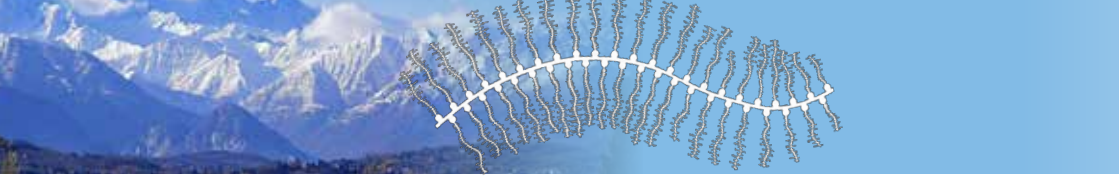
7 LAKES PROTEOGLYCANS conference

Varese, Italy,
10th - 14th September
2017

ABSTRACT BOOK

SESSION 1

NEWS AND FUTURE PERSPECTIVES



THE MAMMALIAN CHONDROITINASE, HYALURONIDASE 4, IS PRODUCED BY HUMAN MAST CELLS AND GENERATES CS CLEAVAGE NEO-EPITOPES

Brooke Farrugia[1], Shuji Mizumoto[2], Megan Lord[1], Shuei Yamada[2], Bruce Caterson[3], John Whitelock[1]

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Introduction: Chondroitin sulfate proteoglycans (CSPGs) are ubiquitous populations of molecules present within virtually all tissues. Members of the hyaluronidase (HYAL) family of enzymes have been shown to have specificity towards cleaving chondroitin sulfate (CS). HYAL4 has been shown to degrade CS structures, but not hyaluronan and shown to be present in the placenta, skeletal muscle and testis.

Objectives: Here, we were interested in determining whether HYAL4 was produced by mast cells and to investigate the CS structures that could potentially be generated following HYAL4 digestion.

Results: Mast cells were identified in human skin by histochemical stains such as Leder stain and toluidine blue and immunohistochemically with tryptase. HYAL4 was shown to be present within cells of similar morphology and localization as those characterized as mast cells, which was confirmed by flow cytometry and Western blot analyses. Interestingly, flow cytometry analysis of mast cells revealed CS structures present including the CS neo-epitope detected with the monoclonal antibody 2B6, which is well characterized for its ability to bind epitopes after digestion with the bacterial lyase, chondroitinase ABC, which leaves an unsaturated bond at the non-reducing end of the resultant disaccharides. Generation of the neo-epitope structure was further investigated by digestion of a CSPG with HYAL4. Degradation of CS chains was observed following incubation of a CSPG with HYAL4 concomitant with the generation of CS neo-epitopes detected with 2B6 and its related antibody 3B3. This observation was investigated further by incubation of CS-A and CS-C alone tethered to a plate via a biotin-streptavidin bridge with HYAL4. HYAL4 was shown to degrade the CS chains, and generate 2B6 and 3B3.

Conclusion: This work shows for the first time that mast cells synthesize HYAL4 and that it cleaves the intracellular CS found in the α-granules of mast cells to generate the 2B6 and 3B3 epitopes.

M1 AND M2 MACROPHAGES IN ATHEROGENESIS: A NOVEL ROLE FOR HEPARAN SULFATE PROTEOGLYCANS IN BINDING LOW DENSITY LIPOPROTEIN

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Introduction: There are two forms of macrophages in atherosclerotic plaques. While M1 are associated with plaque instability, M2 are not; rather they produce stabilizing extracellular matrix (ECM). Some ECM components, particularly the glycosaminoglycan (GAG) chondroitin sulfate (CS) on proteoglycans (PGs) are known to bind atherogenic low density lipoprotein (LDL). However, the ability of M1 and M2 to produce PGs that bind LDL and their contribution to atherogenesis is unknown.

Objectives: This study aimed to characterize the PGs and GAGs produced by M1 and M2 and analyze their ability to bind LDL.

Results: The human monocytic cell line THP-1 derived M1 and M2 produced biglycan, perlecan and versican. The CS profile they produced included C2S, C4S, and C6S disaccharides with a higher level of sulfation exhibited by M1 than M2. Heparan sulfate (HS) levels were significantly higher in M2 than M1-derived PGs. The HS from both cells included uronic acid-N-sulfo-D-glucosamine and uronic acid-N-sulfo-D-glucosamine-6S disaccharides, with the latter being relatively more abundant in M1 than M2 HS. The PGs derived from M1 bound more LDL than M2-derived PGs and surprisingly, this involved HS but not CS. In contrast, M2-derived PGs showed increased LDL binding after GAGs were removed, indicating an interaction between LDL and the core proteins, such as perlecan domain II.

Conclusions: M1 and M2 produce the same PGs, but they differ in their CS and HS composition. HS PGs interact with LDL. This is in contrast to previous reports (1,2) of CS binding LDL, potentially due to the presentation of the HS and CS in the native PGs. M1-derived PGs bind more LDL than those produced by M2, supporting the pro-atherogenic nature of M.

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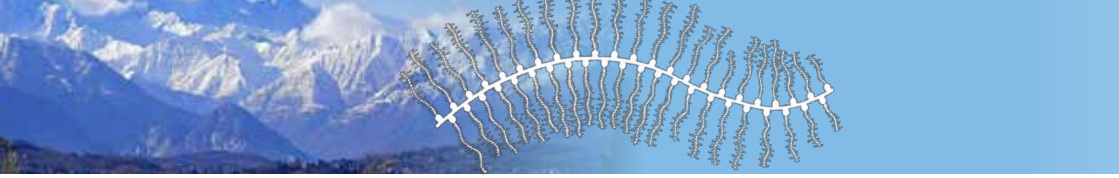

C-TYPE LECTIN 14a BINDS HEPARAN SULFATE TO MODULATE ANGIOGENESIS

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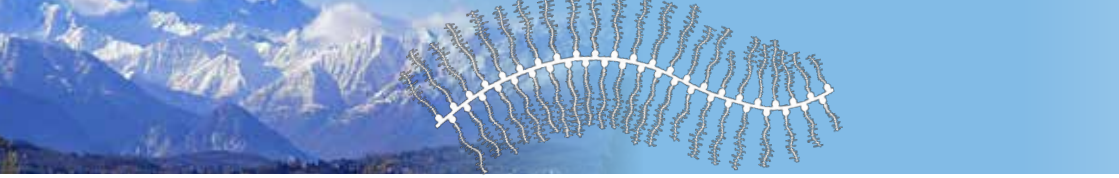
All cell types express heparan sulfate proteoglycans (HSPGs), embedded into the cell membrane or released into the extracellular matrix. HSPGs bind numerous proteins, including cytokines and chemokines, enzymes and enzyme inhibitors, apolipoproteins and lipases, extracellular matrix proteins, and membrane receptors. To identify novel heparan sulfate binding proteins (HSBPs) on endothelial cells, we utilized limited proteolysis to liberate ectodomains of cell surface proteins expressed on human umbilical vein endothelial cells. Chromatography over heparin-Sepharose and mass spectrometry yielded several known HSBPs, including VEGFR1, NRP1, BSG, HHIP and NRG1. Novel endothelial HSPBs were identified as well including PTPRB, CLEC14A, CLEC2B, CD93, and GDF15. We mapped the heparan sulfate-binding domain of CLEC14A by mutagenesis and showed that the binding site resides in the C-type lectin domain. Recombinant CLEC14A ectodomain bound with high affinity to heparan sulfate and heparin oligosaccharides of ≥ dp6. Binding occurred in 1:1 stoichiometry and led to increased thermal stability. Heparin blocked binding of CLEC14A to multimerin-2 (MMRN2), but mutation of a key residue in the heparin-binding domain (Arg161) did not interfere with binding. Overexpression of membrane bound wild-type CLEC14A or the ectodomain had no effect on angiogenic sprouting, whereas the Arg161Ala mutant protein inhibited sprouting. These findings suggest that under resting conditions heparan sulfate binds to CLEC14A and inhibits interaction with MMRN2. A model is proposed in which heparan sulfate modulates the ability of CLEC14A to bind MMRN, resulting in altered endothelial sprouting during embryonic development.

SESSION 2

PROTEOGLYCANS STRUCTURE, METABOLISM, AND ANALYSIS



RECOGNITION OF GLYCOSAMINOGLYCAN ATTACHMENT SITES BY XYLOSYLTRANSFERASE

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Introduction: The first step in the biosynthesis of heparan sulphate (HS), chondroitin sulphate (CS) and dermatan sulphate (DS) is the addition of a xylose monosaccharide to a serine residue in the proteoglycan core protein. In humans and other higher mammals, this initial step is performed by one of two xylosyltransferases (XylTs). Interactions between XylTs and the potential core protein determine whether or not this protein is destined to become a proteoglycan. Previous analyses of experimentally determined proteoglycan attachment sites have revealed a G-S-G consensus motif, with a preference for acidic amino acids upstream of the motif.

Objectives: We wish to understand the molecular basis for acceptor peptide specificity of human xylosyltransferases.

Results: We have determined the crystal structure of XylT1 to 1.8 Å resolution, in complex with both the UDP-xylose donor substrate, and various acceptor peptides. Our structures reveals that XylT1 is a metal-ion independent enzyme, with a deep peptide binding cleft which brings the target serine into the active site. We have used a peptide library to determine acceptor specificity of XylT1 and XylT2, which reveals that the previously described G-S-G motif is overly restrictive, with both enzymes having a greater tolerance for larger nonpolar amino acids, particularly in the position preceding the serine. Additional factors that govern acceptor specificity are also revealed. In addition to this, we have conducted a mutational analysis of xylosyltransferase 1 to determine the catalytic mechanism.

Conclusions: Our structural, mechanistic and functional studies of XylT1 reveal it to have a much broader specificity than previously described. This may aid in the characterisation of novel glycosaminoglycan attachment sites, and determine the effect of disease causing mutations adjacent to these sites.

GAGs BASED GLYCOMICS/INTERACTOME RESEARCH USING SPR

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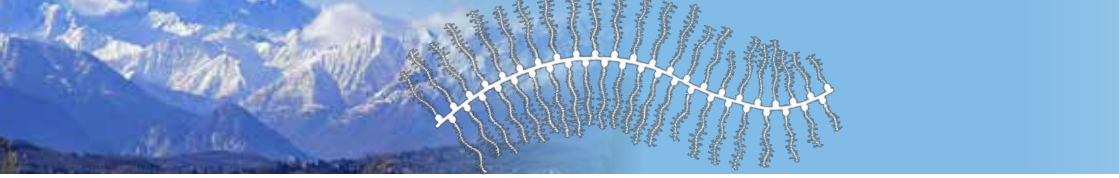
Introduction: Glycosaminoglycans (GAGs) are anionic, polydisperse, linear polysaccharides that are often highly sulfated. Many physiological processes and diseases are modulated through GAG-protein interactions including: blood coagulation, cell growth and differentiation, host defense, viral infection, lipid transport and metabolism, cell-to-cell and cell-to-matrix signaling, inflammation, Alzheimer's disease and cancer. Glycomics/interactome research is currently undergoing rapid development as a result of recent advances in technologies for glycan structural analysis and glycan-protein interactions.

Objectives: Surface plasmon resonance (SPR) is a powerful biosensor technique used to measure biomolecular interactions in real-time and label-free ligands. SPR has been successfully used for quantitative measuring of GAGs/heparin-protein, and biological evaluation of glycotherapeutics (such as heparin and low molecular weight heparins) in our lab.

Results: This presentation reports the application of SPR in GAGs/heparin glycomics/interactome studies in two major areas: i) protein-glycan interaction: we has used SPR to identify heparin-like receptors involved in infectious diseases including dengue virus, hepatitis C virus, malaria parasite and Zika virus providing new approaches for the prevention and treatment of these devastating diseases; SPR has been used in analysis of heparin's interaction with growth factors and receptors in physiological and pathophysiological processes; ii) glyco-pharmaceutical/glycoengineering, we used SPR to assist in solving the heparin contamination crisis. The SPR method we developed provided new insights into the biochemistry behind the adverse reactions of OSCS from contaminated heparins. We also developed SPR methods for heparin related pharmaceutical process monitoring.

Conclusions: SPR is a powerful technique to measure biomolecular interactions in GAGs based glycomics/interactome research, providing important structure/activity and kinetics information.



SESSION 3

PROTEOGLYCANS IN DEVELOPMENT, AGING AND DIFFERENTIATION

CHONDROITIN/DERMATAN SULFATE IS IMPORTANT FOR MIGRATION OF DENDRITIC CELLS AND PHARMACOLOGICAL INHIBITION OF DERMATAN SULFATE MIGHT BE BENEFICIAL IN THE DISEASE MUCOPOLYSACCHARIDOSIS TYPE-I

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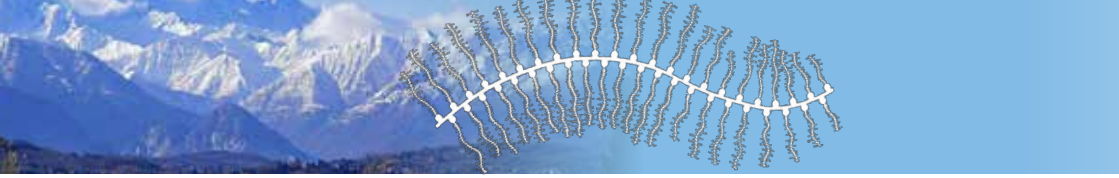
Introduction: We earlier reported in cellular models that chondroitin/dermatan (CS/DS) sulfate located at the cell surface is important for an effective migration. Further, in the in vivo model of Xenopus embryos, genetic reduction of cell-associated CS/DS resulted in alteration of migration of the neural crest cells, resulting in loss of cartilaginous head structures, lack of dorsal fin tissue, and less melanocyte. In attempt to reveal if also the highly mobile dendritic cell population in mouse would be affected in their functions, wild type mice and mice lacking the main dermatan sulfate epimerase, i.e. with reduced iduronic acid in CS/DS, were compared. CS/DS is increased in several pathological conditions as cancer and fibrosis and therefore three DS-specific biosynthetic enzymes could be a suitable drug targets. No inhibitors of these enzymes are known.

Objectives: To see the role of CS/DS in adaptive immunity. To discover inhibitors of dermatan sulfate biosynthesis.

Results: Mice were immunized in the ankle. Skin-derived dendritic cells migration to the draining lymph nodes was reduced in DS-epimerase 1-deficient mice. Consequently, the initiation of the cellular and humoral immune response was impaired, ultimately leading to a severe decrement of antigen-specific serum immunoglobulin levels.
The prospective drug ebselen, obtained after screening of a drug library in enzymatic assays, was effective in reducing DS biosynthesis in cultivated cells. We reasoned that reduction of DS could be beneficial to treat the disease Mucopolysaccharidosis type I (MPS-I), caused by deficiency of the lysosomial enzyme L-iduronidase. Treatment of MPS-I fibroblast with ebselen reduced the pathogenic accumulation of GAGs.

Conclusions: CS/DS regulates the initiation of the adaptive immune response. Ebselen decreases the cellular GAG burden of MPS-I fibroblasts, via a postulated substrate reduction mechanism.



INHIBITION OF ANGIOGENESIS BY CHONDROITIN SULFATE PROTEOGLYCANS FROM SALMON NASAL CARTILAGE

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Introducion: Cartilage lacks neurons and blood and lymphatic vessels, which led to the hypothesis that it contains substances that inhibit the growth of these tissues. Chondroitin sulfate proteoglycans (CSPGs) are major extracellular components in cartilage. CS and CSPG inhibit axonal regeneration; however, their role in blood vessel formation is largely unknown.

Objectives: To clarify whether CSPG functions in blood vessel formation, we tested salmon nasal cartilage PG, a member of the aggrecan family of CSPG, for endothelial capillary-like tube formation and vessel formation in chick chorioallantoic membrane (CAM).

Results: We observed reduction of endothelial cell growth, cell adhesion to fibronectin, and matrix metalloproteinase (MMP) expression following treatment with salmon PG. Salmon PG inhibited in vitro endothelial tube formation and in vivo angiogenesis in CAM in a dose-dependent manner. Enzymatic digestion of salmon PG revealed that the anti-angiogenic activity was derived from attached CS, but not from core protein. Sulfated CS from other sources exhibited similar anti-angiogenic activity, whereas de-sulfated CS did not.

Conclusions: Our results reveal that salmon PG inhibits not only endothelial cell growth and adhesion but also tube formation and MMP expression. Various structures in CS may be essential for its role in angiogenesis. Our findings support that CSPG and CS are anti-angiogenic factors in cartilage.

References

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ENHANCED BIOLOGICAL ACTIVITY OF BMP-2 BOUND TO SURFACE-GRAFTED HEPARAN SULFATE

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Introduction: Over the last decade there has been a growing interest in the development of new materials to improve bone morphogenetic protein-2 (BMP-2) delivery for tissue regeneration. In vivo, BMP-2 is bound to extracellular matrix (ECM) components, mostly to fibronectin (1) and heparan sulfate (2). The development of materials which are able to control BMP-2 molecular presentation through ECM components and local concentration is an essential approach for a deeper understanding of BMP-2 functions and the modulation of its biological activity (3).

Objectives: We aim to unveil the function of exogenous, surface grafted and oriented HS on BMP-2 biological activity and on the antagonistic effect of noggin.

Methods: We develop and apply model surfaces that present BMP-2 via HS. On these surfaces, HS is grafted by its reducing end (4), to mimic the natural arrangement of HS proteoglycans (HSPGs) in the ECM. The binding of each component on these biomimetic surfaces is highly controlled, in terms of stoichiometry of molecules and BMP-2/grafted-HS affinity, as determined by surface sensitive techniques. For comparison, we use surfaces presenting the same amount of BMP-2 immobilized alone. Functional validations of the surfaces are performed using a murine myoblast cell line (C2C12) and primary human mesenchymal stromal cells (hMSCs).

Results: In both cell types, HS-bound BMP-2 and surface-immobilized BMP-2 significantly prolong SMAD 1/5 phosphorylation, compared to BMP-2 added to the culture media. Moreover, BMP-2 bound to grafted HS enhances p-SMAD 1/5 levels in C2C12 cells and reduces noggin antagonistic activity on both C2C12 and hMSCs.

Conclusions: Grafted HS positively affects BMP-2 cellular activity, in vitro, suggesting a potential importance of ECM-HSPGs as regulators of BMP-2 activity. This innovative surface design, which mimics natural interactions of growth factors with ECM components, constitutes a promising candidate for future regenerative medicine applications (5).

References

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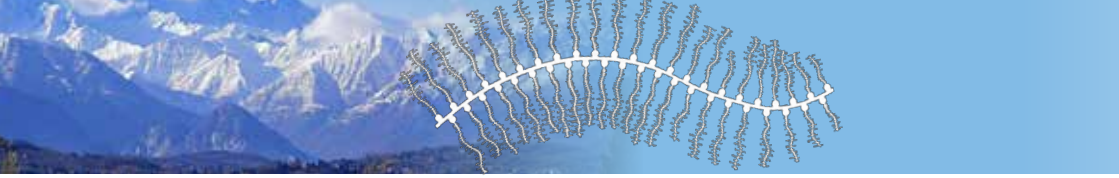

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NT4 REDUCES ANGIOGENESIS AND INVASIVENESS OF TUMOR CELLS BY BINDING TO HSPGS

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Introduction: NT4 is a branched peptide that selectively addresses cancer cells. It proved to be efficient in cancer cell internalizing and tumor tissue selective-binding. It has been successfully used, once conjugate to cytotoxic units to kill cancer cells in vitro and in vivo (1-2), and as a tracer ex-vivo and in vivo, once coupled to fluorescent probes. NT4 selectivity is due to its nanomolar affinity for heparan sulfated proteoglycans (2), HSPGs, which are also well established modulators of angiogenesis (3) and tumor invasiveness.

Objectives: We investigated which kind of effects, mediated by HSPG binding, the peptide produced on endothelial and tumor cells in terms of modulation of invasiveness and angiogenesis.

Results: HUVEC growth was enhanced by FGF2 and thrombin, as expected, and NT4 reduced this triggered-growth to the control levels, in a dose dependent way. HUVEC growth was not influenced by the treatment with heparin or NT4, but it was strongly reduced by the combined challenge of heparin and NT4. HUVEC migration was measured in a wound healing assay, where cells were plated on collagen, fibronectin, or on uncoated wells and a silicon spacer was placed immediately before cell plating. Migration on the different supports was completely inhibited by NT4. NT4 inhibits tube formation, particularly when the phenomenon is increased by FGF2 and thrombin. Thrombin is secreted from human tumor cells and promotes angiogenesis. Heparin, as expected, slightly reduced the ability of HUVEC to form tubes, and NT4, realistically by binding heparin, restored the basal levels and canceled the inhibitory effect. NT4 also inhibited collagen degradation by breast cancer cells MDA MB 231.

Conclusions: NT4 proved to reduce angiogenesis by impairing endothelial cells growth, migration and tubes formation. It also showed to decrease invasive phenotype of tumor cells by reducing migration and collagen degradation.

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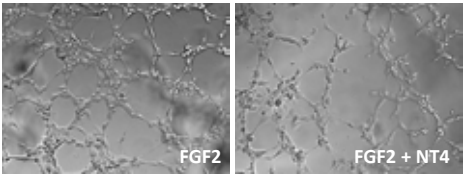
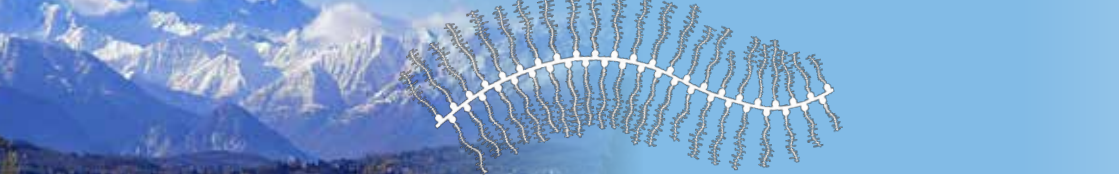
HUVEC Tubes Formation





SESSION 4

PROTEOGLYCANS INFLAMMATION AND SIGNALING

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MACROPHAGE POLARISATION TO ANTI-INFLAMMATORY STATE IN CENTRAL NERVOUS SYSTEM AFTER CHONDROITINASE ABC TREATMENT

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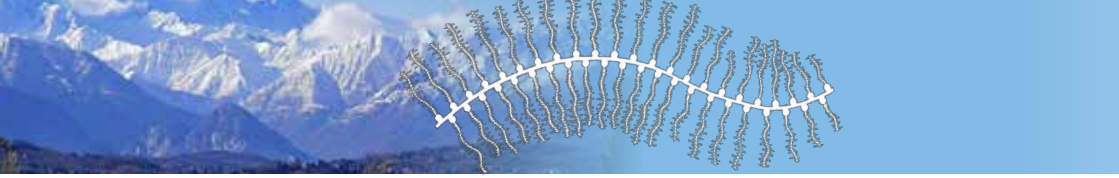
Introduction: The microenvironment of the injured spinal cord activates macrophages to acquire pro-inflammatory M1 polarization with but transient and few acquiring anti-inflammatory M2 polarization.

Objectives: Given our observation of lowered numbers of pro-inflammatory macrophages/microglia following chondroitinase ABC (ChABC) treatment of the hemisected cord in a rat model, we hypothesized that chondroitin sulfate proteoglycans upregulated in the injured environment influences the balance between M1 and M2 polarization states.

Results: To test this, macrophages were derived from collections of bone marrow from adult rats and stimulated with lipopolysaccharide to trigger transition to the pro-inflammatory state. Following acute treatment of the cultures with ChABC versus vehicle control, RNA was recovered from the macrophage cultures for RT-PCR analysis of expression of polarization markers. Such M1 markers as inducible nitric oxide synthase and CD86 were found to be lowered in the ChABC-treated samples. In contrast, such M2 markers as arginase-1, CCL22 and IL10 were found to be higher. Experiments with the rat model revealed that ChABC treatment of the injured cord environment resulted in increased level of the IL10 transcript as compared to the vehicle control.

Conclusions: The results so far support the hypothesis and add impetus to find strategies that shift the M1:M2 polarization as a therapeutic goal.



HEPARAN SULFATE IN RENAL FIBROSIS: WHAT ROLE FOR THE 3-O-SULFOTRANSFERASES?

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For patients with renal failure, kidney transplantation remains by far their best option. Unfortunately following transplantation, the graft can undergo remodelling and become dysfunctional. Heparan Sulfate (HS) and its sulfation patterns participate in the early processes leading to inflammation and fibrosis.

We aim to understand the role of Heparan Sulfate 3-O sulfotransferases (HS3ST) in renal fibrosis and hypothesised that enzyme expression as well as changes in HS structure in the extracellular matrix and at the cell membrane could relate to the level of fibrosis. We studied how HS 3-O-sulfotransferases and sulfatases are modulated by fibrotic factors in vitro and in in vivo using a mouse model of kidney fibrosis.

We observed a significant increase of HS3ST1 expression at 5, 10 and 15 days after Unilateral Ureteral Obstruction in C57BL/6 mice ($p < 0.01$). Additionally, HS3ST3A was downregulated at D5 ($p < 0.05$) and Sulf1 upregulated at D5 ($p < 0.05$). Amongst all HS3STs isoforms, HS3ST1 was the most predominant isoform in primary tubular epithelial cells. HS3ST1 expression was significantly decreased in a renal epithelial cell line (HK2) treated with TGFβ1 (5 ng/mL) and IL1β/TGFβ2 (10 ng/mL) ($p < 0.001$). Renal epithelial cells overexpressing HS3ST1 (R-HS3ST1) and their corresponding mock transfectants (R-CTL) showed no difference in FGF2 signalling. However, R-HS3ST1 demonstrated a different pattern of HB-EGF signalling compared to R-CTL.

In conclusion, Sulf1 levels increase in fibrosis and the expression levels of HS3STs change in response to pro-inflammatory factors and in a renal disease mouse model. In the kidney, HS3ST1 expression seems to relate to the level of fibrosis. These changes could lead to an alteration in cytokine binding and cell signalling. HS 3-O sulfation might be associated with HB-EGF signalling and our current work, including determining the level of HS 3-O sulfation, is examining this possible association.

DETERMINATION OF THE BINDING INTERACTION BETWEEN PROTEOGLYCAN RECEPTORS AND FIBRONECTIN USING DYNAMIC SINGLE MOLECULE FORCE SPECTROSCOPY

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Introduction: Members of the Toll-like and Interleuken-1 Receptor family are central mediators of inflammatory responses. The Interleukin receptor type I (IL-1RI) co-receptor TILRR amplifies NF-kB activation, which controls development of atherosclerosis. TILRR is a cell surface heparan sulfate proteoglycan (HSPG), which associates with the IL-1RI to control signal amplification and is found at focal adhesions. TILRR control of IL-1RI function depends on its glycosaminoglycan (GAG) chain associating with the extracellular matrix component fibronectin (Fn).

Objectives: This study concerns the measurement of binding strength between TILRR and fibronectin, estimating both the dissociation constant (K) and the thermodynamic energy of adhesion (ΔG).

Results: As it has not been possible to obtain an isolated molecular form of TILRR syndecan-4 (SDC4), a physiologically similar cell HSPG was used as a substitute. For comparison decorin (DCN), a Fn binding cell surface PG containing chondroitin and dermatan sulphate chains, and integrin α5β1, a non-PG protein dimer and the primary receptor for Fn, were also measured. Data were collected using dynamic single molecule force spectroscopy (SMFS) by varying the speed of detachment (loading rate). SFMS results from which K and ΔG were deduced are shown in Figure 1.

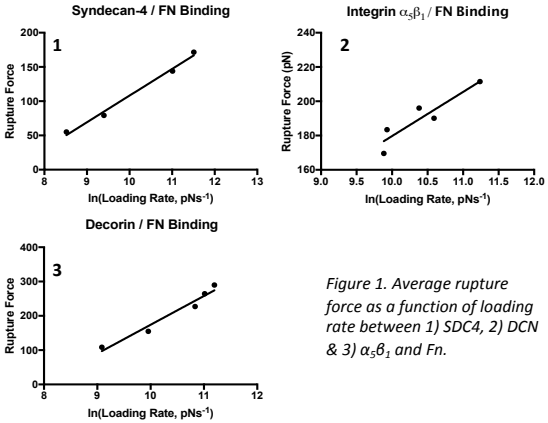
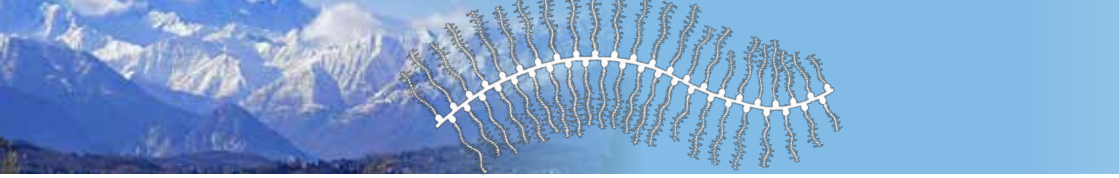


Figure 1. Average rupture force as a function of loading rate between 1) SDC4, 2) DCN & 3) $\alpha_5\beta_1$ and Fn.

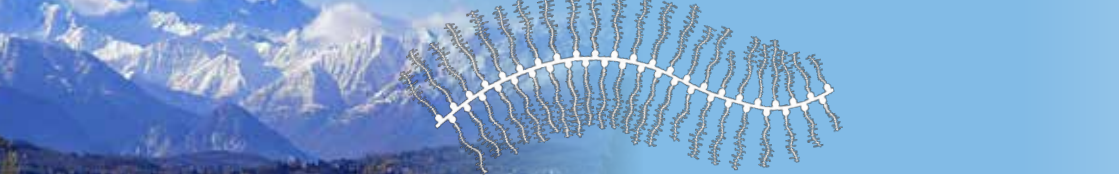
Conclusion: K and ΔG between SDC4, DCN & α5β1 with Fn have been estimated as 35.1



s-1 and 19.5 kBT, 32.9s-1 and 19.5 kBT, and 0.79 s-1 and 23.3 kBT respectively. The measured binding between Fn and each PG receptor is almost identical indicating similar adhesive strength between the three GAG variants. $\alpha 5\beta 1$ was found to form a much stronger ligand-receptor bond with Fn than either PG.

SESSION 5

PROTEOGLYCANS IN STEM CELLS AND THERAPEUTICS



SYNTHETIC PROTEOGLYCAN MIMETICS FOR STEM CELL SURFACE ENGINEERING

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Introduction: Cell surface proteoglycans mediate association of growth factors with their receptors and are, thus, key regulators of growth factor signaling during stem cell differentiation. Glycan engineering at the stem cell-matrix interface may offer a strategy for controlling differentiation post transplantation to enhance tissue repair. However, the lack of tools for controlling glycosylation has greatly limited progress in this area.

Objective: We set to developed synthetic nanomaterials that emulate the architecture and function of cell-surface proteoglycans, engage members of the fibroblast growth factor family and can be introduced to the surfaces of stem cells to promote neural differentiation in murine embryonic stem cells.

Results: This presentation will introduce the synthesis of polyacrylamide based proteoglycan mimetic glycopolymers and their screening in a glycan array format to identify materials with affinity for the fibroblast growth factor 2 and bone morphogenetic protein 4. Functionalization of these materials with lipid anchors enabled their incorporation into membranes of exostosine 1 negative murine embryonic stem cells, where they activated signaling through the MAPK and SMAD pathways and induced differentiation into neuroectoderm and mesoderm, respectively.

Conclusions: Our work demonstrates that synthetic proteoglycan mimetics can be rapidly assembled and rapidly evaluated for their ability bind growth factors. As well, we have developed a general cell-surface engineering strategy that allows for the introduction of these materials into the membranes of living cells, where they could be used to influence the outcome of cellular differentiation.

PERLECAN PROMOTES ENHANCED VASCULARIZATION ON IMPLANTED BIOMATERIALS

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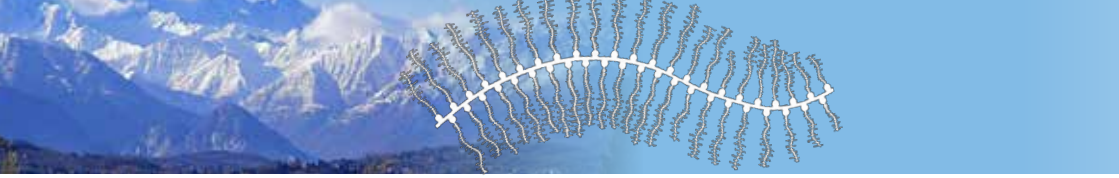
Biomaterials and bioengineered tissues offer a promising solution for the replacement and regeneration of tissues damaged through injury or disease, but their clinical utility has been hampered by the lack of sufficient and timely vascularisation.

The goal of this project is to exploit the biological properties of perlecan (PN), a major extracellular matrix proteoglycan involved in vascular development and homeostasis, to engineer novel biomaterials that mimic the vascular niche and thus promote enhanced vascularization. Perlecan, through its N-terminal heparan sulphate (HS) chains, supports angiogenesis via binding and signaling key vascular growth factors. The C-terminal region is of particular interest in vascular research as it contains the endothelial cell $\alpha 2\beta 1$ integrin-binding site and can be substituted with glycosaminoglycan chains for growth factor delivery.

3D porous silk scaffolds were functionalized with either endothelial cell-derived PN or a recombinant C-terminal region (Leu3626 to Ser4391), which contains domain V and is expressed as a proteoglycan decorated with HS or chondroitin sulphate (DV). Silk biomaterial integration and vascularization was examined by subcutaneous implantation in mice. MRI imaging following contrast agent injection via a tail-vein catheter demonstrated the perfusion of the contrast agents into the scaffolds, indicating the presence of functional, perfused vasculature in the scaffolds. Light sheet imaging following lectin perfusion further confirmed vascular infiltration. Finally, histological examination of explanted scaffolds demonstrated cell infiltration to promote scaffold integration with the surrounding tissue. Scaffold functionalization with PN or DV resulted in an increased number of perfused vessels per unit area compared to silk alone.

Taken together, this study suggests that PN/DV functionalized silk scaffolds present new, promising bioactive biomaterials for use in tissue engineering and in regenerative medicine.



SESSION 6

PROTEOGLYCANS IN PHYSIOLOGICAL SYSTEMS

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THE BRAIN-SPECIFIC LINK PROTEIN, BRAL2, IS A SELECTIVE REGULATOR FOR FORMATION AND TRANSMISSION OF GABAERGIC SYNAPSES BETWEEN PURKINJE AND DEEP CEREBELLAR NUCLEI NEURONS

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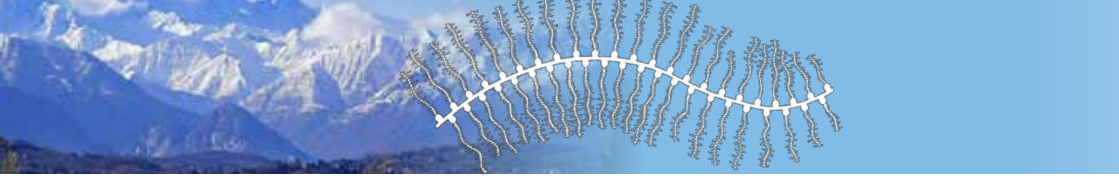
Introduction: The sole output of the cerebellar cortex is conveyed to the deep cerebellar nuclei (DCN) by Purkinje cells (PCs). The DCN neurons are enwrapped by densely organized extracellular matrix structures, called perineuronal nets (PNNs). PNNs are typically displayed around fast-spiking gamma-aminobutyric acid (GABA)ergic interneurons expressing parvalbumin, but interestingly also exist at other neurons such as the neurons in the DCN and medial nucleus of the trapezoid body, which are the postsynaptic neurons of large axo-somatic synapses adapted for fast-signaling. This characteristic localization prompted the hypothesis that PNN might play a role in the maintenance and formation of fast-signaling and large synapses.

Objectives: To elucidate the role of the PNN in those synapses, we investigated the electrophysiological and morphological properties of DCN synapses in the hyaluronan and proteoglycan binding link protein 4 (Hapln4/Bral2) knockout (KO) mice around postnatal day 14. Hapln4/Bral2 is important for PNN structure, as it stabilizes the interaction between hyaluronan and proteoglycan.

Results: Here we show that the Hapln4/Bral2 localized closely with the PC terminals by immunohistochemistry. In the DCN neuron of Hapln4/Bral2 KO mice, the inhibitory synaptic strengths were reduced as compared to wildtype mice, whereas the properties of the excitatory synapses were unaffected. Moreover, Hapln4/Bral2 deficiency caused reduced GABAergic presynaptic terminals of PCs in the DCN.

Conclusions: These results demonstrate that Hapln4/Bral2 is a PNN component that selectively contributes to formation and transmission of PC-DCN synapse in the cerebellum.



TRANSMEMBRANE 165: A NEW PLAYER IN PROTEOGLYCAN SYNTHESIS

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Congenital Disorders of Glycosylation (CDG) are severe inherited diseases in which aberrant protein glycosylation is a hallmark. Transmembrane protein 165 (TMEM165) is a novel Golgi transmembrane protein involved in type II CDG. The TMEM165-CDG patients harbour a peculiar phenotype of major skeletal dysplasia presenting joint anomalies and pronounced dwarfism. Although it has been shown that TMEM165 mutation causes a CDG, sole the N-glycosylation has been studied. To understand the molecular mechanisms by which TMEM165 deficiency may affect skeletal development and bone formation, we investigated whether the lack of TMEM165 impairs the synthesis of proteoglycans. Indeed, proteoglycans play a crucial role in the regulation of several signaling pathways involved in chondrogenesis. To this aim, we generated cell lines that were knock-down for TMEM165 using CRISPR Cas9 technique and discovered that TMEM165 is critical for the biosynthesis of proteoglycans. Next, we explored the levels of activation of different signaling pathways and found significant differences between wild-type and TMEM-165-deficient cells. These results suggest that TMEM165 mutations may affect chondrogenesis and skeletal development through alteration of the proteoglycan synthesis process.

NG2/CSPG4 CONTROLS MALIGNANCY THROUGH ACTIVATION OF CROSS-TALKING SIGNALLING PATHWAYS

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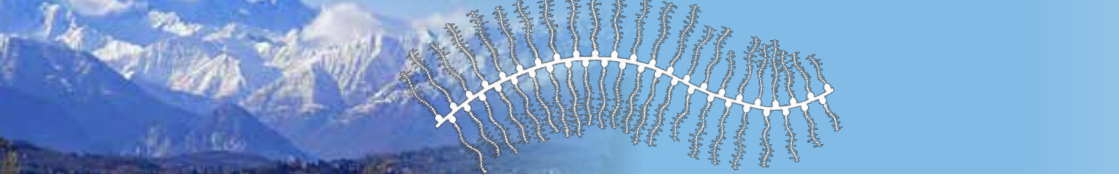
Introduction: NG2/CSPG4 transmembrane proteoglycan (PG) is widely known to promote cancer progression through multivalent interplays with the ECM, ECM-degrading enzymes, soluble signalling molecules, cell surface receptors for these ligands and activation of FAK-Rac/cdc42-PKC-ERK and PI3K-Akt-1-mTOR cell survival pathways.

Objectives: Further elucidate the molecular mechanisms whereby NG2/CSPG4 confers to cancer cells a highly malignant phenotype.

Results: Immunosorted NG2/CSPG4$^{+/-}$ sarcoma and melanoma cells, in which full-length or truncated versions of the PG stably expressed, alongside with cells harbouring stable RNAi-abrogation of NG2/CSPG4 were comparatively assayed for their tumorigenic behaviour in vitro and in immunodeficient mice. Gene expression profiling was performed by DNA microarray, while signal transduction pathways activated in NG2/CSPG4^{+} vs NG2/CSPG4^{-} tumour masses were examined by global phospho-proteomics. NG2/CSPG4^{+} cells were more malignant than NG2/CSPG4^{-} ones, independently of their proliferation status, or the degree of intra-lesional angiogenesis, but showing a significantly lower apoptosis score. Comparative gene profiling in NG2/CSPG4^{+} vs NG2/CSPG4^{-} cells did not highlight upregulation of known tumourigenesis-promoting genes. NG2/CSPG4 promotes tumour nodule-formation by increasing Ca^{2+}-independent cellular adhesion. Augmented cell cohesiveness and enhanced tumour growth is associated with an increased IGF-signalling response, as shown by enhanced phosphorylation of Jak1 and IRS and discrete PKCs, alongside with enhanced phosphorylation of PTEN, mTOR and Akt1.

Conclusion: The findings contribute to a better understanding of the molecular bases for the tumour growth-promoting activity of NG2/CSPG4.



SESSION 7

PROTEOGLYCANS IN PATHOPHYSIOLOGY

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LUMICAN EFFECTIVELY REGULATES THE ESTROGEN RECEPTORS-ASSOCIATED FUNCTIONAL PROPERTIES OF BREAST CANCER CELLS, EXPRESSION OF MATRIX EFFECTORS AND EPITHELIAL-TO-MESENCHYMAL TRANSITION

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Introduction: Lumican is a small leucine-rich proteoglycan that has been shown to contribute in several physiological processes, but also to exert anticancer activity. On the other hand, it has been recently shown that knockdown of the estrogen receptor α (ERα) in low invasive MCF-7 (ERα+) breast cancer cells and the suppression of ERβ in highly aggressive MDA-MB-231 (ERβ+) cells significantly alter the functional properties of breast cancer cells and the gene expression profile of matrix macromolecules related to cancer progression and cell morphology.

Objectives: In this report, we evaluated the effects of lumican in respect to the ERs-associated breast cancer cell behaviour, before and after suppression of ERs, using scanning electron and confocal microscopies, qPCR and functional assays.

Results: Our data pinpointed that lumican significantly attenuated cell functional properties, including proliferation, migration and invasion. Furthermore, it modified cell morphology, inducing cell-cell junctions, evoked EMT/MET reprogramming and suppressed the expression of major matrix effectors (matrix metalloproteinases and EGFR) implicated in breast cancer progression.

Conclusions: The effects of lumican were found to be related to the type of breast cancer cells and the ERα/β type. These data support the anticancer activity of lumican and open a new area for the pharmacological targeting of the invasive breast cancer.

EHLERS-DANLOS SYNDROME CAUSED BY MUTATIONS IN CHST14/D4ST1 RESULTS IN DEFECT OF URINARY DERMATAN SULFATE

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Objective: Dermatan sulfate (DS) plays a number of roles in various biological activities including cell signaling and tissue morphogenesis through interactions with a variety of extracellular matrix proteins such as collagen. An autosomal recessive connective tissue disorder, Ehlers-Danlos syndrome musculocontractural type 1, is caused by mutations in carbohydrate sulfotransferase 14 gene (CHST14) encoding CHST14/dermatan 4-O-sulfotransferase-1 (D4ST1), and is characterized by congenital malformations (specific craniofacial features, and congenital multiple contractures) and progressive fragility-related complications (skin hyperextensibility, bruisability, and fragility with atrophic scars; recurrent dislocations; progressive talipes or spinal deformities; and large subcutaneous hematomas). CHST14/D4ST1 is responsible for the biosynthesis of DS. In an attempt to develop a diagnostic screening method for the various types of Ehlers-Danlos syndrome, the amount of DS in the urine of patients was examined.

Methods: Urinary DS was quantified by an anion-exchange chromatography after treatment with DS-specific degrading enzyme, chondroitinase B.

Results: DS was not detected in the urine of patients with homo- or compound heterozygous mutations in CHST14/D4ST1. These results indicate that the quantitation of urinary DS is applicable to an initial diagnosis of DS-defective EDS.

Conclusions: This is the study to show a urinary DS chains in patients with Ehlers-Danlos syndrome caused by a CHST14/D4ST1 deficiency, and demonstrated the lack of DS chains. This result indicates systemic DS depletion in DS-defective Ehlers-Danlos syndrome, and also proposes the usefulness of a urinary DS chains as a non-invasive screening method for this disorder.

A DROSOPHILA MODEL OF MULTIPLE SULFATASE DEFICIENCY SHOWS DISRUPTION OF AUTOPHAGY REGULATION

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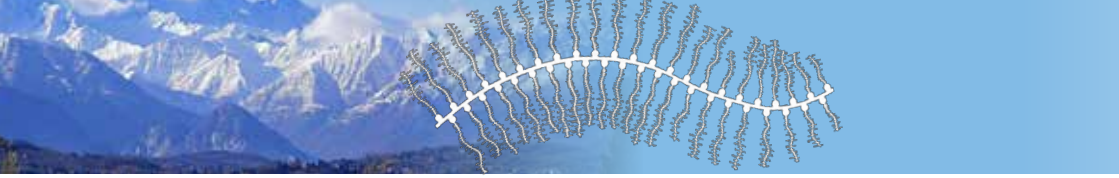
Introduction: The failure to degrade heparan sulfate via the lysosomal system has severe developmental consequences, affecting virtually all organs but with particularly dramatic and tragic effects in the nervous system and bone. Multiple sulfatase deficiency (MSD) is one mucopolysaccharidosis resulting from a defect in a sulfatase-activating enzyme that catalyzes the conversion of an active-site cysteine residue to formylglyine, a feature critical for sulfatase activity. The clinical severity and course is predicted by the level of formylglycine activity, confirming the critical nature of this sulfatase-activating step (1, 2).

Objectives: We wished to devise a Drosophila model of MSD by creating a null mutation in sumf, a homolog of the sulfatase-activating-encoding gene found in vertebrates. Earlier work has demonstrated that loss of heparan sulfate synthesis or sulfation produces an increase in autophagy (3), a critical cellular function involved in mitochondrial turnover and removal of ubiquitinated proteins. We therefore tested the hypothesis that loss of HS degradation (defects in sulfatase activation) leads to a deficiency in autophagy, an outcome that could readily lead to the progressive and severe course of MSD. Autophagy governs a number of cellular processes, including responses to oxidative stress and the levels of ubiquitinated proteins destined for destruction in the lysosomal system. We determined the sensitivity of animals to oxidative stress mediated by exposure to H_2O_2 and the levels of ubiquitinated proteins in the brain both during ageing and upon exposure to oxidative stress using an anti-ubiquitin antibody.

Results: Adult animals bearing mutations in sumf are viable but show wing phenotypes indicative of a defect in sulf function, namely disruption of Wingless signaling mediated by the secreted sulfatase encoded by sulf. This is consistent with the critical role of sumf in sulfatase activation and supports the conservation of this function in Drosophila. To evaluate the role of sumf in autophagy we examined the levels of ubiquitin-modified proteins in adult brains. sumf mutants show elevated levels of ubiquitin-modified proteins as a function of ageing compared to control animals. Autophagy also influences the capacity of animals to resist oxidative stress and sumf mutants show heightened sensitivity to H_2O_2 exposure, consistent with the hypothesis that these animals have reduced levels of autophagy. This is in contrast with animals heterozygous for mutations in HS biosynthetic genes (ttv, sf), which show increased tolerance to oxidative stress compared to wild type controls. The increase in sensitivity to H_2O_2 exposure is accompanied by increases in the level of ubiquitin-modified proteins in the brains of sumf mutants.

Conclusions: These findings suggest that sumf is required for the activation of sulfatases in Drosophila and for the normal regulation of autophagy. sumf mutants provide a model system to determine the molecular physiology of MSD and the degree to which disruption of HS degradation contributes to misregulation of autophagy.

References



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SESSION 8

PROTEOGLYCANS IN LOCOMOTOR SYSTEM AND NEURAL BIOLOGY

GLYPICAN-6 REGULATES THE EMBRYONIC DEVELOPMENT OF LONG BONES, INTESTINE, BRAIN AND EYES

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Introduction: Glypicans regulate several signaling pathways that play critical roles in development. Loss-of-functions mutations of GPC6 cause recessive omodysplasia (OMOD1), a genetic condition characterized by short stature, shortened limbs, and facial dysmorphism.

Objectives: To investigate the role of Glypican-6 (GPC6) in embryonic development.

Results: GPC6-null mice die at birth. To investigate the role of GPC6 in development we used E15 to E18 embryos. Here we show that GPC6-null embryos display most of the abnormalities found in OMOD1 patients, and that Hedgehog (Hh) signaling is strongly reduced in the long bones of these embryos. The Hh-stimulatory activity of GPC6 was also observed in cultured cells, where this glypican increased the binding of Hh to Patched-1. Consistent with this, GPC6 interacts with Hh through its core protein and with Patched-1, through its glycosaminoglycan (GAG) chains. Hh signaling is triggered at the primary cilium. In the absence of Hh, we observe that GPC6 is localized outside of the cilium, but moves into the cilium upon the addition of Hh. Notably, a mutant GPC6 that lacks the GAG chains cannot be induced to migrate into the cilium.

Surprisingly, GPC6-null embryos display a dramatic (70%) reduction in the length of the intestine. Similar reduction of intestinal elongation has been observed in mice with deficient Hh signaling or non-canonical Wnt signaling.

GPC6 promotes the formation of excitatory synapses, but GPC6-null mice do not display obvious developmental abnormalities in the nervous system. Because Glypican-4 (GPC4) also regulates synapses formation, we tried to generate GPC6/GPC4 double knockouts. Unfortunately, these mice die at an early developmental stage. Notably, GPC4-null/GPC6 heterozygote embryos display dramatic abnormalities in brain and eyes, including cyclopia, which has also been observed in Sonic Hh-null mice.

Conclusions: GPC6 plays a critical role in the development of bone, intestine, brain and eyes.

CHONDROCYTE COMPENSATE AND ALTERED HS STRUCTURE BY DISTINCT CHANGES IN HS AND CS

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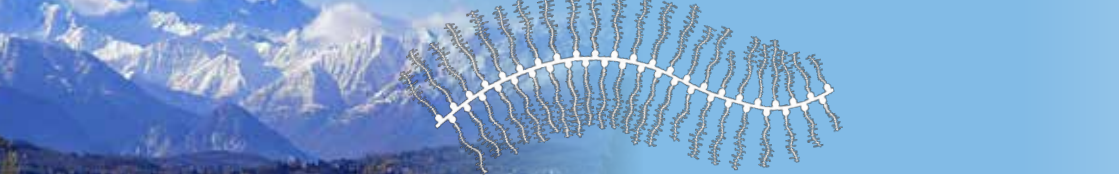
Introduction: Embryonic bone development is regulated by several secreted molecules including Indian hedgehog (Ihh). Several lines of evidence indicate that the distribution and activity of Ihh is dependent on the level and sulfation pattern of Heparan sulfate (HS). Like the closely related Sonic Hedgehog protein, Ihh contains two HS binding sequences, one classical Cardin-Weintraub (CW) motif on the N-terminal arm and a second non-continuous on the globular domain. Nevertheless, an altered HS structure results in relatively mild cartilage phenotypes indicating compensation of HS functions by an altered glycosaminoglycan (GAG) composition.

Objectives: Here we analyze how alterations in the HS binding motifs and the HS sulfation pattern modulate the Ihh/HS interaction and Ihh multimer size. Based on our results, we investigate how changes in the HS pattern alter the GAG composition in chondrocytes.

Results: We found that both HS binding motifs distinctly affect Ihh multimerization and HS-binding in vitro. Surprisingly, although the Ihh multimer size depends on the HS interaction in vitro, an altered HS sulfation pattern seems to be uncritical for the multimer size in chondrocytes in vivo. Based on these results and the relatively mild skeletal phenotypes of HS mutants we analyzed the GAG composition in chondrocytes of $Ext1^{gt/gt}$ mice, which carry reduced HS levels. By immunofluorescence, disaccharide analysis and size exclusion chromatography we found a strong upregulation of CS in mutant chondrocytes. Furthermore, analysis of HS2st mutants, which carry an altered sulfation pattern, revealed that not only reduced HS levels, but also an altered sulfation pattern is compensated by distinct alterations in HS and CS.

Conclusions: Our data demonstrate that chondrocytes react to an altered HS structure not only by a distinctly altered HS synthesis, but also adapt the CS structure accordingly.



GLYPICAN REGULATES SYNAPTIC PLASTICITY AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

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Introduction: The fruit fly, Drosophila, neuromuscular junction (NMJ) is a genetically tractable model of glutamatergic synapses widely used to clarify the molecular mechanisms of synapse formation and plasticity. Recently, it has been demonstrated that, under food deprivation conditions, Drosophila larvae show increases of locomotor activity and synaptic boutons at the NMJ. Such increases depend on octopaminergic innervation of body wall muscles, which is the invertebrate counterpart of mammalian adrenergic system. However, the molecular mechanism by which octopaminergic signaling regulates these behavioral and synaptic plasticities remains elusive.

Objectives: To reveal the roles of heparan sulfate proteoglycans (HSPGs) in synaptic plasticity, we focused on the functions of Drosophila glypican, Dally-like (Dlp), at NMJ.

Results: We found that postsynaptic levels of Dlp at NMJ increased by octopaminergic signaling after food deprivation. In order to examine the significance of such change of Dlp expression, we knocked down dlp in the body wall muscles using GAL4-UAS system. Under normal feeding conditions, knockdown of dlp did not affect crawling behavior. However, locomotor activity and synaptic boutons at NMJ did not increase in the dlp RNAi animals after food deprivation. Furthermore, we found that food deprivation increased the presynaptic BMP signaling and altered the postsynaptic glutamate receptor composition in the NMJ of control animals, both of which were dysregulated in dlp RNAi animals.

Conclusions: Our results suggested that Dlp regulates octopamine-mediated synaptic and behavioral plasticity by modulating the functions of synaptic proteins such as glutamate receptors and BMP.

NEURONAL PENTRAXIN 2 BINDS TO THE PERINEURONAL NETS VIA HYALURONAN

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Introduction: Perineuronal nets (PNNs) are mesh-like structures which form at the end of the critical period for plasticity, in the extracellular matrix of sub-populations of neurons. The PNNs consist of a backbone of hyaluronan (HA) which is linked to a variety of chondroitin sulfate glycosaminoglycans. However, the mechanisms by which PNNs interact with synapses are poorly understood.

Objectives: In this project, I aim to investigate a newly identified binding partner of PNNs, Neuronal Pentraxin 2 (NPTX2) using both molecular biology and microscopy methods. I applied both modified glycan ELISA and Quartz Crystal Microbalance with Disspation (QCM-D) to investigate the binding properties and I investigated the localization of NPTX2 with primary cortical neuronal cultures and fluorescence microscopy.

Results: With the use of modified glycan ELISA, we observed a strong binding of NPTX2 to HA and chondroitin sulphate E, but not to other PNN glycans. QCM-D investigation of the binding of NPTX2 and HA displayed the binding is reversible. In cultured neurons NPTX2 co-localizes with the PNN. When NPTX2 virus is injected in the brain, NPTX2 is located in PNN bearing neurons.

Conclusion: NPTX2 is a binding partner of the PNN. Because NPTX2 and the PNN are both regulators of synaptic plasticity, our results indicate that PNNs may control synaptic plasticity through the binding of NPTX2. Further experiments are necessary to investigate the potential of NPTX2 as a mediator of the effects of the PNNs.

SESSION 9

PROTEOGLYCANS IN CANCERS

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EXTENSIVE DOWNREGULATION OF ANTICOAGULANT HEPARAN SULFATE IN INVASIVE FORMS OF ENDOMETRIOID CARCINOMA

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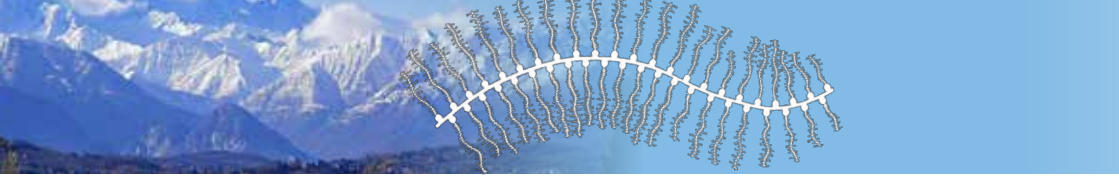
Introduction: Anticoagulant heparan sulfate (aHS) are synthesized by endothelial cells, they bind antithrombin (AT) and promote anticoagulant and anti-inflammatory activity. aHS are also present in endometrial glands but they disappear during the tissue remodelling occurring in the implantation window. Endometrioid adenocarcinoma is the most frequent gynaecologic cancer with a 5-year survival rate of 70 %, stagnant since 30 years.

Objectives: We postulate that tissue remodeling suppresses aHS to facilitate invasion of target tissue. Thus aHS could serve as marker of tissue integrity useful to identify highly invasive endometrial carcinoma. Understanding the mechanism of aHS downregulation through Heparanase (HPSE) degradation will allow us to design a strategy to limit tumorigenesis.

Results: aHS and HPSE expression were analysed on tissue sections of 96 grade 1 human endometrioid adenocarcinoma cases by AT-binding labelling and IHC.
Strong aHS labelling was found in 32% of tumor cases, vs 100% of controls and HPSE labelling was found in 44% of tumor cases, vs 25% of controls. Further, we evaluated if the distribution of aHS and HPSE is specific to the patterns of myometrial invasion. HPSE was strongly positive in all patterns while aHS showed extensive downregulation in the most aggressive patterns: Infiltrating glands and MELF, characterized by widely dispersed tumor glands and cancer cell detachment. Increased distance between tumor front and the first aHS labelled capillary in these patterns reflect the ability of the tumor to remodel its environment to allow invasion.

Conclusions: Tumor progression is related to aHS downregulation with a predominance in stongly invasive tumor phenotypes, conditions allowing cell invasion and tumor angiogenesis. Increased expression of HPSE suggest an involvement in the modulation of aHS. In vitro cell migration, invasion and angiogenesis analysis under aHS treatments and HPSE inhibitors will evaluate their therapeutical potential.



THE ROLE SYNDECAN-1 AND DECORIN AND IN HEPATOCELLULAR CANCER

Kornelia Baghy[1], Eszter Regos[1], Zsolt Horváth[1], Kristóf Rada[1], Ilona Kovalszky[1]

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Introduction: Proteoglycans are composite macromolecules built up by a core-protein to which glycosaminoglycan chain(s) are covalently attached. They fulfill versatile roles from being structural components to regulation of cellular functions. Their distinguished position in cancer biology is already indicated by the observation that their amount and composition is subject to dramatic changes during the events of malignant transformation.

Objectives: The aim of our work was to clarify mechanisms as syndecan-1 and decorin are implicated in the development and progression of liver cancer. To this end surgically removed hepatocellular carcinomas were collected, as well as in vitro hepatoma models were studied. Particular PGs expression was determined by immunohistochemistry followed by semi quantitative or quantitative densitometry. For functional analysis hepatoma cells were transfected with syndecan-1 or treated with decorin.

Results: Quantitation of immunostaining of syndecan and decorin revealed, that increase in syndecan expression is much more related to the cirrhotic remodeling of liver parenchyma than to the malignant transformation, and the amount of decorin is low in the tumor tissue. The amount of syndecan-1 on the surface of cancer cells depends on the shedding of extracellular domain and inhibition of shedding induced cell differentiation, partly by hindering the action of Ets1 and AP1 transcription factors. In spite of cirrhotic remodeling the expression of decorin decreases in the tumorous stroma. Conditioned medium of hepatoma cells downregulated decorin production of myofibroblasts. Considering the potential of decorin to inhibit tyrosin kinase receptor activities of tumor cells this downregulation favours tumor progression.

Conclusion: Both syndecan-1 and decorin play active role in the regulation of hepatocellular cancer behavior, by inhibiting cellular factors critical in the cell proliferation and invasion.

TUMOR CELL SURFACE PROTEOGLYCANS AND MANGANESE AS PARTNERS IN TUMOR PROGRESSION: FROM CELL MIGRATION TO PREMETASTATIC NICHE FORMATION

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Metastasis starts with the acquisition of an invasive phenotype by tumor cells; in parallel, the tumor secretes molecules into the bloodstream that will regulate the formation of premetastatic niches, which will be further populated by migrating tumor cells. Migration via integrin activation by cell surface heparan sulfate proteoglycans (PGs) has been linked to tumor progression. Integrins are cation-dependent molecules, whereas manganese (Mn) promotes integrin activation. Our hypothesis is that Mn exerts direct and indirect effects on tumor cells, involving the binding to PGs and integrins, and the transport to distant organs by exosomes.

This project investigates if Mn is a relevant element for tumor progression, and if exogenous glycosaminoglycans (GAGs) affect Mn binding to tumor cells.

We have investigated the effect of Mn on tumor cells and if exogenous GAGs, such as heparin (Hep) and dermatan sulfate (DS) are able to capture Mn from the tumor cells and control its effects on migration and invasion. Our data shows that Mn may modulate cell migration, and this modulation can be affected by exposure to exogenous GAGs. We also revealed that Hep, DS and chondroitin sulfate are able to bind Mn, and while conditioned media does not present detectable differences in Mn content, microvesicles present high Mn content, indicating that these structures may exert systemic effects in vivo. Finally, we have analyzed mice samples and detected time-dependent differences in Mn distribution within primary tumors, liver and bone marrow, while peripheral blood remains unchanged.

We conclude that Mn, in association with cell surface PGs, such as syndecan-1, promote cell migration, and tumor cell-derived microvesicles may carry this element systemically, reaching distant sites, especially the bone marrow, stimulating the formation of premetastatic niches.

INHIBITION OF HYALURONAN SYNTHESIS BY 4-METHYLUMBELLIFERONE ALTERS BREAST CANCER CELL FUNCTIONAL PROPERTIES

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Introduction: Previous studies have demonstrated that inhibition of hyaluronan (HA), a major extracellular matrix (ECM) heteropolysaccharide, suppresses the tumorigenicity of various malignant tumors including breast cancer. 4-methylumbelliferone (4-MU) has been reported to specifically inhibit HA synthesis in several cell types. However, very few studies have focused on the effects of HA inhibition by 4-MU in breast cancer cells.

Objectives: In this study, we investigated the effects of 4-MU on HA synthesis, certain ECM components synthesis/activity as well as the functional properties of breast cancer cells of different estrogen receptor (ER) status (ERα+/ERβ- low-invasiveness MCF-7 and ERα-/ERβ+ highly aggressive MDA-MB-231 cells).

Results: Immunofluorescence analysis showed strong staining for cell associated HA in MDA-MB-231 (ERα-/ERβ+) cells in contrast to the negligible HA amounts found in MCF-7 (ERα+/ERβ-) cells. Treatment of MDA-MB-231 cells with 4-MU significantly reduced intracellular HA, while it caused an actin cytoskeleton remodeling, as well as a loss of lamellipodia and their spindle-like morphology. 4-MU treatment also significantly inhibited cell growth, motility and invasive capacity of ERα-/ERβ+ MDA-MB-231 cells in a time- and dose-dependent manner. On the other hand, a substantial increase in apoptosis was observed in 4-MU-treated MCF-7 cells. Interestingly, 4-MU markedly changed the transcripts coding for the three HA synthases (HAS1, 2, and 3) as well as the expression and activity of certain matrix metalloproteinases (MMPs) and components of the plasminogen activation system in ERα-/ERβ+ MDA-MB-231 cells.

Conclusions: These data suggest that 4-MU might represent a promising therapeutic candidate for specific breast cancer subtypes with regard to the ER status, a major classification and predictive marker in breast cancers, via suppression of HA synthesis and accumulation, and regulation of matrix-degrading enzymes and inflammatory mediators.

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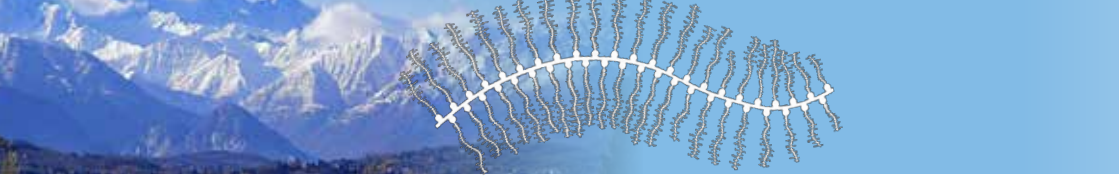
SESSION 10

PROTEOGLYCANS IN

VASCULAR SYSTEM AND TRAFFICKING



INHIBITORY EFFECTS OF HEPARIN ON NEUREGULIN-1β-MEDIATED SIGNALING IN CARDIOMYOCYTES

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Introduction: Heparin, a highly sulfated glycosaminoglycan (GAG), is often used in clinics for its anticoagulant property especially in cases of coronary artery disease. However, recently, there is increasing number of studies which highlighted the complications that arises using heparin such as heparin induced thrombocytopenia, osteoporosis and even severe bleeding. Patients suffering from coronary artery disease would upregulate several growth factors in an attempt for the heart to repair itself. One of the important pro-survival factors being upregulated is Neuregulin-1β (NRG-1β). NRG-1β is a cardiomyocyte pro-survival growth factor that belongs to the epidermal growth factor (EGF) family. It is expressed in cardiac microvascular endothelial cell. NRG-1β binds to the erythroblastic leukemia viral oncogene homolog (ErbB) receptors found on cardiomyocytes and it has been shown to allow cardiomyocyte cell cycle entry, prevent cardiomyocyte apoptosis, and improve myocardial structure.

Objectives: Heparin interaction with NRG-1β in cardiomyocytes has not been thoroughly studied. Therefore, we are interested to look at the structural characteristics of heparin that is essential for NRG-1β binding, its effe,ct on NRG-1β cardiomyocyte surface interaction and subsequently NRG-1β-mediated signaling.

Results: In this study, NRG-1β was identified as a heparin-binding protein requiring a heparin length of dp 22 and N-sulfation for binding. It was also shown that heparan sulfate proteoglycans are crucial for the localization of NRG-1 to the surface of cardiomyocytes. However, exogenous heparin prevents NRG-1 from binding to the cell surface and has an inhibitory effect on NRG-1β-induced Erbb4 signaling pathway in cardiomyocytes.

Conclusion: This study provides insights on the structural properties of heparin that affect NRG-1 β-mediated signaling in cardiomyocytes.

THE PROTEOGLYCANOME OF THE AORTIC WALL: POTENTIAL PATHOGENIC ROLE OF AGGRECAN AND VERSICAN ACCUMULATION IN THORACIC AORTIC ANEURYSM AND DISSECTION

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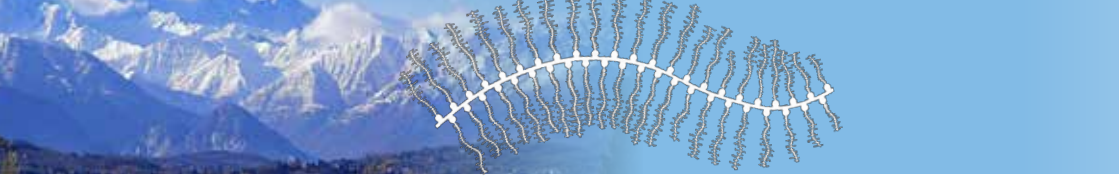
Introduction: Thoracic aortic aneurysm and dissection (TAAD) is a progressive vasculopathy with high mortality. Its histological hallmark is medial degeneration, which includes elastin fragmentation, vascular smooth muscle cell loss, collagen deposition, and proteoglycan pooling. Although proteoglycan pooling is predicted to be of considerable pathobiologic significance, both mechanically and biologically, its role in TAAD is poorly understood.

Objectives: We developed a proteomics workflow for defining the normal and TAAD aortic proteoglycanomes using anion exchange chromatography to isolate aortic proteoglycans, followed by high resolution LC-MS/MS. Aggrecan and versican immunostaining were used to determine their spatial distribution in normal and TAAD human aorta and in Fbn1$^{mgR/mgR}$ mice, a model for severe Marfan syndrome. RT-qPCR of ADAMTS proteases, which mediate their turnover, was also used.

Results: 20 proteoglycans, including aggrecan and versican comprise the proteoglycanomes of normal and TAAD aorta. Because of the swelling pressure they exert, which may be disruptive in TAAD, we determined the distribution of versican and aggrecan in 20 TAADs. Dramatic aggrecan and versican accumulation were observed, especially in areas of medial degeneration. In contrast to human TAAD, Fbn1$^{mgR/mgR}$ aortas had dramatic accumulation of aggrecan but not versican. Despite this difference, aggrecan accumulation in mutant mice is expected to have similar disruptive properties as combined aggrecan and versican accumulation in human TAAD. RT-qPCR showed upregulation of aggrecan and versican mRNA, as well as reduction of ADAMTS protease mRNAs.

Conclusions: These findings implicate excess aggrecan and versican in the disruption of aortic structure and cell function in TAAD. In addition to new mechanistic insights on TAAD provided by the present work, aggrecan and versican may fill the pressing need for biomarkers predicting acute events such as aortic dissection and rupture.



POSTERS

P1
PREPARATION OF PROTEOGLYCAN FROM SALMON NATAL CARTILAGE AND ITS IMMUNE STIMULATING EFFECTS ON CYTOKINE PRODUCTION BY MURINE SPLENOCYTES

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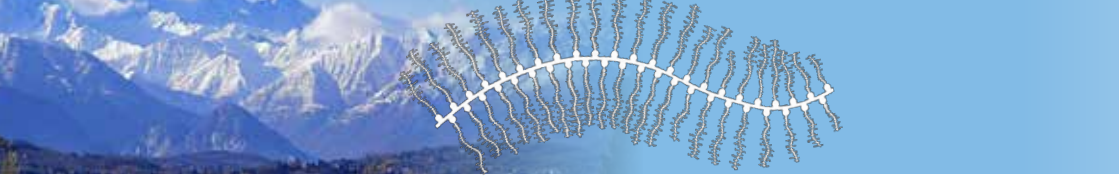
Introduction: Chondroitin sulfate (CS) stabilizes fibrous and cellular elements of the connective tissue and at the same time protects joints. Because of this fact, CS has become increasingly popular as an ingredient in health foods in Japan. On the other hand, biological activities of salmon nasal cartilage CS proteoglycan, aggrecan, are well known and this proteoglycan is used as a nutraceutical for treatment of osteoarthritis. However, the information of functional mechanisms of the proteoglycan for treatment of osteoarthritis is lacking. In this study, at first, a new method for isolation of proteoglycan from salmon nasal cartilage was investigated. Next, effects of orally administered proteoglycan on immune system according to cytokine productions were examined in mice.

Results: The intact proteoglycan from salmon nasal cartilage was extracted and prepared by a new extraction procedure in water containing sugar fatty acid ester as a detergent. This new isolation step suppressed the degradation of proteoglycan and simultaneously afforded to extract proteoglycan – collagen matrix. The prepared proteoglycan was compared with those prepared by different extraction procedures using gel filtration chromatography, polyacrylamide gel electrophoresis. Furthermore, anti-inflammation effects of proteoglycan from salmon nasal cartilage on cytokine production by intestinal immune cells are investigated according to the ribosome profiling techniques. As the results, some cytokine productions by murine splenocytes were significantly affected by oral administration of the proteoglycan.

Conclusion: Proteoglycan from salmon nasal cartilage impacted some cytokine productions compared to CS single chains. Proteoglycan may more powerful than CS as nutraceutical for OA.



P2
DECIPHERING THE SPECIFICITY FOR HEPARAN SULFATE BIOSYNTHESIS

Lars Pedersen[1], Yongmei Xu[2], Andrea Moon[1], Shuqin Xu[2], Juno Krahn[1], Jian Liu[2]

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Introduction: Heparan sulfates (HS) are involved in a wide range of physiological and pathophysiological functions such as assisting in viral/bacterial infection, inflammatory response, blood coagulation, embryonic development and cancer. As such HS show potential for therapeutics for a number of processes beyond the use of Heparin as an anti-coagulant. Specific sulfation patterns help dictate HS interactions with individual binding partners.

Objectives: Our lab studies how HS sulfotransferases recognize their specific substrates for the purpose of designing better therapeutics using a chemoenzymatic technique. To this end, we have solved the structure of the HS 6-O sulfotransferase (6-OST) in complex with three different synthesized homogeneous HS oligosaccharide substrates and performed mutational studies on residues lining the binding pocket to determine their role in specificity and/or catalysis.

Results: The structures reveal significant differences between how 6-OST engages its substrates versus other HS sulfotransferases while maintaining a similar catalytic architecture. Mapping conserved residues from the three human 6-OST isoforms onto the structure reveals residues involved in HS binding are conserved, supporting the notion of similar substrate specificity for the isoforms. Interestingly, mutations at K132 showed significant reduction in activity toward Ido2S-containing substrates while maintain near wild-type activity toward GlcA containing substrates. In addition, mutations at K202, R112, R116, and R329 all showed significantly enhanced activity towards GlcNAc-containing substrates.

Conclusions: The results of this study provide substantial insight into the biosynthesis of HS in the Golgi and suggest mutations that may be useful for the chemoenzymatic synthesis of designer therapeutics.

P3
MASS SPECTROMETRY CHARACTERIZATION OF THE HUMAN ENDOSULFATASE HSULF-2

Ilham Seffouh[1], Cedric Przybylski[1], Amal Seffouh[2], Rana El Masri[2], Diane Lebeau[1], Romain Vivès[2], FLorence Gonnet[1], Régis Daniel[1]

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Introduction: The human heparan sulfate 6-O-endosulfatases (HSulf-1 and HSulf-2) catalyse the regioselective hydrolysis the 6-O- sulfate groups within heparan sulfate chains present on cell surface proteoglycans. Hsulfs exhibit two unique features among human sulfatases, as they are the only known sulfatases to be secreted and active in the extra-cellular medium, and to be active at the polymer level. By specifically modifying the 6-O-sulfation patterns of heparan sulfate (HS), HSulfs affect the polysaccharide modulatory properties towards a large number of growth factors, morphogens and chemokines. Governing an unprecedented post-biosynthetic edition of the HS sulfation, HSulfs are involved in important developmental and tumor processes.

Objectives: Despite their key roles, the HSulf enzymes remain elusive protein objects as regard to their structure and functions. Much effort has been devoted to the understanding the enzyme reaction specificities, while much less is known about their structural organization. HSulf-2 is a heterodimer made of a 75 kDa and 50 kDa subunits likely joined by disulfide bonds, and comprising several potential N-glycosylation sites. While HSulfs are indirectly detected in most reported studies by western-blotting and through the monitoring of their catalytic activity, our goal was to detect and characterize Hsulf-2 by MALDI and electrospray mass spectrometry.

Results: In the present study, we report the first mass spectrometry characterization of HSulf-2, allowing its direct detection at the protein level. We obtained the coverage of the full protein sequence by using a combination of several proteases in a bottom-up proteomics analysis.

Conclusion: Insights into the structural features of HSulfs should help understanding this critical regulatory mechanism of HS biological functions.

P4
ATOMIC-RESOLUTION CONFORMATIONAL DYNAMICS AND THERMODYNAMICS OF CHONDROITIN SULFATE OLIGOMERS

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Introduction: Chondroitin sulfate (CS) glycosaminoglycans (GAGs) are a major constituent of proteoglycans (PGs). Experimental atomic-resolution structural biology on CS GAGs is difficult, in part due to their inherent flexibility and their non-template based biological synthesis. This difficulty imposes a barrier to determining atomic-resolution three-dimensional structures of PGs.

Objectives: All-atom explicit-solvent molecular dynamics simulations were applied to CS 12-mers having a diversity of sulfation patterns to determine their conformational dynamics and thermodynamics. These new data supplement existing studies on the conformational properties of variably sulfated disaccharides corresponding to CS fragments.

Results: As measured by distributions of end-to-end distances, CS 12-mers exhibit significant conformational flexibility in 0.14 M NaCl solution. Glycosidic linkage three-dimensional conformations, as determined by phi/psi distributions, are consistent with those previously determined for CS disaccharides. The flexibility about glycosidic linkages is a major determinant of the overall conformational flexibility.

Conclusions: The present results build on existing data from atomic-resolution molecular simulations toward developing three-dimensional models of PGs with their constituent GAGs. Toward this end, we plan to combine these results with existing experimental structural biology on PG core proteins, previous simulation work on the tetrasaccharide linker and the Xyl-O-Ser linkage, and ongoing work on longer GAG polymers.

P5
REMODELING OF HEPARAN SULFATES: STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF SULFS

Hugues Lortat-Jacob[1], Rana Ismail El Masri[1], Amal Seffouh[1], Zahrat Al Oula Hassoun[1], Romain R. Vivès[1]
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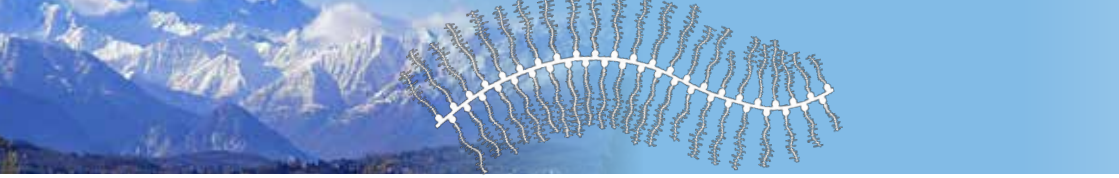
Introduction: Heparan Sulfates (HS) are complexes polysaccharides capable to interact with a broad range of signaling proteins. They are constituted by the repetition of disaccharide units that can be modified by the addition of sulfate groups in different positions. The structure of HS is regulated directly at the cell surface by the action of unique sulfatases (Sulfs). Sulfs are extracellular endosulfatases that dramatically change the HS functional properties in interacting with proteins. Sulfs are therefore implicated in many physiological and pathological processes such as cancer. Sulfs are composed essentially of a catalytic domain CAT that contains the active site of the enzyme, and a hydrophilic domain HD, a unique domain that is responsible on the recognition of HS.

Objectives: Despite their importance, Sulfs are enzymes poorly characterized. The aim of the project is to characterize the structural and the functional properties of the human isoforms HSulf-1 and HSulf-2.

Results: Functional aspects of this study aims at better understanding the implication of HSulfs during tumor progression in vivo. For this, we will transfect MDA-MB-231 cells (that do not express Hsulfs endogenously) with either wild-type (WT) or mutant HSulfs, then use these cells in a nude mouse orthotopic xenograft tumor model. Preliminary data have highlighted a pro-tumoral effect of WT Hsulf-2 expression. The second part of the project will focus on solving the structure of Sulf, which remains a major scientific challenge. We propose to study separately each domain of HSulfs. We will first attempt to crystallize HSulf isolated CAT domain for X-Ray crystallography analysis, as it is predicted to be structured and is highly homologous to previously crystallized sulfatases. In parallel, we will express the isolated HD domain in bacteria to perform NMR analysis, to study its level of structuration, its dynamics and flexibility.

Conclusions: This project will contribute to clarify the structural and functional properties of HSulfs.



P6
DISTINCT ROLES OF DROSOPHILA GLYPICANS IN THE DEVELOPING OVARY

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Introduction: Molecular mechanisms by which HSPGs exert specific functions are poorly understood. Drosophila glypicans, dally and dally-like (dlp), show clear functional specificity. Dally (not Dlp) is a highly active Dpp co-receptor, and Dlp (not Dally) serves as a Hh co-receptor.

Objectives: To understand the functional specificity of dally and dlp, we used the Drosophila ovary, which offers an excellent model system for studying epithelial cell differentiation and function, interactions between somatic and germline cells, and stem cell maintenance and replacement.

Results: Dally is exclusively expressed somatic cells in the developing ovary, including the follicle stem cells (FSCs) and differentiated follicle cells. On the other hand, dlp is expressed in both germline and follicle cells. Both glypicans are expressed in FSCs, which undergo regular turnover and replacement. FSCs are known to compete with FSC daughters for niche occupancy. Mosaic analyses of dally and dlp mutant FSC clones showed that dally is essential for normal FSC maintenance. Unexpectedly, dlp is a hyper-competitive mutation; dlp mutant FSC progenitors often eventually occupy the entire epithelial sheet, which resembles an early phase of cancer formation. These are consistent with previous observations that some human glypicans are oncogenic and others are tumor suppressors.

Conclusions: Dally and dlp play both partially redundant and distinct roles in the developing ovary. The molecular basis for dlp function remains to be elucidated.

P7
SOLUTION STRUCTURE OF CXCL13 AND HEPARAN SULFATE BINDING SHOW THAT GAG BINDING SITE AND BIOLOGICAL ACTIVITY RELY ON DISTINCT DOMAINS

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Introduction: Chemokines promote directional cell migration through binding to G protein-coupled receptors. They also interact with heparan sulfate (HS), the functional consequences of which depend on the respective location of the receptor- and the HS-binding sites (1). The B-lymphocytes trafficking and maturation are controlled by both CXCL12 and CXCL13 (2). While CXCL12 was extensively studied, revealing functional importance of HS-binding, no information was reported yet about CXCL13.

Objectives: In this study, we setup a biochemical framework, including NMR structure resolution, binding assays and in silico ligand screening along with in vitro functional assays, to investigate how HS can regulate CXCL13 activity.

Results: We solved the CXCL13 structure and showed that it comprises, appended to a classical chemokine-like structure, an unusually long and disordered C-terminal domain. Kinetics studies reveal that this latter mainly increases the association rate of CXCL13/HS complex, whereas the core protein helix stabilizes the complex. Using computational approaches we identified HS tetrasaccharide (dp4) sequences that preferentially interact with CXCL13. According to NMR and computational data, we propose a model of CXCL13 dimer in complex with two HS dp4 that could explain the observed CXCL13 dimerization upon dp4 binding. The CXCL13 HS binding sites do not interfere with bioactivity, as inferred from chemotaxis and calcium uptake assays, suggesting that CXCL13 can be productively presented to its signaling receptor in a HS-bound form.

Conclusions: We found that CXCL13 displays a unique association mode to HS and, using computational approaches, we proposed a model that explains the observed induced dimerization of CXCL13 upon HS binding. Importantly, CXCL13 can be functionally presented in a HS-bound form to its receptor, suggesting that it can promote adhesion-dependent cell migration. Finally, we designed mutations that impact HS binding without affecting cognate receptor activation, opening an avenue to study the functional significance of CXCL13/HS binding in vivo.

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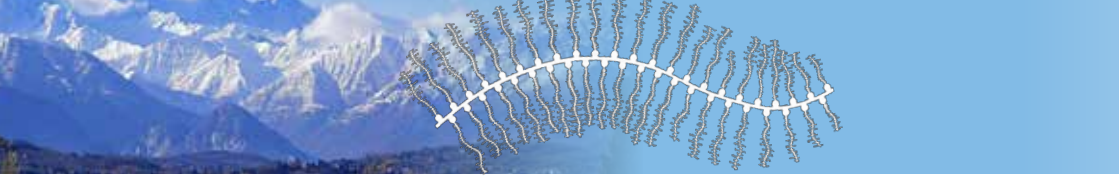

P8
HEPARANASE EXPRESSION IS ASSOCIATED WITH INFLAMMATION IN OVARIAN ENDOMETRIAL CYSTS OF EARLY REPRODUCTIVE-AGE WOMEN

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Introduction: Endoglycosidase Heparanase (HPSE) and one of its substrate heparan sulfate Syndecan-1 (SDC1) are involved in molecular pathways that deregulate cell adhesion during carcinogenesis. Endometriosis is the gynecologic pathology with the heterotopical localization of endometrial loci outside the uterine cavity with some tumor fundamental analogies and persistente activity.

Objective: Was to evaluate the correlation between tissue inflammatory reaction and HPSE and SDC1 expression in paraffin-embedded surgical samples of ovarian endometrial cysts of 10 early reproductive-age women in proliferative menstrual phase using immunohistochemical semi-quantitative (IHC) analysis.

Results: Positive correlation between HPSE expression in the epithelial cells of cysts and the subepithelial inflammation was revealed (Spearman coefficient 0,95, p = 0,0039). The subepithelial inflammatory cell infiltration was accompanied by pain syndrome and was characterized by trend of SDC1 up-regulation in cyst epithelial cells (Mann-Whitney test, p = 0,1649) and by significant HPSE up-regulation in the nuclei (p = 0,0253) comparing samples without leukocyte polymorphic infiltration. Besides, positive correlation between subepithelial inflammatory cell infiltration and the absence of fibrosis (p = 0,0339) was revealed. The presence of fibrosis was accompanied by more expensive HPSE expression in cysts stromal cells (p = 0,0495).

Conclusion: HPSE up-regulation in the ovarian endometrial cyst epithelium is likely to be associated with more pronounced inflammatory reaction and more prominent clinical manifestations of the endometriosis.

P9
DIFFERENTIAL EXPRESSION OF SPECIFIC HEPARAN SULFATE DOMAINS IN RENAL ALLOGRAFT REJECTION

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Heparan sulfate (HS) plays a crucial role in allograft rejection by binding and presenting cytokines and growth factors to their receptors. This raises the possibility that changes in GAG biology might regulate cytokine binding and the development of rejection. We examined the role of HS N, 6-0 and 3-O (Abstract submitted by L Ferreras) sulphation in renal allograft rejection.

In normal human kidney, HS was largely restricted to the tubular basement membrane; chondroitin-4-sulphate and chondroitin-6-sulphate were expressed within the interstitial tissues. The expression of all three GAGs was increased significantly during acute rejection, and heavily sulphated HS remained predominant within the tubular basement membrane. Expression of N-sulfated HS is specifically increased during acute rejection in peritubular capillary vessels, and the chemokine CCL5 binding basement membrane of tubular epithelial cells was shown to co-localize with HS expression.

Furthermore, increased expression of 6-O-sulfated HS domains in tubular epithelial cells was seen during chronic rejection as compared with the controls. To assess the relevance of these data in vivo we established a murine model of fibrosis (unilateral ureteric obstruction). HS-specific phage display antibodies showed significant increase in 6-O-sulfation in fibrotic kidney compared with the control. To examine the role of 6-O-sulfated HS in the development of fibrosis, we generated stable HS6ST1 overexpressing renal epithelial cells. Compared with mock transfectants, the HS6ST1 transfectants showed significantly increased binding of FGF2 ($p < 0.0086$) and pERK activation.

These results suggest an important role of N-sulphation during acute renal allograft rejection and 6-O-sulfation in the pathogenesis of fibrosis associated with chronic rejection.

P10
HEPARAN SULFATE DIFFERENTIALLY CONTROLS CXCL12A- AND CXCL12G-MEDIATED CELL MIGRATION THROUGH DIFFERENTIAL PRESENTATION TO THEIR RECEPTOR CXCR4

Bridgette Connell[1], Rabia Sadir[1], Françoise Baleux[2], Cedric Laguri[1], Jean-Philippe Kleman[1], Lingjie Luo[2], Fernando Arenzana-Seisdedos[2], Hugues Lortat-Jacob[1]

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Introduction: Chemokines have two ligands on cell surface: chemoreceptors (G protein-coupled receptors), and GAGs, usually heparan sulfate (HS). Chemokines stimulate signals in cells by binding to their chemoreceptors. The association with HS provides positional information within tissues in the form of haptotactic gradients along which cells can migrate directionally. Such a mechanism implies that chemokines can simultaneously recognize HS and their specific receptor, however, in many cases the relationships between HS and receptor binding have not been addressed (1).

Objectives: Our aim was to investigate the mechanism by which HS can potentially modulate chemokine functions. For this, we used CXCL12, a chemokine existing in different isoforms, all signaling through CXCR4 but featuring distinct HS binding domains.

Results: Using flow cytometry and surface plasmon resonance with both cell-associated and solubilized CXCR4, we found that although CXCL12g bound to CXCR4 with a higher affinity than did CXCL12a, this isoform displayed reduced signaling and chemotactic activities. These properties were caused by a HS binding domain, localized within the specific carboxyl-terminal region of CXCL12g. HS prevented CXCL12g from interacting with the CXCR4 sulfotyrosines, thereby functionally presenting the chemokine to its receptor such that its activity was similar to that of CXCL12a. HS had no effect on the binding of CXCL12a to CXCR4 nor on its biological activity.

Conclusions: These data demonstrate that free CXCL12g is inactive, due to non-productive interaction with CXCR4, and need to be captured by cell surface HS to be functionally presented to CXCR4 positive cells. In contrast, HS had no effect on CXCL12a binding to CXCR4. This work clearly shows that the polysaccharide controls CXCL12 in an isoform specific manner and modulates receptor ligation and activation (2).

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P11
SYNDECAN-2 EXPRESSION IS UPREGULATED PREDOMINANTLY AT PROXIMAL COLON IN DSS-INDUCED COLITIS MOUSE MODEL

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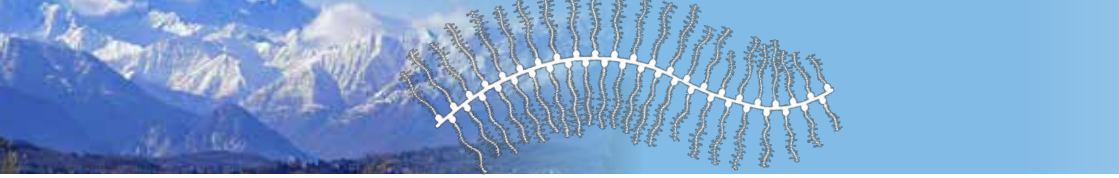
In previous studies, we have reported that during chronic inflammation, inflammatory hypoxia induces syndecan-2 expression in colonic epithelia. Here we report that syndecan-2 expression is site-specific in colon epithelium. Our immunohistochemistry data using the dextran sulfate sodium (DSS)-induced mouse model of acute colitis (3% DSS in 4 days) revealed that syndecan-2 expression was mostly increased at proximal colon, whereas crypt distortion mainly occurred at transverse colon in acute colitis model. In addition, syndecan-2 expression was not correlated with tissue repair in accordance with Ki-67 level, suggesting low correlation between syndecan-2 expression and DSS-induced colitis. However, in DSS-induced mouse model of chronic colitis (2% DSS in 5 days), we observed syndecan-2 expression in both proximal and distal region of colon, suggesting that syndecan-2 expression spread out to transverse colon gradually during chronic inflammation. Together, these data suggest that DSS-induced colitis may increase the expression of syndecan-2 predominantly at proximal colon at the early stage of inflammatory response and it extends to transverse colon.



P12
SYNDECAN-1 POSITIVE PLASMA CELLS BECAME MORE NUMEROUS IN THIN ENDOMETRIUM AFTER PELOIDS APPLICATION FOR TREATMENT OF CHRONIC ENDOMETRITIS

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Introduction: For differential diagnostics of chronic endometritis (CE) in reproductive-age women it is important to identify syndecan-1 (SDC1) positive plasma cells in endometrial pipelle-biopsy during proliferative menstrual phase using immunohystochemical study. Currently, CE is often characterized by so-called thin endometrium (M-echo less than 6 mm) which is not responding to hormone therapy, so the problem of diagnostics and regeneration of such variant of endometrium is not solved for reproductive medicine.

Objective: Was to evaluate the amount of SDC1-plasmocytes and their precursors CD20 B-lymphocytes in the endometrial biopsies of women with CE depending on the thickness of the endometrium after application of Karachi Lake peloids (West Siberian Plain).

Results: Case-control and prospective cohort study of 240 women with CE at the age of 27.0 ± 2.9 years: 118 cases with normal endometrium (M-echo > 6 mm in the proliferative phase of the menstrual cycle) and 122 women with thin endometrium (M-echo ≤ 6 mm), treated with vitamin- and physiotherapy (1st scheme) and in combination with peloids (2nd scheme). Ultrasound and immunohistochemical examination of the endometrium was performed, besides 24 biopsies were analyzed using the non-parametric statistic method and ANOVA for expression pattern of SDC1 plasmocytes and CD20 B-lymphocytes. After treatment CE with normal endometrial thickness in biopsies there was down-regulation of CD20- and SDC1-positive cells. Thin endometrium after treatment with peloids was characterized by more prominent expression of CD20- and SDC1 markers in the biopsies, combined by positive clinical dynamics and the growth of symbiont microflora.

Conclusion: The Siberian Karachi Lake peloids using in the treatment of women with CE and thin endometrium are able to induce regeneration with up-regulation of markers of inflammation.

P13
LUMICAN DELAYS MELANOMA GROWTH IN MICE AND DRIVES TUMOR MOLECULAR ASSEMBLY AS WELL AS RESPONSE TO MATRIX-TARGETED THERAPEUTIC PEPTIDE

Albin Jeanne[1], Valérie Untereiner[2], Corinne Perreau[2], Isabelle Proult[2], Cyril Gobinet[2], Camille Boulagnon-Rombi[2], Christine Terryn[3], Laurent Martiny[1], Stéphane Brézillon[2], Stéphane Dedieu[1]

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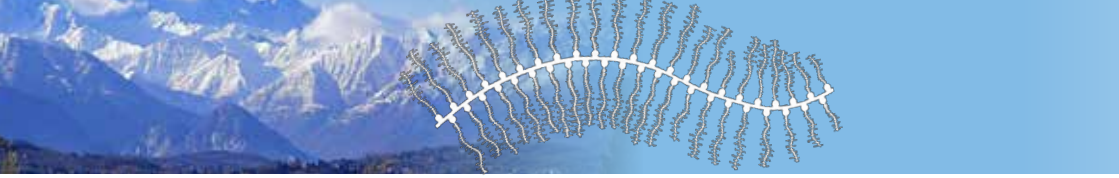
Introduction: Lumican is a small leucine-rich proteoglycan (SLRP) being known as a key regulator of collagen fibrillogenesis. However, little attention has been given so far in studying its influence on tumor-associated matrix architecture.

Objectives: The aim of the present study was to investigate the role of host lumican on tumor matrix organization as well as on disease progression considering an immunocompetent model of melanoma implanted in Lum$^{-/-}$ vs. wild type syngeneic mice. Conjointly, lumican impact on tumor response to matrix-targeted therapy was evaluated considering a previously validated peptide, namely TAX2, that targets matricellular thrombospondin-1.

Results: In the B16 melanoma allograft model, endogenous lumican inhibits tumor growth and modulates response to TAX2 peptide. Indeed, IHC analyses revealed that lumican deficiency impacts intratumoral distribution of matricellular proteins, growth factor and stromal cells. Besides, innovative imaging approaches helped demonstrating that lumican host expression drives biochemical heterogeneity of s.c. tumors, while modulating intratumoral collagen deposition as well as organization.

Conclusion: Altogether, the results obtained present lumican as a strong endogenous inhibitor of tumor growth, while identifying for the first time this proteoglycan as a major driver of tumor matrix coherent assembly.



P14
HOW DOES HEPARIN DIVERT HYPERGLYCEMIC DIVIDING MONOCYTES/MACROPHAGES FROM A PRO-INFLAMMATORY TO AN ANTI-INFLAMMATORY PHENOTYPE?

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Hyperglycemic blood glucose in the Streptozotocin type 1 diabetic rat induces kidney glomerular mesangial cells to divide and initiate abnormal hyaluronan (HA) synthesis in intracellular compartments. This causes a large stress response (autophagy) and extrusion of a monocyte-adhesive HA matrix outside the cells that increases continuously for 6 weeks with an influx of macrophages, and the rat has extensive nephropathy. In contrast, IP treatment daily with heparin prevents the intracellular HA response and reprograms the mesangial cells to make a more extensive monocyte-adhesive HA matrix within the first week, with an influx of macrophages that remove the matrix by 6 weeks, and the rat has no apparent nephropathy (1,2). These results raise the question: How do the macrophages differ when heparin is present? To address this, human U937 monocytes were growth arrested to G0/G1 and stimulated to divide in normal glucose (5 mM), and in high glucose (25 mM) with or without heparin (0.2 mg/ml) for 24 h (one cell division). Monocytes were permeabilized and stained for HA and the HA receptor CD44. Intracellular HA stained extensively and the CD44 showed capping in the high glucose treated monocytes. In contrast the high glucose plus heparin treated monocytes showed no HA, and the CD44 was uniform on the cell surface, the same as for the monocytes that divided in normal glucose. Non-permeabilized monocytes were stained for M1 pro-inflammatory cell surface CD80 and M2 anti-inflammatory CD163 markers. Monocytes in high glucose stained extensively for M1 CD80 while monocytes in high plus heparin stained for M2 CD163. Monocytes in normal glucose showed minimal staining for both. For comparison murine bone marrow macrophages were tested. Macrophages dividing in high glucose promoted a 5-fold increase in M1 TNFa. This was blocked in macrophages dividing in high glucose plus heparin, which instead induced a 2-fold increase in M2 IL10. Untreated U937 monocytes, which are maintained in normal glucose, bound to cryo-sections of diabetic glomeruli at 4° C and phagocytosed HA out of the section with concurrent CD44 capping when warmed to 37° C. These results provide strong evidence that the macrophages in the diabetic kidney have already divided and are M1 proinflammatory when they are recruited into the glomeruli, and are no longer able to phagocytose the monocyte-adhesive matrix. In contrast, the macrophages in the diabetic glomeruli of rats treated with heparin are M2 and maintain their ability to remove the HA matrix being produced by the mesangial cells.

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P15
ROLES OF VERSICAN IN INFLAMMATION AND TUMOR DEVELOPMENT

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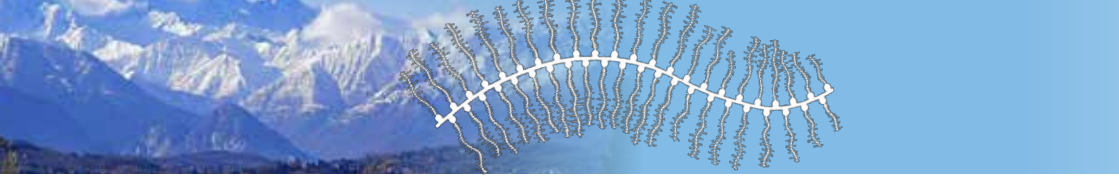
Introduction: Versican (Vcan) is a large chondroitin sulfate proteoglycan in the extracellular matrix (ECM). Based on the observations of expression patterns, Vcan is believed to play a major role in formation of the provisional matrix.

Objective: The aim of our study is to determine in vivo roles of Vcan in diseases, using several Vcan-modified mice.

Results: We generated various pathological conditions in both Vcan-modified and control mice, and analyzed them. To investigate the role of host stromal Vcan in tumor development, we injected QRsP11 fibrosarcoma cells together with cre-expressing adenovirus or GFP-expressing adenovirus as controls into Vcanflox/flox mice. Loss of host stromal Vcan facilitated tumor cell proliferation, and following angiogenesis, decreased cancer-associated fibroblasts, diminished collagen fibers, and altered hyaluronan distribution, concomitant with upregulation of hyaluronan, TGF ƒÀ, and VEGF-mediated signaling. When the Vcan V3 variant consisting of G1 and G3 domains was expressed in tumor cells, it was integrated into the ECM, regained collagen fibers and cancer-associated fibroblasts, and resulted in successful recovery of tumor growth inhibition, indicating that whatever cells produce, the G1 and G3 domains are adequate for Vcan function. These results raise questions as follows: 1) whether this growth inhibition is observed in other tumor cell types, 2) which cell types provide the functional Vcan, 3) whether and how much inflammatory status is involved in tumor behavior, and 4) whether degradation of Vcan is involved in the inhibition of tumor growth. These issues will be addressed discussed.

Conclusion: Collectively, our results indicate a dynamic function of Vcan in the extracellular matrix that regulates tumor cell behavior. A greater understanding of the regulation of Vcan expression may contribute to the development of cancer therapies.



P17
HOW 1,3-GALACTOSYLTRANSFERASE 6 DEFECT PRODUCES A RARE GENETIC DISEASE, THE EHLERS-DANLOS SYNDROME: A FUNCTIONAL AND MOLECULAR INVESTIGATION

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[2]*Center for Medical Genetics, Gent University Hospital, Gent - Belgium*

Introduction: Proteoglycans (PGs) are major components of cell plasma membranes and extracellular matrix. These heteropolysaccharidic macromolecules play an important role in matrix organization of connective tissues and in cell signaling, embryonic and post-natal development. PGs are composed of glycosaminoglycan (GAG) chains covalently attached to a core protein through a tetrasaccharide linkage (Glucuronic acid-β1,3-Galactose-β1,3-Galactose-β1,4-Xylose-β1-O-). The addition of the third residue (galactose) is catalyzed by the β1,3-Galactosyltransferase 6 (β3GalT6), a key glycosyltransferase in GAG initiation. Our laboratories and others discovered that mutations of β3GalT6 are associated to a pleiotropic form of Ehlers-Danlos Syndrome (EDS), a severe connective tissue disorder characterized by skin and bone fragility, musculoskeletal malformations, delayed wound healing, joint hyperlaxity and intellectual disabilities (1, 2).

Objectives: The objectives of this work is to understand the consequence of new β3GalT6 mutations (Asp207His, Gly217Ser and Ala108Glyfs*163) in the development of EDS clinical symptoms, starting with evaluation of GAG anabolism and cell migration in patient dermal fibroblasts. In parallel, the molecular characterization of the human β3GalT6 is being carried out producing a purified truncated soluble form of the enzyme in a bacterial expression system.

Results: We show that β3GalT6 defective fibroblasts exhibited a marked reduction in GAG anabolism and a delay in wound healing in comparison to control cells. Immunofluorescence analysis also showed an alteration HS chains staining in patient cells, confirming that GAG defect is due to β3GalT6 loss of function. These results could explain some phenotypic aspects of the disease, such as defective wound closure in relation to GAG defect. To determine the impact of the β3GalT6 mutations on the enzymatic function, the production and purification of wild-type (and mutated) recombinant human β3GalT6 are in progress towards detailed kinetic β3GalT6 analyses.

Conclusions: This work shows the crucial role of β3GalT6 in EDS pathogeny. β3GalT6 mutations are responsible for a unique combination of severe generalized symptoms, characterized by important connective tissue disorders. We hope from the molecular part of this work to provide better insight into the relationships between β3GalT6 loss of function and the severity of clinical symptoms.

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P18
TARGETING SULFATED GLYCOSAMINOGLYCANS IN CANCER CELLS BY BRANCHED PEPTIDES: FROM BASIC RESEARCH TO DEVELOPMENT OF CANCER THERANOSTICS

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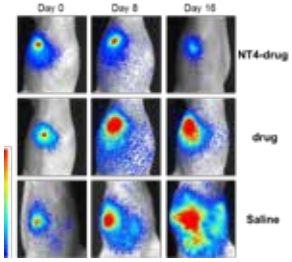
Introduction: Heparan sulfate Proteoglycans (HSPG) are of primary importance in cancer cell differentiation, division, migration and invasiveness. The biological functions of HSPG reside in their ability to interact with various ligands, including growth factors, morphogens, chemokines and extracellular matrix proteins, by means of sulfated glycosaminoglycan (GAG) chains anchored to their protein core. The molecular basis of many HSPG biological functions is still elusive, mainly due to the lack of specific ligands that could enable the role of the glycan chains to be discriminated from that of the core protein and from other co-receptors or ligands. In previous papers we reported the synthesis and biological activity of stable tetra-branched peptides named NT4, which bind with high selectivity to human cancer cells and tissues and can efficiently and selectively deliver drugs or tracers for cancer cell imaging or therapy. We found that the high selectivity of NT4 toward cancer cells and tissues resides in their high affinity binding to sulfated GAGs.

Objectives: 1-use NT4 peptide as tools to enlighten the role of sulfated GAGs in cancer cell migration and invasiveness; 2- exploit the high cancer selectivity of NT4 peptides for developing innovative cancer theranostics.

Results: NT4 peptides inhibit adhesion, migration and invasiveness of different cancer cells, dramatically affecting the directionality and polarity of cell movement. We analyzed modifications induced by NT4 in cancer cell morphology, cytoskeletal organization and mechanochemical signaling in 2D migrating cells. GAG-binding NT4 peptides interfere with integrin activation and produces dramatic reorganization of actin filaments and stress fibers together with an increased number of filopodia.

Conclusions: Taking advantage of NT4 cancer selectivity, we are developing different drug-armed NT4 peptides which allow increasing in vivo activity of the drug and even by-pass cancer cell drug resistance.



P19
NT4-LABELED NEAR-INFRARED QUANTUM DOTS AS CANCER SELECTIVE THERANOSTIC NANODEVICES

Giulia Riolo[1], Chiara Falciani[1], Lorenzo Depau[1], Mandarini Elisabetta[1], Alessandro Pini[1], Luisa Bracci[1], Jlenia Brunetti[1]

[1]*Department of Medical Biotechnology, University of Siena, Siena - Italy*

Introduction: The tetra-branched peptide NT4 targets HSPGs by specifically binding sulfated glycosaminoglycans (1-2). NT4 is a potential cancer theranostic, since it selectively binds human cancer cells and tissues and can efficiently and selectively deliver drugs or liposomes for cancer cell therapy or imaging, in vitro and in vivo (3-5). NT4 conjugated to paclitaxel proved to produce tumor regression in a breast cancer orthotopic mouse model which is not achieved with paclitaxel alone in identical experimental conditions (6).

Objectives: Construction, validation, in vitro and in vivo analysis of novel theranostic nanodevices, for selective diagnosis and imaging of different human malignancies.

Results: We have constructed a near-infrared quantum dots (QDs) functionalized with tetrabranched NT4 peptide (NT4-QDs). We observed specific uptake of the device in human cancer cells in in vitro experiments. Animal models of cancer were also set up to test nano-imaging with NT4-QDs obtaining very promising results.

Conclusions: We will show the ability of NT4 to drive quantum dots tracers selectively to tumor cells in vitro and in vivo, proving promising features of NT4-QDs as theranostics.

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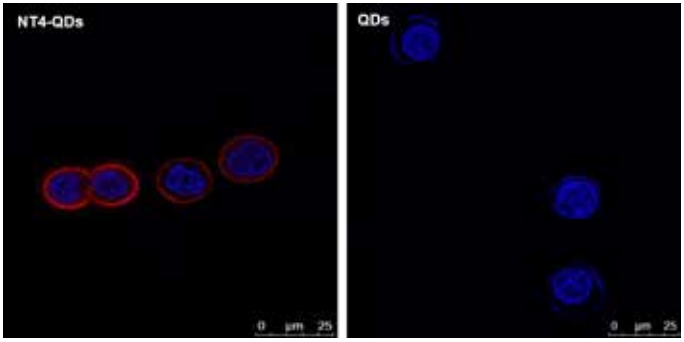

P20
EXPRESSION AND LOCALIZATION OF HEPARAN SULFATE IN HUMAN BRAIN TUMOURS

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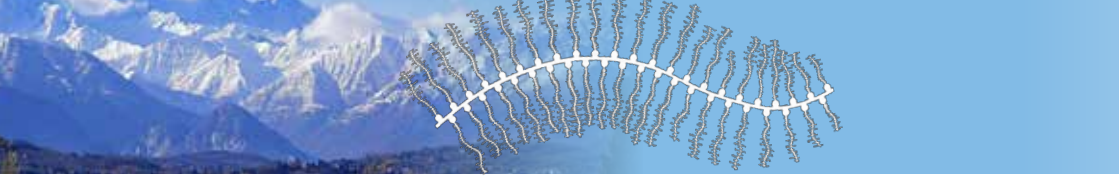
Introduction: Gliomas are the most aggressive brain tumours which are characterized by active invasion of tumour cells into the surrounding brain tissue. The process significantly depends on the extracellular matrix (ECM) structure, which undergoes changes during malignant transformation. Proteoglycans are the main component of brain tissue ECM, however, presence and structure of their polysaccharide chains in normal and cancer brain tissues remain unclear.

Objectives: The aim of the work was to study localization and potential involvement of heparan sulfate (HS) chains in brain malignant transformation.

Results: According to immunohistochemical analysis, HS content was significantly increased in glioblastomas (GBs) compare with normal human brain tissues, and the HS-positive cell populations were heterogeneously distributed over the tissue sample. The cell populations demonstrated a negative association with actively-proliferating Ki-67-positive cells and stem cell marker CD44-positive cells. Surprisingly, the appearance of HS-expressing, slowly proliferating cell populations in glioblastoma tissue was associated with negative prognosis for the patients. Another unexpected fact was intracellular localisation of the HS in glioblastoma tissues possibly related to the revealed disorganization of expression pattern of heparin sulfate biosynthesis-involved genes (EXT1, EXT2, NDST1, NDST2, GLCE, 2OST1, 3OST1, 3OST2, 6OST1, 6OST2, SULF1, SULF2, HPSE) in cancer brain tissue.

Conclusion: Taken together, the results for the first time demonstrate upregulation and heterogeneous distribution of heparan sulfate polysaccharide chains in glioblastoma tissues. Heparan sulfate positive cells seem represent a specific cell population with low proliferative activity but association with a poor prognosis for glioblastoma patients.

This work has been supported by a Russian Science Foundation (RSF grant N16-15-10243).



P21
CELL CONTEXT-DEPENDENT ANTITHETIC ROLE OF GLYPICAN-5 IN TUMOUR GROWTH

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Introduction: Glypicans (GPCs) are frequently misregulated in cancer and immunotargeting of GPC3 has moved through Phase I clinical trials. Whereas GPC1/GPC3 are recognized to be tumour growth promoters, GPC5 exhibits contrasting functions in rhabdomyosarcomas and lung carcinomas.

Objectives: To clarify the molecular bases for the apparently antithetic role of GPC5 in tumour formation and progression.

Results: GPC5 expression levels were manipulated in osteosarcoma and melanoma and the cells were comparatively assayed for matrix adhesion, migration, proliferation and anchorage-independent growth. Diversities in gene expression were defined by DNA microarray. Signal transduction pathways were examined by global phosphoproteomics. In vivo tumorigenesis was evaluated in nude, NOD-SCID, GPC5$^{(-/-)}$, TRAMP and crossed TRAMP-GPC5$^{(-/-)}$ mice. Ectopic expression of GPC5 fairly consistently decrease cellular functions in osteosarcoma cells but increased them in melanoma cells. Tumour growth in immunodeficient mice showed the same trend. Gene expression profiling did not disclose differences in established tumorigenesis gene patterns. GPC5-hampered tumour growth in xenogenic settings was associated with a raise in EGF/IGF-dependent and down-regulation of cytokine-induced signallings. Homozygous TRAMP-GPC5$^{(-/-)}$ developed smaller tumours and survived longer than TRAMP-GPC5$^{(+/+)}$ mice.

Conclusion: The results provide further evidence for an antithetic role of GPC5 in tumorigenesis and highlight cell context-dependent molecular mechanisms sustaining this role.

P22
EXOSTOSIN 1 REGULATES CANCER CELL STEMNESS BY INCREASING HEPARAN SULFATE BIOSYNTHESIS IN DOXORUBICIN RESISTANT BREAST CANCER CELLS

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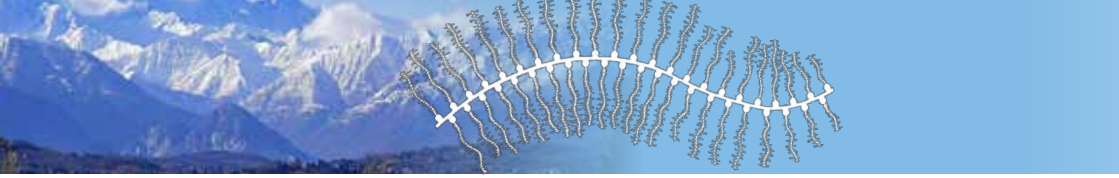
Introduction: Cancer stem cells (CSCs) are associated with cancer recurrence following radio/chemotherapy owing to their high resistance to therapeutic intervention. Exostosin 1 (EXT1) is an endoplasmic reticulum (ER)- residing type II transmembrane glycoprotein that regulates the biosynthesis of heparan sulfate (HS). Based on overexpressed EXT1 in some cancer forms, several reports have emphasized its probable role in cancer progression. However, till date there is no evidence on EXT1 regulating CSC properties.

Objective: The aim of this research is to show the role of EXT1 in the regulation of breast cancer cell stemness through facilitation of HS biosynthesis.

Results: MCF7 and MCF7/ADR, doxorubicin resistant breast cancer cells, were studies. DNA microarray revealed that doxorubicin-resistant MCF7/ADR cells have high levels of EXT1 expression compared to its parental cell line, MCF7. These cells showed significantly higher populations of CSCs; larger populations of aldehyde dehydrogenase ($ALDH^+$) and $CD44^+/CD24^-$ cells together with larger populations of cells with cell surface HS compared to MCF7 cells. Small interfering RNA (siRNA) mediated knockdown of EXT1 in MCF7/ADR cells significantly reduced cell populations with cell surface HS, followed by reduced cancer stem cell markers; populations of $ALDH^+$ and $CD44^+/CD24^-$ cells, mRNA and protein expression for CD44 and mammosphere number. Furthermore, epithelial mesenchymal transition (EMT) markers and migratory behavior were also repressed with reduced EXT1. In an in vitro soft agar colony formation assay, EXT1 knockdown reduced the colony formation ability of these cells.

Conclusion: Based on these results, we suggest that EXT1 could be a promising novel target to overcome cancer cell stemness in anthracycline-based therapeutic resistance by interfering HS biosynthesis process.



P23
HEPARAN SULFATE 3-O-SULFOTRANSFERASES, NEW PRO-TUMORAL FACTORS INVOLVED IN CANCER CELL SURVIVAL?

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Introduction: Heparan sulfates (HS) are sulfated polysaccharides for which the sulfation pattern determines their biological properties. The last step of HS biosynthesis is catalyzed by 3-O-sulfotransferases (3-OSTs), which transfer sulfate group to the position C3 of glucosamine residues. The role of 3-OSTs in cancer is still misunderstood, even if recent studies suggest that 3-O-sulfated HS may act as pro-tumoral factors.

Objectives: Here, we analyzed the effect of the overexpression of 3-OST2, 3B and 4 in the proliferation and survival of human breast tumor MDA-MB-231 cells. The impact of high expression of 3-OSTs on cell signaling was also analyzed.

Results: We showed that the three enzymes similarly enhanced cell proliferation and viability, which was related to an increase in the activation of c-Src and Akt. Overexpression of 3-OST2, 3B and 4 also resulted in an enhanced activation of the NF-κB pathway, as demonstrated by a decrease in the amount of I-κB and related increase in phospho-p65. These results suggesting that 3-OSTs may control pro-survival signals, we analyzed the responses of MDA-MB-231 cells to pro-apoptotic stimuli. We found that cell death was efficiently reduced in transfected cells, thus indicating that high expression of 3-OST2, 3B and 4 was protective against apoptosis.

Conclusion: Overexpression of 3-OST2, 3B and 4 enhanced cell proliferation and viability, via the activation of pro-survival signaling pathways. These effects are similar for the three isozymes, suggesting a common pro-tumoral activity. Taken together, our findings suggest that increase in HS 3-O-sulfation in cancer cells could be associated with a bad prognosis.

P24
MicroRNA TARGETING AS A REGULATORY MECHANISM OF BREAST CANCER CELL PROPERTIES AND ECM COMPOSITION

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Introduction: Estrogens and their receptors have pivotal roles in the development and progression of breast cancer. It is well established that interactions among cancer cells and tumor microenvironment are in dynamic interplay and regulated by extracellular matrix. Even though the role of ERα in breast cancer cells is thoroughly studied, the biological functions of its isoform, ERβ, is less elucidated. Recent data indicated that the strong suppression of ERβ in MDA-MB-231 breast cancer cells reduces their aggressiveness and affects their functional properties as well as certain ECM components.

Objectives: In the present study, we evaluated the effects of ER suppression on the microRNAs expression levels in breast cancer cell lines (MCF-7 and MDA-MB-231 before and after suppression of ERβ).

Results: Our data pinpointed that the different breast cancer cells exhibited alterations in the expression levels of certain microRNAs that are implicated in the inhibition of cancer progression and the retention of EMT, depending on the presence of ERα or ERβ. We demonstrated that the loss of ERβ in shERβ MDA-MB-231 cells resulted in differentiated expression profiles of miR-10b, miR-200b and miR-145, compared to MDA-MB-231 and MCF-7 breast cancer cells. Interestingly, our data revealed that breast cancer cells that lack ERβ exhibited elevated expression levels of miR-145 and decreased levels and miR-10b and that these miRNAs significantly regulate breast cancer cell behavior, EMT process and ECM composition.

Conclusions: These novel results suggest that the alterations in cell behaviour and in ECM composition caused by the suppression of ERβ in aggressive MDA-MB-231 cells are closely related to certain epigenetic miRNA-induced alterations. Targeting the ERβ-regulated miR-10b and miR-145 is a promising tool for diagnosis and pharmaceutical targeting in breast cancer.

P25
SYNDECAN-4 IS OVEREXPRESSED IN GASTRIC CANCER AND ASSOCIATES WITH TUMOUR AGGRESSIVE FEATURES

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Introduction: Gastric cancer's high mortality is mainly due to late diagnosis and metastasis formation. Changes in glycosylation are a hallmark of cancer and aberrant glycans constitute a source of biomarkers and may underlie differences in therapeutic response (1, 2). The gastric carcinogenesis is accompanied by striking changes of the cells glycophenotype (3, 4). We have previously demonstrated that the expression of the heparan sulfate proteoglycan Syndecan-4 (SDC4) is up-regulated upon infection by the gastric carcinogenic bacteria Helicobacter pylori (5).

Objectives: In this work, we evaluated the expression of SDC4 in human gastric cancer cells and addressed the functional implications of SDC4 expression modulation in cancer cell motility features. Furthermore, we assessed the clinical prognostic value of SDC4 expression.

Results: SDC4 is highly expressed on the membrane of different gastric cell line models and its silencing had critical functional implications on gastric cancer cell motility features, including migration and invasion capacity. Human gastric tumours displayed increased expression of SDC4 in comparison with adjacent or healthy gastric mucosa. Importantly, SDC4 expression is associated with gastric cancer patients' poor prognosis.

Conclusions: It is critical to identify unique gastric tumour-associated glycosylation alterations in order to provide diagnosis and prognosis biomarkers to improve the clinical management of gastric cancer. Our data supports that SDC4 participates in the modulation of gastric cancer cell invasion and that SDC4 may constitute a potential biomarker for identification of patients with worse prognosis.

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P27
DECORIN IN HUMAN VULVA CARCINOMA

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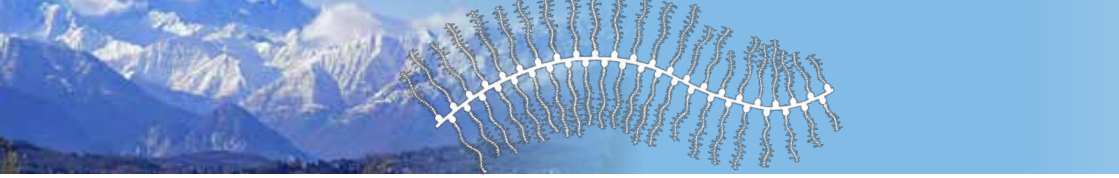
Introduction: The extracellular matrix proteoglycan decorin is well known for its oncosuppressive activity in several types of cancer. Previously we have shown that malignant cells of human epithelial cancers, e.g. breast-, bladder-, and colon cancers are devoid of decorin expression. We have also shown that adenovirus mediated decorin cDNA transduction modulates the behaviour of these cells.

Objectives: In this study we analyzed decorin expression in another epithelial cancer, namely in human vulva carcinoma, using tissue samples and cultured cells of this malignancy. Furthermore, the effects of adenovirus mediated decorin cDNA transduction on the behaviour of cultured human vulva carcinoma cells was examined. Moreover, the potential mechanism modulating the behaviour of these cells was evaluated. Specifically, the effect of transduction on the expression of ErbB receptors was determined.

Results: Malignant cells of human vulva carcinoma tissue samples were shown to lack decorin expression. This was also true for cultured human vulva carcinoma cells. Transfection of human vulva carcinoma cells with adenovirus mediated decorin cDNA resulted in increased apoptosis and reduced proliferation of the cells. RT-qPCR analyses demonstrated that decorin cDNA transduction downregulated the expression of ErbB2 receptor.

Conclusions: Similarly to our previous studies on other human epithelial cancers, in human vulva carcinoma malignant cells are devoid of decorin expression. The malignant behaviour of human vulva carcinoma cells can be attenuated by decorin cDNA transduction. This attenuation is associated with downregulation of ErbB2 receptor expression.



P28
IDENTIFICATION OF XYLT1 MUTATIONS IN DESBUSQUOIS DYSPLASIA TYPE 2 AND FUNCTIONAL CONSEQUENCES ON PROTEOGLYCAN SYNTHESIS

C. Bui[1], C. Huber[2], Y. Alanay[3], B. Tuysuz[4], C. Bole-Feysot[5], J. Leroy[6], G. Mortier[7], P. Nitschke[8], S. Fournel-Gigleux[1] and V. Cormier-Daire[2]

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Introduction: Desbuquois dysplasia (DBQD (MIM 251450)) belongs to the multiple dislocation group of disorders and is a severe condition characterized by short stature, joint laxity and advanced carpal ossification.

Objectives: (i) Identify the molecular basis of DBQD type 2 by studying a group of 20 subjects clinically well characterized, (ii) Assess the functional consequences of gene mutations on the proteoglycan metabolism, (iii) Establish a 3D cellular model to study the pathophysiology of chondrodysplasias.

Results: To identify the DBQD type 2 gene, we first selected 2 siblings from consanguineous parents for whole-exome sequencing analysis and eventually identified 5 homozygous mutations in 7 individuals from 6 consanguineous families in the xylosyltransferase 1 gene (XYLT1). Among the 5 mutations, 4 were expected to result in loss of function and a drastic reduction of XYLT1 cDNA level was demonstrated in 2 cultured individual fibroblasts. Given the major role of xylosyltransferase 1 (XT-I) in proteoglycan (PG) initiation, we studied PG metabolism and showed a significant reduction of cellular PG content in the 2 individual fibroblasts. To further understand the implication of PG machinery during bone/cartilage development, we performed cell differentiation using the mouse chondrogenic cell line ATDC5. Cells were cultured with chondrogenic culture medium during 14 days to promote cell differentiation into chondrocytes[1]. Our preliminary data show a net increase of mRNA expression level of cartilage-specific genes including type 2 collagen and the large PG aggrecan by semiquantitative RT-PCR.

Conclusions: Our findings shed light on the pivotal and specific role of XT-I during the ossification process[2]. For the future, it would be of great interest to use the 3D cellular model to study deeply the functional consequences of PG machinery impairment in chondrodysplasias.

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P29
MUTATIONAL LANDSCAPE AND CO-EXPRESSION OF BIGLYCAN AND CHONDROITIN SULFATE MODIFYING ENZYMES IN BREAST AND BRAIN CANCERS

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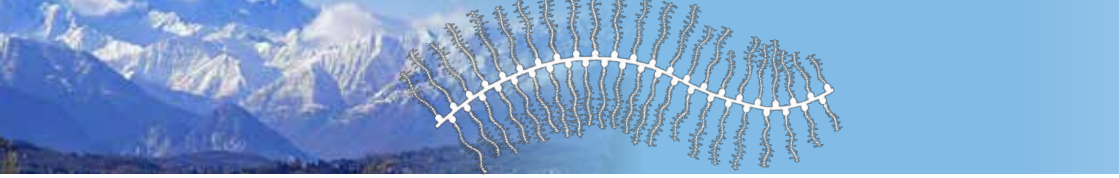
Introduction: Biglycan (BGN), a small leucine-rich proteoglycan (SLRPs), is an extracellular matrix (ECM) protein composed of a protein core with leucine-rich repeat motifs, N-linked oligosaccharides, and two glycosaminoglycan (GAG) side chains. Glycosylation is an enzymatic process that produces glycosidic linkages, which is required to create the GAG chains of chondroitin sulfate (CS) proteoglycans like BGN. In addition to somatic mutations aberrant glycosylation occurs in essentially all types of human cancers. However, the clinical relevance of CS chain modulating enzymes, structural alterations, expression pattern and posttranslational modifications of BGN is limited.

Objectives: It is hypothesized that genetic abnormalities and/or impaired BGN expression and an altered interaction of BGN with different GAG chain modulating enzymes might be associated with neoplastic transformation, disease progression and poor patients' outcome. Therefore the aim of this study is to analyse (i) the structural alteration and expression status of BGN and (ii) CS-modifying enzymes in different cancer types using 'The Cancer Genome Atlas' (TCGA) data portal and (iii) to correlate these data with clinicopathological parameters of the selected tumor entities, such as tumor grading, staging and survival of patients.

Methods: Bioportal analysis (http://www.cbioportal.org/) was used to explore mutations and expression of BGN, its co-occurrence with CS modifying enzymes (e.g. CS synthase (CHSY)-1,3 and chondroitin polymerizing factor (CHPF)-2) in breast (2051 samples) and brain (794 samples) cancers. Using R2 web tool (http://r2.amc.nl) prognostic values of 104 breast and 273 brain cancer samples were determined for BGN and CS modifying enzymes. The significance of differences between groups was assessed using one-way Anova and the Fisher's exact test for the categorical variables.

Results: A tumor type dependent co-expression of BGN and different CS modifying enzymes was found with a coordinated expression of BGN and CHSY3 in breast cancer and of BGN and CHSY1 in glioblastoma. Structural alterations of BGN (0.1% mutation (mut); 1.3% amplification (amp)) and CHSY1 (0.3% mut; 0.5% amp) were detected in glioblastoma, which were associated with worse patients' prognosis in 177/273 cases. In contrast, breast cancer samples lack mutations, but showed amplification in BGN (1.7% amp) and CHSY3 (0.5% amp). The amplification was accompanied with high BGN expression levels, which were associated with a favorable outcome in 94/104 breast cancer patients.

Conclusion: Altered co-expression and mutations in BGN and CS-modifying enzymes are associated with worse outcome of glioblastoma patients, whereas amplification and overexpression of wild type BGN has a tumor suppressive potential in breast cancer. These data suggest that structural alterations and expression patterns of BGN and its CS associated enzymes might represent prognostic biomarkers to assist in risk stratification for cancer patients.



P30
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Conclusions: Our findings shed light on the pivotal and specific role of XT-I during the ossification process2. For the future, it would be of great interest to use the 3D cellular model to study deeply the functional consequences of PG machinery impairment in chondrodysplasias.

References

[1]Swingler TE et al. Arthritis Rheum 2012.
[2]Bui C et al. Am J Hum Genet 2014.

P31
SYNDECAN-2 INDUCES ANGIOGENESIS BY INCREASING HYPOXIA-INDUCIBLE FACTOR-1A THROUGH AKT/MTOR PATHWAY

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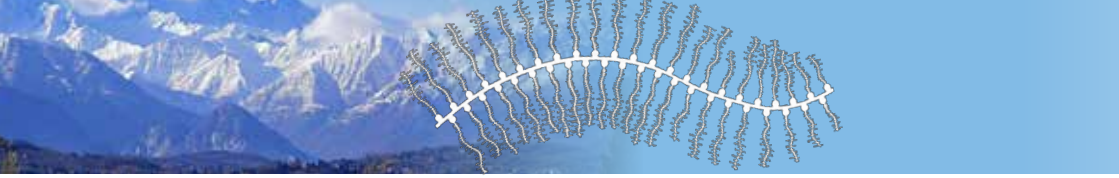
Introduction: Syndecans (SDCs) are type I transmembrane heparan sulphate proteoglycans comprising of syndecan-1,-2,-3, and -4 (SDC-1,-2,-3 and -4) and their extracellular domains interact with various soluble and insoluble factors in the extracellular matrix (ECM). As SDC-2 regulates adhesion, migration, ECM remodeling and angiogenesis, its elevated level is critical in tumorigenesis. Inflammatory hypoxia is reported to induce SDC-2 leading to progression of colon cancer; however, increased angiogenesis on exposure to shed SDC-2 in hypoxic condition is not yet studied.

Objective: The aim of this study is to show the role of shed SDC-2 in neovascularization. The increased vascularization mediated by shed SDC-2 is through increased HIF-1a by activated AKT/mTOR pathway and decreased ubiquitination of HIF-1a in hypoxic condition.

Results: We studied the role of shed SDC-2 in human colon cancer cells and umbilical vein endothelial cells (HUVECs) by using SDC-2 peptide. Colon cancer cells treated with SDC-2 peptide on exposure to hypoxia increased HIF-1a protein. As a molecular mechanism to the increased HIF-1a, activated AKT/mTOR pathway and decreased ubiquitination of HIF-1a were observed. In addition, HUVECs cells exposed to SDC-2 peptide significantly increased the proliferation of these cells in dose dependent manner. Furthermore, migration and tube formation were also found increased significantly with SDC-2 peptide. In support to these data, in vivo chick chorioallantoic membrane (CAM) angiogenesis assay and chick aortic ring assay revealed significantly increased neovascularization in SDC-2 peptide treated group.

Conclusion: Shed SDC-2 can enhance angiogenesis through the stabilization of HIF-1a by upregulation of AKT/mTOR pathway and decreased ubiquitination of HIF-1a in hypoxic environment. Thus shed SDC-2 could be a promising candidate for anticancer or other forms of angiogenesis based disease models.




JHC