the Gordon Conferences, the Gordon Research Seminars. On the GRC webpage you can read: *"The Gordon Research Seminar Program (GRS) is a series of highly successful and unique opportunities for young researchers to share in the GRC experience. Each seminar is held in conjunction with a related GRC and begins the weekend immediately prior to the GRC. Graduate students, post docs, and other scientists with comparable levels of experience and education come together to discuss their current research and build informal networks with their peers that may lead to a lifetime of collaboration and scientific achievement. The 2-day Gordon Research Seminars are organized by young investigators with the support of leading scientists from the associated GRC. The majority, if not all, of the GRS participants are expected to participate in the following GRC."* With the help of several graduate students and young post-docs in attendance at the conference 2009, Billy will now establish such a GRS in conjunction with the Collagen Conference.

In addition, Billy will continue to widen the scope of the Collagen GRC, a trend that has become in-creasingly apparent during the past few meetings. This meeting is more than "just collagen". The response of cells to the mechanical environment was a prominent issue this time. The role of the ex-tracellular matrix as an important part of the stem cell niche was also discussed. Likewise, the programme contained a session on novel therapeutic strategies involving the matrix. You can see that this conference, having a tradition of 40 years next time, still is a dynamic scientific forum. Also, it is a splendid opportunity to meet your friends as well as new faces having a peek into matrix biology. Such personal contacts and experiences still complement our modern electronic communication systems. I am happy that I attended this year and I strongly encourage you to join us next time. It is well worth your effort and expense.

By supporting major conferences in matrix biology, ISMB plays an important role in fostering and promoting the field of Matrix Biology. For example, there is a place for ISMB to be helpful in the career development of our young colleagues. We shall continue to do so at the FECTS-meeting next summer by sponsoring travel expenses and by awarding the Rupert Timpl

prize. Unfortunately, this message still cannot reach many well known matrix biologists, simply because they are not ISMB members, or 'not-yet-members of ISMB'. As an ISMB-member we need your help in making sure that these 'not-yet-members' find the ISMB-webpage http://www.ismb.org and join up. And you will convince them to do so by telling them a few of the many reasons to be an ISMB-member.

Peter Bruckner, ISMB Secretary and Treasurer (for the ISMB Council)

## Awards

Dr. Renato V. Iozzo, Thomas Jefferson University, and Dr. Vincent Hascall, Cleveland Clinic, receive scientific awards from the Hellenic Society of Biochemistry and Molecular Biology and from the Federation of European Biochemical Societies

Congratulations to Renato V. Iozzo, M.D., Professor of Pathology and Cell Biology, Thomas Jefferson University, and Vincent Hascall, Ph.D., Professor of Bioengineering, Lerner Research Center, Cleveland Clinic, for receiving awards from the Hellenic Society of Biochemistry and Molecular Biology together with the Hellenic Research Club for Connective Tissue and Matrix Biology, and from the Federation of European Biochemical Societies, in July 2009 at the University of Patras, Greece. Drs. Iozzo and Hascall received two plaques and two honorary diplomas in recognition of their "Significant Contribution and Scientific Achievements in the fields of Pathobiology and Extracellular Matrices".

Figure: Dr. Iozzo (left panel) and Dr. Hascall receive a plaque depicting Saint Andrew (Saint Andreas), the Patron of Patras, and an Honorary Diploma from Prof. Stavros Koumbias, Rector of the University of Patras (Center) and Prof. Nikos Karamanos, Chairman of the Organizing Committee, FEBS-MSPT 2009.

## Meeting Announcements

***Gordon Research Conference Matrix Metalloproteinases***
Les Diablerets, Switzerland
August 30 - September 4, 2009
Chair: Carl P. Blobel
Vice Chair: Rafael A. Fridman
http://www.grc.org/programs.aspx?year=2009&program=matrixmet

The Fifth Alpbach Workshop on:
***Coiled-coils, collagens and co-proteins***
Boglerhof Hotel, Alpbach, Austria
September 6-12, 2009
For further details contact: j.m.squire@bristol.ac.uk, d.parry@massey.ac.nz or knuppc@cardiff.ac.uk

***25. Ernst Klenk Symposium in Molecular Medicine Extracellular Matrix in Health and Disease***
04. - 06. October 2009 in Cologne
Further information: : http://www.zmmk.uni-koeln.de/content/index_eng.html>www.zmmk.uni-koeln. See poster at end of Newsletter.

***International Bone-Tissue-Engineering Congress***
Hannover, Germany
October 8 -11, 2009
http://www.bone-tec.com

***Matrix Biology Society of Australia and New Zealand annual meeting***
Barossa Valley, South Australia
11-14 October, 2009
http://www.mbsanz.org/index.php?option=com_content&task=view&id=17&Itemid=124

***29th Meeting of the Italian Society for the Study of Connective Tissues (SISC)***
Alghero (Sardinia), Italy
29-30 October 2009

**XXII FECTS meeting** in association with the ISMB
Davos, Switzerland from July 3rd-7th 2010.

## Matrix Research Update

### Meeting abstracts published

**XXVIII Italian Society for the Study of Connective Tissues (SISC) Meeting**
**Pavia, Italy, 6-7 November 2008**
*Connective Tissue Research, v50, issue 2, 2009*

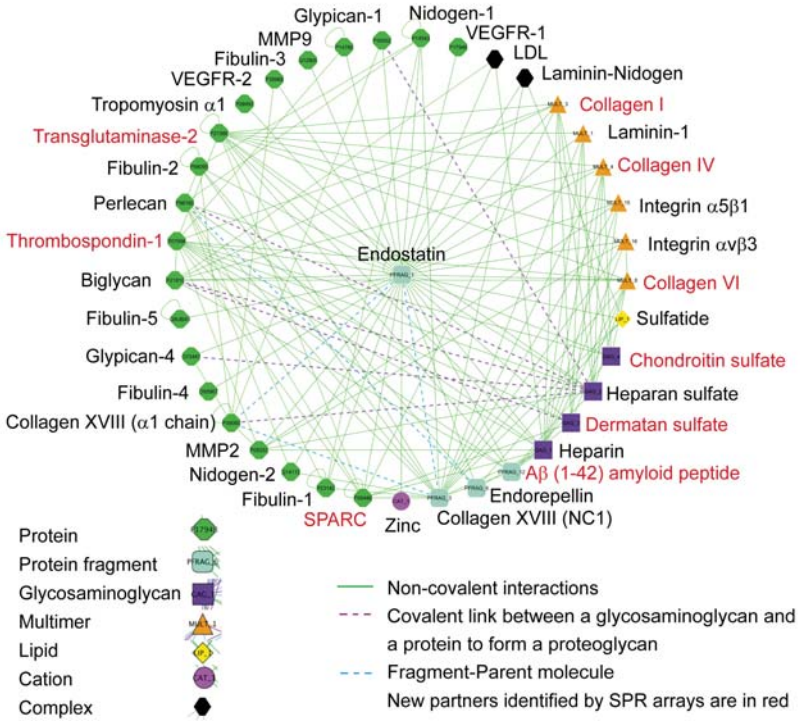
### Papers in press or recently published

**Mutagenesis at the α/β Interface Impairs the Cleavage of the Dystroglycan Precursor**
Francesca Sciandra, Manuela Bozzi, Simona Morlacchi, Antonio Galtieri, Bruno Giardina and Andrea Brancaccio
*FEBS Journal, in press*

The interaction between α-dystroglycan (α-DG) and β-dystroglycan (β-DG), the two subunits composing the adhesion complex dystroglycan, is crucial to maintain the integrity of the dystrophin-glycoprotein complex. The importance of the α/β interface emerges in the skeletal muscle of human patients affected by severe conditions such as Duchenne muscular dystrophy, where the α-DG/β-DG interaction can be secondarily weakened or completely lost, causing sarcolemmal instability and muscular necrosis. The reciprocal binding epitopes of the two subunits reside within the C-terminus of α-DG and the β-DG ectodomain. Since no ultimate structural data are available on the α-DG/β-DG interface yet, site-directed mutagenesis was used to identify which specific amino acids are involved in the interaction. A previous alanine-scanning analysis of the recombinant β-DG ectodomain has allowed the identification of two phenylalanines important for α-DG binding, namely F692 and F718. In this paper, similar experiments performed on the α-DG C-terminal domain have pinpointed two residues, G563 and P565, as possible binding counterparts of the two β-DG phenylalanines. In 293-Ebna cells, the introduction of alanine residues in place of F692, F718, G563 and P565 prevents the cleavage of the DG precursor that liberates α-DG and β-DG, generating a preDG of about 160 kDa. This uncleaved preDG tetramutant is properly targeted at the cell membrane, is partially glycosylated and still binds laminin in pull-down assays. These data reinforce the notion that the DG processing and its membrane targeting are two independent processes and shed new light on the molecular mechanism that drives the DG precursor maturation.

**THE FIRST DRAFT OF THE ENDOSTATIN INTERACTION NETWORK**
Clément Faye, Emilie Chautard, Bjorn R. Olsen, Sylvie Ricard-Blum
*The Journal of Biological Chemistry, in press*

Endostatin is a C-terminal proteolytic fragment of collagen XVIII that is localized in vascular basement membrane zones in various organs. It binds to heparin/heparan sulfate and to a number of proteins, but its molecular mechanisms of action are not fully elucidated. We have used surface plasmon resonance (SPR) arrays to identify new partners of endostatin, and to give further insights on its molecular mechanism of action. New partners of endostatin include glycosaminoglycans (chondroitin and dermatan sulfate), matricellular proteins (thrombospondin-1 and SPARC), collagens (I, IV and VI), the amyloid peptide Aβ(1-42), and transglutaminase-2. The biological functions of the endostatin network involve a number of extracellular proteins containing EGF and EGF-like domains, and able to bind calcium. Depending on the trigger event, and on the availability of its members in a given tissue at a given time, the endostatin network might be involved either in the control of angiogenesis, and tumor growth, or in neurogenesis and neurodegenerative diseases.




**MOLECULAR INTERPLAY BETWEEN ENDOSTATIN, INTEGRINS, AND HEPARAN SULFATE**
Clément Faye, Christophe Moreau, Emilie Chautard, Reidunn Jetne, Naomi Fukai, Florence Ruggiero, Martin J. Humphries, Bjorn R. Olsen, Sylvie Ricard-Blum
*The Journal of Biological Chemistry, in press*

Endostatin is an endogenous inhibitor of angiogenesis. Although several endothelial cell surface molecules have been reported to interact with endostatin, its molecular mechanism of action is not fully elucidated. We used surface plasmon resonance (SPR) assays to characterize interactions between endostatin, integrins and heparin/heparan sulfate (HP/HS). α5β1 and αvβ3 integrins form stable complexes with immobilized endostatin (KD~ 1.8 x10-8 M, 2-state model). Two arginine residues (Arg27 and Arg139) are crucial for the binding of endostatin to integrins and to heparin/heparan sulfate, suggesting that endostatin would not bind simultaneously to integrins and to heparan sulfate. Experimental data and molecular modeling support endostatin binding to the headpiece of the αvβ3 integrin at the interface between the β-propeller domain of the αv subunit and the βA domaiņof the β3 subunit. In addition, we report that α5β1 and αvβ3 integrins bind to HP/HS. The ectodomain of the α5β1 integrin binds to HP with high affinity (KD=15.5 nM). The direct binding between integrins and HP/HS might explain why both HS and α5β1 integrin are required for the localization of endostatin in endothelial cell lipid rafts.

**Migratory chondrogenic progenitor cells from repair tissue during the later stages of osteoarthritis**
Sebastian Koelling*, Jenny Kruegel*, Malte Irmer, Jan Ragnar Path, Boguslawa Sadowski, Xavier Miro, Nicolai Miosge
*Cell Stem Cell, 4, 324-335, 2009*

The regeneration of diseased hyaline cartilage continues to be a great challenge, mainly because degeneration–caused either by major injury or by age-related processes–can overextend the tissue's self-renewal capacity. We show that repair tissue from human articular cartilage during the late stages of osteoarthritis harbors a unique progenitor cell population, termed chondrogenic progenitor cells (CPC). These exhibit stem cell characteristics, such as clonogenicity, multipotency and migratory activity. The isolated CPC, which exhibit a high chondrogenic potential, were shown to populate diseased tissue ex vivo. Moreover, down-regulation of the osteogenic transcription factor runx-2 enhanced the expression of the chondrogenic transcription factor sox-9. This, in turn, increased the matrix synthesis potential of the chondrogenic progenitor cells without altering their migratory capacity. Our results offer new insights into the biology of progenitor cells in the context of diseased cartilage tissue. Our work may be relevant in the development of novel therapeutics for the later stages of osteoarthritis.
*equal contribution

**The integrin α9β1 on hematopoietic stem and progenitor cells: involvement in cell adhesion, proliferation and differentiation**

Thomas D. Schreiber* Carolin Steinl* Mike Essl, Harald Abele, Konstanze Geiger, Claudia A. Müller, Wilhelm K. Aicher, and Gerd Klein

*Haematologica - The Hematology Journal, in press*

Background: Hematopoietic stem and progenitor cells (HSPCs) can interact with their microenvironment via integrins which are adhesion receptors consisting of α and β subunits. Current knowledge suggests that the integrin subunits α4 and α6 expressed on HSPCs have distinct roles in retaining stem cells in the bone marrow. The aim of our study was to gain insight into the expression and functions of the integrin subunits α7-α11 within the endosteal stem cell niche.
Design and methods: Human osteoblasts isolated from trabecular bone and HSPCs purified from umbilical cord blood or bone marrow aspirates were analyzed for the expression of integrin α7-α11 chains by RT-PCR. The involvement of the integrin α9β1 in HSPC cell adhesion, proliferation and differentiation was analyzed in functional assays.
Results: Transcripts for all investigated integrin chains were found in primary osteoblasts. Highly purified HSPCs, however, expressed only transcripts encoding integrin subunits α7 and α9. Flow cytometric analysis verified extracellular expression of the integrin α9β1 on HSPCs. Cell-cell adhesion assays with osteoblasts and dye-labeled CD34+ HSPCs in the presence of function-blocking antibodies revealed a role of integrin α9 in HSPC adhesion to osteoblasts. Furthermore, the addition of anti-integrin α9 antibodies significantly inhibited proliferation and in vitro differentiation of CD34+ HSPCs.
Conclusions: The integrin α9β1 has been identified as a new member of the integriņβ1-subfamily expressed on human hematopoietic stem and progenitor cells. The functional studies strongly suggest that integrin α9β1 contributes to adhesion and differentiation of HSPCs in the endosteal stem cell niche.
* T.D.S. and C.S. contributed equally to this study

**Fibroblast protein profile analysis highlights the role of oxidative stress and vitamin K recycling in the pathogenesis of pseudoxanthoma elasticum.**

Boraldi F., Annovi G., Guerra D., Paolinelli DeVincenzi C., Garcia-Fernandez M.I., Panico F, De Santis G., Tiozzo R., Ronchetti I. and Quaglino D.

*Proteomics - Clinical Applications, in press*

Pseudoxanthoma elasticum (PXE) is a genetic disorder associated to mutations in the ABCC6 gene, however the pathogenetic mechanisms leading to elastic fibre calcifications and to clinical manifestations are still unknown. Dermal fibroblasts, directly involved in the production of the extracellular milieu, have been isolated from healthy subjects and from patients affected by PXE, cultured in vitro and characterized for their ability to produce reactive oxygen species, for structural and functional properties of their cell membranes, for changes in their protein profile. Data demonstrate that oxidative stress has profound and endurable consequences on PXE fibroblast phenotype being responsible for: reduced levels of global DNA methylation, increased amount of carbonylated proteins and of lipid peroxidation products, altered structural properties of cell membranes, modified protein expression. Data shed new light on the pathogenetic pathways in PXE, by identifying a network of proteins affecting elastic fibre calcification through inefficient vitamin K recycling, and highlight the role of differentially expressed proteins as targets for validating the efficacy of future therapeutic strategies aiming to delay and/or revert the pathologic phenotype of PXE fibroblasts. Moreover, data open new perspectives for investigating PXE-like phenotypes in the absence of ABCC6 mutations.

**MULTIPLE CHANGES INDUCED BY FIBROBLASTS ON BREAST CANCER CELLS**

Patrizia Cancemi, Nadia Ninfa Albanese, Gianluca Di Cara, Maria Rita Marabeti, Francesca Costantini, Salvatore Minafra, Ida Pucci-Minafra

*Connective Tissue Research, in press*

It is now widely recognised that the cross-talk between cancer and stromal cells may play a crucial role in cancer progression. However little is known about the complex underlying molecular mechanisms that occur within the tumor microenvironment. Fibroblasts are the major stromal cells with multiple roles, especially towards both the extracellular matrix and the neighbouring cell population, including neoplastic cells. Consequently, proteomic analyses would provide a wider resource for a better understanding of the potential modulating effects exerted by fibroblasts on cancer cells. In this report we describe the effects of fibroblast stimulation on the breast cancer cell line (8701-BC) proteomics, using a trans-well co-culture system. Our results clearly indicate that fibroblasts induce considerable proteomic modulations on 8701-BC, mainly in the cytoskeleton proteins and glycolytic enzymes. Additionally, fibroblast-conditioned medium increased neoplastic cell proliferation and invasion with a concurrent up-regulation of the c-myc oncogene. Collectively these results suggest that fibroblast stimulation may enhance the malignant potential of breast cancer cells in vitro.

**Type XIV Collagen Regulates Fibrillogenesis: Premature Collagen Fibril Growth and Tissue Dysfunction in Null mice**

Ansorge HL, Meng X, Zhang G, Veit G, Sun M, Klement JF, Beason DP, Soslowsky LJ, Koch M, Birk DE. *J. Biol Chem. 284:8427-8438, 2009*

Type XIV collagen is a fibril-associated collagen with interrupted triple helix. This collagen interacts with the fibril surface and has been implicated as a regulator of fibrillogenesis; however, a specific role has not been elucidated. Functional roles for type XIV collagen were defined utilizing a new type XIV collagen deficient mouse line. This line was produced using a conventional targeted knockout approach. Col14a1-/- mice were devoid of type XIV collagen while heterozygous mice had reduced synthesis. Both mutant Col14a1 genotypes were viable with a grossly normal phenotype; however, mature skin exhibited altered mechanical properties. Prior to evaluating tendon fibrillogenesis in type XIV collagen deficient mice, the developmental expression patterns were analyzed in wild type flexor digitorum longus (FDL) tendons. Analyses of mRNA and protein expression indicated tissue-specific temporal expression that was associated with the early stages in fibrillogenesis. Ultrastructural analyses of wild type and null tendons demonstrated premature fibril growth and larger fibril diameters in tendons from null mice at post-natal day 4 (P4). However, fibril structure in mature tendons was normal. Biomechanical studies established a direct structure/function relationship with reduced strength in P7 null tendons. However, the biomechanical properties in P60 tendons were comparable in null and wild type mice. Our results indicate a regulatory function for type XIV collagen in early stages of collagen fibrillogenesis with tissue differences.

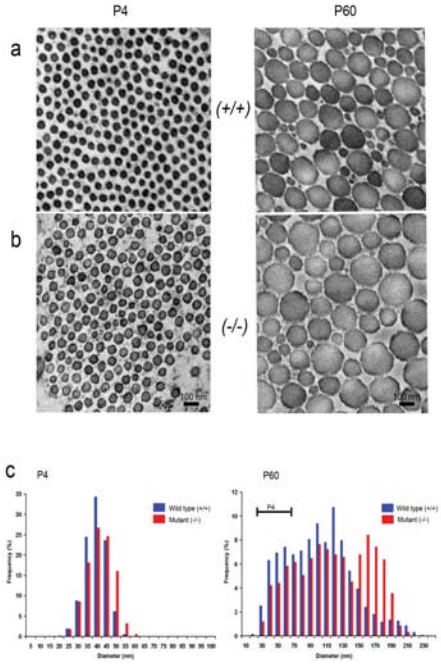



Figure: Altered collagen fibril formation in Col14a1-/- tendons. Transmission electron micrographs of FDL tendon at postnatal day 4 (P4) from (a) Col14a1+/+ and (b) Col14a1-/- mice. At P4, the fibrils of the wild type control are more uniform in shape and smaller than the fibrils in the Col14a1-/- tendon which are more irregular and larger. At P60, the mature fibrils from the wild type tendon are of variable size with small to larger cross sectional profiles. In the Col14a1-/- tendon, there are fewer smaller fibrils and more fibrils of larger diameter as compared to the wild type control. Bar = 100 nm. (c) Histograms representing fibril diameter measurements of tendons at P4 and P60. In the P4 wild type tendon.

**The melanocortin system in articular chondrocytes**

Detection of melanocortin receptors, proopiomelanocortin and precursor proteases - Evidence for a regulatory effect on pro-inflammatory cytokines and extracellular matrix components

Susanne Grässel, Alfred Opolka, Sven Anders, Rainer H. Straub, Joachim Grifka, Thomas A. Luger and Markus Böhm *Arthritis and Rheumatism, in press*

Objective. α-Melanocyte-stimulating hormone (α-MSH) is a proopiomelanocortin (POMC)-derived neuropeptide which mediates its effects via melanocortin receptors (MC-Rs). Here, we investigated the expression of the melanocortin system and the effects of α-MSH in human articular chondrocytes.

Methods. Articular chondrocytes established from osteoarthritic joint cartilage were analysed for expression of MC-Rs, POMC and prohormone convertases by RT-PCR and Western blotting. MC-1R expression in articular cartilage was studied by immunohistochemistry. α-MSH signalling was monitored by cAMP and Ca2+ assays. Functional studies were performed using chondrocyte micromass pellets stimulated with α-MSH. Expression of cytokines and extracellular matrix (ECM) components was determined by real-time RT-PCR, Western immunoblotting and ELISA.

Results. MC-1R was detected in articular chondrocytes in vitro and in articular cartilage in situ. Articular chondrocytes expressed also transcripts for MC-2R, MC-5R, POMC and prohormone convertases. α-MSH increased intracellular cAMP but not Ca2+ in chondrocytes. mRNA and protein expression of various pro-inflammatory cytokines, collagens, matrix metalloproteases (MMPs) and Sox9 were modulated by α-MSH.

Conclusion. Human articular chondrocytes are target cells for α-MSH. The effects of α-MSH on cytokine- and MMP expression suggest a role of this neuropeptide in inflammatory and degenerative processes in cartilage. It is conceivable that inflammatory reactions can be mitigated by induction of endogenous melanocortins or administration of α-MSH to the

affected joints. The induction pattern of regulatory and structural ECM components as collagens, Sox9, anabolic and catabolic cytokines points towards a function of α-MSH as a trophic factor in skeletal development during endochondral ossification rather than in homeostasis of permanent cartilage.

**Enzymatic Breakdown of Type II Collagen in the Human Vitreous.**
van Deemter M, Pas HH, Kuijer R, van der Worp RJ, Hooymans JM, Los LI.
*Invest Ophthalmol Vis Sci., in press*

Purpose: To investigate whether enzymatic collagen breakdown is an active process in the human vitreous. Methods: Human donor eyes were used for immunohistochemistry in order to detect the possible presence of the matrix metalloproteinase (MMP)-induced type II collagen breakdown product col2-3/4C-short in the vitreous. Western blotting and slot blotting were used to further identify vitreal type II collagen breakdown products in three age-groups with average ages of 25, 45 and 65 years. Purified type II collagen was cleaved by MMPs that are known to occur naturally in the vitreous in order to elucidate what possible type II collagen breakdown products could thus be formed in the human vitreous. Results: By means of both immunohistochemistry and slot blotting col2-3/4C-short was detected in the vitreous. Using Western blotting, a range of type II collagen breakdown products was found, mostly in younger eyes, but none of these products contained the neoepitope that characterizes the col2-3/4C-short molecule. Digestion of purified type II collagen by MMPs did not give the same breakdown products as found in the vitreous. Conclusions: The presence of collagen degradation products in the human vitreous supports the hypothesis that enzymatic breakdown is most likely an active process in this extracellular matrix. Based on the size of the degradation products found by Western blotting it is likely that in addition to MMPs, other proteolytic enzymes that are able to digest type II collagen are also active.

**Genetic Evidence For The Coordinated Regulation Of Collagen Fibrillogenesis In The Cornea By Decorin And Biglycan**
Zhang G, Chen S, Goldoni S, Calder BW, Simpson HC, Owens RT, McQuillan DJ, Young MF, Iozzo RV, Birk DE.
*J Biol Chem. 284:8888-8897, 2009*

Decorin and biglycan are class I small leucine-rich proteoglycans (SLRPs) involved in regulation of collagen fibril and matrix assembly. We hypothesize that tissue-specific matrix assembly, such as in the cornea, requires a coordinate regulation involving multiple SLRPs. To this end, we investigated the expression of decorin and biglycan in the cornea of mice deficient in either SLRP gene and in -mutant mice. Decorin and biglycan exhibited overlapping spatial expression patterns throughout the corneal stroma with differential temporal expression. Whereas decorin was expressed at relatively high levels in all developmental stages, biglycan expression was high early, decreased during development and was present at very low levels in the mature cornea. Ultrastructural analyses demonstrated comparable fibril structure in the decorin- and biglycan-null corneas compared to wild type controls. We found a compensatory up-regulation of biglycan gene expression in the decorin-deficient mice, but not the reverse. Notably, the corneas of compound decorin/biglycan-null mice showed severe disruption in fibril structure and organization, especially affecting the posterior corneal regions, corroborating the idea that biglycan compensates for the loss of decorin. Fibrillogenesis assays using recombinant decorin and biglycan confirmed a functional compensation, with both having similar effects at high SLRP/collagen ratios. However, at low ratios decorin was a more efficient regulator. The use of proteoglycan or protein core yielded comparable results. These findings provide firm genetic evidence for an interaction of decorin and biglycan during corneal development and further suggest that decorin has a primary role in regulating fibril assembly, a function that can be fine-tuned by biglycan during early development.

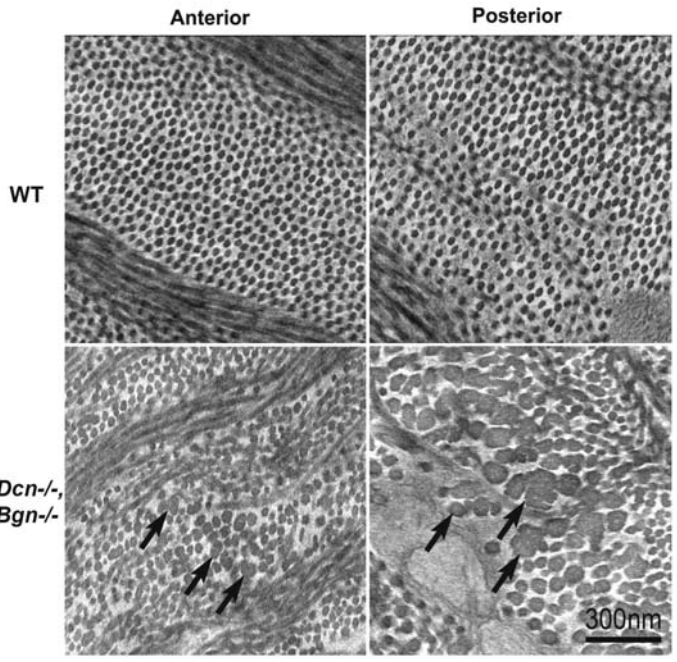



Figure: Aberrant fibril structure in the compound Dcn/Bgn-null corneas. Transmission electron micrographs of the posterior and anterior stroma from: wild type (WT) and double-null (Dcn-/-,Bgn -/-) mice at P60.

**Decorin is a Novel Antagonistic Ligand of the Met Receptor**

Silvia Goldoni, Ashley Humphries, Alexander Nyström, Sampurna Sattar, Rick T. Owens, David J. McQuillan, Keith Ireton, and Renato V. Iozzo

*J. Cell Biol. 185:743-754, 2009*.

See also faculty of 1000: http://www.f1000biology.com/article/id/1159659/evaluation

Decorin, a member of the small leucine-rich proteoglycan gene family, regulates tumor cell growth by downregulating the epidermal growth factor receptor. Due to the complex binding repertoire of decorin, we predicted that decorin would modulate the bioactivity of other tyrosine kinase receptors. We discovered that decorin binds directly and with high affinity (Kd~2 nM) to Met, the receptor for hepatocyte growth factor (HGF). Binding of decorin to Met was efficiently displaced by HGF, and less efficiently by internalin B, a bacterial leucine-rich protein that binds to Met with similar affinities. Interaction of decorin with Met induced transient activation of the receptor, recruitment of the E3 ubiquitin ligase c-Cbl, followed by rapid intracellular degradation of Met (T1/2~6 min). Decorin suppressed intracellular levels of β-catenin, a known downstream Met effector, and inhibited cell migration and growth via Met receptor-mediated events. These findings indicate that decorin exerts its cytostatic activity by antagonistically targeting multiple tyrosine kinase receptors, thereby contributing to reduction in primary tumor growth and metastastic spreading.

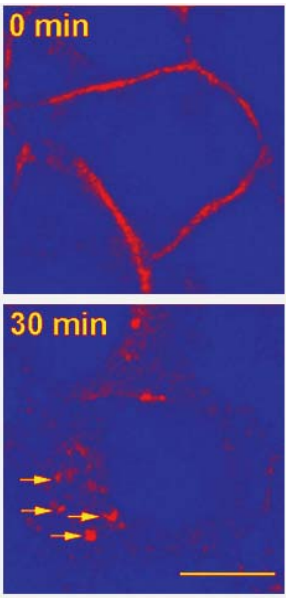



Figure: Confocal fluorescence images of HeLa cells (blue pseudocolor) immunostained for Met receptor (red) before or after a 30-min incubation with decorin core. Notice the rapid loss of cell surface Met and its translocation into endosomal degradative vesicles (arrows). Bar = 10 μm.

**Mice lacking the extracellular matrix protein WARP develop normally but have compromised peripheral nerve structure and function.**

Allen JM, Zamurs L, Brachvogel B, Schlötzer-Schrehardt U, Hansen U, Lamandé SR, Rowley L, Fitzgerald J, Bateman JF.

*J Biol Chem. 2009 284(18):12020-30*

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 we also demonstrate here that WARP binds 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 WARP-null mice exhibited a significantly delayed response to acute painful stimulus and impaired fine motor coordination, although general motor function was not affected, suggesting compromised peripheral nerve function. Immunostaining of WARP-interacting ligands demonstrated that the collagen VI microfibrillar matrix was severely reduced and mislocalized in peripheral nerves of WARP-null mice. Further ultrastructural analysis revealed reduced fibrillar collagen deposition within the peripheral nerve extracellular matrix and abnormal partial fusing of adjacent Schwann cell basement membranes, suggesting an important function for WARP in stabilizing the association of the collagenous interstitial matrix with the Schwann cell basement membrane. In contrast, other WARP-deficient tissues such as articular cartilage, intervertebral discs, and skeletal muscle showed no detectable abnormalities, and basement membranes formed normally. Our data demonstrate that although WARP is not essential for basement membrane formation or musculoskeletal development, it has critical roles in the structure and function of peripheral nerves.

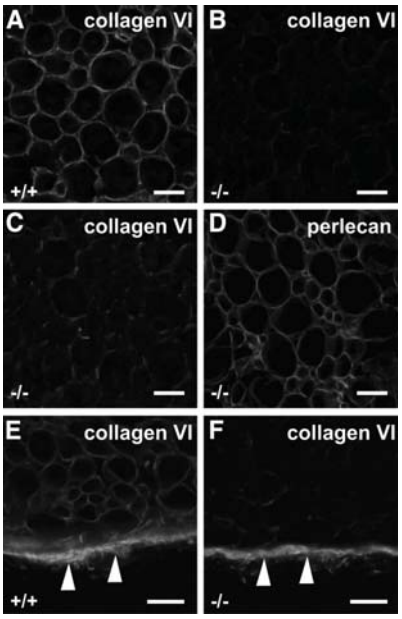



Figure: Confocal analysis of sciatic nerve endoneurial matrix in wild type and WARP-null mice. Immunofluorescent staining for collagen VI is significantly weaker in WARP-deficient mice (B) than in wild type (A) mice. Visualization of the field in image B with increased gain (C) reveals that the collagen VI matrix is disorganized as well as reduced in the WARP-null mouse. Perlecan still forms an intact basement membrane structure in the WARP-deficient endoneurium (D). The collagen VI structures in the outer perineurium layer (arrowheads) are unaffected in the WARP knock-out (F) compared with the wild type (E) mouse. Bar, 10 μm.

## Thank you Larry Rosenberg

Larry Rosenberg is an ideal model for a clinician researcher. When I was a graduate student at Rockefeller University and beginning to work on cartilage proteoglycans, I knew of the work that he was doing with Maxwell Schubert at New York University. I contacted this laboratory to learn some of the techniques that they were using to isolate proteoglycans. This introduced me to Larry and began a wonderful friendship that has continued to the present. Maxwell Schubert retired soon after, and Larry initiated his innovative studies to visualize the cartilage proteoglycans using electron microscopy. He connected with Albrecht Kleinschmidt, who worked in the same building and who had developed the Kleinschmidt procedure for visualizing DNA molecules. The application of this procedure provided the first pictures of the molecule we now know as aggrecan and its aggregates. Larry was captivated by research and began to devote his full time to research after his move to Montefiore Hospital. Early in his career Jody Buckwalter also approached Larry as described below. This led to the famous electron micrograph of the aggrecan aggregate that is featured in the attached montage, which was a gift to Larry to celebrate his 80th birthday, November 23, 2008. Larry's artistic abilities are clear in his famous 'blue dot' model of the aggregate that is the centerpiece of the montage. The picture of Larry, comfortably perched on the wall, is from the group photograph of the first Proteoglycan Gordon Conference in 1984. The ancient Australian aboriginal petroglyph is remarkably similar to a monomer from Larry's model. Throughout his career, Larry's contributions to orthopaedic research before and after I met him are well known by the extracellular matrix community. His sharing and thoughtful interactions with his colleagues are equally well known. Thank you Larry for your friendship and the many contributions you have made to our research world.

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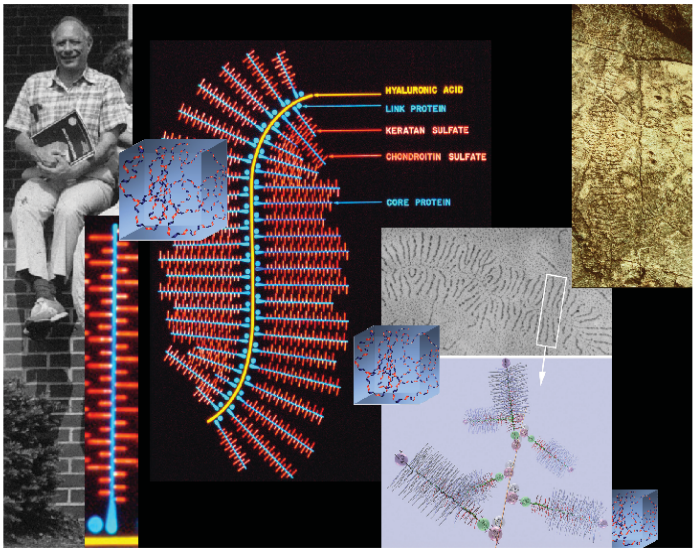


*Vincent Hascall,*
The Cleveland Clinic

I treasure many memories of Larry Rosenberg's inspirational scientific work as well as his warmth, kindness, and thoughtfulness. He helped advance the entire field of orthopaedic research through his service to the Orthopaedic Research Society including his year as President, his role in the establishment of the Journal of Orthopaedic Research and his mentoring of young investigators. When I was a first year resident in Orthopaedic Surgery at the University of Iowa, I submitted an abstract for the 1976 meeting of the Orthopaedic Research Society in New Orleans in Louisiana. It described electron microscopic studies of the human intervertebral disc. I was pleased, but worried, when it was accepted for presentation at the meeting. I was pleased that the program committee felt it was worthy of presentation, but worried when I learned that the moderator of my session was the President of the Orthopaedic Research Society, Dr. Larry Rosenberg. I was well aware of Larry's many scientific contributions and in particular his studies of proteoglycans. Since I would be presenting work that was crude compared with Larry's work, I was concerned. When I arrived at the room where the session would be held, Larry Rosenberg sought me out and greeted me warmly. I somehow managed to get through the presentation without committing a major error. Larry thoughtfully asked some easy questions. After the session I shared with him my admiration for his work. He invited me to his room where he showed me his remarkable darkfield electron micrographs of proteoglycan aggregates. This was one of the most exciting moments in my career, to see the actual structure of these molecules and discuss the images with the scientist who made them possible. Larry invited me to visit NYU to learn the spreading technique for the study proteoglycans, and I spent a week with Larry and Willimena Hellman. They were generous and helpful. I returned to Iowa and began to pursue further studies of cartilage, meniscal, intervertebral disc, and chondrosarcoma proteoglycans. This work, inspired and made possible by Larry, led to my receiving a 1980 Kappa Delta Award. I felt strongly that it should also go to Larry, but he, in his firm but pleasant way, insisted that I not include his name on the award. He told me it was important that in that stage of my career that I be recognized. I doubt there are many, if any other scientists, who would be so thoughtful, insightful and generous.

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Buckwalter JA, Rosenberg LC, Teng L-H: The effect of link protein on proteoglycan aggregate structure. JBC 259:5361, 1984.

*Jody Buckwalter*
University of Iowa

## 25. Ernst Klenk Symposium in Molecular Medicine
## Extracellular Matrix in Health and Disease
## 04. – 06. October 2009

Dear friends and colleagues

We would like to invite you to the 25. Ernst Klenk Symposium in Molecular Medicine on "Extracellular Matrix in Health and Disease" which will be held from 04. to 06. October 2009 at the Main Lecture Hall of the Medical Faculty of the University of Cologne.

We are very pleased to announce that Prof. Bjorn R. Olsen (Department of Developmental Biology, Harvard School of Dental Medicine, Boston, USA) was substantially involved in the scientific coordination of the program. Prof. Olsen will also present the Ernst Klenk Lecture entitled "Translational Cell and Matrix Biology of Vascular Disease" on Monday, 05. Oct. 2009 at 6 p.m.

The list of speakers includes internationally leading researchers who will speak on a variety of pertinent subjects including extracellular matrix proteins, their processing, their cellular receptors and their role in development and in disease.

The Klenk Symposium - organized by the Center for Molecular Medicine of the University of Cologne (CMMC) - is intended to provide a forum for discussion of state-of-the-art research in these fields for interested scientists and students from academia and industry.

The symposium will start on Sunday, 04. Oct. 2009 at c. 1.30 p.m. and will end on Tuesday, 06. Oct. 2009 at around 2.00 p.m. Please note that the participation is free of charge; for free registration please click here.

We expect lively and fruitful discussions and look forward to welcoming you at the 25. Ernst Klenk Symposium in Cologne.

Yours faithfully,

Thomas Krieg
Chairman
Center for Molecular Medicine Cologne

Mats Paulsson
Executive Board Member
Center for Molecular Medicine Cologne

# 25. Ernst Klenk Symposium
## in Molecular Medicine
## 04. - 06. October 2009

# Extracellular Matrix in Health and Disease

**Invited Speakers**

**Suneel Apte** - Cleveland, USA
**Leena Bruckner-Tuderman** - Freiburg, DE
**Peter Carmeliet** - Leuven, BE
**Kathryn S. E. Cheah** - Hong Kong, HK
**David A. Cheresh** - San Diego, USA
**Benoit de Crombrugghe** - Houston, USA
**Harry Dietz** - Baltimore, USA
**Reinhard Fässler** - Martinsried, DE
**Dick Heinegård** - Lund, SE
**Renato V. Iozzo** - Philadelphia, USA
**Gerard Karsenty** - New York, USA
**Birgit Leitinger** - London, UK
**Robert Mecham** - St.Louis, USA
**Stefan Mundlos** - Berlin, DE
**Raili Myllylä** - Oulu, FI
**Bjorn R. Olsen** - Boston, USA
**Leena Peltonen** - Cambridge, UK
**Vicki Rosen** - Boston, USA
**Samuel I. Stupp** - Evanston, USA
**Kenneth M. Yamada** - Bethseda, USA

## Ernst Klenk Lecture - Bjorn R. Olsen - Boston, USA

**Venue**
Main Lecture Hall of the
Centers for Biochemistry and Physiology
Building 44b, Joseph-Stelzmann-Str. 52
Medical Faculty, University of Cologne, DE

**Scientific Organizer**
**Bjorn R. Olsen** - Boston, USA

**Organizers**
Thomas Krieg - Cologne, DE
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**Program and further information:**
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**- Participation is free of charge; free registration at: klenk-symposium@uni-koeln.de -**

**Organization**
Center for Molecular Medicine, University of Cologne (CMMC/ZMMK)

CuBiTuS

*Welcome to the*

*Science knows no country, because knowledge belongs to humanity, and is the light which illuminates the world - Louis Pasteur (1822-1895)*

6th International Conference on Proteoglycan

Aix-les-Bains, France

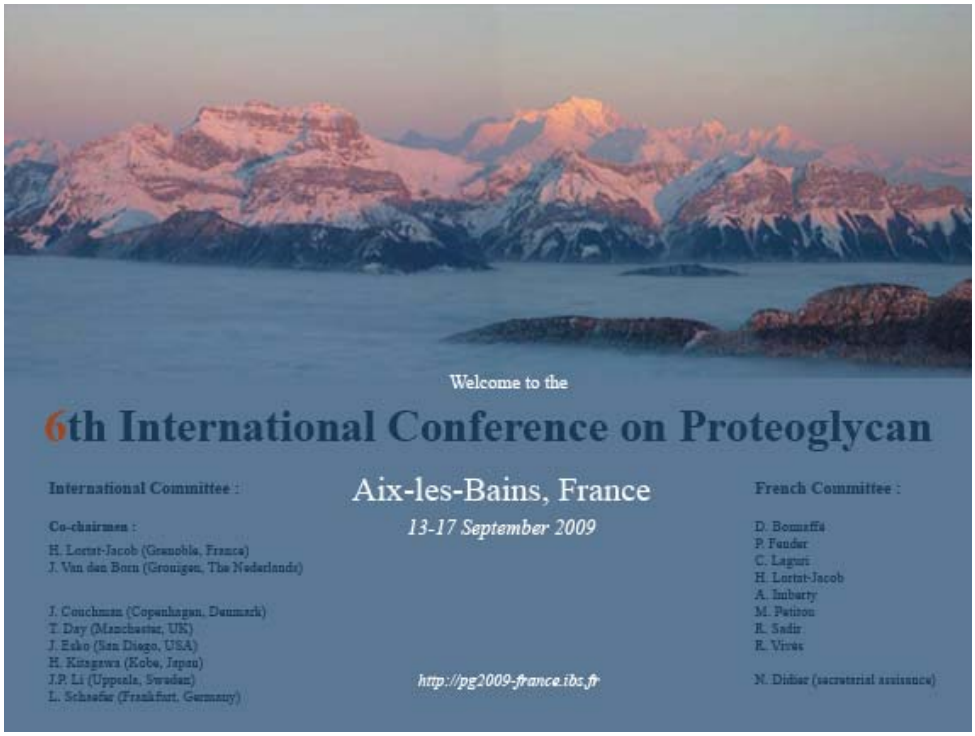
13-17 September 2009

PROTEOGLYCAN 2009



## Welcome to the 6th international Conference on Proteoglycan

*11 December 2008*



Matrix Biology 28 (2009) 63–64

Contents lists available at ScienceDirect

Matrix Biology

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From the Editor's Desk

# Insights into endothelial mechanisms through the lens of vascular anomalies

*Dear readers of Matrix Biology,*

I have frequently reminded graduate students and postdoctoral fellows in my laboratory that science is more like a marathon than a sprint in that hard, steady work on important problems over a long period of time is the best way to prepare for success.

In the case of most projects such periods fall well within the lifetime of a grant or a fellowship, allowing the work to be published before the support ends. However, in a few cases, progress in the study of hard problems can be painfully slow and stress both financial and intellectual resources. These are the studies that truly test the commitment of talented students and postdoctoral fellows and benefit from stable, long-term funding mechanisms. Two recent publications that describe endothelial pathogenetic mechanisms associated with two different human vascular anomalies are examples of such studies.

One of these papers, by Limaye et al. (2009) provides compelling evidence for a combination of germline and somatic mutations in the endothelial receptor tyrosine kinase TIE2 in familial cases of venous malformations and for combinations of somatic mutations in the same receptor in sporadic cases of the disorder. The work builds on studies initiated more than a decade ago in my laboratory when M. Vikkula et al. (1996) demonstrated that familial cases of venous malformations are associated with heterozygous activating missense mutations in TIE2. Since then, additional studies have confirmed this finding and several different missense mutations in this tyrosine kinase receptor are now known to cause familial venous maformations (Limaye et al., 2009).

Venous malformations are multifocal (the rare familial form) or single (the most common sporadic form) cutaneous or mucosal lesions consisting of tortuous small veins with incomplete covering of the endothelium by smooth muscle cells. The localized nature of the lesions suggests that the formation of the defects in the familial form requires a localized triggering event in addition to the germline mutations in TIE2. The nature of this event and what causes the common sporadic lesions have remained a mystery — until now.

By studying cDNA isolated from the lesion of a member of a family with the "original" (Vikkula et al., 1996) heterozygous R849W missense mutation, Limaye et al. (2009) found an in-frame somatic deletion in the allele that did not carry the R849W mutation. The deletion causes loss of 43 amino acid residues from the ligand-binding domain of the receptor, loss of cell surface expression and loss of activation (phosphorylation) in the presence of ligand. Therefore, the somatic lesion-restricted mutation turns the affected TIE2 allele into a loss-of-function allele so that only the mutant R849W TIE 2 protein is functioning in the lesion. This finding led to the speculation that similar somatic changes in TIE2 may be associated with sporadic venous malformations. By sequencing blood-derived DNA and lesion-derived DNA (both genomic and cDNA) from a number of individuals, the authors discovered both single and double somatic mutations in sporadic venous malformations. In general, the mutations are activating mutations and in cases where two mutations have occurred (in the same allele!) the mutations have added effects on receptor activation. The study firmly establishes that abnormal activation of TIE2 signaling is the underlying cause of both the rare familial and the common sporadic forms of venous malformations and provides strong support for the idea that therapies aimed at inhibiting TIE2 dependent processes may be effective in both inherited and sporadic forms.

The Limaye et al. (2009) paper also provides an example of (or a contrast to, see below) genetic mechanisms that may be responsible for the discrete lesions seen in other vascular anomalies. One such disorder is the most common tumor of infancy called infantile hemangioma. This sporadic endothelial tumor, occurring in up to 10% of infants of European descent, has fascinated investigators and clinicians for a long time, for several reasons. Hemangiomas have a unique life cycle with rapid growth (proliferating phase) during infancy, followed by a longer period (months to years) of involution, resulting in complete regression. The tumors occur more frequently in females than in males, in premature babies and in cases where the pregnancy has been associated with placental abnormalities.

Many hypotheses have been proposed for the cellular and molecular mechanisms responsible for the appearance and rapid growth of the hemangioma lesion in the early postnatal period, but based on the available evidence it has not been possible to identify one specific pathogenetic mechanism that explains all the cellular and molecular properties of hemangiomas. With a recent paper from my own laboratory, this situation has now changed. Our study (Jinnin et al., 2008) builds on a previous investigation of X-chromosome inactivation patterns in sporadic hemangioma lesions. In collaboration with the laboratory of Joyce Bischoff, we found (Boye et al., 2001) that endothelial cells from the lesions of female patients in all cases tested, showed complete skewing of X-chromosome inactivation, clearly consistent with clonality; in contrast, non-endothelial cells from the lesions were just as clearly not clonal. During the past 6 years we have therefore examined the biochemical properties of hemangioma-derived endothelial cells with the aim of identifying molecular properties that are unique to hemangioma endothelial cells and distinguish these cells from several different types of control endothelial cells.

The paper by Jinnin et al. (2008) provides, I believe, justification for saying that we were quite successful in reaching this aim. We found that transcript and protein levels of the decoy receptor VEGFR1 are abnormally low in hemangioma endothelial cells compared to control endothelial cells (several different kinds), resulting in constitutive,

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doi:10.1016/j.matbio.2009.01.001

VEGF-dependent activation of VEGFR2 receptor and its downstream targets. Jinnin et al. (2008) further demonstrated that low levels of VEGFR1 expression in hemangioma is a consequence of repressed activation of the transcription factor NFATc2 in hemangioma endothelial cells. Consistent with this, we found that the expression of other known NFAT target genes is also low in the cells. The repressed activation of NFAT could be traced back to abnormalities in an endothelial surface protein complex containing of VEGFR2, β1 integrin and the "integrin-like" transmembrane protein known as TEM8 or Anthrax toxin receptor 1. Sequencing of several candidate genes with DNA from nine different hemangioma patients resulted in identification of heterozygous missense mutations in TEM8 and VEGFR2 in three patients. These mutations result in abnormal VEGFR2/β1 integrin/TEM8 complex formation, suppression of β1 integrin activation and loss of the ability of the complex to induce NFAT-dependent VEGFR1 expression when cells are treated with VEGF or β1 integrin stimulatory antibodies.

Interestingly, we found that abnormal VEGFR2/β1 integrin /TEM8 complex formation is a characteristic feature of all hemangioma endothelial cells, even those derived from patients where mutations in TEM8 or VEGFR2 have not been identified. We believe, therefore, that this complex may contain additional components and that some of these components may be candidates for hemangioma-causing mutations as well.

Since the mutations already identified in TEM8 and VEGFR2 are heterozygous germline mutations, they cannot explain the clonality of endothelial cells within the lesions of these patients. Like the heterozygous germline TIE2 mutations in familial venous malformations, they also do not explain the localized nature of the lesions. Whether hemangioma lesions in patients with germ-line mutations in TEM8 or VEGFR2 are initiated because of somatic mutations similar to what happens in familial venous malformations is not known. Since venous malformations do not regress like hemangiomas, we have argued (Jinnin et al., 2008) that the initiating event in hemangioma formation may be a reversible pathophysiological event rather than a somatic mutation, but a somatic mutation cannot be ruled out. However, we speculate that most sporadic hemangiomas, like venous malformations, may be caused by somatic mutations in genes, such as TEM8 and VEGFR2, that have already been found to carry a germline mutation in some cases. With access to large a number of DNA and lesional tissue samples collected from hemangioma patients, these possibilities can hopefully be addressed faster than the time we have spent so far to open the door to the pathogenetic "mystery" of proliferating hemangiomas. However, we are also preparing ourselves for another "marathon run" in that we plan studies of the mechanisms by which hemangiomas undergo the slow process of involution leading to regression. While the current studies suggest that anti-VEGF therapy may be effective in treating clinically problematic hemangiomas in the proliferating phase, only a complete understanding of the involution process may set the stage for attempts to accelerate the process in patients with already established tumors. Understanding the involution mechanisms may even help in developing better strategies for inducing vascular regression in angiogenesis-dependent diseases other than hemangiomas.

So, I keep reminding my students and postdoctoral fellows that science is not a sprint…"

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**THOMAS JEFFERSON UNIVERSITY**
**PHILADELPHIA, PA**

## POSTDOCTORAL POSITIONS IN PROTEOGLYCAN RESEARCH, CANCER AND ANGIOGENESIS

Two postdoctoral positions are available to investigate the biology of perlecan and decorin in cancer and angiogenesis. The candidates will join a multi-disciplinary team of researchers involved in investigating the molecular mechanisms through which these two proteoglycans affect tumor biology, EGFR and Met receptor signaling, growth and angiogenesis, both *in vitro* and *in vivo* (*J. Cell Biol.* **166**:97-109, 2004; *J. Biol. Chem.* **280**:32468-32479, 2005; *Nature Rev. Mol. Cell. Biol.* **6**:646-656, 2005; *J. Natl. Cancer Inst.* **15**: 1634-1646, 2006; *Blood* **109**: 3745-3749, 2007; *J. Biol.Chem.* **283**:2335-2344, 2008; *J. Cell Biol.* **181**:381-394, 2008.; *Biochemistry* **47**:11174-11183, 2008; *Am. J. Pathol*. **173**:844-855. 2008; *J. Cell Biol.* **185**:743-754, 2009).

Requirements include a Ph.D. or an M.D./Ph.D. in biochemistry or cell biology. A molecular biology background is highly desirable. Send resume and three letters of reference to:

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