



International Society for Matrix Biology Newsletter

2005, No. 1 - September

Contents

From the President's Desk	1
Meeting Announcements	2
Matrix Research Update	2
Job Advertisements	4
ISMB Officers and Council	4
Matrix Biology - From the Editors' Desk (v24, issue 6, p387-388)	5

From the President's Desk

For many years the International Society for Matrix Biology was fortunate in having a dedicated newsletter editor, Ray Dalglish, to provide regular communication between the ISMB membership. The importance of this interaction has become obvious since the cessation of the newsletter.

This is the first in a new series of regular ISMB newsletters to be circulated by email every three months - March, June, September and December. Jamie Fitzgerald (Melbourne) and Dieter Reinhardt (Montreal) have enthusiastically accepted the roles of Newsletter Editors, and no doubt you will be hearing from them regularly as they solicit content. The idea is that the newsletter will contain a number of regular features including a message from the President on current ISMB activities and future plans, meeting announcements, jobs and scholarships. In addition, Bjorn Olsen's "From the Editor's Desk" comments from Matrix Biology will be included prior to publication to stimulate interest in topical matrix biology issues, and no less importantly, in our journal *Matrix Biology*. The newsletters will also provide the opportunity for ISMB members to bring their latest publications to the attention of their peers in a "Matrix Research Update" section.

I hope the ISMB Newsletter will evolve into an important vehicle for communication between the ISMB membership, and I urge you to take ownership of the Newsletter by contributing ideas and content. The structure is fluid and meant to serve members needs, so I'm sure Jamie and Dieter will be prepared to take on any suggestions you may have on improving it.

ISMB to participate in the FECTS

At the recent General Assembly of ISMB, held in conjunction with the Collagen Gordon Conference, the role of the Society in organising International meetings was discussed. This is an important initiative for ISMB and the decision was taken to achieve this by becoming involved in co-organisation of major matrix biology meetings such as FECTS. ISMB will provide funding of 15,000 Euros and participate in the program organisation of the 2006 FECTS meeting in Oulu, Finland. The focus of ISMB funding will be matrix biologists outside of the usual FECTS 'catchment', such those in the USA and the Asia/Pacific region and thus further "internationalize" the meeting. The funding will be provided in a mixture of competitive awards to young researchers and invitations for keynote speakers. Details of the awards will be sent out to ISMB members in the next few months and will be also publicized in the December newsletter. ISMB has formed a program organizing committee who will work with the FECTS organizers to develop the program. ISMB is also having discussions with the ASMB and

hope that the ISMB sponsored international matrix meetings will alternate between FECTS and ASMB. In the case of ASMB, ISMB travel funding would be directed to researchers outside of the USA. More on this in the next newsletter.

Matrix Biology

As noted above, Matrix Biology *is our journal*, and it continues to grow in stature under the outstanding stewardship of Bjorn Olsen as Editor-in-Chief, with the support of the Editorial Board. The number of submissions is increasing each year, as is the quality and significance of the publications. ISMB is currently having discussions with Elsevier with the goal of securing a royalty stream from subscriptions to the journal. This will allow us to provide more support for conferences and other initiatives. I would encourage you all to seriously consider Matrix Biology for your next best paper. You may be interested to learn that Matrix Biology has a very healthy impact factor of **4.104** (ISI-2004).

Rupert Timpl Award

The "Rupert Timpl Award" was instituted by ISMB to recognise the research achievements and potential of young scientists active in the field of matrix biology. The award is made every two years and is based on the "best paper" on a subject in matrix biology published in the preceding two years. The inaugural "Rupert Timpl Award" in 2005 was made to Dr Claus Werner Franzke for his paper in EMBO Journal entitled "Transmembrane collagen XVI, an epithelial adhesion protein, is shed from the cell surface by ADAMs". The next award will be made in conjunction with the 2006 FECTS meeting. Nominations can be made by members of ISMB and self-nominations are excluded. The closing date for nominations for the 2006 award is April 30th, 2006 and papers published in 2004 and 2005 will be considered. Submissions should be made to Peter Bruckner [peter.bruckner@uni-muenster.de].

As always, your comments and suggestions are welcome and appreciated.

Regards,

John Bateman [john.bateman@mcri.edu.au]

Meeting Announcements

The 6th Pan Pacific Connective Tissue Societies Symposium

November 30th - December 5th, 2005, at the Hilton Waikoloa Village, Hawaii. More information can be found at: <http://www.shcc.org/6ppets/>.

Please note that abstracts are due on September 30th 2005.

52nd Annual Meeting of the Orthopaedic Research Society

The 2005 ORS meeting has been moved from New Orleans to Chicago, Illinois and held on March 19-22, 2005. Please note that the date of the meeting has also been changed. Details of the meeting can be found at: <http://www.ors.org/Meetings/52ndAnnualMeeting/AnnualMeeting.asp>

The XXth meeting of the Federation of European Connective Tissue Societies

Arranged jointly with the **International Society of Matrix Biology** in Oulu, Finland on July 1-7, 2006. The conference venue is the Main Building at the Medical Campus of the University of Oulu. Further information can be found at www.facts-ismb.org

The abstract deadline: March 15th 2006

Also keep your diaries free for the **ASMB Biannual Meeting 2006** held from November 1-4, 2006 in Nashville, Tennessee.

Matrix Research Update

In Press Publications

Modification of the Structure and Function of Fibrillin-1 by Homocysteine Suggests a Potential Pathogenetic Mechanism in Homocystinuria

D. Hubmacher, K. Tiedemann, R. Bartels, J. Brinckmann, T. Vollbrandt, B. Bätge, H. Notbohm, and [D.P. Reinhardt](#)

Journal of Biological Chemistry

Homocystinuria, a disorder originating in defects in the methionine metabolism, is characterized by an elevated plasma concentration of homocysteine. Most patients have a defect in the cystathionine- β -synthase, the key enzyme in the conversion of homocysteine to cysteine. Many abnormalities in the connective tissue of patients with homocystinuria resemble those seen in Marfan syndrome, caused by mutations in fibrillin-1. These observations led to the hypothesis, that the structure and function of fibrillin-1 is compromised in patients with homocystinuria. To test this hypothesis, we produced recombinant human fibrillin-1 fragments spanning the central portion of the molecule (8-Cys/transforming growth factor- β binding domain 3 to calcium binding EGF domain 22) and analyzed extensively the potential of homocysteine to modify structural and functional properties of these proteins. Circular dichroism spectroscopy revealed moderate changes of their secondary structures after incubation with homocysteine. Equilibrium dialysis demonstrated a number of high affinity calcium binding sites in the tandemly repeated calcium binding EGF domains 11-22. Calcium binding of

homocysteine-modified fragments was completely abolished. Incubation of the recombinant proteins with homocysteine rendered the analyzed calcium binding EGF domains as well as the 8-Cys/TB domain significantly more susceptible to proteolytic degradation. Furthermore, data were obtained demonstrating that homocysteine can covalently modify fibrillin-1 via disulfide-bonds. These data strongly suggest that structural and functional modifications as well as degradation processes of fibrillin-1 in the connective tissues of patients with homocystinuria play a major role in the pathogenesis of this disorder.

Absence of autoantibodies against correctly folded recombinant fibrillin-1 protein in systemic sclerosis patients

J. Brinckmann, N. Hunzelmann, E. El-Hallous, T. Krieg, L.Y. Sakai, S. Krenzel, D.P. Reinhardt

Arthritis Research & Therapy

Autoantibodies to short recombinant fragments of fibrillin-1 produced in bacterial expression systems have been found in tight-skin mouse, systemic sclerosis, mixed connective tissue disease, and primary pulmonary hypertension syndrome. In patients with scleroderma, the frequency of anti-fibrillin-1 antibodies was 42% in Caucasians. Until now it is unclear whether this immune response plays a primary role in the disease pathogenesis or is a secondary phenomenon. In the present study, we analyzed the frequency of autoantibodies against two overlapping recombinant polypeptides spanning the N-terminal- and the C-terminal half of human fibrillin-1, which were produced in human embryonic kidney cells 293. Correct three-dimensional structures of the recombinant fibrillin-1 polypeptides were shown by electron microscopy and immunoreactivity with antibodies. Screening of fibrillin-1 antibodies was performed in 41 sera from systemic sclerosis patients and in 44 healthy controls with a Caucasian background. Microtiter plates were coated with the recombinant polypeptides of fibrillin-1 and incubated with 1:100 diluted sera. Positive binding was declared as > 2 SD above the mean of the control group. Enzyme linked immunosorbent assays showed that none of the sera of patients with systemic sclerosis contained autoantibodies neither to the N-terminal nor the C-terminal recombinant fibrillin-1 polypeptide. The data presented show the absence of autoantibodies against recombinant fibrillin-1 protein in Caucasian systemic sclerosis patients. Since the correct three-dimensional folding of the recombinant proteins has been substantiated by several independent methods, we conclude that autoantibodies against correctly folded fibrillin are not a primary phenomenon in the pathogenesis of systemic sclerosis.

Perlecan from Human Epithelial Cells is a Hybrid Heparan/Chondroitin/Keratan Sulfate Proteoglycan

S. Knox, A.J. Fosang, K. Last, J. Melrose, & J. Whitlock.

FEBS Letters

Perlecan is a multidomain proteoglycan, usually substituted with heparan sulphate (HS), and sometimes substituted with both HS and chondroitin sulphate (CS). In this paper we describe perlecan purified from HEK-293 cells substituted with HS, CS and keratan sulphate (KS). KS substitution was confirmed by immunoreactivity with antibody 5D4, sensitivity to keratanase treatment, and fluorophore-assisted carbohydrate electrophoresis. HEK-293 perlecan failed to promote FGF-dependent cell growth in an *in vitro* assay. This study is the first to report perlecan containing (KS), and makes perlecan one of only a very few proteoglycans substituted with three distinct types of glycosaminoglycan chains.

The accumulation of intracellular ITEGE and DIPEN neoepitopes in bovine articular chondrocytes is mediated by CD44 internalization of hyaluronan

Jennifer J. Embry Flory, Amanda J. Fosang and Warren Knudson

Arthritis & Rheumatism

Objective. A dramatic loss of aggrecan proteoglycan from cartilage is associated with osteoarthritis. The fate of residual G1 domains of aggrecan is unknown but, inefficient turnover of these domains may impede subsequent repair and retention of newly-synthesized aggrecan. Thus the objective of this study was to determine whether ITEGE and DIPEN-containing G1 domains, generated *in situ*, are internalized by articular chondrocytes and, whether these events are dependent on hyaluronan (HA) and its receptor, CD44.

Methods. ITEGE and DIPEN neoepitopes were detected by immunofluorescent staining of bovine articular cartilage chondrocytes treated without or with IL-1. Additionally, purified ITEGE or DIPEN-containing G1 domains were aggregated with HA and then added to articular chondrocytes, articular chondrocytes transfected with CD44⁶⁷ or, COS-7 cells transfected with or without full length CD44. Internalized epitopes were distinguished by their resistance to extensive trypsinization of the cell surface.

Results. Both ITEGE and DIPEN were visualized within the extracellular cell-associated matrix of chondrocytes as well as within intracellular vesicles. Following trypsinization, the intracellular accumulation of both epitopes was clearly visible. IL-1 treatment increased extracellular as well as intracellular ITEGE epitope accumulation. Once internalized, the ITEGE neoepitope became localized within the nucleus and displayed little co-localization with HA, DIPEN, or other G1 domain epitopes. The internalization of both ITEGE and DIPEN G1 domains was dependent on the presence of HA and CD44.

Conclusion. One important mechanism for the elimination of residual G1 domains following extracellular degradation of aggrecan is CD44-mediated co-internalization with HA.

Job Advertisements

Position: Postdoctoral fellow (immediately available)

Affiliation: McGill University, Dept. of Anatomy and Cell Biology, Montreal, Canada

Project: Functional aspects of fibrillin-containing microfibrils

Requirements: Experience in proteomic analysis desired but not absolutely necessary

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From the Editor's Desk

Dear readers of Matrix Biology,

In a previous editorial in *Matrix Biology* (Olsen, 2004) I described how anthrax toxin takes advantage of two integrin-like cell surface molecules, CMG2 and TEM8, on vascular endothelium to gain entry into the cells and accomplish its deadly mission. Now another pathogen, this time an emerging paramyxovirus called Nipah virus, has been shown to gain access to cells by binding to the cell surface molecule ephrin B2, present not only on endothelial cells, but on smooth muscle cells in arterioles and small arteries and on neurons as well (Bonaparte et al., 2005; Negrete et al., 2005). Outbreaks of Nipah virus infections have been reported in Malaysia, Singapore and Bangladesh since 1998–1999; the virus causes fatal encephalitis in up to 70% of infected patients and induces the formation of syncytia of multinucleated endothelial cells. The formation of these endothelial syncytia is mediated by the fusion and attachment proteins in the viral envelope.

Negrete et al. (2005) took advantage of the properties of the envelope proteins to go fishing for the cell surface receptor by generating a fusion protein between the ectodomain of the viral attachment protein and the Fc-region of human IgG1 and comparing the binding of this recombinant protein “hook” to cells that are permissive or non-permissive for fusion. The recombinant Fc-attachment protein immunoprecipitated a 48 kDa protein from permissive cells and following trypsin fragmentation and mass spectrometry this protein was uniquely identified as ephrin B2. The approach used by Bonaparte et al. (2005) was somewhat different; these authors used microarrays with mRNA isolated from fusion-non-permissive and fusion-permissive cell lines to search for cell membrane proteins common to the fusion-permissive cells. Ten candidate genes were tested for their ability to convert cells that were non-permissive for fusion into fusion-permissive cells, and only one gene, ephrin B2, was found to make non-fusing cells competent to fuse when exposed to viral attachment and fusion proteins.

Negrete et al. (2005) also showed that ephrin B2 had the ability to make non-fusing cells fusogenic and both groups demonstrated (using different assays) not only that ephrin

B2 can bind directly to the viral attachment protein, but also that a soluble version of ephrin B2 or antibodies against ephrin B2 inhibit the fusion of cells when they are exposed to viral fusion and attachment proteins. Furthermore, Bonaparte et al. (2005) showed that a soluble form of ephrin B2 can block infection of susceptible cells with infectious virus, that non-susceptible cells become susceptible to viral infection following transfection with ephrin B2, and that this infection can also be blocked with soluble ephrin B2. Instead of using infectious virus (requiring a BSL-4 type facility), Negrete et al. (2005) generated a recombinant vesicular stomatitis virus expressing red fluorescent protein and the Nipah viral fusion and attachment proteins instead of its own envelope proteins. The entry of this recombinant virus into susceptible cells resulted in cellular production of red fluorescent protein, and this was inhibited by antiserum against the Nipah viral coat proteins or by soluble ephrin B2, but not by soluble ephrin B1; infection with recombinant vesicular stomatitis virus carrying its own envelope proteins was not inhibited by either ephrin. Negrete et al. (2005) ruled out the involvement of cell surface heparan sulfate proteoglycans in viral entry into cells by demonstrating that a mutant CHO cell line, non-permissive for viral entry and carrying no heparan sulfate proteoglycans on the cell surface, became infected following transfection with ephrin B2. Finally, these authors demonstrated that entry of vesicular stomatitis virus carrying Nipah envelope proteins into neurons and microvascular endothelial cells was ephrin B2 dependent and that this entry could be inhibited by soluble Eph B4 or Eph B2, the physiological receptors for ephrin B2.

Ephrin B2 plays important roles in vasculogenesis and axonal guidance (Adams et al., 1999; Nakagawa et al., 2000; Palmer and Klein, 2003; Wang et al., 1998; Williams et al., 2003). The use by Nipah virus of ephrin B2 as the cellular receptor explains the finding of viral antigen in endothelial cells, neurons and arteriolar smooth muscle cells in patients who have died from Nipah infection, since these cells express high levels of ephrin B2. A recent review by Peter Carmeliet and Marc Tessier-Lavigne (Carmeliet and Tessier-Lavigne, 2005)

contains an excellent discussion of the roles of ephrins and their Eph receptors as well as other common signaling mechanisms by which blood vessel and nerve morphogenesis is controlled. Ephrin B2 is one of three transmembrane proteins (ephrin B1-3) that bind to EphB receptors (Eph B1-4, Eph B6). Ephrins A1-5 are linked to cell membranes by glycosyl-phosphatidyl inositol anchors; these ephrins bind to the receptors Eph A1-8. Ephrin B2 and the receptor Eph B4 are expressed in developing veins and arteries and loss-of-function analyses in mice indicate that signaling through ephrin B2-Eph B4 prevents mixing of arterial and venous endothelial cells and helps define boundaries between arteries and veins (Adams et al., 1999). Cells expressing Eph B4 show repulsive and anti-adhesive behavior when in contact with ephrin B2-expressing cells; in contrast, cells expressing ephrin B2 show propulsive adhesion when in contact with Eph B4-expressing cells. Negrete et al. (2005) speculate that if the attachment protein of the Nipah viral envelope binds to ephrin B2 and activates it in a similar manner as Eph B4, it could help recruit more endothelial cells into areas of viral infection. If this turns out to be the case, one wonders whether the Nipah viral attachment protein, in some modified form, may one day be used to stimulate endothelial cell migration into tissues of poor vascularity.

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