

Biodiversity of CS-proteoglycan Sulphation Motifs: Chemical Messenger Recognition Modules with Roles in Information Transfer, Control of Cellular Behaviour and Tissue Morphogenesis.

Anthony Hayes^a, Kazuyuki Sugahara^{b, c}, Brooke Farrugia^d, John M Whitelock^d, Bruce Caterson^e, James Melrose^{e, ¶}

^aBioimaging Research Hub, Cardiff School of Biosciences, Cardiff University, Cardiff CF10

3AX, Wales, UK. ^bGraduate School of Life Science, Hokkaido University, Sapporo, Japan.

^cDepartment of Pathobiochemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan.

^dGraduate School of Biomedical Engineering, University of New South Wales, Sydney 2052,

NSW, Australia. ^eSchool of Biosciences, Cardiff University, Cardiff, CF10 1AX, Wales, UK.

[¶]Raymond Purves Bone and Joint Research Laboratories, Kolling Institute of Medical Research, Royal North Shore Hospital and University of Sydney, St. Leonards, NSW 2065, Australia.

¶Address correspondence to: -

Dr. J. Melrose,

Raymond Purves Bone and Joint Research Laboratories,

Level 10, Kolling Institute of Medical Research B6,

The Royal North Shore Hospital,

St. Leonards, NSW 2065, Australia.

Ph +61 2 9926-4806,

Fax +61 2 9926-5266

Email: james.melrose@sydney.edu.au

Abstract

Chondroitin sulphate glycosaminoglycan chains on cell and ECM proteoglycans can no longer be regarded as merely hydrodynamic space fillers. Overwhelming evidence over recent years indicates that sulphation motif sequences within the chondroitin sulphate chain structure are a source of significant biological information to cells and their surrounding environment. Chondroitin sulphate sulphation motifs have been shown to interact with a wide variety of bioactive molecules e.g. cytokines, growth factors, chemokines, morphogenetic proteins, enzymes and enzyme inhibitors, as well as structural components within the extracellular milieu. They are therefore capable of modulating a panoply of signalling pathways thus controlling diverse cellular behaviours including proliferation, differentiation, migration and matrix synthesis. Consequently, through these motifs, chondroitin sulphate proteoglycans play significant roles in the maintenance of tissue homeostasis, morphogenesis, development, growth and disease. Here we review (i) the biodiversity of chondroitin sulphate proteoglycans and their sulphation motif sequences and (ii) the current understanding of the signalling roles they play in regulating cellular behaviour during tissue development, growth, disease and repair.

1. Introduction

Chondroitin sulphate (CS) and its sulphation motifs on cell associated, pericellular and extracellular matrix (ECM) proteoglycans (PGs) represent a significant repository of information in tissues with the capacity to encode functional information rivalling that of RNA, DNA and proteins [1]. This information is realised when CS and its sulphation motifs interact with growth factors, cytokines, morphogenetic proteins, enzymes, inhibitors and pericellular matrix (PCM) and ECM stabilising glycoproteins. Such interactions have diverse effects on cellular metabolism, proliferation and differentiation, cell migration, matrix synthesis/stabilisation and tissue remodelling in development and are critical to the cellular control of tissue homeostasis. CS sulphation motifs on cell-associated, PCM and ECM proteoglycans also provide important molecular recognition and activity signals to stem/progenitor cell niches facilitating the sequestration of combinations of growth factors, cytokines and chemokine's which maintain the niche microenvironment ensuring stem cell survival and their maintenance in a state of quiescent self-renewal within the niche environment. Perturbations in the signals which stem cells receive in this niche can also orchestrate stem/progenitor cell differentiation and pluripotency resulting in stem cell activation and proliferation into specific cell lineages with migratory properties facilitating their participation in tissue growth, development and repair processes.

Virtually every cell in the human body is surrounded by a dense glycocalyx of glycoconjugates consisting of mixtures of glycoproteins, proteoglycans and glycolipids which provide a protective and interactive barrier [2]. CS is a prominent glycosaminoglycan (GAG) component of many of these molecules and is the most abundant GAG in the human body [3]. The glycocalyx connects the cell to its external microenvironment and components in the glycocalyx act as biosensors through which cells perceive and respond to changes in the environment they live in [4, 5]. The endothelial glycocalyx also has mechanosensory shear and compression responsive functions, which regulate mechanotransductive effects on endothelial cell signalling and vascular permeability, which are important in the nutrition and development of tissues [2, 5-9]. In brain, the glycocalyx of microglia and oligodendrocytes contain cell surface sialic acid binding immunoglobulin like lectins (SIGLECS), which identify sialic acids in cell surface glycoconjugates in adjacent neurons facilitating cellular communication and signalling through an intracellular immunoreceptor tyrosine based inhibition motif (ITIM) which maintains a homeostatic balance in

neuronal cells [4]. Embryonic stem cells also assemble a glycocalyx containing cell surface epitopes which not only can be used to identify specific stages of stem cell differentiation but serve as interactive modules that can network with regulatory cues received from the ECM influencing stem cell differentiation [2]. The endothelial and epithelial glycocalyx also have important roles to play in inflammation and immunomodulation [10]. MUC1 (CD227), a high molecular weight (>400 kDa) widely distributed, multifunctional, type-1 membrane tethered epithelial glycoprotein, has roles in dendritic cells, monocytes, T and B cells in immune mediated inflammatory processes [11]. MUC1 in the mucosal lining also provides a protective lubricative barrier to microbial infection [12, 13]. The cerebrovascular glycocalyx also has important roles to play in neural protection; SIGLECS protect neurons from acute toxicity through interaction with glycolipids, which provide barrier functions [14, 15]. The glycocalyx displays brain specific functions through its participation in interactions with cell surface receptors, which undertake protein-phosphorylation mediated signalling by neurons and can also influence apoptosis and amyloid deposition [7]. GAG components in the glycocalyx have important roles in neuroprotection through interactions with CS-receptors and participation in cell signalling events which maintain cellular integrity and also preserve the tissue hydration provided by GAGs to the PCM and ECM.

2. The biodiversity of ECM and cell associated molecules decorated with CS GAG chains.

CS (Fig 1) is composed of β 1-3 and β 1-4 linked D-glucuronic acid and *N*-acetyl D-galactosamine repeat disaccharide units which can be O-sulphated at the 2, 4 and C6 position [16]. Furthermore, the D-glucuronic acid moiety may also be epimerised to α -L-Iduronic acid in the related GAG dermatan sulphate (DS) leading to a considerable degree of structural diversity in CS/DS and the ability to interact with a large range of cytokines, chemokines, morphogens and growth factors which regulate cellular proliferation and differentiation and tissue development [16-25]. CS also has indispensable roles to play in stem cell differentiation and the attainment of pluripotency [26]

CS (Fig 1) decorates a remarkably diverse collection of matrix and cell associated macromolecules (Fig 2-4). Their functional properties are summarised in Table 1 and their structural features shown diagrammatically in Figs 2-4. CS occurs as a number of isomeric forms, referred to as CS-A, CS-C, or CS-D based on the mono- or disulphate positions (Figure 1j). CS-B, also known

as dermatan sulphate, like CS-A is sulphated at the C4 position of N-acetyl D-galactosamine, but differs due to epimerization of D-glucuronic acid to L-Iduronic acid and this is sulphated at C2.

2.1 The CS-proteoglycans represent a bio-diverse group of molecules.

2.1.1 The Hyalactans

The Hyalactans are a group of large HA interactive CS-proteoglycans [27-31] (Fig 2a). Aggrecan and versican form aggregate structures with HA which provide tissues with an ability to act as weight bearing and tension resisting structures while neurocan, brevican and aggrecan hyalactans form perineural-net structures through interaction with HA and tenascin-R (Fig 5a). Perineuronal nets have neuroprotective roles [32, 33] but also inhibit neuronal repair processes by inhibiting neurite outgrowth, both of these functions are due to the particular GAGs, which decorate these proteoglycans. Similar network structures between aggrecan, link protein and hyaluronan are also prominently featured in cartilaginous tissues where they have roles in weight bearing (Fig 5b).

2.1.2 The SLRPs

The small leucine rich proteoglycan (SLRP) family have well known functional roles in the regulation of collagen fibrillogenesis but have additional cell regulatory roles through their interactive properties with cytokines, growth factors and morphogens [34-38] Decorin and biglycan are two SLRPs which contain one or two CS chains and in specific contexts DS (Fig 2b).

2.1.3 SPACRCAN

SPACRCAN is a novel 400 kDa CS-proteoglycan of the inter-photoreceptor ECM providing an interface between the photoreceptors and the pigmented retinal epithelium in the fundus of the eye [39]. SPACRCAN contains 6-sulphated CS chains and a number of *N*- and *O*-linked oligosaccharides which collectively constitute ~60 % of its total mass (Fig 2c) . SPACRCAN has two RHAMM like binding domains through which it interacts with HA to form an aggregate structure which organises the ECM and is also important in the hydration of this tissue [40].

2.1.4 Perlecan

Perlecan is a HS-proteoglycan in vascular tissues however chondrocytes and smooth muscle cells synthesise a hybrid form of perlecan where CS chains replace some of its HS chains [41-46]. Epithelial perlecan is also a hybrid perlecan and a unique proteoglycan containing HS, CS and KS chains [47]. The form of perlecan synthesised by foetal IVD progenitor cells contains 7-D-4 CS sulphation motifs [48]. The GAG side chains of perlecan in growth plate cartilage contain

embedded 4, 6-disulphated CS-E disaccharides that direct collagen fibrillogenesis[49] and are also found in the brain proteoglycan, appican interacting with neuroregulatory factors, which direct neuritogenesis [50-52].

2.1.5 *Appican*

Over sulphated disaccharides of CS-D and CS-E (Fig 1j) regulate neuronal adhesion, cell migration, and neurite outgrowth in the CNS. Several brain CS-proteoglycans including phosphacan (DSD-1) and bikunin contain embedded CS-D motifs within their CS side chains. Appican is the only brain proteoglycan identified, with embedded CS-E [51]. Appican is produced exclusively by astrocytes, which regulate neural cell adhesion and outgrowth. Appican also contains Alzheimer amyloid precursor protein (APP) as a core protein component, which is a Kunitz protease inhibitor/Protease nexin 2 domain [50] with voltage gated ion channel blocking properties relevant to neurite regulation [53]. The CS-E motif is essential for the interaction of the appican CS-chain with growth/differentiation factors, and the regulation of neuronal cell adhesion, migration and neurite outgrowth.

2.1.6 *NG2/CSPG4*

Chondroitin sulphate proteoglycan-4 (CSPG-4) (Fig 3a), also known as high molecular weight melanoma associated antigen in humans and nerve-glia antigen-2 (NG2) in rodents is a transmembrane CS-proteoglycan expressed by immature progenitor cells including oligodendrocyte, chondroblasts/osteoblasts, myofibroblasts, smooth muscle cells, pericytes, interfollicular epidermal and hair follicle cells [54, 55]. CSPG4 is a single pass type 1 transmembrane protein, occurring as a 250kDa glycoprotein, a 450kDa C4S-proteoglycan or can be non-glycanated [56, 57]. The CS side chain of CSPG-4 facilitates interactions with $\alpha4\beta1$ integrin and fibronectin and has roles in the activation of proMMP2 by transmembrane MMPs. This ability to influence integrin and MMP activation implicates CSPG-4 in melanoma migration and invasion in skin [58, 59]. CSPG-4 may participate in cell signalling as a co-receptor or by association with cytoplasmic kinases such as FAK or ERK-1, 2. CSPG-4 binds FGF-1 and PDGF AA and presents these to their cognate receptors to influence cellular proliferation and differentiation [60]. The central non-globular domain of CSPG-4 binds to collagen V and VI [61, 62] facilitating cellular attachment and ECM stabilisation and may induce cytoskeletal reorganisation conducive to cell spreading and migration [63, 64]. In NG2 knockout mice the epidermis is very thin due to reduced

basal keratinocyte proliferation providing clues as to the likely role of this proteoglycan in skin development and homeostasis and insightful as to its possible roles in melanoma spread [54, 57].

2.1.7 *Thrombomodulin*

Thrombomodulin (TM- β , CD141) is a multifunctional 74-105 kDa cell-surface CS-proteoglycan mediator of endothelial anticoagulant activity, activator of Protein-C and a thrombin receptor (Fig 3b). The presence of CS on TM- β decreases the Kd for thrombin binding and significantly accelerates thrombin inhibition [65, 66]. The C-4-S chains on TM are relatively small (10-12 kDa) [67, 68] but essential for its anticoagulant activities [69]. TM acts as an anticoagulant protein through its actions on thrombin and by participating in the generation of activated protein C (APC) [66]. Once APC is formed it binds to protein-S on the cell surface and the APC-protein-S complex inactivates factors Va and VIIIa. [70-72]. TMs domain structure and multi-component interactions with thrombin, Protein-C, Thrombin-Activatable Fibrinolysis Inhibitor, Complement, Lewis^Y antigen, and HMGB1, a chromosomal protein which regulates transcriptional replication, facilitates TM's physiologically significant anti-inflammatory, anti-coagulant, and anti-fibrinolytic properties [73, 74].

2.1.8 *Phosphacan*

Receptor-type protein tyrosine phosphatase beta (RPTP- β) is a transmembrane CS-proteoglycan expressed in the developing nervous system and contains an extracellular carbonic anhydrase (CAH) and fibronectin type III repeat domain, both of these domains foster protein-protein interactions. RPTP is expressed in 3 alternatively spliced forms RPTP- γ , RPTP- β ζ , and a truncated form of RPTP- β with an 860 amino acid deletion (Fig 3c). Phosphacan is the proteolytically released ecto-domain of the transmembrane protein tyrosine phosphatase receptor- ζ of neurons and glial cells [75] and is a principal CNS proteoglycan promoting neuron-glia interactions, neuronal differentiation, myelination and axonal repair. The transient nature of cell signalling by phosphorylation requires specific phosphatases for regulatory control. Phosphorylation of tyrosine residues in cellular proteins plays an important role in the control of cell growth and differentiation in the brain [76-79]. The complexity of this regulatory system is evident in the spectrum and widespread distribution of spatially and temporally expressed protein tyrosine phosphatases. The CAH domain of RPTP- β /□□□□□□□□□□ promotes protein-protein

recognition, induces cell adhesion and neurite outgrowth of primary neurons, and differentiation of neuroblastoma cells. Interaction of phosphacan with contactin may generate unidirectional or bidirectional signals which direct neural development and axonal repair [80].

2.1.9 The Syndecan family

The GAG side chains of the syndecan proteoglycans provide subtle variation in their binding properties with ligands (Fig 3d). The core protein of the syndecans have a protease sensitive site close to the transmembrane attachment region, its cleavage results in the release of a soluble ecto-domain form of these proteoglycans. Although widely categorised as HS-proteoglycans, syndecan-1, 3 and 4 can also be substituted with CS chains [81]. HS chains have an invariant structure between syndecan family members however their CS chains may contain non-sulphated, 4-*O*-, 6-*O*-, and 4,6-*O*-disulfated *N*-acetylgalactosamine-CS-E. The CS chains of syndecan-4 generally display a greater overall sulphation level than the CS chains in syndecan-1 [82, 83]. The HS and CS chains of syndecan-1 and 4 bind FGF-2, midkine (MK) and pleiotrophin (PTN). The HS and CS side chains of syndecan-4 are found localised with integrins in focal adhesions in fibroblasts indicating that they have roles in cellular attachments and promote cellular migration [84] and may also influence cell signalling .

2.1.10 CD44

CD44 is the major HA receptor in the human body and is a ubiquitously distributed cell surface receptor (Fig 3e). CD44 can also occur as a part-time proteoglycan called epican, which is substituted with HS or CS chains. Epican is expressed by keratinocytes and mediates cell adhesive properties between keratinocytes in the epidermis [85, 86].

2.1.11 Bikunin

Bikunin is a 30-39 kDa serum proteinase inhibitor synthesized in the liver and is a member of the inter- α -trypsin inhibitor (220 kDa) (ITI) and pre- α -trypsin inhibitor (125 kDa) (Pre- α -TI) families [87, 88] (Fig 4a). A retrospective assessment of the Kunitz serine proteinase inhibitory proteins present in ovine articular cartilage, meniscus and intervertebral disc indicated that the 250, 120, 86, 58, 34-36 and 6-12 kDa SPIs in these tissues were related to ITI and pre- α -TI [89, 90]. Bikunin's CS chains contain regions which are sulphated and non-sulphated, the sulphated region contain embedded CS-D disaccharides [91]. The CS chain in bikunin is relatively small but heterogenous

(8-25 kDa) [92, 93]. Bikunin inhibits trypsin, thrombin, chymotrypsin, kallikrein, plasmin, elastase, cathepsins, Factors IXa, Xa, XIa, XIIa inhibitory activity and contains two 6 kDa Kunitz inhibitory domains. Bikunin counters inflammatory processes during a number of physiological processes and also has anti-tumour and anti-viral and neuroregulatory activities.

2.1.12 Type IX collagen

Type IX collagen [94, 95] contains a CS chain attached to the α 2-chain of the type IX NC3 domain [96-98] and is the PG-Lt proteoglycan isolated from chick embryonic tibia and femur [99] and chick embryo sternal cartilage [100, 101] (Fig 4b). CS-substituted type IX collagen has also been isolated from chondrosarcoma [102] but is present as a minor glycanated form in articular cartilage [103]. The Type IX collagen of chick vitreous humour contains an extraordinarily large CS chain of 350kDa in size [104-107]. The related type XII [108, 109] and XIV collagen [110] which are basement membrane components, also bear CS chains and homology to type IX collagen.

2.1.13 Testican

The SPOCK gene encodes the protein core of a seminal plasma testican proteoglycan containing CS- and HS chains (Fig 4c). This protein's function is unknown, although similarity to thyropin-type cysteine protease-inhibitors suggests its function may be related to protease inhibition. Testican-1 inhibits cathepsin-L [111]. Testican-2, 3 also regulates MMP activation at the cell surface abrogating MT1 MMP activity and proMMP-2 processing [112, 113]. Testican is produced by endothelial cells [114] and has a widespread distribution, the brain is a particularly rich source of testican [115].

2.1.14 Serglycin

Serglycin is the only intracellular proteoglycan so far identified. Serglycin localizes to the α -secretory granules of platelets and mast cells, where it binds and regulates the activity of platelet factor-4 in platelets or tryptase and chymase in mast cells [116-119]. Serglycin is decorated with CS chains in the secretory granules of circulating basophils, but with heparin in resident tissue mast cells [116, 120]. Mast cell serglycin displays a 2-B-6 (-) epitope on the CS chains which decorate this proteoglycan [121]. Trypstatin, the Kunitz protease inhibitor domain 2 of bikunin/ITI is localized complexed with serine proteases in the α -granules of mast cells.

2.1.15 Colony stimulating factor

Human monocytes secrete two CS-proteoglycan forms of colony stimulating factor (CSF) containing two CS chains attached at the C-terminus of CSF-1 [122, 123].

2.1.16 Leparcan

Leparcan is a basement membrane CS-proteoglycan; it contains an N-terminal leucine and proline rich domain, a C-terminal globular domain containing two CS chains and a 2-oxoglutarate-Fe dependant dioxygenase and prolyl-3-hydroxylase enzymatic activity [124]. Prolyl hydroxylases 1-3 (PHD1-3) are oxygen-sensing enzymes which catalyse the hydroxylation of conserved prolyl residues in the HIF-1 α sub-unit in normoxia targeting it for proteasomal degradation. HIF-1 α and NF- κ B are stabilised in hypoxia regulating a diverse range of ~200 genes in erythropoiesis, angiogenesis, cardiovascular function, inflammation, apoptosis and cellular metabolism [125-128].

2.1.17 Identification of neuroendocrine Pro-hormones as CS-Proteoglycans.

A number of CS-DS pro-hormones have been identified using a proteomics screen involving isolation by anion exchange chromatography, pre-digestion of the isolated anionic proteins with chondroitinase ABC and identification of the CS linkage tetrasaccharide by mass spectrometry. Many of these pro-hormones are stored in intracellular granules in neuroendocrine cells. Granule proteins such as Chromogranin-A are processed into hormone peptides such as secretogranin-1, 2, 3, cholecystokinin or neuropeptide W.

3. Aggregated proteoglycan structures in cartilage and brain.

Members of the hyalactan proteoglycan family including aggrecan, brevican, neurocan and versican form massive supramolecular perineural net structures in the CNS (Fig 5a) through interactions with HA and tenascin-R. Perineural nets protect neurons from oxidative stress and mechanical damage but also provide inhibitory signals preventing neural outgrowth. Aggrecan and versican also form massive supramolecular aggregate structures by interaction with HA and link protein in cartilage and fibrocartilaginous tissues (Fig 5b). These aggregated structures have impressive water regain properties equipping cartilage and IVD with the ability to withstand compressive forces.

4. CS sulphation motifs as molecular markers of tissue morphogenesis

During tissue morphogenesis several proteoglycans contain native 4-C-3, 7-D-4, 3-B-3[-] and 6-C-3 sulphation motifs (see Fig 1 for explanation). These native CS sulphation motifs are expressed in the surface regions of developing articular cartilages in the knee joint (Fig 6 a, b) perichondrial growth plate, and in vascular ingrowth and stromal vascular niches of transitional tissues associated with diarthrodial joint and IVD development.[129-132]. Confocal colocalisation of the aforementioned CS sulphation motifs with aggrecan, versican and perlecan in neonatal cartilages has demonstrated that aggrecan and perlecan in these tissues bear these sulphation motifs while versican does not [133]. Confocal studies in the human foetal elbow also demonstrated perlecan associated with perichondrial stem cell niches (Fig 7a) and with progenitor cell populations in the perichondrium (Fig 7c, g) and surface regions of the developing elbow joint cartilages (Fig 7c, h, i).

4.1 Cartilage proteoglycans containing CS sulphation motifs with roles in tissue development.

Aggrecan is the major CS-proteoglycan of cartilaginous tissues, with well-known space-filling and water imbibing properties that equip these tissues with resilience to compressive loading. Correct sulphation of CS-proteoglycans is essential for proper Indian hedgehog signalling in the developing growth plate [134], perlecan, a hybrid CS-HS proteoglycan in cartilage is also responsible for the localization and activity of the related Sonic hedgehog protein [135]. Native CS sulphation motifs such as 7-D-4 on proteoglycans may serve to immobilise growth factors/morphogens actively involved in tissue development [17]. The unique distributions of native CS motifs such as 7-D-4 with surface zone progenitor cells in articular cartilage [132, 136, 137] and within the developmental intervertebral disc (IVD) [129] and human foetal elbow [130] is suggestive of an early stage of progenitor cell differentiation and indicates that native CS sulphation motifs have functional roles in chondrogenesis and in IVD development [129, 136, 137].

4.2 Focal expression of the 7-D-4 CS sulphation motif in human foetal paraspinal blood vessels.

Perlecan produced by endothelial and smooth muscle cells is a prominent component of capillaries and larger blood vessels (Fig 8a, b). The 7-D-4 CS sulphation motif displays a focal distribution in the luminal surfaces of capillaries and between the endothelial cells lining human foetal paraspinal blood vessels (Fig 8b). Pericytes are contractile cells that wrap around the abluminal surface of endothelial cells that line the capillaries and venules throughout the body (Fig

8d-g). Caplan proposed that all stem cells were pericytes emphasising their vascular origins [138-141]. Pericytes are embedded in basement membrane where they communicate with endothelial cells of the body's smallest blood vessels by means of both direct physical contact and paracrine signalling [142-145]. Blood flow generated shear forces are also important functional determinants of the differentiation of stem cells in the luminal surfaces of blood vessels [8, 146].

4.3 The tissue distribution and function of oversulphated CS isomers CS-D and CS-E

Developmental studies on the whole rat brain have correlated changes in the CS side chain structure of phosphacan with measurable changes in the binding affinity of PTN and functional consequences on the cell signalling response. Phosphacan isolated from whole rat brain from various developmental stages was examined using the CS antibodies MO225, CS56 and 2H6 in a plasmon resonance study [147]. P7-phosphacan strongly reacted with CS56 and 2H6 but not MO225. P12-phosphacan showed moderate reactivity with CS56 and 2H6 but no reactivity to MO225 contrasting with P20-phosphacan which was strongly reactive with MO225 but low reactivity with CS56 and 2H6. mAb 2H6 is sold as an anti CS-A Ab due to its high reactivity with whale cartilage CS-A however its reactivity with phosphacan of a defined CS-A content does not correlate with this. P7 phosphacan with a CS-A content of 64% had the highest reactivity with mAb 2H6 while P20 phosphacan with a CS-A content of 86% had very low reactivity. This showed that the 2H6 epitope was not to a simple CS-A unit but to a more extended binding epitope. Subsequent studies have shown that mAb CS-56 and MO-225 specifically recognize octasaccharides containing an A-D tetrasaccharide sequence, whereas 2H6 preferred sequences with A- and C-units such as C-C-A-C for strong binding but no D-unit, mAb MO225 also recognised the CS-E disaccharide motif from squid cartilage in an extended E-E-E-E-C binding motif [148, 149]. The development of CS oligosaccharide libraries [150] of defined structure has further enhanced the precision of such structure-function studies. These show that the CS motifs are differentially regulated in brain development and modulation in CS structure occurs in a spatiotemporal manner.

5. Cell regulatory proteoglycans are involved in neural development and repair.

Oversulphated CS/DS promotes neural development with variation in sulphation profiles of proteoglycans regulating vertebrate CNS development. The disulphated disaccharide D-unit promotes neurite outgrowth through the DSD-1 epitope embedded in the CS chains of DSD-1-PG/phosphacan [150-155]. Oversulphated DS displays neurite outgrowth activity [156]. The short

isoform, non-proteoglycan variant form of phosphacan/receptor protein tyrosine phosphatase- β also interacts with neuronal receptors and promotes neurite outgrowth [80]. Bikunin is also expressed in brain tissue [157, 158] and accumulates in brain tumours [159]. Like phosphacan, bikunin contains disulphated embedded CS-D motifs within the repeating disaccharide region of its CS chain [91]. Such motifs promote neurite outgrowth, suggesting that bikunin may also have similar roles to play in neural development. Appican is another brain CS-proteoglycan [50, 160] produced by astrocytes [161], which direct neural development. CS-E motifs embedded within the CS chains of appican [51] interact with neuroregulatory factors [52] inducing morphological change in C6 glioma cells and directed adhesion of neural cells to the ECM [53]. CS-E motifs also promote chondrocytic differentiation of ATDC5 cells. ATDC5 cells produce monosulphated CS-A or disulphated disaccharides (CS-E) in their ECM proteoglycans. Exogenously added CS-E also affects chondrogenic differentiation of ATDC5 cells, promoting chondrogenic differentiation demonstrating the existence of cell surface receptors for CS-E [162]. Embedded CS-E in the CS side chains of growth plate perlecan also promote collagen fibrillogenesis [49].

NG2 proteoglycan, phosphacan and syndecan-1-4 have roles in cellular regulation and tissue development, which may be of application in tissue repair strategies. For example NG2 proteoglycan stimulates endothelial cell proliferation and promotes migration during micro-vascular morphogenesis. NG2 is also expressed by chondroblasts and chondrocytes and acts as a cell surface $\alpha 2$ -VI collagen receptor conferring cellular motility and $\alpha 4\beta 1$ integrin mediated cell spreading by activation of FAK and ERK1/ERK2 signalling cascades. A better understanding of the CS-sulphation motifs and their binding partners and how these regulate cellular processes in tissue remodelling and repair may allow the development of improved therapeutic procedures in repair biology.

5.1 The balance between stimulatory and inhibitory signals in neural development

SRPX2 (Sushi repeat protein, X linked 2) (Fig 2a) is a novel secreted CS-proteoglycan, which promotes synaptogenesis in the cerebral cortex and is found as an embedded domain in some members of the lectican proteoglycan family. The SRPX2 gene is a target of the foxhead box protein P2 transcription factor (FoxP2) that modulates synapse formation [163]. Mutations in SRPX2 causes Rolandic epilepsy and speech impairment (RESDX syndrome). Interactome/cell surface binding/plasmon resonance studies have identified SRPX2 as a ligand for uPAR, the urokinase type

plasminogen activator (uPA) receptor [164]. uPAR knockout mice exhibit an enhanced susceptibility to epileptic seizures and anomalous cortical organization consistent with altered neuronal migration during brain development [165, 166]. uPAR is a crucial component of the extracellular plasminogen-plasmin system, which remodels the ECM during brain development. Cathepsin B and ADAMTS4 are also SRPX2 ligands and also likely participants in developmental processes in the brain [167]. ADAMTS-4 has been localised to regions of the spinal cord undergoing repair. ADAMTS-4 degrades aggrecan and versican in the CNS thus removing the inhibitory signals provided by the CS side chains of these PGs [168, 169].

Cathepsin B is a well known activator of pro uPA thus SRPX2 and its ligands represent a network of proteins with critical roles in brain development and specifically in the centres of speech and cognitive learning. The Rolandic and Sylvian fissures bisect the human cerebral hemispheres and it is the adjacent areas of the brain, which are responsible for speech processing. Ordered neuronal migration is therefore essential for the correct development of these areas of the brain. SRPX2 protein expression occurs in neurons from birth and has central roles to play in developmental processes in the centres of speech and cognitive learning. Two mutations have been identified in SRPX2 in RESDX patients. One mutation (N327S) results in altered glycosylation while a second mutation (Y72S) affects the first sushi domain of SRPX2 [170], 3D modelling indicates that the Y72S mutation affects an area of the SRPX2 core protein normally involved in protein-protein interactions [171]. Cultured cells from RESDX patients display alterations in the intracellular processing of proteins and likely misfolding which may have functional consequences [172].

Specific CS sulphation motifs are involved in interactions between neurons and glial cells to regulate the development and regeneration of the CNS. Migrating neurons are guided by glial cells through ECM proteoglycans they assemble such as phosphacan, and the CS-lectican proteoglycan family. Phosphacan promotes neurite outgrowth whereas versican, neurocan, and brevican inhibit this process thus collectively these proteoglycans direct neurite growth. This is a function of their differing GAG CS sulphation motifs. CS-D motifs in phosphacan promote neurite outgrowth while lectican CS-A and CS-C motifs inhibits neuronal migration [152] and regulate neural tissue morphogenesis [153] [150, 173]. Glucuronyl transferase-1 knockout embryonic stem cells (ESCs) lack CS resulting in a significantly altered ability to differentiate and reduced ability to develop into pluripotent cell lineages [26]. HS maintains ESCs in a state primed for differentiation

however CS maintains ESC pluripotency and promotes ESC differentiation. Binding of CS-A and CS-E to E-cadherin to overcome cell inhibitory signals enhances ESC differentiation [26]. The highly charged CS-D and CS-E sulphate motifs can mimic HS in terms of growth factor and cytokine binding however the less highly charged CS-A and CS-C isomers should not be discounted in such interactions. Surface plasmon resonance studies have demonstrated CS-A and CS-C bind with significant affinity to midkine, pleiotrophin, HGF and stromal cell derived factor-1 β but with a lower affinity than CS-D, CS-E and HS [174] regulating the growth, differentiation and migration of neural precursor cells [175]. Such lower affinity interactions may provide a more subtle control mechanism than the strong on-off signals supplied by HS. FGF-2 and EGF dependant proliferation of glial cells regulates neurogenesis during CNS development [176-178]. Chondroitin-6-sulphate synthesis is upregulated in the injured CNS, induced by injury related cytokines and enhanced in axon-growth inhibitory glia [179] and of relevance to nerve regeneration through glial scar formations [180].

6. CS sulphation motifs and pathological remodelling of connective tissues

Several years ago [181, 182] it was noted that mAbs 3-B-3 (-) and 7-D-4 identified chondrocyte “cell-clusters” in pathological (osteoarthritic) canine and human articular cartilage and at that time these were considered a classical feature of the onset of late stage degenerative joint disease. In these early publications a lack of knowledge of stem/progenitor cells in cartilage and expression of proteoglycans (aggrecan) with CS GAG chains recognised by mAbs 3-B-3 (-) and 7-D-4 were interpreted to indicate a failed, late-stage, attempt to repair cartilage and replacement of new proteoglycans in a matrix that had been extensively degraded by MMPs. An alternative hypothesis now is that these ‘chondrocyte clusters’ arose from adult stem/progenitor cells in these tissues [183]. Tesche and Miosge [184, 185] showed that adult stem cell clusters were surrounded by a pericellular matrix containing perlecan. This is also a feature of stem cell niches in foetal knee, hip, IVD and elbow cartilage [129-133, 186]. It is expected that in different connective tissues, the CS sulphation motifs will be present on different matrix and cell surface proteoglycans, if this is the case, an important feature of the stem/progenitor cell niche may be the sulphation of the GAGs rather than the core proteins to which they are attached. Expression of different levels of GAG sulphotransferases in stem/progenitor cells would therefore also contribute to tissue repair [187-189]. More recently, cell clusters within the superficial zone of healthy articular cartilage have been

shown positive for both Notch 1 and CD166 [190], cell surface markers that are synonymous with the stem cell niche environment [136, 191].

6.1 CS expression and tumour development

Neuroendocrine tumours with different degrees of histological differentiation have correlative alterations in associated CS but little change in HS. Normal stroma contains no staining with anti-CS Abs while staining in tumour is significantly elevated and highest in advanced tumour grades [192]. CS-proteoglycan levels are elevated in liver cancer [193], renal [194], hepatocellular [195, 196] and gastric carcinoma [197], pancreatic cancer [198] and in mammary tumours [199]. In gastric and pancreatic cancer non-sulphated and 6-sulphated CS predominate over other GAG isoforms and the GAG chains display a smaller hydrodynamic size than normal tissues. Elevated levels of 4- and 6-sulphated CS are found in renal cancer. Decorin and versican levels are elevated 7-27 fold in pancreatic cancer, and contain non-sulphated and 6-sulphated CS. This contrasts with the normal pancreas where DS is the predominant GAG decorating versican and decorin core proteins. Tumour proteoglycans have altered interactive properties further impairing the normal functional properties of tumour affected tissues. Proteoglycans and GAGs modulate cellular processes relevant to all stages of tumour progression, including cell proliferation, cell-matrix interaction, cell motility and invasive growth. HS, CS/DS and HA all have well documented roles in tumour pathobiology [200]. CS is abundantly present in the ECM in ovarian cancer. Alterations in the sulphation of CS also influences cancer development and its aggressive status. The *CHST15* gene is responsible for the biosynthesis of highly sulphated CS-E [269]. The single chain phage Ab GD3A11 to highly sulphated CS facilitates identification of biomarkers in aggressive tumour development. The GD3A11 epitope is minimally expressed in normal tissues but is intensely expressed in a number of ovarian cancer sub-types but not in benign ovarian tumours [201]. Serum over-sulphated CS levels measured using mAb WF-6 are also elevated in ovarian cancer and may also be a useful biomarker [202]. Silencing of *CHST15* in-vitro and in a xenograft model of pancreatic cancer down-regulates tumour invasion in pancreatic ductal adenocarcinoma (PDAC). CS-E is detected in both tumour and stromal cells in PDAC and is considered to have multistage involvement in its development [203]. A single intra-tumoural injection of CHST15 siRNA almost completely silenced tumour growth providing evidence of the direct involvement of CHST15 in the proliferation of pancreatic tumour cells identifying a novel therapeutic target. The phage display antibody GD3G7 also reacts with the rare CS-E and DS-E epitopes in normal tissues, where DS-E epitope represents IdoUA-GalNAc (4,6-*O*-disulphate) [368]. CS-E is strongly up-regulated in

ovarian adenocarcinomas. Thus Ab GD3G7 is useful in defining tumour tissue alterations [204]. Quantitation of GAGs in colorectal tumour tissue using electrospray ionization mass spectrometry showed that neoplastic tissues displayed greater levels of CS and DS than non-neoplastic tissue where HS was decreased [205]. NEDD9 (CAS-L, HEF-1) cells have key roles in the migration and proliferation of MDA-MB-231 breast cancer cells [206]. Microarray studies in breast cancer samples demonstrate elevated CD44 and serglycin and down-regulation of syndecan-1, syndecan-2 and versican whereas *CHST11*, *CHST15* and *CSGALNACT1* were all up-regulated in NEDD9 cells, an increase in CS-E attached to CD-44 was also evident in tumour cells. Removal of CS using chondroitinase ABC inhibited colony formation by NEDD9 cells whereas exogenous application of CS-E enhanced NEDD9 cell proliferation and tumour development clearly demonstrating roles for CS-E in tumorigenesis [206]. A number of tumour cells express GAGs with alterations in sulphation level. Altered expression of CS and HS on tumour cells has a key role to play in malignant transformation and tumour metastasis [207, 208]. Receptor for Advanced Glycation End products (RAGE) is a receptor for CS-E in Lewis lung carcinoma (LLC) cells [273, 278]. RAGE binds strongly to CS-E and HS and to LLC cells and has roles in tumour development. Serglycin is the major proteoglycan produced by multiple myeloma (MM) cells. Knockdown of serglycin dramatically attenuated MM tumour growth [209]. Tumours, which develop in serglycin knockdown animals, display lower levels of HGF and reduced blood vessel development indicating that serglycin has roles in angiogenesis. The CS chains on serglycin are at least partly responsible for cellular attachment to CD44. Serglycin was originally considered to be a product of haematopoietic cells, recent studies have shown that it is also synthesized by a number of non-haematopoietic cells [210]. Serglycin is expressed by tumour cells, promotes an aggressive phenotype and confers resistance to drugs and inactivation by the complement system. Serglycin promotes inflammatory conditions through inflammatory mediators, which are normally complexed in intracellular granules thereby contributing to tumour development. The CS-lectican proteoglycan versican accumulates in the tumour stroma and has key roles in malignant transformation and tumour progression. Elevated expression of versican in malignant tumours is associated with cancer relapse and poor clinical outcome in prostate, breast and many other cancer types. Versican (so named from 'versatile') regulates cell adhesion, proliferation, apoptosis, migration, angiogenesis, cell invasion and metastasis. These processes involve interactions mediated by the CS and DS chains of versican and its G1 and G3 globular domains. Versican therefore represents a logical therapeutic target in tumour pathobiology [211]. Versican G3 domain regulates neurite growth

[212]. Versican Vo and V1 regulate neural crest cell migration [213]. Versican G3 domain promotes tumour growth and angiogenesis [214, 215].

CD44 regulates apoptosis in chronic lymphocytic leukaemia (CLL) and its expression is mediated by the tumour microenvironment. Interaction of CD44 with HA and CS protects CLL cells from apoptosis. Specific antibodies to CD44 (IM7, A3D8) impair the viability of CLL cells and represent a potential therapeutic target [216]. CS chains in the microenvironment of breast cancer cells have been suggested as appropriate molecular therapeutic targets given that they promote many aspects of carcinogenesis in-vitro [217]. Dramatically elevated CS levels have been observed in the stromal microenvironment of many solid tumours. Intra-tumoural injection of chondroitinase ABC was ineffective in promoting primary tumour regression but led to development of secondary tumours indicating that the CS chains associated with primary tumours had a metastasis inhibitory role exploitable in therapeutic interventions. Cell surface HS and CS chains have roles in the infective stages of viral mediated carcinomas such as Merkel cell carcinoma, a highly lethal but rare form of skin cancer. HS and CS chains act as cellular receptors in the infective stages of Merkel cell polyomavirus. Modulation or removal of such CS or HS entry points may provide an approach to combat viral attachment to cells during these initial infective stages [218]. CS on the surface of breast cancer cells function as P-selectin ligands. CHST11 and CSPG4 are highly expressed in aggressive breast cancer cell lines and correlate with P-selectin binding levels. The CS chains of CSPG4 facilitate binding of P-selectin to highly metastatic breast cancer cell lines. Targeting of CS and its biosynthesis represents an attractive approach in anti-metastatic therapy [219, 220]. Therapeutic targeting of CSPG4 has been used to specifically target myeloma tumour cells using mAb based therapies [58, 221]. Adoptive transfer of genetically modified T cells is emerging as a powerful anti-cancer biotherapeutic. CSPG4 is an attractive target molecule in this approach due to its high expression in cancer cells in several types of human malignancies but restricted distribution in normal tissues [222] and helps to minimise any potential toxic side effects using such approaches. T-cells expressing a CSPG4-specific chimeric antigen receptor offer the possibility of targeting a broad spectrum of solid tumours for which no curative treatment is currently available [222-224]. The treatment of rhabdomyosarcoma (RMS) remains particularly challenging, with metastatic and alveolar RMS offering a particularly poor prognosis. CSPG4 is specifically expressed on RMS cells. The immunotoxin α MCSP-ETA', specifically recognizes CSPG4 on the RMS cell lines RD, FL-OH1, TE-671 and Rh30 and is internalized rapidly, induces apoptosis and kills RMS cells

selectively. Preliminary studies have demonstrated promising results with the specific binding of this immunotoxin to RMS primary tumours [225]. Deterioration of liver function in liver cancer is accompanied by an increase in the amount of CS-proteoglycans. This alteration in proteoglycan composition interferes with the physiologic function of the liver. Glypicans, agrin, and versican also play significant roles in the development of liver cancer [193]. CS-proteoglycans have essential roles to play in tissue morphogenesis and in cancer development involving interactions with growth factors, morphogens, cytokines, cell surface receptors, and a number of matrix proteins [226].

GAGs play vital roles in every step of tumour progression. Tumour samples with different degrees of histological differentiation demonstrate important alterations in the CS chains of a number of proteoglycans. Immunolocalisations conducted with anti-CS antibodies consistently showed normal stroma was negative whereas tumoural stroma were positive with elevated staining in the higher grade cancer samples while the tumour cells themselves were negative. Syndecan-2 levels were low or undetectable in normal tissues but significantly elevated in endocrine tumours. Glypican-5 was overexpressed in high-grade tumours with epithelial differentiation, but not in neuroendocrine tumours. Normal neuroendocrine cells displayed positive cytoplasmic and membrane staining for glypican-1 but elevated expression in low-grade tumours and reduced in high grade tumours [192]. Use of a therapeutic CSPG4 specific antibody (225.28) enhanced and prolonged the inhibitory response of PLX4032 (Vemurafenib) in combination therapy suggesting Ab 225.28 may be useful as a delivery system in the treatment of melanoma [227].

7. CS sulphation motifs regulate cell behaviour - can they be used to promote tissue repair?

This review has demonstrated pivotal roles for CS-proteoglycans in developmental processes in cell migration, cellular recognition and tissue morphogenesis [1-4]. Novel CS sulphation sequences also occur in the functionally distinct layers of skin [228]; are associated with the long bone growth plates in endochondral ossification and occur at important growth zones in the developing intervertebral disc, diarthrodial joints and tendon [229, 230]. During lymphopoiesis, CS chains are also differentially modified at sites of B-cell differentiation and maturation [229, 231] and in the brain CS sulphation plays an important role in neurite outgrowth, synaptic plasticity and neurological development [232]. Accumulated evidence therefore points to specific GAG sequences in CS having roles in cell interactions and developmental processes. A greater understanding of

these processes through sustained basic research could eventually lead to their use in advanced therapeutic applications in regenerative medicine. With the advances in oligosaccharide synthesis methodology now available, the CS sulphation motifs discussed in this review can be synthesised and CS oligosaccharide microarrays prepared to answer structure-function questions relevant in tissue repair strategies. A number of lipid-derivatized CS oligosaccharides with well-defined sulphation features have been synthesised and used in CS oligosaccharide microarrays to characterise the preferred binding sequences of the anti-CS mAbs 2H6, MO225, 473HD and LY111 [167] and to assess prospective binding partners (growth factors, cytokines) and many of the effects of these CS oligosaccharides on cellular behaviour have also been determined in-vitro. This supports their therapeutic application in tissues such as the brain and CNS, and may lead to the re-establishment of nerve function in glial scar formations.

7.1 Development of smart CS-bioscaffolds to improve tissue repair

The development of CS-bioscaffolds and their applications with stem cells in cartilage, bone, cornea, skin and nerve repair strategies represent a significant advance in bioscaffold design and performance in tissue repair strategies. CS has indispensable roles to play in stem cell differentiation and attainment of pluripotency [26]. Accumulated evidence points to CS sulphation motifs having critical roles in cell interactions, cell differentiation, proliferation and matrix assembly. A greater understanding of these glyco-code mediated processes could lead to improved repair biology therapeutics. Cartilage is a particularly difficult tissue to repair and many biomatrices have been developed in order to perfect an effective repair strategy [233], these have focussed on MSCs as a therapeutic cell type. CS-bioscaffolds promote proliferation of bone marrow stromal MSCs and their differentiation to a chondrogenic phenotype appropriate for cartilage repair. Combinations of CS, gelatin, chitosan, HA incorporated into polyvinyl alcohol (PVA), polylactic-co-glycolic acid (PLGA) hydrogels [233-237] have been developed. A thermoresponsive photopolymerizable CS hydrogel has been used to prepare a chondrocyte matrix suitable for 3D printing [234]. CS tethered on silk fibroin, silk-gelatin-CS-HA biocomposites or CS biomimetic scaffolds [238] have proved suitable for induction of a chondrogenic phenotype in MSCs [239, 240]. Porous CS-alginate foams and chitosan-gelatin-C6S-HA cryogels promote the chondrogenic differentiation of MSCs [241-243] as do CS-HA-Silk-lentiviral inserted TGF- β 3 gene, HA-CS-Heparin-Collagen scaffolds, and multilayered 3D CS chitosan constructs [244-246]. Atellocollagen-CS, collagen-CS-HA 3D hydrogels, cross-linked type II collagen-CS scaffolds [247-

249], PLGA-gelatin-CS-HA-TGF- β 3 and elastic copolymer-CS-TGF- β 3 scaffolds provide superior induction of chondrogenic cells from seeded MSCs [250, 251].

Fibrous tissue formed in response to implanted materials has been shown to contain CS [252], with increased infiltration of inflammatory mast cells. The mast cell proteoglycans serglycin and perlecan display a 2-B-6 (-) epitope on their CS chains [121]. Like 3-B-3 (-), 2-B-6 (-) is not generated by chondroitinase ABC digestion. The presence of this 2-B-6 (-) epitope has previously been reported in osteoarthritic cartilage, however how the epitope is generated, or its function remain to be established [253]. Generation of this epitope is due to the action of a member of the hyaluronidase (HYAL) family, HYAL-1 or HYAL-4, depolymerise CS via a hydrolytic cleavage reaction at the β 1 \rightarrow 4 disaccharide glycosidic linkage [254, 255]. HYAL-1 or HYAL-4 may also generate the 3-B-3 (-) 'native' CS epitope. The 2-B-6 (-) epitope, and production of HYAL-4 by mast cells are both associated with tissue remodelling and repair in inflammatory conditions.

7.2 Cell surface CS-receptors and CS interactive molecules that control cellular behaviour

Only a few cell interactive oligosaccharide sequences in CS-DS have so far been identified due to inherent difficulties in decoding their complicated structures. CS-DS hexasaccharide and octasaccharide motifs, which facilitate interactions with heparin cofactor-II and pleiotrophin, have been determined [256-258]. A major difficulty in the identification of these interactive CS-DS modules is due to them not being a well defined saccharide sequence, but rather several heterogeneous modification patterns, the so called 'wobble CS-DS motifs' [25]. The nomenclature for CS isoforms CS-A, CS-C, CS-D, CS-E, and DS, is confusing and misleading in that naturally occurring CS-A, for example, is not a homogenous polymer composed of CS-A disaccharide units only, but may contain a mixture of A, C, and unsulphated chondroitin units and embedded CS-D or CS-E motifs within the repeating disaccharide regions of CS-A GAG chains or the D-glucuronic acid can be epimerized to L-iduronic acid [259, 260]. HS has historically been considered to play more important roles in GAG-mediated cellular regulation than CS due to its higher propensity to interact with growth factors, morphogens and ECM components [261]. Recent studies have now demonstrated essential roles for CS-DS in a number of biological processes, especially in events, which regulate CNS development, and in tumorigenesis/metastasis.

The soluble ecto-domain of PTPR β/ζ , phosphacan interacts with the cell surface receptor contactin-1 (Fig 9a). Further cell signalling membrane proteins include the syndecan proteoglycan family (SDC 1-4), CSPG-4, betaglycan or endoglycan, these interact with cell surface receptors such as neuropilin-1, leukocyte common antigen-related phosphatase (LAR) and the related receptor protein tyrosine phosphatase β/ζ (Fig 9b, c). The neuronal Nogo axonal guidance receptor family consist of three GPI-anchored receptors (NgR1, R2, R3) (Fig 9d, e) [262], the semaphorins, and neuropilins are further receptors which interact with CS-ligands (Fig 9f, g) P75 is a transmembrane co-receptor which interacts with a number of receptors including the Nogo-1 receptor (NgR1) and acts as a signal transducer, converting signals initiated upon binding of myelin associated inhibitory proteins (MAIs) or CS-DS GAG to NgR1 converting this into intracellular signals via p75's cytoplasmic domains and the Ras/MAPK and JNK pathways to inhibit neurite outgrowth.

7.4 E-Cadherin

The cadherins are calcium-dependent type-1 transmembrane proteins which form adherens junctions, binding cells tightly together within tissues and have essential roles to play during embryonic development and critical in the induction of stem cell pluripotency [263-266]. Cell adhesion is mediated by extracellular cadherin domains, intracellular cytoplasmic domains associate with a large number of adaptor and cytoskeletal signalling proteins which constitute the cadherin adhesome [267]. The cadherin membrane-spanning adherens junction proteins have crucial roles in cell-cell contact formation and are also connected to cytoplasmic proteins which regulate signalling pathways and relay information regarding cell interactions to the nucleus [268-272]. E-cadherin and LRP 5/6 interact cooperatively with cell surface CSPGs and frizzled to regulate intracellular signalling through effects mediated by the catenin system which affects actin polymerisation/depolymerisation regulating ERK phosphorylation and cell signalling (Fig 10 a-b). CS-DS contributes to several signalling pathways and biological events [273]. A CS-E isoform binds strongly to Wntless/int-3a (Wnt-3a) and to a number of growth factors, neurotrophic factors, and cytokines in-vitro [274-276] (Fig 10c). *Wnt* signalling controls a number of developmental processes, tissue renewal and regeneration, and the development of several diseases, particularly cancers [274-276]. Specific arrangement of sulphation motifs on CS-DS chains modulate *Wnt* signalling and diffusion. Early stages of embryonic stem cell differentiation are promoted or repressed, by CS-E, but not by CS-A through Wnt/ β -catenin signalling pathways [275]. The migration of breast cancer cells in-vitro is reduced by CS-E, but not CS-DS [276]. CS-E regulates

type I collagen fibrillogenesis and expression, and is a positive regulator of breast carcinoma, through *Wnt* signalling [276]. Collectively, these findings provide insights into how cancer development is mediated through CS-E and *Wnt/β-catenin* signalling. However, it is still unclear what specific sulphation pattern(s) or length of CS-E saccharide is required to activate and regulate such development processes.

7.3 CS-E interactions with RAGE involved in tumour metastasis

The biosynthesis of stromal CS-DS proteoglycans is up-regulated in many tumours causing their accumulation in stromal tissues with attendant effects on tumour progression [277, 278]. The proportion of CS-E disaccharide units in CS-DS chains is elevated in ovarian and pancreatic cancers [212, 275], resulting in alterations in neoplastic growth and cell motility. Tumour cell signalling is also controlled by VEGF and cleavage of CD44 [279, 280]. The stronger expression of disulphated CS-E disaccharide units on CS-DS chains on the surface of metastatic Lewis lung carcinoma (LLC) cells correlates with their invasive properties in lung tissue [281]. In the lung RAGE acts as a receptor for cell surface CS-DS chains containing CS-E units expressed by LLC cells [282] (Fig 11a, b). RAGE recognizes CS-E unit containing deca-saccharides [282], these markedly inhibit the pulmonary metastasis of LLC cells [281], most probably by competitive inhibition. Binding of CS-DS ligands to RAGE leads to downstream effects on intracellular proteins such as Rap1 and PKC which effect the activation of NFκB and CREB signalling and transcriptional regulation (Fig 11c).

8. Conclusions

While advances in detection methodologies continue to improve the characterization of an ever-expanding repertoire of complex glycans in small amounts of sample, certain unifying principles have emerged with regard to how these entities regulate cellular metabolism. The sulphate motifs within glycosaminoglycans represent a key information storage and transfer medium, which cells can interpret, to effect tissue homeostasis. The glyco-code contained in glycosaminoglycans is an IT system which nature has developed over many hundreds of millions of years of evolution. However, despite the complexity and biodiversity of glycosaminoglycan structures it is the sulphate motifs which are key cell-directive players in the glyco-code and the sophisticated structures to which they are attached may be viewed as molecular scaffolds whereby varied planar orientations or densities of the sulphate groups can be explored to achieve optimal interactions with their respective ligands. Significant inroads have been made in the sequencing of glycosaminoglycans

and encoded sequences linked with biological processes continue to be identified. A greater understanding of this glyco-code will undoubtedly continue to improve our understanding of the development and regulation of connective tissues and may lead to significant improvements in how this information is applied in advanced strategies in repair biology.

Acknowledgements

No funding was received for this study other than the infrastructure provided by The Institute of Bone and Joint Research, Kolling Institute of Medical Research, The University of Sydney.

Disclosures

The authors have no conflicts or financial disclosures to make.

Author Contributions

All authors contributed to the writing of this manuscript, JM co-ordinated the review comments and final content of the manuscript. All authors endorsed the final version of the manuscript.

Table 1. Form and Functions of CS/DS Proteoglycans

Protein alternative name Gene	Distribution	Function	Ref
Aggrecan CSPG1 <i>ACAN</i>	Widespread in ECM, cartilage, tendon, IVD	Tissue hydration, space-filling, weight bearing proteoglycan in cartilage, IVD, forms protective perineural nets with HA and tenascin-C, has, roles in heart development.	[283, 284]
Versican CSPG2 <i>VCAN</i>	Widespread in ECM, CNS	So named as a "versatile " proteoglycan based on its ability to promote cell proliferation, differentiation, cell migration in tissue remodelling and in connective tissue morphogenesis	[285-287]
Neurocan CSPG3 <i>NCAN</i>	CNS	Brain lecticans HA binding proteoglycans interactive with NCAM, Ng-CAM/L1. Modulate cell binding in CNS development and neurite outgrowth activity in CNS/PNS. Upregulated in glial scars, inhibits astrocyte and neuronal growth, may act antagonistically with other brain PGs to regulate neurogenesis.	[288, 289]
Brevican CSPG7 <i>BCAN</i>	CNS, One of the most abundant brain proteoglycans	Primary astrocytes and neural cell lines bind brevican independently of HA, controlling infiltration of axons and dendrites into maturing glomeruli in brain development.	
Chondroitin sulphate-4 NG2 <i>CSPG4</i>	Widespread distribution in ECM and CNS with roles in development. Integral transmembrane proteoglycan.	Found on the surface of immature oligodendrocyte and chondroblastic progenitor cells. Roles in cell-PCM stabilisation, cellular proliferation, migration, inhibits neurite outgrowth during axonal regeneration. May sequester FGF-2/PDGF. CSPG4 is a collagen VI transduction receptor activating FAK/ERK1/ERK2. Widely expressed by tumour cells and is specifically targeted by therapeutic measures combatting tumourogenesis. up-regulated in spinal cord injury and in chondrogenesis	[141] [56]
Neuroglycan-C <i>CSPG5</i>	CNS	Transmembrane CS-proteoglycan bearing an EGF ECM domain, acts as an active growth factor and ligand for ErbB3, sixth member (neuroregulin-6) of the neuroregulin family. Contains CS-E, CS-C	[290] [291]
Syndecan-1 <i>SDC1</i> , Syndecan-4 <i>SDC4</i>	Widespread distribution in vascular, epithelial and weight bearing connective tissues and brain	CS-E chains found in Syndecan-1, 3. Widely distributed cell surface CS and HS substituted PGs also containing CS-E and CS-C. Midkine interacts with CS-E motif and participates in neural development and repair but interacts weakly with CS-A and CS-C.	[82, 292, 293]
Phosphacan <i>PTPRZ1</i>	CS-KS-HNK-1 proteoglycan, known as receptor-type tyrosine-protein phosphatase zeta (PTPR- ζ) Widespread distribution in CNS/PNS	CS-KS-HNK-1 proteoglycan also known as receptor-type tyrosine-protein phosphatase zeta (PTPR- ζ) single pass type 1 membrane protein with cytoplasmic tyrosine protein phosphatase, carbonic anhydrase and fibronectin type III domains. Alternative splice forms exist. DSD-1 is the mouse homologue. Roles in embryonic spinal cord development/neurogenesis. Contains CS-D which promotes embryonic axonal growth in CNS in mice. 473HD CS epitope in phosphacan has roles in neural precursor cell proliferation [294]	[147, 152, 294, 295]
Chondrocyte and SMC perlecan <i>HSPG2</i>	CS/HS hybrid proteoglycan found in cartilage, IVD,	CS replaces some HS chains in perlecan domain 1 in articular and growth plate cartilage, 4,6-disulphated CS found in growth plate perlecan regulates collagen	[43, 44]

	meniscus, tendon and blood vessels	fibrillogenesis. Roles in ECM stabilisation, growth factor-receptor transfer and activation. Cell proliferation and differentiation, cell signalling.	
Leprecan <i>PRHI</i>	Basement membrane	100kDa Leu-Pro enriched CS-proteoglycan of basement membrane of cardiac and skeletal muscle, central nervous system (cerebral cortex and cerebellum), intestinal tract, trachea, ear, skin, liver, and kidney. Localises to the vascular basement membrane/smooth muscle in each organ. Also expressed in the notochord during embryonic chordate development. May also have roles in the secretory pathway and as a growth suppressor.	[296-298]
Thrombomodulin <i>THBD</i>	Endothelial cell membrane	Endothelial cofactor proteoglycan with roles in the thrombin induced activation of Protein C anticoagulant pathway.	[299, 300]
Decorin <i>DCN</i>	CS substituted decorin and biglycan are found in bone, but DS forms are normally found in cartilage and skin	SLRPs have roles in ECM organisation/stabilisation/collagen fibrillogenesis. Facilitates cell signalling through interaction with inflammatory cytokines (IL-1, TNF- α) and growth factors (BMPs, WISP-1) and their receptors (EGF-R, IGFIR) affecting cell proliferation, survival, adhesion, migration, matrix synthesis. Controls the bioavailability of TGF- β regulates tissue fibrosis. Biglycan interacts with complement system and TLR4 in innate immune regulation.	[38, 301-303]
Biglycan <i>BGN</i>			
Asporin <i>ASP</i>	Susceptibility gene in OA	ASPN is unique among the SLRPs in not having a GAG at its N terminus but contains an Aspartic acid repeat region which binds TGF- β BMP-2 to negatively regulate chondrogenesis and osteogenesis.	[304]
Epiphycan <i>EPYC</i>	DS SLRP found in epiphyseal cartilage	Epiphycan contains 7 LRRs instead of 10-11 LRRs like other SLRP members, related to osteoglycin	[305]
Bikunin/ITI <i>AMB</i>	Liver serum serine proteinase inhibitor, also synthesised by IVD cells, chondrocytes and meniscal cells	Stabilises the condensed HA layer in growth plate hypertrophic region, and around oocytes, proteinase inhibitory activity, anti-bacterial, antiviral, anti-metastatic, immune-modulatory and growth promoting properties	[91, 306, 307]
Appican <i>APP</i>	Brain	CS-A and CS-E brain proteoglycan (Amyloid precursor protein)	[50-53, 161]
Sushi repeat protein X-linked 2 <i>SRPX2</i>	SRPX2 is a CS-proteoglycan which is overexpressed in gastrointestinal cancer and has roles in synaptogenesis	SRPX2 is significantly upregulated in colon cancer and its expression levels correlates with tumour aggressiveness. SRPX2 siRNA markedly down regulates β -catenin, MMP-2 and -9 expression reducing tumour cell proliferation, adhesion and migration via the Wnt/ β -catenin pathway. SRPX2 promotes synaptogenesis in the cerebral cortex. Mutations in SRPX2 result in Roland epilepsy and speech impairment (RESDX syndrome). Cathepsin B, ADAMTS-4 and uPAR - binding partners of SRPX2 in neural tissues.	[308, 309] [163-165, 167, 171, 308]
interphotoreceptor matrix proteoglycan 2 (SPACRCAN) <i>IMPG2</i>	Eye interphotoreceptor matrix	IMPG2, interphotoreceptor matrix proteoglycan-2	[39]

[120, 121]

Serglycin <i>SRGN</i>	Mast cells, platelets, macrophages, T-lymphocytes, leucocytes	Mast cell serglycin is substituted with heparin side chains, macrophage serglycin has CS (CS-A, CS-E) side chains	
Endoglycan <i>PODXL2</i>	CD-34 sialomucin transmembrane proteoglycan family member	Contains extensive substitution with sialic acid and N- and O- linked glycan	[310]
CD44 <i>CD44</i>	CD44 V3 splice variants bearing CS chains have reduced affinity for HA	CD44 binds Ezrin, fibrin/fibrinogen, fibronectin, HA, osteopontin, Selectins-P, -E, -L. Ubiquitous HA receptor	[311]
Miscellaneous			
Neuroendocrine cell granule-pro-hormones			
Dermcidin proteolysis inducing factor <i>DCD</i>		Anion exchange, Chondroitinase ABC MS proteomics screen used to identify intracellular CS-DS proteins. Chromogranin-A, Secretogranin-1, 2, 3. Dermcidin, Neuropeptide W, Cholecystokinin, granule bone marrow cell CS-PGs and collagen and calcium-binding EGF domain-containing protein-1.	[312-321]
Parathyroid secretory protein-1 /Chromogranin-A <i>CHGA</i>	Neurons and endocrine cells		
Cholecystokinin Pancreozymin <i>CCK</i>			

Abbreviations: CNS, central nervous system; NCAM, neural cell adhesion molecule; PCM, pericellular matrix; FAK, focal adhesion kinase; ERK, extracellular regulated kinase; LRR, leucine rich repeat; SLRP, small leucine repeat proteoglycan; TLR4, Toll-like receptor-4, MS, mass spectrometry

References

- 1 Melrose, J. (2016) The glycosaminoglycan/glycan interactome: a bioinformatic platform. An evolutionary conserved biosensor platform controlling cellular behaviour, tissue morphogenesis, tissue assembly. Scholars Press, Schaltungsdienst Lange OHG., Saarbrücken, Berlin
- 2 Furukawa, J., Okada, K. and Shinohara, Y. (2016) Glycomics of human embryonic stem cells and human induced pluripotent stem cells. *Glycoconj J.* **33**, 707-715
- 3 Sugahara, K., Mizumoto, S., Yamada, S. (2014) Chondroitin sulphate. *Encyclopedia of Polymeric Nanomaterials*
- 4 Linnartz-Gerlach, B., Mathews, M. and Neumann, H. (2014) Sensing the neuronal glycocalyx by glial sialic acid binding immunoglobulin-like lectins. *Neuroscience.* **275**, 113-124
- 5 Tarbell, J. M. and Ebong, E. E. (2008) The endothelial glycocalyx: a mechano-sensor and -transducer. *Sci Signal.* **1**, pt8
- 6 Curry, F. E. and Adamson, R. H. (2012) Endothelial glycocalyx: permeability barrier and mechanosensor. *Ann Biomed Eng.* **40**, 828-839
- 7 Dawson, G. (2014) Glycosignaling: a general review. *Adv Neurobiol.* **9**, 293-306
- 8 Tarbell, J. M., Simon, S. I. and Curry, F. R. (2014) Mechanosensing at the vascular interface. *Annu Rev Biomed Eng.* **16**, 505-532
- 9 Fu, B. M. and Tarbell, J. M. (2013) Mechano-sensing and transduction by endothelial surface glycocalyx: composition, structure, and function. *Wiley Interdiscip Rev Syst Biol Med.* **5**, 381-390
- 10 Chignalia, A. Z., Yetimakman, F., Christiaans, S. C., Unal, S., Bayrakci, B., Wagener, B. M., Russell, R. T., Kerby, J. D., Pittet, J. F. and Dull, R. O. (2016) THE GLYCOCALYX AND TRAUMA: A REVIEW. *Shock.* **45**, 338-348
- 11 Apostolopoulos, V., Stojanovska, L. and Gargosky, S. E. (2015) MUC1 (CD227): a multi-tasked molecule. *Cell Mol Life Sci.* **72**, 4475-4500
- 12 Corfield, A. P. (2015) Mucins: a biologically relevant glycan barrier in mucosal protection. *Biochim Biophys Acta.* **1850**, 236-252
- 13 Ouwkerk, J. P., de Vos, W. M. and Belzer, C. (2013) Glycobiome: bacteria and mucus at the epithelial interface. *Best Pract Res Clin Gastroenterol.* **27**, 25-38
- 14 Dudas, B. and Semeniken, K. (2012) Glycosaminoglycans and neuroprotection. *Handb Exp Pharmacol.* 325-343
- 15 Haeren, R. H., van de Ven, S. E., van Zandvoort, M. A., Vink, H., van Overbeeke, J. J., Hoogland, G. and Rijkers, K. (2016) Assessment and Imaging of the Cerebrovascular Glycocalyx. *Curr Neurovasc Res.* **13**, 249-260
- 16 Sugahara, K., Mikami, T., Uyama, T., Mizuguchi, S., Nomura, K. and Kitagawa, H. (2003) Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate. *Curr Opin Struct Biol.* **13**, 612-620
- 17 Caterson, B. (2012) Fell-Muir Lecture: chondroitin sulphate glycosaminoglycans: fun for some and confusion for others. *Int J Exp Pathol.* **93**, 1-10
- 18 Cummings, R. D. (2009) The repertoire of glycan determinants in the human glycome. *Mol Biosyst.* **5**, 1087-1104
- 19 Maeda, N., Fukazawa, N. and Hata, T. (2006) The binding of chondroitin sulfate to pleiotrophin/heparin-binding growth-associated molecule is regulated by chain length and oversulfated structures. *J Biol Chem.* **281**, 4894-4902
- 20 Pufe, T., Bartscher, M., Petersen, W., Tillmann, B. and Mentlein, R. (2003) Expression of pleiotrophin, an embryonic growth and differentiation factor, in rheumatoid arthritis. *Arthritis Rheum.* **48**, 660-667
- 21 Pufe, T., Bartscher, M., Petersen, W., Tillmann, B. and Mentlein, R. (2003) Pleiotrophin, an embryonic differentiation and growth factor, is expressed in osteoarthritis. *Osteoarthritis Cartilage.* **11**, 260-264
- 22 Pufe, T., Groth, G., Goldring, M. B., Tillmann, B. and Mentlein, R. (2007) Effects of pleiotrophin, a heparin-binding growth factor, on human primary and immortalized chondrocytes. *Osteoarthritis Cartilage.* **15**, 155-162
- 23 Malavaki, C., Mizumoto, S., Karamanos, N. and Sugahara, K. (2008) Recent advances in the structural study of functional chondroitin sulfate and dermatan sulfate in health and disease. *Connect Tissue Res.* **49**, 133-139
- 24 Nandini, C. D. and Sugahara, K. (2006) Role of the sulfation pattern of chondroitin sulfate in its biological activities and in the binding of growth factors. *Adv Pharmacol.* **53**, 253-279
- 25 Purushothaman, A., Sugahara, K. and Faissner, A. (2012) Chondroitin sulfate "wobble motifs" modulate maintenance and differentiation of neural stem cells and their progeny. *J Biol Chem.* **287**, 2935-2942
- 26 Izumikawa, T., Sato, B. and Kitagawa, H. (2014) Chondroitin sulfate is indispensable for pluripotency and differentiation of mouse embryonic stem cells. *Sci Rep.* **4**, 3701

- 27 Milev, P., Maurel, P., Chiba, A., Mevissen, M., Popp, S., Yamaguchi, Y., Margolis, R. K. and Margolis, R. U. (1998) Differential regulation of expression of hyaluronan-binding proteoglycans in developing brain: aggrecan, versican, neurocan, and brevican. *Biochem Biophys Res Commun.* **247**, 207-212
- 28 Howell, M. D. and Gottschall, P. E. (2012) Lectican proteoglycans, their cleaving metalloproteinases, and plasticity in the central nervous system extracellular microenvironment. *Neuroscience.* **217**, 6-18
- 29 Yamaguchi, Y. (2000) Lecticans: organizers of the brain extracellular matrix. *Cell Mol Life Sci.* **57**, 276-289
- 30 Iozzo, R. V. and Schaefer, L. (2015) Proteoglycan form and function: A comprehensive nomenclature of proteoglycans. *Matrix Biol.* **42**, 11-55
- 31 Ruoslahti, E. (1988) Structure and biology of proteoglycans. *Annu Rev Cell Biol.* **4**, 229-255
- 32 Sorg, B. A., Berretta, S., Blacktop, J. M., Fawcett, J. W., Kitagawa, H., Kwok, J. C. and Miquel, M. (2016) Casting a Wide Net: Role of Perineuronal Nets in Neural Plasticity. *J Neurosci.* **36**, 11459-11468
- 33 Suttkus, A., Morawski, M. and Arendt, T. (2016) Protective Properties of Neural Extracellular Matrix. *Mol Neurobiol.* **53**, 73-82
- 34 Dellett, M., Hu, W., Papadaki, V. and Ohnuma, S. (2012) Small leucine rich proteoglycan family regulates multiple signalling pathways in neural development and maintenance. *Dev Growth Differ.* **54**, 327-340
- 35 Iozzo, R. V. and Schaefer, L. (2010) Proteoglycans in health and disease: novel regulatory signaling mechanisms evoked by the small leucine-rich proteoglycans. *FEBS J.* **277**, 3864-3875
- 36 Moreth, K., Iozzo, R. V. and Schaefer, L. (2012) Small leucine-rich proteoglycans orchestrate receptor crosstalk during inflammation. *Cell Cycle.* **11**, 2084-2091
- 37 Nikitovic, D., Aggelidakis, J., Young, M. F., Iozzo, R. V., Karamanos, N. K. and Tzanakakis, G. N. (2012) The biology of small leucine-rich proteoglycans in bone pathophysiology. *J Biol Chem.* **287**, 33926-33933
- 38 Schaefer, L. and Iozzo, R. V. (2008) Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction. *J Biol Chem.* **283**, 21305-21309
- 39 Acharya, S., Foletta, V. C., Lee, J. W., Rayborn, M. E., Rodriguez, I. R., Young, W. S., 3rd and Hollyfield, J. G. (2000) SPACRCAN, a novel human interphotoreceptor matrix hyaluronan-binding proteoglycan synthesized by photoreceptors and pinealocytes. *J Biol Chem.* **275**, 6945-6955
- 40 Chen, Q., Cai, S., Shadrach, K. G., Prestwich, G. D. and Hollyfield, J. G. (2004) Spacrcan binding to hyaluronan and other glycosaminoglycans. *Molecular and biochemical studies. J Biol Chem.* **279**, 23142-23150
- 41 Gubbiotti, M. A., Neill, T. and Iozzo, R. V. (2017) A current view of perlecan in physiology and pathology: A mosaic of functions. *Matrix Biol.* **57-58**, 285-298
- 42 Iozzo, R. V. (1994) Perlecan: a gem of a proteoglycan. *Matrix Biol.* **14**, 203-208
- 43 Lord, M. S., Chuang, C. Y., Melrose, J., Davies, M. J., Iozzo, R. V. and Whitelock, J. M. (2014) The role of vascular-derived perlecan in modulating cell adhesion, proliferation and growth factor signaling. *Matrix Biol.* **35**, 112-122
- 44 Melrose, J., Roughley, P., Knox, S., Smith, S., Lord, M. and Whitelock, J. (2006) The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal, postnatal, and mature hyaline cartilages. *J Biol Chem.* **281**, 36905-36914
- 45 Whitelock, J. M., Melrose, J. and Iozzo, R. V. (2008) Diverse cell signaling events modulated by perlecan. *Biochemistry.* **47**, 11174-11183
- 46 Zoeller, J. J., McQuillan, A., Whitelock, J., Ho, S. Y. and Iozzo, R. V. (2008) A central function for perlecan in skeletal muscle and cardiovascular development. *J Cell Biol.* **181**, 381-394
- 47 Knox, S., Fosang, A. J., Last, K., Melrose, J. and Whitelock, J. (2005) Perlecan from human epithelial cells is a hybrid heparan/chondroitin/keratan sulfate proteoglycan. *FEBS Lett.* **579**, 5019-5023
- 48 Shu, C., Hughes, C., Smith, S. M., Smith, M. M., Hayes, A., Caterson, B., Little, C. B. and Melrose, J. (2013) The ovine newborn and human foetal intervertebral disc contain perlecan and aggrecan variably substituted with native 7D4 CS sulphation motif: spatiotemporal immunolocalisation and co-distribution with Notch-1 in the human foetal disc. *Glycoconj J.* **30**, 717-725
- 49 Kvist, A. J., Johnson, A. E., Morgelin, M., Gustafsson, E., Bengtsson, E., Lindblom, K., Aszodi, A., Fassler, R., Sasaki, T., Timpl, R. and Aspberg, A. (2006) Chondroitin sulfate perlecan enhances collagen fibril formation. Implications for perlecan chondrodysplasias. *J Biol Chem.* **281**, 33127-33139

- 50 Pangalos, M. N., Shioi, J., Efthimiopoulos, S., Wu, A. and Robakis, N. K. (1996) Characterization of appican, the chondroitin sulfate proteoglycan form of the Alzheimer amyloid precursor protein. *Neurodegeneration*. **5**, 445-451
- 51 Tsuchida, K., Shioi, J., Yamada, S., Boghosian, G., Wu, A., Cai, H., Sugahara, K. and Robakis, N. K. (2001) Appican, the proteoglycan form of the amyloid precursor protein, contains chondroitin sulfate E in the repeating disaccharide region and 4-O-sulfated galactose in the linkage region. *J Biol Chem*. **276**, 37155-37160
- 52 Umehara, Y., Yamada, S., Nishimura, S., Shioi, J., Robakis, N. K. and Sugahara, K. (2004) Chondroitin sulfate of appican, the proteoglycan form of amyloid precursor protein, produced by C6 glioma cells interacts with heparin-binding neuroregulatory factors. *FEBS Lett*. **557**, 233-238
- 53 Wu, A., Pangalos, M. N., Efthimiopoulos, S., Shioi, J. and Robakis, N. K. (1997) Appican expression induces morphological changes in C6 glioma cells and promotes adhesion of neural cells to the extracellular matrix. *J Neurosci*. **17**, 4987-4993
- 54 Kadoya, K., Fukushi, J., Matsumoto, Y., Yamaguchi, Y. and Stallcup, W. B. (2008) NG2 proteoglycan expression in mouse skin: altered postnatal skin development in the NG2 null mouse. *J Histochem Cytochem*. **56**, 295-303
- 55 Trotter, J., Karram, K. and Nishiyama, A. (2010) NG2 cells: Properties, progeny and origin. *Brain Res Rev*. **63**, 72-82
- 56 Nishiyama, A., Dahlin, K. J., Prince, J. T., Johnstone, S. R. and Stallcup, W. B. (1991) The primary structure of NG2, a novel membrane-spanning proteoglycan. *J Cell Biol*. **114**, 359-371
- 57 Price, M. A., Colvin Wanshura, L. E., Yang, J., Carlson, J., Xiang, B., Li, G., Ferrone, S., Dudek, A. Z., Turley, E. A. and McCarthy, J. B. (2011) CSPG4, a potential therapeutic target, facilitates malignant progression of melanoma. *Pigment Cell Melanoma Res*. **24**, 1148-1157
- 58 Campoli, M., Ferrone, S. and Wang, X. (2010) Functional and clinical relevance of chondroitin sulfate proteoglycan 4. *Adv Cancer Res*. **109**, 73-121
- 59 Mayayo, S. L., Prestigio, S., Maniscalco, L., La Rosa, G., Arico, A., De Maria, R., Cavallo, F., Ferrone, S., Buracco, P. and Iussich, S. (2011) Chondroitin sulfate proteoglycan-4: a biomarker and a potential immunotherapeutic target for canine malignant melanoma. *Vet J*. **190**, e26-30
- 60 Goretzki, L., Burg, M. A., Grako, K. A. and Stallcup, W. B. (1999) High-affinity binding of basic fibroblast growth factor and platelet-derived growth factor-AA to the core protein of the NG2 proteoglycan. *J Biol Chem*. **274**, 16831-16837
- 61 Burg, M. A., Tillet, E., Timpl, R. and Stallcup, W. B. (1996) Binding of the NG2 proteoglycan to type VI collagen and other extracellular matrix molecules. *J Biol Chem*. **271**, 26110-26116
- 62 Tillet, E., Ruggiero, F., Nishiyama, A. and Stallcup, W. B. (1997) The membrane-spanning proteoglycan NG2 binds to collagens V and VI through the central nonglobular domain of its core protein. *J Biol Chem*. **272**, 10769-10776
- 63 Fang, X., Burg, M. A., Barritt, D., Dahlin-Huppe, K., Nishiyama, A. and Stallcup, W. B. (1999) Cytoskeletal reorganization induced by engagement of the NG2 proteoglycan leads to cell spreading and migration. *Mol Biol Cell*. **10**, 3373-3387
- 64 Stallcup, W. B. (2002) The NG2 proteoglycan: past insights and future prospects. *J Neurocytol*. **31**, 423-435
- 65 Bourin, M. C. and Lindahl, U. (1990) Functional role of the polysaccharide component of rabbit thrombomodulin proteoglycan. Effects on inactivation of thrombin by antithrombin, cleavage of fibrinogen by thrombin and thrombin-catalysed activation of factor V. *Biochem J*. **270**, 419-425
- 66 Esmon, C. T., Esmon, N. L. and Harris, K. W. (1982) Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. *J Biol Chem*. **257**, 7944-7947
- 67 Bourin, M. C., Lundgren-Akerlund, E. and Lindahl, U. (1990) Isolation and characterization of the glycosaminoglycan component of rabbit thrombomodulin proteoglycan. *J Biol Chem*. **265**, 15424-15431
- 68 Bourin, M. C., Ohlin, A. K., Lane, D. A., Stenflo, J. and Lindahl, U. (1988) Relationship between anticoagulant activities and polyanionic properties of rabbit thrombomodulin. *J Biol Chem*. **263**, 8044-8052
- 69 Nawa, K., Sakano, K., Fujiwara, H., Sato, Y., Sugiyama, N., Teruuchi, T., Iwamoto, M. and Marumoto, Y. (1990) Presence and function of chondroitin-4-sulfate on recombinant human soluble thrombomodulin. *Biochem Biophys Res Commun*. **171**, 729-737
- 70 Nguyen, M., Arkell, J. and Jackson, C. J. (2000) Activated protein C directly activates human endothelial gelatinase A. *J Biol Chem*. **275**, 9095-9098
- 71 Xue, M., March, L., Sambrook, P. N. and Jackson, C. J. (2007) Differential regulation of matrix metalloproteinase 2 and matrix metalloproteinase 9 by activated protein C: relevance to inflammation in rheumatoid arthritis. *Arthritis Rheum*. **56**, 2864-2874

- 72 Xue, M., Thompson, P., Kelso, I. and Jackson, C. (2004) Activated protein C stimulates proliferation, migration and wound closure, inhibits apoptosis and upregulates MMP-2 activity in cultured human keratinocytes. *Exp Cell Res.* **299**, 119-127
- 73 Conway, E. M. (2012) Thrombomodulin and its role in inflammation. *Semin Immunopathol.* **34**, 107-125
- 74 Shi, C. S., Shi, G. Y., Hsiao, H. M., Kao, Y. C., Kuo, K. L., Ma, C. Y., Kuo, C. H., Chang, B. I., Chang, C. F., Lin, C. H., Wong, C. H. and Wu, H. L. (2008) Lectin-like domain of thrombomodulin binds to its specific ligand Lewis Y antigen and neutralizes lipopolysaccharide-induced inflammatory response. *Blood.* **112**, 3661-3670
- 75 Dwyer, C. A., Katoh, T., Tiemeyer, M. and Matthews, R. T. (2015) Neurons and glia modify receptor protein-tyrosine phosphatase zeta (RPTPzeta)/phosphacan with cell-specific O-mannosyl glycans in the developing brain. *J Biol Chem.* **290**, 10256-10273
- 76 Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. (1991) Oncogenes and signal transduction. *Cell.* **64**, 281-302
- 77 Hunter, T. (1991) Cooperation between oncogenes. *Cell.* **64**, 249-270
- 78 Hunter, T. (1995) Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell.* **80**, 225-236
- 79 Ullrich, A. and Schlessinger, J. (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell.* **61**, 203-212
- 80 Garwood, J., Heck, N., Reichardt, F. and Faissner, A. (2003) Phosphacan short isoform, a novel non-proteoglycan variant of phosphacan/receptor protein tyrosine phosphatase-beta, interacts with neuronal receptors and promotes neurite outgrowth. *J Biol Chem.* **278**, 24164-24173
- 81 Kokenyesi, R. and Bernfield, M. (1994) Core protein structure and sequence determine the site and presence of heparan sulfate and chondroitin sulfate on syndecan-1. *J Biol Chem.* **269**, 12304-12309
- 82 Deepa, S. S., Yamada, S., Zako, M., Goldberger, O. and Sugahara, K. (2004) Chondroitin sulfate chains on syndecan-1 and syndecan-4 from normal murine mammary gland epithelial cells are structurally and functionally distinct and cooperate with heparan sulfate chains to bind growth factors. A novel function to control binding of midkine, pleiotrophin, and basic fibroblast growth factor. *J Biol Chem.* **279**, 37368-37376
- 83 Ueno, M., Yamada, S., Zako, M., Bernfield, M. and Sugahara, K. (2001) Structural characterization of heparan sulfate and chondroitin sulfate of syndecan-1 purified from normal murine mammary gland epithelial cells. Common phosphorylation of xylose and differential sulfation of galactose in the protein linkage region tetrasaccharide sequence. *J Biol Chem.* **276**, 29134-29140
- 84 Woods, A., Longley, R. L., Tumova, S. and Couchman, J. R. (2000) Syndecan-4 binding to the high affinity heparin-binding domain of fibronectin drives focal adhesion formation in fibroblasts. *Arch Biochem Biophys.* **374**, 66-72
- 85 Kugelman, L. C., Ganguly, S., Haggerty, J. G., Weissman, S. M. and Milstone, L. M. (1992) The core protein of epican, a heparan sulfate proteoglycan on keratinocytes, is an alternative form of CD44. *J Invest Dermatol.* **99**, 886-891
- 86 Milstone, L. M., Hough-Monroe, L., Kugelman, L. C., Bender, J. R. and Haggerty, J. G. (1994) Epican, a heparan/chondroitin sulfate proteoglycan form of CD44, mediates cell-cell adhesion. *J Cell Sci.* **107 (Pt 11)**, 3183-3190
- 87 Enghild, J. J., Salvesen, G., Thogersen, I. B., Valnickova, Z., Pizzo, S. V. and Hefta, S. A. (1993) Presence of the protein-glycosaminoglycan-protein covalent cross-link in the inter-alpha-inhibitor-related proteinase inhibitor heavy chain 2/bikunin. *J Biol Chem.* **268**, 8711-8716
- 88 Zhuo, L., Hascall, V. C. and Kimata, K. (2004) Inter-alpha-trypsin inhibitor, a covalent protein-glycosaminoglycan-protein complex. *J Biol Chem.* **279**, 38079-38082
- 89 Melrose, J., Shen, B. and Ghosh, P. (2001) Affinity and Western blotting reveal homologies between ovine intervertebral disc serine proteinase inhibitory proteins and bovine pancreatic trypsin inhibitor. *Proteomics.* **1**, 1529-1533
- 90 Rodgers, K. J., Melrose, J. and Ghosh, P. (1996) Purification and characterisation of 6 and 58 kDa forms of the endogenous serine proteinase inhibitory proteins of ovine articular cartilage. *Biol Chem.* **377**, 837-845
- 91 Lord, M. S., Day, A. J., Youssef, P., Zhuo, L., Watanabe, H., Caterson, B. and Whitelock, J. M. (2013) Sulfation of the bikunin chondroitin sulfate chain determines heavy chain-hyaluronan complex formation. *J Biol Chem.* **288**, 22930-22941
- 92 Chi, L., Wolff, J. J., Laremore, T. N., Restaino, O. F., Xie, J., Schiraldi, C., Toida, T., Amster, I. J. and Linhardt, R. J. (2008) Structural analysis of bikunin glycosaminoglycan. *J Am Chem Soc.* **130**, 2617-2625
- 93 Ly, M., Leach, F. E., 3rd, Laremore, T. N., Toida, T., Amster, I. J. and Linhardt, R. J. (2011) The proteoglycan bikunin has a defined sequence. *Nat Chem Biol.* **7**, 827-833

- 94 Mayne, R., van der Rest, M., Ninomiya, Y. and Olsen, B. R. (1985) The structure of type IX collagen. *Ann N Y Acad Sci.* **460**, 38-46
- 95 van der Rest, M., Mayne, R., Ninomiya, Y., Seidah, N. G., Chretien, M. and Olsen, B. R. (1985) The structure of type IX collagen. *J Biol Chem.* **260**, 220-225
- 96 Bruckner, P., Vaughan, L. and Winterhalter, K. H. (1985) Type IX collagen from sternal cartilage of chicken embryo contains covalently bound glycosaminoglycans. *Proc Natl Acad Sci U S A.* **82**, 2608-2612
- 97 Irwin, M. H. and Mayne, R. (1986) Use of monoclonal antibodies to locate the chondroitin sulfate chain(s) in type IX collagen. *J Biol Chem.* **261**, 16281-16283
- 98 McCormick, D., van der Rest, M., Goodship, J., Lozano, G., Ninomiya, Y. and Olsen, B. R. (1987) Structure of the glycosaminoglycan domain in the type IX collagen-proteoglycan. *Proc Natl Acad Sci U S A.* **84**, 4044-4048
- 99 Noro, A., Kimata, K., Oike, Y., Shinomura, T., Maeda, N., Yano, S., Takahashi, N. and Suzuki, S. (1983) Isolation and characterization of a third proteoglycan (PG-Lt) from chick embryo cartilage which contains disulfide-bonded collagenous polypeptide. *J Biol Chem.* **258**, 9323-9331
- 100 Vaughan, L., Winterhalter, K. H. and Bruckner, P. (1985) Proteoglycan Lt from chicken embryo sternum identified as type IX collagen. *J Biol Chem.* **260**, 4758-4763
- 101 Douglas, S. P. and Kadler, K. E. (1998) Specific glycanforms of type IX collagen accumulate in embryonic chick sterna after 17 days of development. *Glycobiology.* **8**, 1013-1019
- 102 Arai, M., Yada, T., Suzuki, S. and Kimata, K. (1992) Isolation and characterization of type IX collagen-proteoglycan from the Swarm rat chondrosarcoma. *Biochim Biophys Acta.* **1117**, 60-70
- 103 Diab, M., Wu, J. J. and Eyre, D. R. (1996) Collagen type IX from human cartilage: a structural profile of intermolecular cross-linking sites. *Biochem J.* **314 (Pt 1)**, 327-332
- 104 Yada, T., Arai, M., Suzuki, S. and Kimata, K. (1992) Occurrence of collagen and proteoglycan forms of type IX collagen in chick embryo cartilage. Production and characterization of a collagen form-specific antibody. *J Biol Chem.* **267**, 9391-9397
- 105 Yada, T., Suzuki, S., Kobayashi, K., Kobayashi, M., Hoshino, T., Horie, K. and Kimata, K. (1990) Occurrence in chick embryo vitreous humor of a type IX collagen proteoglycan with an extraordinarily large chondroitin sulfate chain and short alpha 1 polypeptide. *J Biol Chem.* **265**, 6992-6999
- 106 Skandalis, S. S., Theocharis, D. A. and Noulas, A. V. (2007) Chondroitin sulphate proteoglycans in the vitreous gel of sheep and goat. *Biomed Chromatogr.* **21**, 451-457
- 107 Theocharis, D. A., Skandalis, S. S., Noulas, A. V., Papageorgakopoulou, N., Theocharis, A. D. and Karamanos, N. K. (2008) Hyaluronan and chondroitin sulfate proteoglycans in the supramolecular organization of the mammalian vitreous body. *Connect Tissue Res.* **49**, 124-128
- 108 Gordon, M. K., Gerecke, D. R., Dublet, B., van der Rest, M. and Olsen, B. R. (1989) Type XII collagen. A large multidomain molecule with partial homology to type IX collagen. *J Biol Chem.* **264**, 19772-19778
- 109 Gordon, M. K., Gerecke, D. R., Dublet, B., van der Rest, M., Sugrue, S. P. and Olsen, B. R. (1990) The structure of type XII collagen. *Ann N Y Acad Sci.* **580**, 8-16
- 110 Aubert-Foucher, E., Font, B., Eichenberger, D., Goldschmidt, D., Lethias, C. and van der Rest, M. (1992) Purification and characterization of native type XIV collagen. *J Biol Chem.* **267**, 15759-15764
- 111 Bocock, J. P., Edgell, C. J., Marr, H. S. and Erickson, A. H. (2003) Human proteoglycan testican-1 inhibits the lysosomal cysteine protease cathepsin L. *Eur J Biochem.* **270**, 4008-4015
- 112 Nakada, M., Yamada, A., Takino, T., Miyamori, H., Takahashi, T., Yamashita, J. and Sato, H. (2001) Suppression of membrane-type 1 matrix metalloproteinase (MMP)-mediated MMP-2 activation and tumor invasion by testican 3 and its splicing variant gene product, N-Tes. *Cancer Res.* **61**, 8896-8902
- 113 Nakada, M., Miyamori, H., Yamashita, J. and Sato, H. (2003) Testican 2 abrogates inhibition of membrane-type matrix metalloproteinases by other testican family proteins. *Cancer Res.* **63**, 3364-3369
- 114 Marr, H. S., Basalamah, M. A. and Edgell, C. J. (1997) Endothelial cell expression of testican mRNA. *Endothelium.* **5**, 209-219
- 115 Marr, H. S., Basalamah, M. A., Bouldin, T. W., Duncan, A. W. and Edgell, C. J. (2000) Distribution of testican expression in human brain. *Cell Tissue Res.* **302**, 139-144
- 116 Kolset, S. O. and Tveit, H. (2008) Serglycin--structure and biology. *Cell Mol Life Sci.* **65**, 1073-1085
- 117 Ronnberg, E., Melo, F. R. and Pejler, G. (2012) Mast cell proteoglycans. *J Histochem Cytochem.* **60**, 950-962
- 118 Ronnberg, E. and Pejler, G. (2012) Serglycin: the master of the mast cell. *Methods Mol Biol.* **836**, 201-217

- 119 Scully, O. J., Chua, P. J., Harve, K. S., Bay, B. H. and Yip, G. W. (2012) Serglycin in health and diseases. *Anat Rec (Hoboken)*. **295**, 1415-1420
- 120 Mulloy, B., Lever, R. and Page, C. P. (2016) Mast cell glycosaminoglycans. *Glycoconj J*
- 121 Farrugia, B. L., Whitelock, J. M., O'Grady, R., Caterson, B. and Lord, M. S. (2016) Mast Cells Produce a Unique Chondroitin Sulfate Epitope. *J Histochem Cytochem*. **64**, 85-98
- 122 Chang, M. Y., Olin, K. L., Tsoi, C., Wight, T. N. and Chait, A. (1998) Human monocyte-derived macrophages secrete two forms of proteoglycan-macrophage colony-stimulating factor that differ in their ability to bind low density lipoproteins. *J Biol Chem*. **273**, 15985-15992
- 123 Suzu, S., Kimura, F., Yamada, M., Yanai, N., Kawashima, T., Nagata, N. and Motoyoshi, K. (1994) Direct interaction of proteoglycan macrophage colony-stimulating factor and basic fibroblast growth factor. *Blood*. **83**, 3113-3119
- 124 Aravind, L. and Koonin, E. V. (2001) The DNA-repair protein AlkB, EGL-9, and leprecan define new families of 2-oxoglutarate- and iron-dependent dioxygenases. *Genome Biol*. **2**, RESEARCH0007
- 125 Jaakkola, P. M. and Rantanen, K. (2013) The regulation, localization, and functions of oxygen-sensing prolyl hydroxylase PHD3. *Biol Chem*. **394**, 449-457
- 126 Pientka, F. K., Hu, J., Schindler, S. G., Brix, B., Thiel, A., Jöhren, O., Fandrey, J., Berchner-Pfannschmidt, U. and Depping, R. (2012) Oxygen sensing by the prolyl-4-hydroxylase PHD2 within the nuclear compartment and the influence of compartmentalisation on HIF-1 signalling. *J Cell Sci*. **125**, 5168-5176
- 127 Place, T. L. and Domann, F. E. (2013) Prolyl-hydroxylase 3: Evolving Roles for an Ancient Signaling Protein. *Hypoxia (Auckl)*. **2013**, 13-17
- 128 Wong, B. W., Kuchnio, A., Bruning, U. and Carmeliet, P. (2013) Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes. *Trends Biochem Sci*. **38**, 3-11
- 129 Hayes, A. J., Hughes, C. E., Ralphs, J. R. and Caterson, B. (2011) Chondroitin sulphate sulphation motif expression in the ontogeny of the intervertebral disc. *Eur Cell Mater*. **21**, 1-14
- 130 Hayes, A. J., Hughes, C. E., Smith, S. M., Caterson, B., Little, C. B. and Melrose, J. (2016) The CS Sulfation Motifs 4C3, 7D4, 3B3[-]; and Perlecan Identify Stem Cell Populations and Their Niches, Activated Progenitor Cells and Transitional Areas of Tissue Development in the Fetal Human Elbow. *Stem Cells Dev*. **25**, 836-847
- 131 Melrose, J., Smith, S. M., Hughes, C. E., Little, C. B., Caterson, B., Hayes, A. J. (2016) The 7D4, 4C3 and 3B3 (-) Chondroitin Sulphation Motifs are expressed at Sites of Cartilage and Bone Morphogenesis during Foetal Human Knee Joint Development. *Journal of Glycobiology*. **5**, 1
- 132 Melrose, J., Isaacs, M. D., Smith, S. M., Hughes, C. E., Little, C. B., Caterson, B. and Hayes, A. J. (2012) Chondroitin sulphate and heparan sulphate sulphation motifs and their proteoglycans are involved in articular cartilage formation during human foetal knee joint development. *Histochem Cell Biol*. **138**, 461-475
- 133 Hayes, A. J., Tudor, D., Nowell, M. A., Caterson, B. and Hughes, C. E. (2008) Chondroitin sulfate sulfation motifs as putative biomarkers for isolation of articular cartilage progenitor cells. *J Histochem Cytochem*. **56**, 125-138
- 134 Cortes, M., Baria, A. T. and Schwartz, N. B. (2009) Sulfation of chondroitin sulfate proteoglycans is necessary for proper Indian hedgehog signaling in the developing growth plate. *Development*. **136**, 1697-1706
- 135 Palma, V., Carrasco, H., Reinchisi, G., Olivares, G., Faunes, F. and Larrain, J. (2011) SHh activity and localization is regulated by perlecan. *Biol Res*. **44**, 63-67
- 136 Douthwaite, G. P., Bishop, J. C., Redman, S. N., Khan, I. M., Rooney, P., Evans, D. J., Houghton, L., Bayram, Z., Boyer, S., Thomson, B., Wolfe, M. S. and Archer, C. W. (2004) The surface of articular cartilage contains a progenitor cell population. *J Cell Sci*. **117**, 889-897
- 137 Hollander, A. P., Dickinson, S. C. and Kafienah, W. (2010) Stem cells and cartilage development: complexities of a simple tissue. *Stem Cells*. **28**, 1992-1996
- 138 Caplan, A. I. (2008) All MSCs are pericytes? *Cell Stem Cell*. **3**, 229-230
- 139 Caplan, A. I. (2017) The new MSC: MSCs as pericytes are sentinels and gatekeepers. *J Orthop Res*
- 140 da Silva Meirelles, L., Caplan, A. I. and Nardi, N. B. (2008) In search of the in vivo identity of mesenchymal stem cells. *Stem Cells*. **26**, 2287-2299
- 141 Crisan, M., Yap, S., Casteilla, L., Chen, C. W., Corselli, M., Park, T. S., Andriolo, G., Sun, B., Zheng, B., Zhang, L., Norotte, C., Teng, P. N., Traas, J., Schugar, R., Deasy, B. M., Badyrak, S., Buhring, H. J., Giacobino, J. P., Lazzari, L., Huard, J. and Peault, B. (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. **3**, 301-313
- 142 Bergers, G. and Song, S. (2005) The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol*. **7**, 452-464

- 143 Diaz-Flores, L., Gutierrez, R., Madrid, J. F., Varela, H., Valladares, F., Acosta, E., Martin-Vasallo, P. and Diaz-Flores, L., Jr. (2009) Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. *Histol Histopathol.* **24**, 909-969
- 144 Fukushi, J., Makagiansar, I. T. and Stallcup, W. B. (2004) NG2 proteoglycan promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and alpha3beta1 integrin. *Mol Biol Cell.* **15**, 3580-3590
- 145 Ribatti, D., Nico, B. and Crivellato, E. (2011) The role of pericytes in angiogenesis. *Int J Dev Biol.* **55**, 261-268
- 146 Zhang, C., Zeng, L., Emanuelli, C. and Xu, Q. (2013) Blood flow and stem cells in vascular disease. *Cardiovasc Res.* **99**, 251-259
- 147 Maeda, N., He, J., Yajima, Y., Mikami, T., Sugahara, K. and Yabe, T. (2003) Heterogeneity of the chondroitin sulfate portion of phosphacan/6B4 proteoglycan regulates its binding affinity for pleiotrophin/heparin binding growth-associated molecule. *J Biol Chem.* **278**, 35805-35811
- 148 Deepa, S. S., Kalayanamitra, K., Ito, Y., Kongtawelert, P., Fukui, S., Yamada, S., Mikami, T. and Sugahara, K. (2007) Novel sulfated octa- and decasaccharides from squid cartilage chondroitin sulfate E: sequencing and application for determination of the epitope structure of the monoclonal antibody MO-225. *Biochemistry.* **46**, 2453-2465
- 149 Deepa, S. S., Yamada, S., Fukui, S. and Sugahara, K. (2007) Structural determination of novel sulfated octasaccharides isolated from chondroitin sulfate of shark cartilage and their application for characterizing monoclonal antibody epitopes. *Glycobiology.* **17**, 631-645
- 150 Ito, Y., Hikino, M., Yajima, Y., Mikami, T., Sirko, S., von Holst, A., Faissner, A., Fukui, S. and Sugahara, K. (2005) Structural characterization of the epitopes of the monoclonal antibodies 473HD, CS-56, and MO-225 specific for chondroitin sulfate D-type using the oligosaccharide library. *Glycobiology.* **15**, 593-603
- 151 Dobbertin, A., Rhodes, K. E., Garwood, J., Properzi, F., Heck, N., Rogers, J. H., Fawcett, J. W. and Faissner, A. (2003) Regulation of RPTPbeta/phosphacan expression and glycosaminoglycan epitopes in injured brain and cytokine-treated glia. *Mol Cell Neurosci.* **24**, 951-971
- 152 Faissner, A., Heck, N., Dobbertin, A. and Garwood, J. (2006) DSD-1-Proteoglycan/Phosphacan and receptor protein tyrosine phosphatase-beta isoforms during development and regeneration of neural tissues. *Adv Exp Med Biol.* **557**, 25-53
- 153 Garwood, J., Rigato, F., Heck, N. and Faissner, A. (2001) Tenascin glycoproteins and the complementary ligand DSD-1-PG/ phosphacan--structuring the neural extracellular matrix during development and repair. *Restor Neurol Neurosci.* **19**, 51-64
- 154 Garwood, J., Schnadelbach, O., Clement, A., Schutte, K., Bach, A. and Faissner, A. (1999) DSD-1-proteoglycan is the mouse homolog of phosphacan and displays opposing effects on neurite outgrowth dependent on neuronal lineage. *J Neurosci.* **19**, 3888-3899
- 155 Margolis, R. U. and Margolis, R. K. (1997) Chondroitin sulfate proteoglycans as mediators of axon growth and pathfinding. *Cell Tissue Res.* **290**, 343-348
- 156 Hikino, M., Mikami, T., Faissner, A., Vilela-Silva, A. C., Pavao, M. S. and Sugahara, K. (2003) Oversulfated dermatan sulfate exhibits neurite outgrowth-promoting activity toward embryonic mouse hippocampal neurons: implications of dermatan sulfate in neuritogenesis in the brain. *J Biol Chem.* **278**, 43744-43754
- 157 Takano, M., Mori, Y., Shiraki, H., Horie, M., Okamoto, H., Narahara, M., Miyake, M. and Shikimi, T. (1999) Detection of bikunin mRNA in limited portions of rat brain. *Life Sci.* **65**, 757-762
- 158 Yoshida, K., Suzuki, Y., Yamamoto, K. and Sinohara, H. (1999) Guinea pig alpha 1-microglobulin/bikunin: cDNA sequencing, tissue expression and expression during acute phase. *Comp Biochem Physiol B Biochem Mol Biol.* **122**, 165-172
- 159 Yoshida, E., Maruyama, M., Sugiki, M. and Mihara, H. (1994) Immunohistochemical demonstration of bikunin, a light chain of inter-alpha-trypsin inhibitor, in human brain tumors. *Inflammation.* **18**, 589-596
- 160 Pangalos, M. N., Shioi, J. and Robakis, N. K. (1995) Expression of the chondroitin sulfate proteoglycans of amyloid precursor (appican) and amyloid precursor-like protein 2. *J Neurochem.* **65**, 762-769
- 161 Shioi, J., Pangalos, M. N., Ripellino, J. A., Vassilacopoulou, D., Mytilineou, C., Margolis, R. U. and Robakis, N. K. (1995) The Alzheimer amyloid precursor proteoglycan (appican) is present in brain and is produced by astrocytes but not by neurons in primary neural cultures. *J Biol Chem.* **270**, 11839-11844
- 162 Kawamura, D., Funakoshi, T., Mizumoto, S., Sugahara, K. and Iwasaki, N. (2014) Sulfation patterns of exogenous chondroitin sulfate affect chondrogenic differentiation of ATDC5 cells. *J Orthop Sci.* **19**, 1028-1035

- 163 Sia, G. M., Clem, R. L. and Huganir, R. L. (2013) The human language-associated gene SRPX2 regulates synapse formation and vocalization in mice. *Science*. **342**, 987-991
- 164 Royer-Zemmour, B., Ponsole-Lenfant, M., Gara, H., Roll, P., Leveque, C., Massacrier, A., Ferracci, G., Cillario, J., Robaglia-Schlupp, A., Vincentelli, R., Cau, P. and Szepetowski, P. (2008) Epileptic and developmental disorders of the speech cortex: ligand/receptor interaction of wild-type and mutant SRPX2 with the plasminogen activator receptor uPAR. *Hum Mol Genet*. **17**, 3617-3630
- 165 Bruneau, N. and Szepetowski, P. (2011) The role of the urokinase receptor in epilepsy, in disorders of language, cognition, communication and behavior, and in the central nervous system. *Curr Pharm Des*. **17**, 1914-1923
- 166 Spalice, A., Parisi, P., Nicita, F., Pizzardi, G., Del Balzo, F. and Iannetti, P. (2009) Neuronal migration disorders: clinical, neuroradiologic and genetics aspects. *Acta Paediatr*. **98**, 421-433
- 167 Archinti, M., Britto, M., Eden, G., Furlan, F., Murphy, R. and Degryse, B. (2011) The urokinase receptor in the central nervous system. *CNS Neurol Disord Drug Targets*. **10**, 271-294
- 168 Lemarchant, S., Pruvost, M., Hebert, M., Gauberti, M., Hommet, Y., Briens, A., Maubert, E., Gueye, Y., Feron, F., Petite, D., Mersel, M., do Rego, J. C., Vaudry, H., Koistinaho, J., Ali, C., Agin, V., Emery, E. and Vivien, D. (2014) tPA promotes ADAMTS-4-induced CSPG degradation, thereby enhancing neuroplasticity following spinal cord injury. *Neurobiol Dis*. **66**, 28-42
- 169 Tauchi, R., Imagama, S., Natori, T., Ohgomori, T., Muramoto, A., Shinjo, R., Matsuyama, Y., Ishiguro, N. and Kadomatsu, K. (2012) The endogenous proteoglycan-degrading enzyme ADAMTS-4 promotes functional recovery after spinal cord injury. *J Neuroinflammation*. **9**, 53
- 170 Roll, P., Rudolf, G., Pereira, S., Royer, B., Scheffer, I. E., Massacrier, A., Valenti, M. P., Roedel-Trevisiol, N., Jamali, S., Beclin, C., Seegmuller, C., Metz-Lutz, M. N., Lemainque, A., Delepine, M., Caloustian, C., de Saint Martin, A., Bruneau, N., Depetris, D., Mattei, M. G., Flori, E., Robaglia-Schlupp, A., Levy, N., Neubauer, B. A., Ravid, R., Marescaux, C., Berkovic, S. F., Hirsch, E., Lathrop, M., Cau, P. and Szepetowski, P. (2006) SRPX2 mutations in disorders of language cortex and cognition. *Hum Mol Genet*. **15**, 1195-1207
- 171 Royer, B., Soares, D. C., Barlow, P. N., Bontrop, R. E., Roll, P., Robaglia-Schlupp, A., Blancher, A., Levasseur, A., Cau, P., Pontarotti, P. and Szepetowski, P. (2007) Molecular evolution of the human SRPX2 gene that causes brain disorders of the Rolandic and Sylvian speech areas. *BMC Genet*. **8**, 72
- 172 Soleman, S., Filippov, M. A., Dityatev, A. and Fawcett, J. W. (2013) Targeting the neural extracellular matrix in neurological disorders. *Neuroscience*. **253**, 194-213
- 173 Nadanaka, S., Clement, A., Masayama, K., Faissner, A. and Sugahara, K. (1998) Characteristic hexasaccharide sequences in octasaccharides derived from shark cartilage chondroitin sulfate D with a neurite outgrowth promoting activity. *J Biol Chem*. **273**, 3296-3307
- 174 Mizumoto, S., Fongmoon, D. and Sugahara, K. (2013) Interaction of chondroitin sulfate and dermatan sulfate from various biological sources with heparin-binding growth factors and cytokines. *Glycoconj J*. **30**, 619-632
- 175 Gu, W. L., Fu, S. L., Wang, Y. X., Li, Y., Lu, H. Z., Xu, X. M. and Lu, P. H. (2009) Chondroitin sulfate proteoglycans regulate the growth, differentiation and migration of multipotent neural precursor cells through the integrin signaling pathway. *BMC Neurosci*. **10**, 128
- 176 Sirko, S., von Holst, A., Weber, A., Wizenmann, A., Theocharidis, U., Gotz, M. and Faissner, A. (2010) Chondroitin sulfates are required for fibroblast growth factor-2-dependent proliferation and maintenance in neural stem cells and for epidermal growth factor-dependent migration of their progeny. *Stem Cells*. **28**, 775-787
- 177 Sirko, S., von Holst, A., Wizenmann, A., Gotz, M. and Faissner, A. (2007) Chondroitin sulfate glycosaminoglycans control proliferation, radial glia cell differentiation and neurogenesis in neural stem/progenitor cells. *Development*. **134**, 2727-2738
- 178 Pyka, M., Wetzel, C., Aguado, A., Geissler, M., Hatt, H. and Faissner, A. (2011) Chondroitin sulfate proteoglycans regulate astrocyte-dependent synaptogenesis and modulate synaptic activity in primary embryonic hippocampal neurons. *Eur J Neurosci*. **33**, 2187-2202
- 179 Properzi, F., Carulli, D., Asher, R. A., Muir, E., Camargo, L. M., van Kuppevelt, T. H., ten Dam, G. B., Furukawa, Y., Mikami, T., Sugahara, K., Toida, T., Geller, H. M. and Fawcett, J. W. (2005) Chondroitin 6-sulphate synthesis is up-regulated in injured CNS, induced by injury-related cytokines and enhanced in axon-growth inhibitory glia. *Eur J Neurosci*. **21**, 378-390
- 180 Silver, J. and Miller, J. H. (2004) Regeneration beyond the glial scar. *Nat Rev Neurosci*. **5**, 146-156
- 181 Slater, R. R., Jr., Bayliss, M. T., Lachiewicz, P. F., Visco, D. M. and Caterson, B. (1995) Monoclonal antibodies that detect biochemical markers of arthritis in humans. *Arthritis Rheum*. **38**, 655-659

- 182 Visco, D. M., Johnstone, B., Hill, M. A., Jolly, G. A. and Caterson, B. (1993) Immunohistochemical analysis of 3-B(-) and 7-D-4 epitope expression in canine osteoarthritis. *Arthritis Rheum.* **36**, 1718-1725
- 183 Brown, S., Matta, A., Erwin, W. M., Roberts, S., Gruber, H. H., Hanley, E. N., Little, C. B. and Melrose, J. (2017) Cell clusters are indicative of stem cell activity in the degenerate intervertebral disc: can their properties be manipulated to improve intrinsic repair of the disc? *Stem Cells Dev*
- 184 Tesche, F. and Miosge, N. (2004) Perlecan in late stages of osteoarthritis of the human knee joint. *Osteoarthritis Cartilage.* **12**, 852-862
- 185 Tesche, F. and Miosge, N. (2005) New aspects of the pathogenesis of osteoarthritis: the role of fibroblast-like chondrocytes in late stages of the disease. *Histol Histopathol.* **20**, 329-337
- 186 Smith, S., Melrose, J. (2016) Perlecan Delineates stem cell niches in Human Foetal Hip, Knee and Elbow Cartilage Rudiments and has potential roles in the regulation of Stem Cell Differentiation. *J Stem Cell Res Devel Ther.* **3**, 9-16
- 187 Akatsu, C., Mizumoto, S., Kaneiwa, T., Maccarana, M., Malmstrom, A., Yamada, S. and Sugahara, K. (2011) Dermatan sulfate epimerase 2 is the predominant isozyme in the formation of the chondroitin sulfate/dermatan sulfate hybrid structure in postnatal developing mouse brain. *Glycobiology.* **21**, 565-574
- 188 Akita, K., von Holst, A., Furukawa, Y., Mikami, T., Sugahara, K. and Faissner, A. (2008) Expression of multiple chondroitin/dermatan sulfotransferases in the neurogenic regions of the embryonic and adult central nervous system implies that complex chondroitin sulfates have a role in neural stem cell maintenance. *Stem Cells.* **26**, 798-809
- 189 Mitsunaga, C., Mikami, T., Mizumoto, S., Fukuda, J. and Sugahara, K. (2006) Chondroitin sulfate/dermatan sulfate hybrid chains in the development of cerebellum. Spatiotemporal regulation of the expression of critical disulfated disaccharides by specific sulfotransferases. *J Biol Chem.* **281**, 18942-18952
- 190 Hiraoka, K., Grogan, S., Olee, T. and Lotz, M. (2006) Mesenchymal progenitor cells in adult human articular cartilage. *Biorheology.* **43**, 447-454
- 191 Hayes, A. J., Dowthwaite, G. P., Webster, S. V. and Archer, C. W. (2003) The distribution of Notch receptors and their ligands during articular cartilage development. *J Anat.* **202**, 495-502
- 192 Garcia-Suarez, O., Garcia, B., Fernandez-Vega, I., Astudillo, A. and Quiros, L. M. (2014) Neuroendocrine tumors show altered expression of chondroitin sulfate, glypican 1, glypican 5, and syndecan 2 depending on their differentiation grade. *Front Oncol.* **4**, 15
- 193 Baghy, K., Tatrai, P., Regos, E. and Kovalszky, I. (2016) Proteoglycans in liver cancer. *World J Gastroenterol.* **22**, 379-393
- 194 Ucakturk, E., Akman, O., Sun, X., Baydar, D. E., Dolgun, A., Zhang, F. and Linhardt, R. J. (2016) Changes in composition and sulfation patterns of glycoaminoglycans in renal cell carcinoma. *Glycoconj J.* **33**, 103-112
- 195 Jia, X. L., Li, S. Y., Dang, S. S., Cheng, Y. A., Zhang, X., Wang, W. J., Hughes, C. E. and Caterson, B. (2012) Increased expression of chondroitin sulphate proteoglycans in rat hepatocellular carcinoma tissues. *World J Gastroenterol.* **18**, 3962-3976
- 196 Lv, H., Yu, G., Sun, L., Zhang, Z., Zhao, X. and Chai, W. (2007) Elevate level of glycosaminoglycans and altered sulfation pattern of chondroitin sulfate are associated with differentiation status and histological type of human primary hepatic carcinoma. *Oncology.* **72**, 347-356
- 197 Theocharis, A. D., Vynios, D. H., Papageorgakopoulou, N., Skandalis, S. S. and Theocharis, D. A. (2003) Altered content composition and structure of glycosaminoglycans and proteoglycans in gastric carcinoma. *Int J Biochem Cell Biol.* **35**, 376-390
- 198 Skandalis, S. S., Kletsas, D., Kyriakopoulou, D., Stavropoulos, M. and Theocharis, D. A. (2006) The greatly increased amounts of accumulated versican and decorin with specific post-translational modifications may be closely associated with the malignant phenotype of pancreatic cancer. *Biochim Biophys Acta.* **1760**, 1217-1225
- 199 Hinrichs, U., Rutteman, G. R. and Nederbragt, H. (1999) Stromal accumulation of chondroitin sulphate in mammary tumours of dogs. *Br J Cancer.* **80**, 1359-1365
- 200 Viola, M., Bruggemann, K., Karousou, E., Caon, I., Carava, E., Vigetti, D., Greve, B., Stock, C., De Luca, G., Passi, A. and Gotte, M. (2016) MDA-MB-231 breast cancer cell viability, motility and matrix adhesion are regulated by a complex interplay of heparan sulfate, chondroitin-/dermatan sulfate and hyaluronan biosynthesis. *Glycoconj J*
- 201 van der Steen, S. C., van Tilborg, A. A., Vallen, M. J., Bulten, J., van Kuppevelt, T. H. and Massuger, L. F. (2016) Prognostic significance of highly sulfated chondroitin sulfates in ovarian cancer defined by the single chain antibody GD3A11. *Gynecol Oncol.* **140**, 527-536

- 202 Pothacharoen, P., Siriaunkgul, S., Ong-Chai, S., Supabandhu, J., Kumja, P., Wanaphirak, C., Sugahara, K., Hardingham, T. and Kongtawelert, P. (2006) Raised serum chondroitin sulfate epitope level in ovarian epithelial cancer. *J Biochem.* **140**, 517-524
- 203 Takakura, K., Shibazaki, Y., Yoneyama, H., Fujii, M., Hashiguchi, T., Ito, Z., Kajihara, M., Misawa, T., Homma, S., Ohkusa, T. and Koido, S. (2015) Inhibition of Cell Proliferation and Growth of Pancreatic Cancer by Silencing of Carbohydrate Sulfotransferase 15 In Vitro and in a Xenograft Model. *PLoS One.* **10**, e0142981
- 204 ten Dam, G. B., van de Westerlo, E. M., Purushothaman, A., Stan, R. V., Bulten, J., Sweep, F. C., Massuger, L. F., Sugahara, K. and van Kuppevelt, T. H. (2007) Antibody GD3G7 selected against embryonic glycosaminoglycans defines chondroitin sulfate-E domains highly up-regulated in ovarian cancer and involved in vascular endothelial growth factor binding. *Am J Pathol.* **171**, 1324-1333
- 205 Marolla, A. P., Waisberg, J., Saba, G. T., Waisberg, D. R., Margeotto, F. B. and Pinhal, M. A. (2015) Glycomics expression analysis of sulfated glycosaminoglycans of human colorectal cancer tissues and non-neoplastic mucosa by electrospray ionization mass spectrometry. *Einstein (Sao Paulo).* **13**, 510-517
- 206 Iida, J., Dorchak, J., Clancy, R., Slavik, J., Ellsworth, R., Katagiri, Y., Pugacheva, E. N., van Kuppevelt, T. H., Mural, R. J., Cutler, M. L. and Shriver, C. D. (2015) Role for chondroitin sulfate glycosaminoglycan in NEDD9-mediated breast cancer cell growth. *Exp Cell Res.* **330**, 358-370
- 207 Basappa, Murugan, S., Sugahara, K. N., Lee, C. M., ten Dam, G. B., van Kuppevelt, T. H., Miyasaka, M., Yamada, S. and Sugahara, K. (2009) Involvement of chondroitin sulfate E in the liver tumor focal formation of murine osteosarcoma cells. *Glycobiology.* **19**, 735-742
- 208 Basappa, Rangappa, K. S. and Sugahara, K. (2014) Roles of glycosaminoglycans and glycanmimetics in tumor progression and metastasis. *Glycoconj J.* **31**, 461-467
- 209 Purushothaman, A. and Toole, B. P. (2014) Serglycin proteoglycan is required for multiple myeloma cell adhesion, in vivo growth, and vascularization. *J Biol Chem.* **289**, 5499-5509
- 210 Korpetinou, A., Skandalis, S. S., Labropoulou, V. T., Smirlaki, G., Noulas, A., Karamanos, N. K. and Theocharis, A. D. (2014) Serglycin: at the crossroad of inflammation and malignancy. *Front Oncol.* **3**, 327
- 211 Du, W. W., Yang, W. and Yee, A. J. (2013) Roles of versican in cancer biology--tumorigenesis, progression and metastasis. *Histol Histopathol.* **28**, 701-713
- 212 Xiang, Y. Y., Dong, H., Wan, Y., Li, J., Yee, A., Yang, B. B. and Lu, W. Y. (2006) Versican G3 domain regulates neurite growth and synaptic transmission of hippocampal neurons by activation of epidermal growth factor receptor. *J Biol Chem.* **281**, 19358-19368
- 213 Dutt, S., Kleber, M., Matasci, M., Sommer, L. and Zimmermann, D. R. (2006) Versican V0 and V1 guide migratory neural crest cells. *J Biol Chem.* **281**, 12123-12131
- 214 Touab, M., Villena, J., Barranco, C., Arumi-Uria, M. and Bassols, A. (2002) Versican is differentially expressed in human melanoma and may play a role in tumor development. *Am J Pathol.* **160**, 549-557
- 215 Zheng, P. S., Wen, J., Ang, L. C., Sheng, W., Vilorio-Petit, A., Wang, Y., Wu, Y., Kerbel, R. S. and Yang, B. B. (2004) Versican/PG-M G3 domain promotes tumor growth and angiogenesis. *FASEB J.* **18**, 754-756
- 216 Fedorchenko, O., Stiefelhagen, M., Peer-Zada, A. A., Barthel, R., Mayer, P., Ecke, L., Breuer, A., Crispatz, G., Rosen, N., Landwehr, T., Lilienthal, N., Mollmann, M., Montesinos-Rongen, M., Heukamp, L., Durig, J., Hallek, M., Fingerle-Rowson, G. and Herling, M. (2013) CD44 regulates the apoptotic response and promotes disease development in chronic lymphocytic leukemia. *Blood.* **121**, 4126-4136
- 217 Prinz, R. D., Willis, C. M., Vilorio-Petit, A. and Kluppel, M. (2011) Elimination of breast tumor-associated chondroitin sulfate promotes metastasis. *Genet Mol Res.* **10**, 3901-3913
- 218 Schowalter, R. M., Pastrana, D. V. and Buck, C. B. (2011) Glycosaminoglycans and sialylated glycans sequentially facilitate Merkel cell polyomavirus infectious entry. *PLoS Pathog.* **7**, e1002161
- 219 Cooney, C. A., Jousheghany, F., Yao-Borengasser, A., Phanavanh, B., Gomes, T., Kieber-Emmons, A. M., Siegel, E. R., Suva, L. J., Ferrone, S., Kieber-Emmons, T. and Monzavi-Karbassi, B. (2011) Chondroitin sulfates play a major role in breast cancer metastasis: a role for CSPG4 and CHST11 gene expression in forming surface P-selectin ligands in aggressive breast cancer cells. *Breast Cancer Res.* **13**, R58
- 220 Monzavi-Karbassi, B., Stanley, J. S., Hennings, L., Jousheghany, F., Artaud, C., Shaaf, S. and Kieber-Emmons, T. (2007) Chondroitin sulfate glycosaminoglycans as major P-selectin ligands on metastatic breast cancer cell lines. *Int J Cancer.* **120**, 1179-1191
- 221 Amoury, M., Mladenov, R., Nachreiner, T., Pham, A. T., Hristodorov, D., Di Fiore, S., Helfrich, W., Pardo, A., Fey, G., Schwenkert, M., Thepen, T., Kiessling, F., Hussain, A. F., Fischer,

- R., Kolberg, K. and Barth, S. (2016) A novel approach for targeted elimination of CSPG4-positive triple-negative breast cancer cells using a MAP tau-based fusion protein. *Int J Cancer*. **139**, 916-927
- 222 Wang, Y., Geldres, C., Ferrone, S. and Dotti, G. (2015) Chondroitin sulfate proteoglycan 4 as a target for chimeric antigen receptor-based T-cell immunotherapy of solid tumors. *Expert Opin Ther Targets*. **19**, 1339-1350
- 223 Beard, R. E., Zheng, Z., Lagisetty, K. H., Burns, W. R., Tran, E., Hewitt, S. M., Abate-Daga, D., Rosati, S. F., Fine, H. A., Ferrone, S., Rosenberg, S. A. and Morgan, R. A. (2014) Multiple chimeric antigen receptors successfully target chondroitin sulfate proteoglycan 4 in several different cancer histologies and cancer stem cells. *J Immunother Cancer*. **2**, 25
- 224 Wang, X., Osada, T., Wang, Y., Yu, L., Sakakura, K., Katayama, A., McCarthy, J. B., Brufsky, A., Chivukula, M., Khoury, T., Hsu, D. S., Barry, W. T., Lyerly, H. K., Clay, T. M. and Ferrone, S. (2010) CSPG4 protein as a new target for the antibody-based immunotherapy of triple-negative breast cancer. *J Natl Cancer Inst*. **102**, 1496-1512
- 225 Brehm, H., Niesen, J., Mladenov, R., Stein, C., Pardo, A., Fey, G., Helfrich, W., Fischer, R., Gattenlohner, S. and Barth, S. (2014) A CSPG4-specific immunotoxin kills rhabdomyosarcoma cells and binds to primary tumor tissues. *Cancer Lett*. **352**, 228-235
- 226 Mizumoto, S., Yamada, S. and Sugahara, K. (2015) Molecular interactions between chondroitin-dermatan sulfate and growth factors/receptors/matrix proteins. *Curr Opin Struct Biol*. **34**, 35-42
- 227 Yu, L., Favoino, E., Wang, Y., Ma, Y., Deng, X. and Wang, X. (2011) The CSPG4-specific monoclonal antibody enhances and prolongs the effects of the BRAF inhibitor in melanoma cells. *Immunol Res*. **50**, 294-302
- 228 Sorrell, J. M., Mahmoodian, F., Schafer, I. A., Davis, B. and Caterson, B. (1990) Identification of monoclonal antibodies that recognize novel epitopes in native chondroitin/dermatan sulfate glycosaminoglycan chains: their use in mapping functionally distinct domains of human skin. *J Histochem Cytochem*. **38**, 393-402
- 229 Caterson, B., Mahmoodian, F., Sorrell, J. M., Hardingham, T. E., Bayliss, M. T., Carney, S. L., Ratcliffe, A. and Muir, H. (1990) Modulation of native chondroitin sulphate structure in tissue development and in disease. *J Cell Sci*. **97 (Pt 3)**, 411-417
- 230 Hayes, A. J., Benjamin, M. and Ralphs, J. R. (2001) Extracellular matrix in development of the intervertebral disc. *Matrix Biol*. **20**, 107-121
- 231 Sorrell, J. M., Lintala, A. M., Mahmoodian, F. and Caterson, B. (1988) Epitope-specific changes in chondroitin sulfate/dermatan sulfate proteoglycans as markers in the lymphopoietic and granulopoietic compartments of developing bursae of Fabricius. *J Immunol*. **140**, 4263-4270
- 232 Rhodes, K. E. and Fawcett, J. W. (2004) Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? *J Anat*. **204**, 33-48
- 233 Melrose, J., Chuang, C., Whitelock, J. . (2008) Tissue engineering of Cartilages using Biomatrices. *J Chemical Technology and Biotechnology* **83**, 444-463
- 234 Abbadessa, A., Blokzijl, M. M., Mouser, V. H., Marica, P., Malda, J., Hennink, W. E. and Vermonden, T. (2016) A thermo-responsive and photo-polymerizable chondroitin sulfate-based hydrogel for 3D printing applications. *Carbohydr Polym*. **149**, 163-174
- 235 Fan, M., Ma, Y., Tan, H., Jia, Y., Zou, S., Guo, S., Zhao, M., Huang, H., Ling, Z., Chen, Y. and Hu, X. (2017) Covalent and injectable chitosan-chondroitin sulfate hydrogels embedded with chitosan microspheres for drug delivery and tissue engineering. *Mater Sci Eng C Mater Biol Appl*. **71**, 67-74
- 236 Gupta, V., Tenny, K. M., Barragan, M., Berkland, C. J. and Detamore, M. S. (2016) Microsphere-based scaffolds encapsulating chondroitin sulfate or decellularized cartilage. *J Biomater Appl*. **31**, 328-343
- 237 Xu, H., Yan, Y. and Li, S. (2011) PDLA/chondroitin sulfate/chitosan/NGF conduits for peripheral nerve regeneration. *Biomaterials*. **32**, 4506-4516
- 238 Corradetti, B., Taraballi, F., Minardi, S., Van Eps, J., Cabrera, F., Francis, L. W., Gazze, S. A., Ferrari, M., Weiner, B. K. and Tasciotti, E. (2016) Chondroitin Sulfate Immobilized on a Biomimetic Scaffold Modulates Inflammation While Driving Chondrogenesis. *Stem Cells Transl Med*. **5**, 670-682
- 239 Bhattacharjee, M., Chawla, S., Chameettachal, S., Murab, S., Bhavesh, N. S. and Ghosh, S. (2016) Role of chondroitin sulphate tethered silk scaffold in cartilaginous disc tissue regeneration. *Biomed Mater*. **11**, 025014
- 240 Sawatjui, N., Damrongrungruang, T., Leeansaksiri, W., Jearanaikoon, P., Hongeng, S. and Limpiboon, T. (2015) Silk fibroin/gelatin-chondroitin sulfate-hyaluronic acid effectively enhances in vitro chondrogenesis of bone marrow mesenchymal stem cells. *Mater Sci Eng C Mater Biol Appl*. **52**, 90-96
- 241 Huang, Z., Noeaid, P., Kohl, B., Roether, J. A., Schubert, D. W., Meier, C., Boccaccini, A. R., Godkin, O., Ertel, W., Arens, S. and Schulze-Tanzil, G. (2015) Chondrogenesis of human

- bone marrow mesenchymal stromal cells in highly porous alginate-foams supplemented with chondroitin sulfate. *Mater Sci Eng C Mater Biol Appl.* **50**, 160-172
- 242 Kuo, C. Y., Chen, C. H., Hsiao, C. Y. and Chen, J. P. (2015) Incorporation of chitosan in biomimetic gelatin/chondroitin-6-sulfate/hyaluronan cryogel for cartilage tissue engineering. *Carbohydr Polym.* **117**, 722-730
- 243 Ni, Y., Tang, Z., Cao, W., Lin, H., Fan, Y., Guo, L. and Zhang, X. (2015) Tough and elastic hydrogel of hyaluronic acid and chondroitin sulfate as potential cell scaffold materials. *Int J Biol Macromol.* **74**, 367-375
- 244 Hortensius, R. A. and Harley, B. A. (2013) The use of bioinspired alterations in the glycosaminoglycan content of collagen-GAG scaffolds to regulate cell activity. *Biomaterials.* **34**, 7645-7652
- 245 Silva, J. M., Georgi, N., Costa, R., Sher, P., Reis, R. L., Van Blitterswijk, C. A., Karperien, M. and Mano, J. F. (2013) Nanostructured 3D constructs based on chitosan and chondroitin sulphate multilayers for cartilage tissue engineering. *PLoS One.* **8**, e55451
- 246 Sun, L., Li, H., Qu, L., Zhu, R., Fan, X., Xue, Y., Xie, Z. and Fan, H. (2014) Immobilized lentivirus vector on chondroitin sulfate-hyaluronate acid-silk fibroin hybrid scaffold for tissue-engineered ligament-bone junction. *Biomed Res Int.* **2014**, 816979
- 247 Chen, W. C., Wei, Y. H., Chu, I. M. and Yao, C. L. (2013) Effect of chondroitin sulphate C on the in vitro and in vivo chondrogenesis of mesenchymal stem cells in crosslinked type II collagen scaffolds. *J Tissue Eng Regen Med.* **7**, 665-672
- 248 Guo, Y., Yuan, T., Xiao, Z., Tang, P., Xiao, Y., Fan, Y. and Zhang, X. (2012) Hydrogels of collagen/chondroitin sulfate/hyaluronan interpenetrating polymer network for cartilage tissue engineering. *J Mater Sci Mater Med.* **23**, 2267-2279
- 249 Tamaddon, M., Walton, R. S., Brand, D. D. and Czernuszka, J. T. (2013) Characterisation of freeze-dried type II collagen and chondroitin sulfate scaffolds. *J Mater Sci Mater Med.* **24**, 1153-1165
- 250 Fan, H., Tao, H., Wu, Y., Hu, Y., Yan, Y. and Luo, Z. (2010) TGF-beta3 immobilized PLGA-gelatin/chondroitin sulfate/hyaluronic acid hybrid scaffold for cartilage regeneration. *J Biomed Mater Res A.* **95**, 982-992
- 251 Park, J. S., Yang, H. J., Woo, D. G., Yang, H. N., Na, K. and Park, K. H. (2010) Chondrogenic differentiation of mesenchymal stem cells embedded in a scaffold by long-term release of TGF-beta 3 complexed with chondroitin sulfate. *J Biomed Mater Res A.* **92**, 806-816
- 252 Farrugia, B. L., Whitelock, J. M., Jung, M., McGrath, B., O'Grady, R. L., McCarthy, S. J. and Lord, M. S. (2014) The localisation of inflammatory cells and expression of associated proteoglycans in response to implanted chitosan. *Biomaterials.* **35**, 1462-1477
- 253 Asari, A., Akizaki, S., Itoh, T., Kominami, E. and Uchiyama, Y. (1996) Human osteoarthritic cartilage exhibits the 2B6 epitope without pretreatment with chondroitinase ABC. *Osteoarthritis Cartilage.* **4**, 149-152
- 254 Kaneiwa, T., Miyazaki, A., Kogawa, R., Mizumoto, S., Sugahara, K. and Yamada, S. (2012) Identification of amino acid residues required for the substrate specificity of human and mouse chondroitin sulfate hydrolase (conventional hyaluronidase-4). *J Biol Chem.* **287**, 42119-42128
- 255 Kaneiwa, T., Mizumoto, S., Sugahara, K. and Yamada, S. (2010) Identification of human hyaluronidase-4 as a novel chondroitin sulfate hydrolase that preferentially cleaves the galactosaminidic linkage in the trisulfated tetrasaccharide sequence. *Glycobiology.* **20**, 300-309
- 256 Bao, X., Muramatsu, T. and Sugahara, K. (2005) Demonstration of the pleiotrophin-binding oligosaccharide sequences isolated from chondroitin sulfate/dermatan sulfate hybrid chains of embryonic pig brains. *J Biol Chem.* **280**, 35318-35328
- 257 Bao, X., Nishimura, S., Mikami, T., Yamada, S., Itoh, N. and Sugahara, K. (2004) Chondroitin sulfate/dermatan sulfate hybrid chains from embryonic pig brain, which contain a higher proportion of L-iduronic acid than those from adult pig brain, exhibit neuritogenic and growth factor binding activities. *J Biol Chem.* **279**, 9765-9776
- 258 Maimone, M. M. and Tollefsen, D. M. (1991) Structure of a dermatan sulfate hexasaccharide that binds to heparin cofactor II with high affinity. *J Biol Chem.* **266**, 14830
- 259 Bartolini, B., Thelin, M. A., Svensson, L., Ghiselli, G., van Kuppevelt, T. H., Malmstrom, A. and Maccarana, M. (2013) Iduronic acid in chondroitin/dermatan sulfate affects directional migration of aortic smooth muscle cells. *PLoS One.* **8**, e66704
- 260 Thelin, M. A., Bartolini, B., Axelsson, J., Gustafsson, R., Tykesson, E., Pera, E., Oldberg, A., Maccarana, M. and Malmstrom, A. (2013) Biological functions of iduronic acid in chondroitin/dermatan sulfate. *FEBS J.* **280**, 2431-2446
- 261 Bishop, J. R., Schuksz, M. and Esko, J. D. (2007) Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature.* **446**, 1030-1037

- 262 Dickendeshner, T. L., Baldwin, K. T., Mironova, Y. A., Koriyama, Y., Raiker, S. J., Askew, K. L., Wood, A., Geoffroy, C. G., Zheng, B., Liepmann, C. D., Katagiri, Y., Benowitz, L. I., Geller, H. M. and Giger, R. J. (2012) NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. *Nat Neurosci.* **15**, 703-712
- 263 Chen, T., Yuan, D., Wei, B., Jiang, J., Kang, J., Ling, K., Gu, Y., Li, J., Xiao, L. and Pei, G. (2010) E-cadherin-mediated cell-cell contact is critical for induced pluripotent stem cell generation. *Stem Cells.* **28**, 1315-1325
- 264 Larue, L., Antos, C., Butz, S., Huber, O., Delmas, V., Dominis, M. and Kemler, R. (1996) A role for cadherins in tissue formation. *Development.* **122**, 3185-3194
- 265 Larue, L., Ohsugi, M., Hirchenhain, J. and Kemler, R. (1994) E-cadherin null mutant embryos fail to form a trophoblast epithelium. *Proc Natl Acad Sci U S A.* **91**, 8263-8267
- 266 Redmer, T., Diecke, S., Grigoryan, T., Quiroga-Negreira, A., Birchmeier, W. and Besser, D. (2011) E-cadherin is crucial for embryonic stem cell pluripotency and can replace OCT4 during somatic cell reprogramming. *EMBO Rep.* **12**, 720-726
- 267 Zaidel-Bar, R. (2013) Cadherin adhesome at a glance. *J Cell Sci.* **126**, 373-378
- 268 Bhatt, T., Rizvi, A., Batta, S. P., Kataria, S. and Jamora, C. (2013) Signaling and mechanical roles of E-cadherin. *Cell Commun Adhes.* **20**, 189-199
- 269 Huveneers, S. and de Rooij, J. (2013) Mechanosensitive systems at the cadherin-F-actin interface. *J Cell Sci.* **126**, 403-413
- 270 Katoh, M. (2006) Cross-talk of WNT and FGF signaling pathways at GSK3beta to regulate beta-catenin and SNAIL signaling cascades. *Cancer Biol Ther.* **5**, 1059-1064
- 271 Nelson, W. J. and Nusse, R. (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. *Science.* **303**, 1483-1487
- 272 Stepniak, E., Radice, G. L. and Vasioukhin, V. (2009) Adhesive and signaling functions of cadherins and catenins in vertebrate development. *Cold Spring Harb Perspect Biol.* **1**, a002949
- 273 Sugahara, K. and Mikami, T. (2007) Chondroitin/dermatan sulfate in the central nervous system. *Curr Opin Struct Biol.* **17**, 536-545
- 274 Nadanaka, S., Kinouchi, H., Taniguchi-Morita, K., Tamura, J. and Kitagawa, H. (2011) Down-regulation of chondroitin 4-O-sulfotransferase-1 by Wnt signaling triggers diffusion of Wnt-3a. *J Biol Chem.* **286**, 4199-4208
- 275 Prinz, R. D., Willis, C. M., van Kuppevelt, T. H. and Kluppel, M. (2014) Biphasic role of chondroitin sulfate in cardiac differentiation of embryonic stem cells through inhibition of Wnt/beta-catenin signaling. *PLoS One.* **9**, e92381
- 276 Willis, C. M. and Kluppel, M. (2014) Chondroitin sulfate-E is a negative regulator of a pro-tumorigenic Wnt/beta-catenin-Collagen 1 axis in breast cancer cells. *PLoS One.* **9**, e103966
- 277 Mizumoto, S. and Sugahara, K. (2013) Glycosaminoglycans are functional ligands for receptor for advanced glycation end-products in tumors. *FEBS J.* **280**, 2462-2470
- 278 Theocharis, A. D., Gialeli, C., Bouris, P., Giannopoulou, E., Skandalis, S. S., Aletras, A. J., Iozzo, R. V. and Karamanos, N. K. (2014) Cell-matrix interactions: focus on proteoglycan-proteinase interplay and pharmacological targeting in cancer. *FEBS J.* **281**, 5023-5042
- 279 Sugahara, K. N., Hirata, T., Tanaka, T., Ogino, S., Takeda, M., Terasawa, H., Shimada, I., Tamura, J., ten Dam, G. B., van Kuppevelt, T. H. and Miyasaka, M. (2008) Chondroitin sulfate E fragments enhance CD44 cleavage and CD44-dependent motility in tumor cells. *Cancer Res.* **68**, 7191-7199
- 280 Vallen, M. J., Massuger, L. F., ten Dam, G. B., Bulten, J. and van Kuppevelt, T. H. (2012) Highly sulfated chondroitin sulfates, a novel class of prognostic biomarkers in ovarian cancer tissue. *Gynecol Oncol.* **127**, 202-209
- 281 Li, F., Ten Dam, G. B., Murugan, S., Yamada, S., Hashiguchi, T., Mizumoto, S., Oguri, K., Okayama, M., van Kuppevelt, T. H. and Sugahara, K. (2008) Involvement of highly sulfated chondroitin sulfate in the metastasis of the Lewis lung carcinoma cells. *J Biol Chem.* **283**, 34294-34304
- 282 Mizumoto, S., Takahashi, J. and Sugahara, K. (2012) Receptor for advanced glycation end products (RAGE) functions as receptor for specific sulfated glycosaminoglycans, and anti-RAGE antibody or sulfated glycosaminoglycans delivered in vivo inhibit pulmonary metastasis of tumor cells. *J Biol Chem.* **287**, 18985-18994
- 283 Kiani, C., Chen, L., Wu, Y. J., Yee, A. J. and Yang, B. B. (2002) Structure and function of aggrecan. *Cell Res.* **12**, 19-32
- 284 Roughley, P. J. and Mort, J. S. (2014) The role of aggrecan in normal and osteoarthritic cartilage. *J Exp Orthop.* **1**, 8
- 285 Aspberg, A., Miura, R., Bourdoulous, S., Shimonaka, M., Heinegard, D., Schachner, M., Ruoslahti, E. and Yamaguchi, Y. (1997) The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein-protein interactions independent of carbohydrate moiety. *Proc Natl Acad Sci U S A.* **94**, 10116-10121

- 286 Wight, T. N. (2002) Versican: a versatile extracellular matrix proteoglycan in cell biology. *Curr Opin Cell Biol.* **14**, 617-623
- 287 Wu, Y. J., La Pierre, D. P., Wu, J., Yee, A. J. and Yang, B. B. (2005) The interaction of versican with its binding partners. *Cell Res.* **15**, 483-494
- 288 Rauch, U., Feng, K. and Zhou, X. H. (2001) Neurocan: a brain chondroitin sulfate proteoglycan. *Cell Mol Life Sci.* **58**, 1842-1856
- 289 Spicer, A. P., Joo, A. and Bowling, R. A., Jr. (2003) A hyaluronan binding link protein gene family whose members are physically linked adjacent to chondroitin sulfate proteoglycan core protein genes: the missing links. *J Biol Chem.* **278**, 21083-21091
- 290 Kinugasa, Y., Ishiguro, H., Tokita, Y., Oohira, A., Ohmoto, H. and Higashiyama, S. (2004) Neuroglycan C, a novel member of the neuregulin family. *Biochem Biophys Res Commun.* **321**, 1045-1049
- 291 Shuo, T., Aono, S., Matsui, F., Tokita, Y., Maeda, H., Shimada, K. and Oohira, A. (2004) Developmental changes in the biochemical and immunological characters of the carbohydrate moiety of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan. *Glycoconj J.* **20**, 267-278
- 292 Pap, T. and Bertrand, J. (2013) Syndecans in cartilage breakdown and synovial inflammation. *Nat Rev Rheumatol.* **9**, 43-55
- 293 Cheng, B., Montmasson, M., Terradot, L. and Rousselle, P. (2016) Syndecans as Cell Surface Receptors in Cancer Biology. A Focus on their Interaction with PDZ Domain Proteins. *Front Pharmacol.* **7**, 10
- 294 von Holst, A., Sirko, S. and Faissner, A. (2006) The unique 473HD-Chondroitinsulfate epitope is expressed by radial glia and involved in neural precursor cell proliferation. *J Neurosci.* **26**, 4082-4094
- 295 Milev, P., Friedlander, D. R., Sakurai, T., Karthikeyan, L., Flad, M., Margolis, R. K., Grumet, M. and Margolis, R. U. (1994) Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. *J Cell Biol.* **127**, 1703-1715
- 296 Wassenhove-McCarthy, D. J. and McCarthy, K. J. (1999) Molecular characterization of a novel basement membrane-associated proteoglycan, leprecan. *J Biol Chem.* **274**, 25004-25017
- 297 Capellini, T. D., Dunn, M. P., Passamaneck, Y. J., Selleri, L. and Di Gregorio, A. (2008) Conservation of notochord gene expression across chordates: insights from the Leprecan gene family. *Genesis.* **46**, 683-696
- 298 Kaul, S. C., Sugihara, T., Yoshida, A., Nomura, H. and Wadhwa, R. (2000) Gros1, a potential growth suppressor on chromosome 1: its identity to basement membrane-associated proteoglycan, leprecan. *Oncogene.* **19**, 3576-3583
- 299 Sadler, J. E. (1997) Thrombomodulin structure and function. *Thromb Haemost.* **78**, 392-395
- 300 Esmon, C. (2005) Do-all receptor takes on coagulation, inflammation. *Nat Med.* **11**, 475-477
- 301 Chen, S. and Birk, D. E. (2013) The regulatory roles of small leucine-rich proteoglycans in extracellular matrix assembly. *FEBS J.* **280**, 2120-2137
- 302 Merline, R., Schaefer, R. M. and Schaefer, L. (2009) The matricellular functions of small leucine-rich proteoglycans (SLRPs). *J Cell Commun Signal.* **3**, 323-335
- 303 Neame, P., Kay, C.J. (2000) Small leucine rich proteoglycans In proteoglycans: Structure, Biology and Molecular interactions (RV, I., ed.). pp. 201-236, CRC Press. Marcell Dekker, NY, Basel
- 304 Ikegawa, S. (2008) Expression, regulation and function of asporin, a susceptibility gene in common bone and joint diseases. *Curr Med Chem.* **15**, 724-728
- 305 Johnson, H. J., Rosenberg, L., Choi, H. U., Garza, S., Hook, M. and Neame, P. J. (1997) Characterization of epiphycan, a small proteoglycan with a leucine-rich repeat core protein. *J Biol Chem.* **272**, 18709-18717
- 306 Bost, F., Diarra-Mehrpour, M. and Martin, J. P. (1998) Inter-alpha-trypsin inhibitor proteoglycan family--a group of proteins binding and stabilizing the extracellular matrix. *Eur J Biochem.* **252**, 339-346
- 307 Fries, E. and Kaczmarczyk, A. (2003) Inter-alpha-inhibitor, hyaluronan and inflammation. *Acta Biochim Pol.* **50**, 735-742
- 308 Liu, K. L., Wu, J., Zhou, Y. and Fan, J. H. (2015) Increased Sushi repeat-containing protein X-linked 2 is associated with progression of colorectal cancer. *Med Oncol.* **32**, 99
- 309 Tanaka, K., Arao, T., Tamura, D., Aomatsu, K., Furuta, K., Matsumoto, K., Kaneda, H., Kudo, K., Fujita, Y., Kimura, H., Yanagihara, K., Yamada, Y., Okamoto, I., Nakagawa, K. and Nishio, K. (2012) SRPX2 is a novel chondroitin sulfate proteoglycan that is overexpressed in gastrointestinal cancer. *PLoS One.* **7**, e27922

- 310 Sasseti, C., Van Zante, A. and Rosen, S. D. (2000) Identification of endoglycan, a member of the CD34/podocalyxin family of sialomucins. *J Biol Chem.* **275**, 9001-9010
- 311 Jackson, D. G., Bell, J. I., Dickinson, R., Timans, J., Shields, J. and Whittle, N. (1995) Proteoglycan forms of the lymphocyte homing receptor CD44 are alternatively spliced variants containing the v3 exon. *J Cell Biol.* **128**, 673-685
- 312 Bartolomucci, A., Pasinetti, G. M. and Salton, S. R. (2010) Granins as disease-biomarkers: translational potential for psychiatric and neurological disorders. *Neuroscience.* **170**, 289-297
- 313 Burian, M. and Schitteck, B. (2015) The secrets of dermcidin action. *Int J Med Microbiol.* **305**, 283-286
- 314 Dockray, G. J. (2012) Cholecystokinin. *Curr Opin Endocrinol Diabetes Obes.* **19**, 8-12
- 315 Huttner, W. B., Gerdes, H. H. and Rosa, P. (1991) The granin (chromogranin/secretogranin) family. *Trends Biochem Sci.* **16**, 27-30
- 316 Noborn, F., Gomez Toledo, A., Sihlbom, C., Lengqvist, J., Fries, E., Kjellen, L., Nilsson, J. and Larson, G. (2015) Identification of chondroitin sulfate linkage region glycopeptides reveals prohormones as a novel class of proteoglycans. *Mol Cell Proteomics.* **14**, 41-49
- 317 Rehfeld, J. F. (2016) Cholecystokinin expression in tumors: biogenetic and diagnostic implications. *Future Oncol.* **12**, 2135-2147
- 318 Schitteck, B. (2012) The multiple facets of dermcidin in cell survival and host defense. *J Innate Immun.* **4**, 349-360
- 319 Schroder, J. M. and Harder, J. (2006) Antimicrobial skin peptides and proteins. *Cell Mol Life Sci.* **63**, 469-486
- 320 Shooshtarizadeh, P., Zhang, D., Chich, J. F., Gasnier, C., Schneider, F., Haikel, Y., Aunis, D. and Metz-Boutigue, M. H. (2010) The antimicrobial peptides derived from chromogranin/secretogranin family, new actors of innate immunity. *Regul Pept.* **165**, 102-110
- 321 Taupenot, L., Harper, K. L. and O'Connor, D. T. (2003) The chromogranin-secretogranin family. *N Engl J Med.* **348**, 1134-1149

Legends to Figures

Figure 1. Organisation of GAG saccharides in full length, partially depolymerised, terminal (a) and stub epitopes (b) of the CS side chains of proteoglycans and in the GAG disaccharides in CS types A, B, C, D, E (c) and simplified diagrams of the sulphate presentations in a typical CS side chain (e-g) In the example shown the chain is terminated in a 3-B-3(-) epitope and contains internal 7-D-4 and 4-C-3 epitopes as shown. A 3-B-3(+) stub epitope attached to the linkage tetrasaccharide is also shown, this epitope is generated by chondroitinase ABC. The CS side chains can also be terminated in an alternate 2-B-6(-) epitope and have a 2-B-6(+) stub epitope.

Figure 2. Diagrammatic representation of the sub-domain structural organisation of SRPX2 (Sushi repeat protein, X linked 2) and members of the hyalectan family (a), Decorin and biglycan SLRP members (b) and the inter photoreceptor ECM proteoglycan SPACRCAN (c). SUSHI complement control protein modular data was obtained from the public SMART database (<http://smart.embl-heidelberg.de/>).

Figure 3. Structural organisation of cell associated CS-proteoglycans. CSPG4 (a), thrombomodulin (b), RPTP/Phosphacan (c), syndecan family (d), and a CS substituted variant of the HA receptor CD44 (e). Abbreviations not covered in key, ED, extracellular domain; TMD, transmembrane domain; CD, cytoplasmic domain.

Figure 4. Organisation of the Kunitz protease inhibitor CS-proteoglycan, bikunin (a), type IX collagen (b), and testican, seminal plasma CS-proteoglycan (c).

Figure 5. Diagrammatic CS-proteoglycans assembled into protective perineural nets in brain tissue (a) and ternary link-protein stabilised macro-aggregate structures with hyaluronan in articular cartilage which convey important hydrodynamic weight bearing and self lubricative properties to this tissue.

Figure 6. Confocal immunolocalisation of the 4C3 (a) and 7D4 (b) native CS sulphation motifs and perlecan using anti-domain IV antibody A7L6 (c) in human foetal (14 week gestational age) knee tibial cartilage. Cell nuclei are stained red with propidium iodide in (a) and (b) and with DAPI in (c). The primary antibody localisations were stained green using FITC conjugated anti mouse or rat IgG. Perlecan identifies stem cell niches in the surface regions of the developing cartilage. Figure modified from [130]

Figure 7. Perlecan and 4C3/7D4 immunolocate the perichondrial stem cell niche and activated progenitor cells involved in foetal elbow joint development. Immunolocalisation of perlecan and the CS sulphation motifs 4C3 and 7D4 using indirect fluorescent confocal microscopy of human foetal elbow (14 week gestational age). Perlecan is immunolocalised to the outer layers of the perichondrium (a, b) while the 7D4 (c-e) and 4C3 CS sulphation motifs (f-i) are located on cell associated proteoglycans deeper in the elbow cartilage rudiment and in the surface regions of the interzone cartilage of the developing elbow joint (f-i).

Figure 8. Focal expression of perlecan (a) and 7-D-4 CS sulphation motif (b) in foetal human paraspinal blood vessels. Perlecan is a well known vascular HS-proteoglycan, produced by endothelial cells. The 7-D-4 CS sulphation motif is focally expressed in the luminal surfaces of these small blood vessels (b) and may provide evidence of a vascular progenitor cell population directed by signals from pericytes on the abluminal surfaces of these vessels (c). Diagram of a small capillary showing the relationship of the endothelial cells and pericytes (d, e). Type IV collagen delineates the blood vessel the pericyte resides on (f) while NG2 proteoglycan is a pericyte marker (g).

Figure 9. Neuronal cell CS-interactive surface molecules with regulatory roles in neuronal development. Contactin-1 (a), leukocyte common antigen-related (LAR) tyrosine phosphatase receptor (b), protein tyrosine phosphatase receptors (PTP- σ and PTP- ζ) (c), $\square o \square \square$ receptor-1 and 3 (NgR1 and NgR3) (d, e), semaphorin-5A (Sem 5A) (f) and neuropilin-1 (NRP-1) (g).

Figure 10. CS-Interactive properties of E-cadherin (a), syndecan proteoglycan family (b) and the frizzled LRP-5/6 co-receptor complex (c) and resultant effects on cytoskeletal re-organisation and gene regulation.

Figure 11. Interactive properties of CS-DS GAG chains of proteoglycans (a), HMBG1, AGEs, S100 proteins and amyloid peptides with the extracellular domains of the RAGE receptor and effects on cytoskeletal proteins (b), activation of NF κ B and CREB transcriptional regulation (c).