Heparan Sulfate Proteoglycans

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Heparan sulfate proteoglycans are found at the cell surface and in the extracellular matrix, where they interact with a plethora of ligands. Over the last decade, new insights have emerged regarding the mechanism and biological significance of these interactions. Here, we discuss changing views on the specificity of protein–heparan sulfate binding and the activity of HSPGs as receptors and coreceptors. Although few in number, heparan sulfate proteoglycans have profound effects at the cellular, tissue, and organismal level.

Heparan sulfate proteoglycans (HSPGs) are glycoproteins, with the common characteristic of containing one or more covalently attached heparan sulfate (HS) chains, a type of glycosaminoglycan (GAG) (Esko et al. 2009). Cells elaborate a relatively small set of HSPGs (~17) that fall into three groups according to their location: membrane HSPGs, such as syndecans and glycosylphosphatidylinositol-anchored proteoglycans (glypicans), the secreted extracellular matrix HSPGs (agrin, perlecan, type XVIII collagen), and the secretory vesicle proteoglycan, serglycin (Table 1). Much of the early work in the field concentrated on composition (size, chain number, and structure of the HS chains), biosynthesis, and binding properties of the chains. In 1985, the first somatic cell mutants altered in HSPG expression were identified (Esko et al. 1985), which allowed functional studies in the context of a cell culture model (Zhang et al. 2006). A decade later, the first HSPG mutants in a model organism

(*Drosophila melanogaster*) were identified (Rogalski et al. 1993; Nakato et al. 1995; Häcker et al. 1997; Bellaiche et al. 1998; Lin et al. 1999), which was followed by identification of mutants in nematodes, tree frogs, zebrafish, and mice (Tables 2 and 3). HS is evolutionarily ancient and its composition has remained relatively constant from *Hydra* to humans (Yamada et al. 2007; Lawrence et al. 2008).

Figure 1 shows in pictorial form many of the systems in which HSPGs participate.

1. HSPGs are present in basement membranes (perlecan, agrin, and collagen XVIII), where they collaborate with other matrix components to define basement membrane structure and to provide a matrix for cell migration.
2. HSPGs are found in secretory vesicles, most notably serglycin, which plays a role in packaging granular contents, maintaining proteases in an active state, and regulating various biological activities after secretion

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Table 1. Heparan sulfate proteoglycans

Class	Proteoglycan	Core mass (kDa) ^a	Chain type (number) ^b	Tissue	Human disease
Membrane-bound	Syndecan-1–syndecan-4	31–45	HS (2–3) in Sdc2 and Sdc4; HS/CS (3–4 HS/1–2 CS) in Sdc1 and Sdc3	Epithelial cells, fibroblasts	
	Glypican-1–glypican-6	57–69	HS (1–3)	Epithelial cells, fibroblasts	Simpson–Golabi–Behmel syndrome (overgrowth) (GPC3) (Pilia et al. 1996); omodysplasia (skeletal dysplasia) (GPC6) (Campos-Xavier et al. 2009)
	Betaglycan (part-time PG)	110	HS/CS (1–2)	Fibroblasts	
Secretory vesicles	Neuropilin-1 (part-time PG)	130	HS or CS (1)	Endothelial cells	
	CD44v3	37	HS (1)	Lymphocytes	
	Serglycin	10–19	Heparin/CS (10–15)	Mast cells, hematopoietic cells	
Extracellular matrix	Perlecan	400	HS (1–4)	Basement membranes	Schwartz–Jampel syndrome (skeletal dysplasia) (Nicole 2000; Arikawa-Hirasawa et al. 2001)
	Agrin	212	HS (2–3)	Basement membranes	
	Collagen XVIII	150	HS (1–3)	Epithelial cells, basement membranes	Knobloch syndrome type I (Sertie et al. 2000)

HS, heparan sulfate; CS, chondroitin sulfate; PG, proteoglycan.
^aThe variation in core mass is because of species differences.
^bThe number of chains is based on the number of putative attachment sites for chain initiation as well as data from the literature; the actual number of chains varies by method, tissue, and species.

- such as coagulation, host defense, and wound repair.
3. HSPGs can bind cytokines, chemokines, growth factors, and morphogens, protecting them against proteolysis. These interactions provide a depot of regulatory factors that can be liberated by selective degradation of the HS chains. They also facilitate the formation of morphogen gradients essential for
- cell specification during development and chemokine gradients involved in leukocyte recruitment and homing.
4. HSPGs can act as receptors for proteases and protease inhibitors regulating their spatial distribution and activity.
5. Membrane proteoglycans cooperate with integrins and other cell adhesion receptors

to facilitate cell-ECM attachment, cell–cell interactions, and cell motility.

6. Membrane HSPGs act as coreceptors for various tyrosine kinase-type growth factor receptors, lowering their activation threshold or changing the duration of signaling reactions.
7. Membrane HSPGs act as endocytic receptors for clearance of bound ligands, which is especially relevant in lipoprotein metabolism in the liver and perhaps in the formation of morphogen gradients during development.

This article is divided into 10 subsections. The first three are written for investigators

outside the field who may need some background information on the diversity of HSPGs and the interactions that occur with protein ligands. The subsequent sections describe seven systems that illustrate general principles or ideas that have undergone a significant shift over the last decade. Because of space limitations not all subjects can be considered or treated in appropriate depth and therefore the reader is referred to excellent recent review articles (Tkachenko et al. 2005; Bulow and Hobert 2006; Bishop et al. 2007; Lamanna et al. 2007; Bix and Iozzo 2008; Filmus et al. 2008; Ori et al. 2008; Rodgers et al. 2008; Sanderson and Yang 2008; Iozzo et al. 2009; Couchman 2010).

Table 2. Mutants altered in HSPG core proteins

Gene	Proteoglycan	Phenotype (references)
<i>Sdc1</i>	Syndecan-1	Null allele: viable; increase in inflammation-mediated corneal angiogenesis (Gotte et al. 2002, 2005); corneal epithelial cells migrate more slowly, show reduced localization of $\alpha 9$ integrin during wound closure and fail to increase in proliferation after wounding (Stepp et al. 2002); enhanced leukocyte-endothelial interaction in the retina (Gotte et al. 2002, 2005); increase in medial and intimal smooth muscle cell replication and neointimal lesion after injury (Fukai et al. 2009); reduced cardiac fibrosis and dysfunction during angiotensin II–induced hypertension (Schellings et al. 2010); not required for follicle initiation and development (Richardson et al. 2009); accumulates plasma triglycerides, and shows prolonged circulation of injected human VLDL and intestinally derived chylomicrons (Stanford et al. 2009); juvenile mice resistant to carcinogen-induced tumorigenesis (McDermott et al. 2007); increased basal protein leakage and more susceptible to protein loss induced by combinations of IFN- γ , TNF- α , and increased venous pressure (Bode et al. 2008); exacerbates anti-GBM nephritis shifting Th1/Th2 balance toward a Th2 response (Rops et al. 2007); no role in hepatocyte infection by <i>Plasmodium yoelii</i> sporozoites (Bhanot 2002); normal larval development of <i>Trichinella spiralis</i> , but modestly reduced Th2 responses during infection (Beiting et al. 2006); less susceptible to <i>Pseudomonas aeruginosa</i> infection (Haynes et al. 2005); reduced <i>P. aeruginosa</i> infection rate and virulence (Park et al. 2001); protected from <i>Staphylococcus aureus</i> beta-toxin-induced lung injury (Hayashida et al. 2009a); exaggerated airway hyperresponsiveness, eosinophilia, and lung IL-4 responses to allergens (Xu et al. 2005); exaggerated CXC chemokines, neutrophilic inflammation, organ damage, and lethality in LPS endotoxemia (Hayashida et al. 2009b); prolonged recruitment of inflammatory cells in dextran sodium sulfate (DSS)-induced colitis and delayed type hypersensitivity (Masouleh et al. 2009; Floer et al. 2010).

Continued

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Table 2. *Continued*

Gene	Proteoglycan	Phenotype (references)
<i>Sdc2</i>	Syndecan-2	No mutants reported. <i>Sdc2</i> antisense impairs angiogenesis in human microvascular endothelial cells (Noguer et al. 2009); morpholinos inhibit cell migration and fibrillogenesis during embryogenesis in zebrafish (Arrington and Yost 2009).
<i>Sdc3</i>	Syndecan-3	Null allele: viable; altered feeding behavior (Strader et al. 2004); no phenotype in synovial endothelial cells (Patterson et al. 2005); enhanced long-term potentiation (LTP) in area CA1 (brain) and impaired performance in tasks assessing hippocampal function (Kaksonen et al. 2002); more sensitive to inhibition of food intake by the melanocortin agonist MTII (Reizes et al. 2003); perturbs laminar structure of the cerebral cortex as a result of impaired radial migration, and neural migration in the rostral migratory stream is impaired (Hienola et al. 2006); novel form of muscular dystrophy characterized by impaired locomotion, fibrosis, and hyperplasia of myonuclei and satellite cells (Cornelison et al. 2004).
<i>Sdc4</i>	Syndecan-4	Null allele: viable; enhanced fibrin deposition in degenerating fetal vessels in the placental labyrinth (Ishiguro et al. 2000); delayed angiogenesis in wound granulation tissue (Echtermeyer et al. 2001); defective subcellular localization of mTOR Complex2 and Akt activation in endothelial cells, affecting endothelial cell size, NOS, and arterial blood pressure (Partovian et al. 2008); decreased macrophage uptake of phospholipase A2-modified LDL (Boyanovsky et al. 2009); mesangial expansion, enhanced matrix collagens I and IV, fibronectin and focal segmental glomerulosclerosis in males, and induction of <i>Sdc2</i> in glomeruli (Cevikbas et al. 2008); more susceptible to hepatic injury, and thrombin-cleaved form of osteopontin is significantly elevated after concanavalin-A injection (Kon et al. 2008); less damage in osteoarthritic cartilage in a surgically induced model of osteoarthritis (Echtermeyer et al. 2009); explanted satellite cells fail to reconstitute damaged muscle and are deficient in activation, proliferation, MyoD expression, myotube fusion, and differentiation (Cornelison et al. 2004); vibrissae are shorter and have a smaller diameter because of suboptimal response to fibroblast growth factors (Iwabuchi and Goetinck 2006); lower phosphorylation levels of focal adhesion kinase (Wilcox-Adelman et al. 2002); random migration of fibroblasts as a result of high delocalized Rac1 activity (Bass et al. 2007); defective RGD-independent cell attachment to transglutaminase-fibronectin matrices (Telci et al. 2008); impaired suppression of production of IL-1 β by TGF- α (Ishiguro et al. 2002); decreased neutrophil recruitment and increased myofibroblast recruitment and interstitial fibrosis after bleomycin-treatment, no inhibition of fibrosis with recombinant CXCL10 protein (Jiang et al. 2010); hypersensitivity to LPS because of decreased TGF- β suppression of IL-1 production in monocytes and neutrophils (Ishiguro et al. 2001).
<i>Gpc1</i>	Glypican-1	Null allele: viable; reduced brain size (Jen et al. 2009). Athymic mutant mice show decreased tumor angiogenesis and metastasis (Aikawa et al. 2008).
<i>Gpc2</i>	Glypican-2	No mutants reported.

Continued

Heparan Sulfate Proteoglycans

Table 2. Continued

Gene	Proteoglycan	Phenotype (references)
<i>Gpc3</i>	Glypican-3	Null allele: viable; resembles Simpson–Golabi–Behmel overgrowth syndrome, including somatic overgrowth, renal dysplasia, accessory spleens, polydactyly, and placentomegaly (Cano-Gauci et al. 1999; Chiao et al. 2002); defects in cardiac and coronary vascular development (Ng et al. 2009); alterations in Wnt signaling, in vivo inhibition of the noncanonical Wnt/JNK signaling, activation of canonical Wnt/ β -catenin signaling (Song et al. 2005); increased Hedgehog signaling (Capurro et al. 2008); abnormal rates of proliferation and apoptosis in cortical and medullary collecting duct cells (Grisaru et al. 2001); delay in endochondral ossification, impairment in the development of the myelomonocytic lineage (Viviano et al. 2005).
<i>Gpc4</i>	Glypican-4	Zebrafish <i>knypek</i> controls cell polarity during convergent extension (Topczewski et al. 2001); craniofacial skeletal defects in adult fish (LeClair et al. 2009).
<i>Gpc5</i>	Glypican-5	No mutants reported.
<i>Gpc6</i>	Glypican-6	Impaired endochondral ossification and omodysplasia (Campos-Xavier et al. 2009).
<i>Tgfbβ3</i>	Betaglycan	Null allele: embryonic lethal; heart and liver defects (Stenvers et al. 2003); defect in seminiferous cord formation in E12.5–13.5 embryos (Sarraj et al. 2010).
<i>Hspg2</i>	Perlecan	Null allele: embryonic lethal (E10–12); developmental angiogenesis altered in zebrafish (Zoeller et al. 2009); high incidence of malformations of the cardiac outflow tract, lack of well-defined spiral endocardial ridges (Costell et al. 2002); lower amounts of collagen IV and laminins in embryonic hearts, reduced function in infarcted hearts from heterozygous mice (Sasse et al. 2008); absence of acetylcholinesterase at the neuromuscular junctions (Arikawa-Hirasawa et al. 2002); cephalic and skeletal abnormalities (Arikawa-Hirasawa et al. 1999); cerebral ectopias, exencephaly (Girós et al. 2007); increased cross-sectional area of myosin heavy chain type IIb fibers in the tibialis anterior muscle (Xu et al. 2010b); diminished osteocyte canalicular pericellular area (Thompson et al. 2011). Exon 3 deletion (<i>Hspg2</i> ^{3/3}) viable: proteinuria after protein loading (Morita et al. 2005); monocyte/macrophage influx impaired in <i>Hspg2</i> ^{3/3} <i>Col18a1</i> ^{-/-} mice in a model of renal ischemia/reperfusion (Celie et al. 2007). Secreted as CSPG in some tissues (Danielson et al. 1992; Govindraj et al. 2002; Vogl-Willis and Edwards 2004; West et al. 2006), but relationship of CSPG isoform to phenotypes not established.
<i>Prg1</i>	Serglycin	Null allele: viable; secretory granule defects in mast cells (Abrink et al. 2004); dense core formation is defective in mast cell granules (Henningsson et al. 2006); defective secretory granule maturation and granzyme B storage in cytotoxic T cells (Grujic et al. 2005); no effect on macrophages (Zernichow et al. 2006); platelets and megakaryocytes contain unusual scroll-like membranous inclusions (Woulfe et al. 2008); enlargement of multiple lymphoid organs, decrease in the proportion of CD4 ⁺ cells, more pronounced airway inflammatory response in older mice (Wernersson et al. 2009); increased virulence of <i>Klebsiella pneumoniae</i> (Niemann et al. 2007); defective regulation of antiviral CD8 ⁺ T-cell responses (Grujic et al. 2008).

Continued

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Table 2. *Continued*

Gene	Proteoglycan	Phenotype (references)
<i>Agrn</i>	Agrin	Null allele: embryonic lethal; reduced number, size, and density of postsynaptic acetylcholine receptor aggregates in muscles; abnormal intramuscular nerve branching and presynaptic differentiation (Gautam et al. 1996,1999); smaller brains (Serpinskaya et al. 1999); abnormal development of interneuronal synapses (Gingras et al. 2007); increased resistance to excitotoxic injury (Hilgenberg et al. 2002); reduced number of cortical presynaptic and postsynaptic specializations (Ksiazek et al. 2007). Floxed allele: Inactivation in podocytes does not affect glomerular charge selectivity or glomerular architecture (Harvey et al. 2007).
<i>Col18a1</i>	Collagen XVIII	Null allele: viable; increased microvascular growth (Li and Olsen 2004); increased angiogenesis associated with atherosclerotic plaques (Moulton et al. 2004); delayed regression of blood vessels in the vitreous along the surface of the retina after birth and lack of or abnormal outgrowth of retinal vessels (Fukai et al. 2002); larger choroidal neovascularization lesions and increased vascular leakage (Marneros et al. 2007); accelerated healing and vascularization rate of excisional dorsal skin wounds (Seppinen et al. 2008); anomalous anastomoses of vasculature; disruption of the posterior iris pigment epithelial cell layer with release of melanin granules, severe thickening of the stromal iris basement membrane zone (Marneros and Olsen 2003); increase in the amount of retinal astrocytes (Hurskainen et al. 2005); more severe glomerular and tubulointerstitial injury in induced anti-GBM glomerulonephritis (Hamano et al. 2010); monocyte/macrophage influx impaired in <i>Hspg2</i> ^{3/3} <i>Col18a1</i> ^{-/-} mice in a model of renal ischemia/reperfusion (Celie et al. 2007); mild chylomicronemia (Bishop et al. 2010).

Table 3. Mouse mutants altered in HS biosynthesis

Gene	Enzyme	Phenotype
<i>Xt1</i>	Xylosyltransferase-1	No mutants reported.
<i>Xt2</i>	Xylosyltransferase-2	Null allele: viable; polycystic kidney and livers (Condac et al. 2007).
<i>GalTI</i> (β 4GalT7)	Galactosyltransferase I	Human mutants: defective chondroitin substitution of decorin and biglycan in an Ehlers–Danlos patient (Gotte and Kresse 2005; Seidler et al. 2006).
<i>GalTII</i> (β 3GalT6)	Galactosyltransferase II	No mutants reported.
<i>Glc1</i>	Glucuronyltransferase I	Null allele: embryonic lethal (4–8-cell stage) (Izumikawa et al. 2010).
<i>Extl3</i>	<i>N</i> -acetylglucosaminyl transferase I	Floxed allele: Inactivation in islets decreases growth and insulin secretion (Takahashi et al. 2009).
<i>Ext1/Ext2</i>	HS Copolymerase (<i>N</i> -acetylglucosaminyl-glucuronyltransferase)	Null allele: embryonic lethal (E6–7.5); lack of mesoderm differentiation (Lin et al. 2000; Stickens et al. 2005); heterozygotes develop rib growth plate exostoses (Stickens et al. 2005; Zak et al. 2011); unaltered vascular permeability in heterozygous mice (Xu et al. 2010a).

Continued

Heparan Sulfate Proteoglycans

Table 3. Continued

Gene	Enzyme	Phenotype
		Floxed allele of <i>Ext1</i> : defective brain morphogenesis and midline axon guidance after <i>nestin-Cre</i> inactivation (Inatani et al. 2003); no effect on adaptive immune response in CD15Cre mice (Garner et al. 2008); altered T-cell and dendritic cell homing to lymph nodes in <i>Tie2Cre</i> mice (Bao et al. 2010); rib growth plate exostosis formation in <i>Col2Cre</i> mice (Jones et al. 2010; Matsumoto et al. 2010; Zak et al. 2011).
<i>Ndst1</i>	N-acetylglucosaminyl N-deacetylase/N-sulfotransferase-1	Null allele: Perinatal lethal; lung hypoplasia, defective forebrain, lens, and skull development (Fan et al. 2000; Ringvall et al. 2000; Grobe et al. 2005; Pan et al. 2006). Floxed allele: decreased chemokine transcytosis and presentation and neutrophil infiltration in <i>Tie2Cre</i> mice (Wang et al. 2005); decreased allergen-induced airway hyperresponsiveness and inflammation because of reduction in recruitment of eosinophils, macrophages, neutrophils, and lymphocytes in <i>Tie2Cre</i> mice (Zuberi et al. 2009); decreased pathological angiogenesis in <i>Tie2Cre</i> mice (Fuster et al. 2007); decreased vascular VEGF-induced hyperpermeability (Xu et al. 2010a); decreased vascular smooth muscle cell proliferation, vessel size, and vascular remodeling after arterial injury in <i>SM22αCre</i> mice (Adhikari et al. 2010a); mild effect on T-cell response in <i>Tie2Cre;Ndst2^{-/-}</i> mice (Garner et al. 2008); defective lacrimal gland development and Fgf10-Fgfr2b complex formation and signaling in <i>LeCre</i> mice (Pan et al. 2008); defective lobuloalveolar development in mammary gland (Crawford et al. 2010).
<i>Ndst2</i>	N-acetylglucosaminyl N-deacetylase/N-sulfotransferase-2	Null allele: viable; mast cell deficiency and defective storage of proteases (Forsberg et al. 1999; Humphries et al. 1999); compounding mutation with <i>Ndst1</i> reduces L-selectin interactions (Wang et al. 2005).
<i>Ndst3</i>	N-acetylglucosaminyl N-deacetylase/N-sulfotransferase-3	Null allele: viable; floxed allele available (Pallerla et al. 2008).
<i>Ndst4</i>	N-acetylglucosaminyl N-deacetylase/N-sulfotransferase-4	No mutants reported.
<i>Glee</i>	Uronyl C5 epimerase	Null allele: perinatal lethal; renal agenesis (Li et al. 2003).
<i>H2st</i>	Uronyl 2-O-sulfotransferase	Null allele: perinatal lethal; renal agenesis; skeletal and ocular defects (Bullock et al. 1998; Merry et al. 2001); defective cerebral cortical development (McLaughlin et al. 2003); altered lacrimal gland development (Qu et al. 2011). Floxed allele: altered lipoprotein clearance in <i>AlbCre</i> mice (Stanford et al. 2010); altered branching morphogenesis in the mammary gland (Garner et al. 2011).

Continued

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Table 3. *Continued*

Gene	Enzyme	Phenotype
<i>H3st1</i>	Glucosaminyl 3-O-sulfotransferase 1	Null allele: partially penetrant lethality; no alteration in coagulation (HajMohammadi et al. 2003); fertility defects because of impaired ovarian function and placenta development (Shworak et al. 2002; HajMohammadi et al. 2003).
<i>H3st2</i>	Glucosaminyl 3-O-sulfotransferase 2	Null allele; viable; no neuronal phenotype (Hasegawa and Wang 2008).
<i>H3st3</i>	Glucosaminyl 3-O-sulfotransferase 3	No mutants reported.
<i>H3st4</i>	Glucosaminyl 3-O-sulfotransferase 4	No mutants reported.
<i>H3st5</i>	Glucosaminyl 3-O-sulfotransferase 5	No mutants reported.
<i>H3st6</i>	Glucosaminyl 3-O-sulfotransferase 6	No mutants reported.
<i>H6st1</i>	Glucosaminyl 6-O-sulfotransferase 1	Null allele: embryonic lethal (Habuchi et al. 2007; Sugaya et al. 2008). Gene trap allele: embryonic lethal; retinal axon guidance defects (Pratt et al. 2006). Floxed allele: systemic inactivation embryonic lethal (Izvolosky et al. 2008); no change in plasma triglycerides in AlbCre mice (Stanford et al. 2010).
<i>H6st2</i>	Glucosaminyl 6-O-sulfotransferase 2	Null allele: viable (Sugaya et al. 2008); HS6ST-2, but not HS6ST-1, morphants in zebrafish show abnormalities in the branching morphogenesis of the caudal vein (Chen et al. 2005).
<i>H6st3</i>	Glucosaminyl 6-O-sulfotransferase 3	No mutants reported.
<i>Hpa</i>	Heparanase, transgene	Accelerated wound angiogenesis, enhanced delayed type hypersensitivity response (Zcharia et al. 2005; Edovitsky et al. 2006; Ilan et al. 2006); accumulation of intracellular crystals of protein Ym1 in macrophages (Waern et al. 2010); resistance to amyloid protein A amyloidosis (Li et al. 2005); age-related enlargement of lymphoid tissue and altered leukocyte composition (Wernersson et al. 2009).
<i>Hpa</i>	Heparanase	Null allele: viable; altered MMP-2 and MMP-14 expression (Zcharia et al. 2009).
<i>Sulf1</i>	Endo-6-sulfatase 1	Null allele: viable; esophageal defect (Ai et al. 2007; Ratzka et al. 2008); enhanced osteoarthritis, MMP-13, ADAMTS-5, and noggin elevated, col2a1 and aggrecan reduced in cartilage and chondrocytes (Otsuki et al. 2010).
<i>Sulf2</i>	Endo-6-sulfatase 2	Null allele: viable; behavioral defects (Lamanna et al. 2006); enhanced osteoarthritis, MMP-13, ADAMTS-5, and noggin elevated, col2a1 and aggrecan reduced in cartilage and chondrocytes (Otsuki et al. 2010). Gene trap allele: <i>Sulf2</i> GT(pGT1TMPfs)155Ska, no phenotype (Lum et al. 2007).

Figure 1. HSPGs have multiple activities in cells and tissues. (Adapted from Bishop et al. 2007; reprinted with permission from Nature Publishing Group © 2007.)

A BIRD'S-EYE VIEW OF STRUCTURE AND ASSEMBLY

An idealized picture of a HSPG is shown in Figure 2. Each proteoglycan consists of a protein and one or more covalently attached HS chains. Comprehensive reviews have appeared on the assembly process and structural characterization of the chains, and therefore these subjects will not be discussed further here (Esko and Selleck 2002; Sugahara and Kitagawa 2002; Sasisekharan et al. 2006; Ori et al. 2008; Laremore et al. 2009). However, several features are important to consider in the context of their

biological activities. (1) HSPGs are polyanionic and have unusual hydrodynamic volume because of the presence of long HS chains (40–300 sugar residues, ~20–150 nm), sulfate groups, and uronic acids. Thus, different HSPGs often copurify by techniques that rely simply on the anionic characteristics of the chains or gel filtration. HS and other sulfated GAGs are amongst the most highly negatively charged biopolymers in nature and variation in the number and length of the chains gives rise to enormous polydispersity. (2) Some proteoglycans contain only one GAG chain (e.g., CD44v3 and betaglycan), whereas others have

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Figure 2. Heparan sulfate (HS) structure. HS biosynthesis initiates by the attachment of xylose to specific serine residues in HSPG core proteins followed by the formation of a linkage tetrasaccharide, glucuronic acid-galactose-galactose-xylose (GlcA-Gal-Gal-Xyl). Ext1/3 attaches the first *N*-acetyl-D-glucosamine (GlcNAc) residue and an enzyme complex composed of Ext1 and Ext2 alternately adds GlcA and GlcNAc to the nascent chain. The chains simultaneously undergo a series of processing reactions that begins by the removal of the acetyl groups from clusters of GlcNAc residues and substitution of the free amino groups with sulfate, catalyzed by one or more *N*-deacetylase-*N*-sulfotransferases (Ndst). The C5 epimerase (HsGlcE) epimerizes D-glucuronic acids immediately adjacent to *N*-sulfoglucosamine units to L-iduronic acid (IdoA). A series of *O*-sulfotransferases can then add sulfate: uronyl 2-*O*-sulfotransferase (Hs2st) adds sulfate at C2 of the iduronic acids (and less frequently to glucuronic acids), 6-*O*-sulfotransferases (Hs6st1-3) add sulfate at C6 of the *N*-sulfoglucosamine units and less frequently to *N*-acetylglucosamine, and 3-*O*-sulfotransferases (Hs3st1, 2, 3a, 3b, 4, 5, 6) add sulfate at C3 of glucosamine units (*N*-sulfated or *N*-unsulfated). As shown in the top of the figure by red shading, the modifications occur in clusters of variable length (*N*-sulfated or NS domains), which are interspersed by unmodified domains (*N*-acetylated or NA domains). The regions at the junction of these domains are sometimes called NA/NS domains (not shown) because the extent of processing is less. The modified domains make up binding sites for protein ligands as depicted for antithrombin, FGF and FGF receptor. The HS chains can be further modified once they arrive at the cell surface or in the ECM by two endo-sulfatases (Sulf1 and Sulf2), which remove specific sulfate groups located at C6 of glucosamine units, or by the action of extracellular heparanase or extracellular proteases (not shown). (Figure adapted from Bishop et al. 2007; reprinted with permission from Nature Publishing Group © 2007.)

three to five chains (e.g., syndecan). Furthermore, the stoichiometry of GAG chain substitution can vary depending on source and growth conditions. “Part-time” proteoglycans can exist with or without a GAG chain (Table 1). (3) Some proteoglycans contain other types of glycans (e.g., asparagine-linked [*N*-linked] and serine/threonine-linked [*O*-linked] mucin-type chains). Some proteoglycans, such as syndecan-1, contain both HS and chondroitin/dermatan sulfate, another type of GAG. Other types of posttranslational modifications can occur (e.g., phosphorylation on cytoplasmic

domains of *trans*-membrane proteoglycans). (4) The number and sulfation state of the chains can vary according to growth conditions and in response to growth factors. (5) The arrangement of negatively charged sulfate groups and the orientation of the carboxyl groups specify the location of ligand-binding sites. Furthermore, the sulfated residues are clustered in regions along the chain containing mixtures of iduronic acid and glucuronic acid (NS domains, Fig. 2) and are separated by nonsulfated domains rich in glucuronic acid (NA domains). (6) The pattern of sulfation, extent of uronic

acid epimerization, and organization of the modified residues is generally thought to depend on the cell type in which HS is expressed rather than on the nature of the core protein (Kato et al. 1994). Thus, the overall composition of HS on different core proteins expressed by the same cell appears to be similar, but great variation occurs between cell types. This concept might be an oversimplification, as some variation has been suggested to occur in ligand-binding properties and composition dependent on the core protein (Shworak et al. 1993; Tveit et al. 2005).

GENERALIZATIONS ABOUT THE INTERACTION OF PROTEIN LIGANDS WITH HS PROTEOGLYCANS

HSPGs bind to many ligands, usually via the HS chains. In fact heparin, a highly sulfated form of HS, is often used as an “affinity” matrix for purifying proteins, and many of the growth factors in use today were purified by heparin affinity chromatography. Heparin-binding ligands include growth factors, cytokines, chemokines, enzymes, enzyme inhibitors, and extracellular matrix proteins. In other areas of glycobiology, glycan-binding proteins are referred to as “lectins,” a designation based on the presence of a carbohydrate recognition domain defined by characteristic protein folds or sequence motifs indicating their membership in an evolutionarily conserved gene family (Varki et al. 2009). In contrast, proteins that bind to HS appear to have evolved by convergent evolution; that is, they do not possess a specific fold or recognizable amino acid sequence pattern (Esko and Linhardt 2009). Heparin-binding sites often occur on the external surface of proteins or in shallow grooves lined with positively charged amino acids. Attempts have been made to define “consensus” sequences in heparin-binding proteins based on content and spacing of positively charged amino acid residues within linear sequences (Cardin and Weintraub 1989; Hileman et al. 1998; Capila and Linhardt 2002). However, the binding site for HS is often defined by positive residues contributed by noncontiguous segments of the protein.

Although electrostatic interactions contribute much of the binding energy, hydrogen-bonding, van der Waal interactions, and hydrophobic effects also participate (Conrad 1998; Capila and Linhardt 2002).

The binding of a ligand to HS follows the same principles that underlie the interaction of other macromolecules, but the following considerations are important.

- Dissociation constants for HS-dependent ligands range from millimolar to nanomolar values. Many growth factors bind with high affinity (e.g., fibroblast growth factors, FGFs), whereas many matrix proteins bind with low affinity (e.g., fibronectin). However, affinity does not dictate selectivity; some low affinity ligands achieve high avidity through dimerization (e.g., chemokines) or by clustering (e.g., fibronectin fibrils).
- The HS chains can facilitate diffusion of ligands by allowing them to bind and slide or dissociate/reassociate through adjacent binding sites (mass action).
- Binding can lead to a conformational change in the protein. The best-studied example is the allosteric effect of heparin on anti-thrombin.
- HS can act as a template to approximate two proteins next to each other. Antithrombin inactivation of thrombin serves as the paradigm for this type of interaction, but other examples include the association of some growth factors with their receptor tyrosine kinases (e.g., FGF with FGF receptors).

In addition to the HS chains, the protein core of HSPGs can also bind ligands. For example, the *Drosophila* glypican ortholog, Dally, can directly interact with a number of morphogens, such as decapentaplegic (Dpp) and bone morphogenetic factor 4, in the absence of HS chains (Kirkpatrick et al. 2006). Expression of HS-deficient Dally can rescue several mutant phenotypes in Dally-deficient flies, indicating that a number of biologically relevant functions are mediated by the glypican protein core independently of HS. In mammals, the glypican-3

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core protein interacts with hedgehog (Hh) independently of HS, and *Gpc3*-null embryos display increased Hh signaling, which might explain the overgrowth phenotype observed in patients lacking glypican-3 (Simpson–Golabi–Behmel syndrome) (Capurro et al. 2008). As discussed below, a peptide sequence in the core protein of syndecan-1 interacts with $\alpha V\beta 3$ and $\alpha V\beta 5$ integrins and modulates cell adhesion (Beauvais et al. 2004; McQuade et al. 2006). Finally, perlecan, collagen XVIII, and agrin are large proteins composed of multiple, functionally independent domains that can bind to other matrix components and growth factors (Iozzo et al. 2009).

ON THE SPECIFICITY OF BINDING

Although some ligands bind directly to the HSPG core proteins, the vast majority interact with sulfated domains within HS chains. Early studies of heparin and its interaction with antithrombin guided much of our thinking about the specificity of protein–HS interactions, but recent studies have broadened our view considerably. Heparin has high anticoagulant activity and higher-than-average overall sulfation (heparin contains approximately 2.3 sulfate groups per disaccharide, whereas typical HS contains approximately 0.8 sulfate groups per disaccharide). The anticoagulant properties of heparin, however, do not depend on overall charge, but instead depend on a unique pentasaccharide with a specific arrangement of sulfate groups and uronic acid epimers, and requires a sulfate group positioned at C3 of the central glucosamine residue as shown in Figure 2 (Lindahl et al. 1980; Atha et al. 1985). This discovery suggested that the interaction of HS with other protein ligands might show similar selectivity.

Interestingly, 3-*O*-sulfated glucosamine residues are quite rare in HS, occurring about 1/20 disaccharides in heparin and less than 1/100 disaccharides in HS or not at all. Nevertheless, the 3-*O*-sulfotransferases comprise the largest family of HS sulfotransferases, with seven members (Fig. 2). Two of the enzymes can produce the antithrombin-binding sequence, whereas the others generate 3-*O*-sulfated sequences

distinct in structure from the antithrombin-binding site, suggesting their purpose lies in the formation of binding sites for other ligands. One class of these sites interacts with glycoprotein gD of Herpes simplex virus-1 and appears to be required for infection (Shukla et al. 1999). Endogenous ligands include cyclophilin B (Vanpouille et al. 2007), FGF7 (Ye et al. 2001), and possibly the ectodomain of FGFR1 (McKeehan et al. 1999), but based on the large size of the 3-*O*-sulfotransferase family, other ligands undoubtedly exist.

Most ligands do not require 3-*O*-sulfation, but the study of heparin–antithrombin interaction set the stage conceptually for searching for specific arrangements of sulfated sugars to achieve selective binding. This technically challenging problem was initially approached by fractionating HS into pools that bound to the ligand or that did not, followed by compositional analysis or partial sequencing. Unfortunately, little variation in composition was noted, most likely because binding sites for most proteins represent only five to 12 sugars. To circumvent this problem, partially cleaved preparations were employed, eventually leading to the identification of minimally sized oligosaccharides that bound with reasonable affinity and that in some cases would initiate a biological response. Examples include, but are not limited to, FGF2 (Guimond et al. 1993; Maccarana et al. 1993), platelet-derived growth factor (Feyzi et al. 1997), platelet factor 4 (Maccarana and Lindahl 1993; Stringer and Gallagher 1997), MIP1 α (Stringer et al. 2002, 2003), hepatocyte growth factor (scatter factor) (Lyon et al. 1994; Ashikari et al. 1995), vascular endothelial growth factor (Soker et al. 1994; Ono et al. 1999; Ashikari-Hada et al. 2005; Robinson et al. 2006), lipoprotein lipase (Parthasarathy et al. 1994; Spillmann et al. 2006), amyloid (Lindahl and Lindahl 1997), and L-selectin (Norgard-Sumnicht and Varki 1995; Wang et al. 2002). When FGF1 was studied in detail, it was noted that a range of HS octasaccharides that varied in the number as well as the positions of individual sulfate groups could bind with varying affinity (Kreuger et al. 2001). Furthermore, the formation of complexes between

FGF1 and FGF receptors was promoted by a variety of saccharides of differing overall sulfate content. This apparent lack of specificity has a corollary *in vivo*; in *Drosophila* deletion of 2-O- or 6-O-sulfotransferases has no effect on FGF signaling and tracheal development, which depends on FGF (Kamimura et al. 2006). Inactivation of either enzyme results in elevated sulfation at other positions, suggesting a form of molecular compensation. Lack of both enzymes impairs FGF signaling and causes multiple patterning deficiencies, indicating that HS is essential but that the receptor ligand complex can accommodate differently sulfated oligosaccharides. It would appear that other ligand-receptor pairs might behave similarly, because many organs and tissues in mice bearing mutations in these sulfotransferases (or the epimerase that interconverts glucuronic acid to iduronic acid) develop normally (Table 3).

Nevertheless, specificity does exist. For example, mice lacking the 2-O-sulfotransferase suffer renal agenesis (Bullock et al. 1998), but other tissues develop normally. In the lacrimal gland, a decrease in overall sulfation of HS affects Fgf10-Fgfr2b signaling required for branching morphogenesis (Pan et al. 2008). Similar reduction in overall sulfation alters vasculogenesis (Jakobsson et al. 2006), tumor angiogenesis (Fuster et al. 2007), and vascular hyperpermeability (Xu et al. 2010a). Wnt signaling is specifically affected by removal of 6-O-sulfate groups on HS by a pair of cell surface endolytic-6-O-sulfatases (the Sulfos) that act at very restricted sites in the chain (Ai et al. 2003; Lamanna et al. 2008). Although the range of biological processes regulated *in vivo* by the Sulfos remains to be elucidated, growth factors including FGF, Wnt, and GDNF are clearly affected by their loss, resulting in developmental defects and early postnatal lethality (Ai et al. 2007; Holst et al. 2007). Thus, determining whether specific sequences mediate binding and biological action remains an active area of investigation.

Chemokines are a subset of cytokines that interact with HS (Lortat-Jacob 2009). These small (8–10-kDa) secreted proteins signal through G protein-coupled receptors on cell

surfaces to control cell migration in development, lymphocyte homing, inflammation, and wound repair. Binding of chemokines to HSPGs allows their local retention, protection from degradation (Sadir et al. 2004), activation by oligomerization (Proudfoot et al. 2003), and presentation to leukocytes (Handel et al. 2005). Interestingly, chemokines usually occur as dimers with positively charged domains oriented in different configurations (Fig. 3). Although no cocrystal structures are currently available, molecular docking suggests that an HS chain might fit along the dimer interface or can span the heparin-binding domains oriented on opposite sides of the dimer. In the latter case, the distance between the two binding sites in the dimer exceeds the length of a typical sulfated domain in HS (around four to eight sugars). Thus, the preferred organization of the bioactive segment of a chain might consist of two short sulfated domains (NS domains, Fig. 2) separated by a nonsulfated linker of a specific length (NA domain) (Lortat-Jacob et al. 2002). Examples of other ligands in which binding sites require extended or discontinuous domains include IFN- γ (Lortat-Jacob et al. 1995) and platelet factor 4 (Stringer and Gallagher 1997), and may include other paired systems, such as FGF/FGFR (Mulloy and Linhardt 2001) or VEGF/VEGFR/Nrp1 (Grunewald et al. 2010). Generating this arrangement would presumably require spatial control over the assembly process, but how this is achieved *in vivo* is not known (Lindh and Li 2009).

PROTEOGLYCANS AS CORECEPTORS

In 1991, Yayon et al. (1991) and Rapraeger et al. (1991) reported that cell surface HSPGs facilitate the formation and signaling of FGF2-FGF receptor complexes (Fig. 4A). As described above, exogenous heparin or HS can also potentiate the formation of complexes of FGF with FGF receptors. In this context, the HSPG or heparin is considered a “coreceptor,” because its function is to aid the formation of ligand-receptor complexes either through conformational change of ligand and/or receptor or by acting as a template to approximate ligand

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Figure 3. Docking of HS to chemokines. Molecular modeling was used to dock a fully sulfated heparin-like chain to several chemokines. The proteins are represented by ribbons except for the side chains of the basic amino acids directly involved in polysaccharide binding (green). The heparin molecule is represented by sticks. (Data from Lortat-Jacob et al. 2002.)

and receptor. In cells, activation usually occurs *in cis* by HSPGs expressed on the same cell as the signaling receptor (Fig. 4A). For example, altering HS sulfation selectively in endothelial cells using the Cre-lox system decreases pathological angiogenesis *in vivo* (Fuster et al. 2007). Similarly, altering HS in mammary epithelial cells has a striking impact on lobuloalveolar development, in spite of expression of HS in surrounding stromal cells (Crawford et al. 2010). Studies of lens development (Pan et al. 2006), branching morphogenesis in the lacrimal gland (Pan et al. 2008; Qu et al.

2011), axon guidance, and development of the central nervous system (Inatani et al. 2003; Yamaguchi et al. 2010) also suggest that HSPGs act as coreceptors in a cell-autonomous manner.

Nevertheless, the observation that exogenous heparin, HS or HSPGs can activate FGF signaling raised the possibility that HSPGs on one cell type might activate signaling *in trans* on adjacent cells. Kraemer and Yost provided the first *in vivo* evidence for *trans*-activation in their studies of left–right development in *Xenopus*. In this system ectodermal syndecan-2 transmits in a non-cell-autonomous fashion

Figure 4. Model for *trans*-activation of VEGF receptor by heparan sulfate proteoglycans. (A) Resident plasma membrane HSPGs can mediate VEGF interactions with its receptor in *cis*, inducing cell signaling and subsequent internalization of the complex. (B) HSPGs from an adjacent cell can also mediate VEGF interactions with its receptor in *trans*, delaying internalization of the signaling complex and enhancing VEGF response. (From Jakobsson et al. 2006; reprinted with permission from Elsevier © 2006.)

left–right information to migrating mesoderm via a growth factor signaling pathway (Kramer and Yost 2002). More recently, Jakobsson et al. analyzed VEGF signaling in embryoid bodies derived from mutant stem cell populations that were either deficient in HS biosynthesis or in VEGF receptor expression (Jakobsson et al. 2006). Although both mutant stem cell lines were unable to support VEGF signaling, generation of chimeras restored VEGF signaling and response. A model for how HSPGs might activate VEGF receptors in *trans* is shown in Figure 4B. As discussed below, germline stem cells are maintained in a stem cell niche by short-range *trans*-signaling mediated by glypicans expressed in niche cells acting on the stem cells (Hayashi et al. 2009). *Trans*-activation of receptors by HSPGs could potentially elicit stronger signaling responses by trapping the receptor at the cell surface in an activated state. Overall, the ability of HSPGs to *trans*-activate adjacent cells represents a novel type of cellular cross talk and may play an important role in regulating cellular differentiation and response during development.

Secreted HSPGs also can act in a non-cell-autonomous manner (e.g., by directly eliciting signaling responses in nearby cells). The HSPG agrin acts in this way to induce postsynaptic differentiation at the neuromuscular junction

(Bezakova and Ruegg 2003). To carry out this function, agrin is secreted by motor neurons where it activates the receptor muscle-specific receptor tyrosine kinase (MuSK) on adjacent muscle cells. Signaling cascades induced by MuSK signaling result in cytoskeletal reorganization and subsequent aggregation of acetylcholine receptors on muscle cells, priming the neuromuscular junction for activation. Recent studies have shown that proteolytic processing of agrin at the neurological synapses by neurotrypsin releases an active carboxy-terminal fragment that subsequently induces the formation of dendritic filopodia on hippocampal neurons (Matsumoto-Miyai et al. 2009). Other proteoglycans can also undergo proteolytic processing to release bioactive domains that can act in an endocrine fashion, including collagen XVIII (Marneros and Olsen 2001) and perlecan (Bix and Iozzo 2008).

The coreceptor function of some proteoglycans, such as the *trans*-membrane syndecans, can be dynamically regulated by modulating their association with the cell surface through a process known as shedding (Bernfield et al. 1999; Manon-Jensen et al. 2010). Syndecan shedding is mediated by matrix metalloproteinases (MMP1, MMP7, MMP9, ADAM17) (Fitzgerald et al. 2000; Li et al. 2002; Endo et al. 2003; Ding et al. 2005; Brule et al. 2006;

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Pruessmeyer et al. 2010). Mechanistically, induced shedding of syndecan-1 appears to involve its cytoplasmic tail, which binds to Rab5, a small GTPase that regulates intracellular trafficking and signaling events (Hayashida et al. 2008). Rab5 may regulate shedding by inducing dissociation of syndecan-1 from $\beta 1$ integrin (see below), thus exposing a normally cryptic cleavage site. Shedding has a major effect on the localization and signaling capacity of HS-bound ligands (Kato et al. 1998; Park et al. 2000b). For example, syndecan-1 shedding modulates chemokine-dependent inflammatory processes in models of tissue damage caused by noninfectious agents (Li et al. 2002; Xu et al. 2005b; Hayashida et al. 2009b). Syndecan-1 shed by implanted tumor cells can exert biologic effects distal to the primary tumor, driving formation of osteoclasts and bone destruction via bound heat-labile factors (Kelly et al. 2010). Some microorganisms can enhance host cell proteolytic shedding of syndecan-1 resulting in enhanced bacterial colonization (Park et al. 2000a, 2001). In all of these models, the activity of shed syndecan ectodomains depends on the HS chains, suggesting their activity depends on ligands bound to the chains.

PROTEOGLYCANS AS ENDOCYTIC RECEPTORS

Although often overlooked, membrane HSPGs also act as endocytic receptors, and undergo constitutive as well as ligand-induced endocytosis (Williams and Fuki 1997; Belting 2003). Although the precise mechanism of endocytosis is unclear, it appears to occur independently of clathrin, caveolin, and dynamin, but in a lipid-raft-dependent manner involving vesicles of unusual composition (Zimmermann et al. 2005; Wittrup et al. 2010). Ligands bound to the HS chains “piggy back” into the cell through this route. The endocytic activity of HSPGs plays a significant physiological role in lipid metabolism. Recent genetic evidence showed that mice lacking syndecan-1 accumulate both liver-derived and dietary triglycerides in the form of remnant lipoprotein particles (Stanford

et al. 2009). Altering the structure of HS by selective inactivation of sulfotransferases in hepatocytes led to the same phenotype and showed that the HS chains on syndecan-1 represent the binding site for the lipoproteins (MacArthur et al. 2007; Stanford et al. 2010).

Other membrane HSPGs, such as syndecan-2 and syndecan-4 and glypicans, also can mediate uptake of ligands in cultured cells (Fuki et al. 2000). Furthermore, uptake can be induced by FGF2 or by antibody-induced clustering (Fuki et al. 1997; Tkachenko et al. 2004; Zimmermann et al. 2005). Although recycling of internalized HSPGs has been observed (Fransson et al. 1995; Zimmermann et al. 2005), most HSPGs end up in lysosomes, where they undergo degradation by lysosomal proteases and exolytic glycosidases and sulfatases. Bound ligands also are degraded, which provides a mechanism for delivering nutrients to cells or for removal of bioactive factors from the environment. The formation of tissue gradients of morphogens (discussed below) may depend in part on continuous clearance of the ligands through an endocytic mechanism (Lander et al. 2002; Marois et al. 2006; Ren et al. 2009).

Viruses and other pathogens can exploit HSPGs to transit from the extracellular environment to the inside of cells. The membrane-penetrating peptide, HIV-tat, is released from HIV-infected cells and then enters surrounding cells using HSPGs (Green and Loewenstein 1988; Frankel and Pabo 1988). Based in part on HIV-tat, a number of cell-penetrating-peptides have been generated, typically rich in arginine or lysine residues that will facilitate interaction with HSPGs (Poon and Gariepy 2007). In addition, synthetic positively charged transporters, such as guanidynylated aminoglycosides have been prepared. Conjugation of these carriers to drugs, toxins, enzymes, oligonucleotides, as well as quantum dots can mediate delivery of cargo into the cells via HSPGs (Elson-Schwab et al. 2007; Sarrazin et al. 2010).

HSPGs can also mediate transcellular transport. Wang et al. showed HSPG-mediated chemokine transport across endothelial cells and its dependence on the sulfation state of the HS chains (Wang et al. 2005). As discussed below,

cell surface HSPGs are also engaged in cell adhesion, which is dependent on the interaction of HS chains with ECM proteins such as fibronectin. Whether cell attachment and endocytosis are mutually exclusive or perhaps mediated through different membrane proteoglycans is unclear.

PROTEOGLYCANS AS ADHESION RECEPTORS

HSPGs play several roles in cell adhesion and in the determination of cell shape. These processes depend on binding of cell surface HS to “heparin-binding” domains present in matrix proteins, such as fibronectin, laminins, vitronectin, thrombospondin, and some fibrillar collagens (Bernfield et al. 1999). Syndecan-4 provides an interesting example of a mechanical and functional link between the ECM and the actin cytoskeleton. Syndecan-4 is widely expressed during development and in most adult tissues and is a central component of focal adhesions (Oh and Couchman 2004). Fibroblasts lacking syndecan-4 have an altered actin cytoskeleton and multiple HS chains are required to cluster syndecan-4 on the plasma membrane (Gopal et al. 2010). Consistent with this idea, overexpression of syndecan-4 in CHO cells results in increased focal adhesion formation, organization of cytoskeletal stress fibers, and decreased cell motility (Longley et al. 1999). The activity of syndecan-4 depends on multimerization, which occurs via recruitment of PIP₂, activation of PKC- α , and downstream signaling through the RhoA pathway (Oh et al. 1997a,b,c). Interestingly, multimerization of syndecan-4 is prevented by phosphorylation of its cytoplasmic tail by PKC- δ in response to FGF2 signaling (Murakami et al. 2002). FGF2 is a mitogen and during proliferation, cells need to detach to undergo cytokinesis. Thus, syndecan-4 has a central role in coordinating cytoskeletal changes that take place during adhesion and cell proliferation.

As described in other articles (e.g., Schwartz 2010; Campbell and Humphries 2011; Geiger and Yamada 2011; Watt and Fujiwara 2011), integrins mediate various interactions between

cells and ECM components. Integrins can recognize short peptide sequences (e.g., RGD) present in many ECM proteins, and binding leads to activation, intracellular signaling via kinases and other enzymes, and focal adhesion formation (Cox et al. 2006). When fibroblasts attach and spread in response to fibronectin fragments containing the RGD site, the formation of focal adhesions and stress fibers can often take place only if the heparin-binding domain of the fibronectin is also present (Saoncella et al. 1999; Woods et al. 2000; Morgan et al. 2007). The HS chains of syndecan-4 bind to fibronectin, which together with integrin, induce formation of focal adhesions and stress fibers (Fig. 5).

Syndecan-1 (and syndecan-4) also can regulate activation of $\alpha v \beta 3$ and $\alpha v \beta 5$ integrin by way of interaction of the extracellular domain of the proteoglycan with the β -integrin subunit (Beauvais et al. 2004, 2009; McQuade et al. 2006). The engagement of these receptors occurs outside the cell via a defined peptide segment in syndecan-1, but the activation mechanism is cytoplasmic and occurs via a talin-dependent, inside-out signaling pathway that requires syndecan-1 clustering. The HS chains of syndecan-1 are required, presumably by facilitating syndecan-1 clustering along aggregated ECM components.

HSPGs also can facilitate cell–cell adhesion. During the inflammatory response, endothelial HS interacts with L-selectin on passing leukocytes to aid in the initial tethering of leukocytes to the luminal surface of the endothelium (Wang et al. 2005; Celie et al. 2009). This interaction may depend on the presence of an unusual N-unsubstituted glucosamine unit in HS (Norgard-Sumnicht and Varki 1995) perhaps in combination with fully sulfated domains (Smits et al. 2010). Interestingly, heparinoids administered intravenously to mice dramatically reduce leukocyte infiltration in response to inflammation (Wang et al. 2002) by disruption of endogenous HS-selectin (Wang et al. 2005) or sialyl Lewis^x-selectin interactions (Koenig et al. 1998; Stevenson et al. 2007). After passing the endothelial cell layer, leukocytes encounter the vascular

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Figure 5. The role of syndecan-4 in focal adhesion. (A) Fibroblasts attach and spread through $\alpha 5 \beta 1$ integrin on coverslips coated with the integrin-binding domain of fibronectin but they do not form focal adhesions. (B) Focal adhesions (arrows) form only after engagement of syndecan-4 HS chains after the addition of the heparin-binding domain (HepII) from fibronectin. (Data for image from Okina et al. 2009.)

basement membrane. Leukocyte migration through this barrier is considered to involve local degradation by matrix metalloproteinases and secreted heparanase, which may be necessary for dissolution of HSPG in the basement membrane (Vreys and David 2007; Li and Vlodavsky 2009).

PROTEOGLYCANS REGULATE GROWTH FACTOR BINDING TO ECM AND CELL MIGRATION

The ECM provides a structural network for mediating and regulating cellular movement (e.g., during development and wound repair). One of the ways the ECM regulates cell migration is to directly bind growth factors, such as platelet-derived growth factor (PDGF), providing directional and stimulatory cues for moving cells (Smith et al. 2009). Interestingly, the association of PDGF with ECM appears to be dependent on HS, but does not involve direct binding to HS (Symes et al. 2010). Similarly, HS-dependent interactions between fibronectin and VEGF have been reported (Mitsi et al. 2006). The mechanism by which HS regulates the binding of growth factors to fibronectin appears to stem from its ability to induce the transition of fibronectin from a globular form to a more stable extended form, revealing growth factor-binding sites (Mitsi et al. 2008).

This activity depends on the size and composition of the chains, as shown by studies in which only heparin chains longer than 22 saccharides and with sulfation at the 6-O- and N-positions of glucosamine units retained the ability to modify fibronectin structure and allow VEGF binding (Mitsi et al. 2006).

Tissue-specific expression of different proteoglycans during zebrafish embryogenesis also has been shown to play a role in determining the structure and function of the extracellular matrix. Syndecan-2 expression in the extraembryonic yolk syncytial layer induces fibronectin and laminin matrix assembly throughout the embryo and directs primordial cell migration (Arrington and Yost 2009). Interestingly, overexpression of syndecan-2 in the embryo does not rescue embryonic defects resulting from yolk syncytial layer deficiency, suggesting that proteoglycans in specific cell types can act in a unique manner because of either positional or structural differences. Other studies have also shown that the loss of specific proteoglycans such as syndecan-4 in *Xenopus laevis* can have adverse effects on neural crest cell migration (Matthews et al. 2008) and convergent extension movements (Munoz et al. 2006). These model systems provide a powerful empirical approach for determining the participation of HSPGs in matrix deposition and cell migration.

PROTEOGLYCANS AND BARRIER ACTIVITY

HSPGs were long thought to be a filtration barrier for charged macromolecules in the kidney, but recent studies cast doubt on this idea. The basement membrane of the kidney filtration structure, the glomerulus, contains HSPGs such as agrin, perlecan, and collagen XVIII, and HS accounts for much of the negative charge in the glomerular basement membrane (GBM) (Miner 1999; Raats et al. 2000). Early studies in which HS was removed by perfusion of rat kidneys with heparinase suggested that HS was essential for filtration of large charged proteins such as ferritin and albumin (Kanwar et al. 1980). Correlations of reduced HS levels in the GBM, increased heparanase expression and proteinuria were also made in patients with various kidney diseases such as diabetic nephropathy, further suggesting a putative barrier function for GBM HS (Makino et al. 1992; Tamsma et al. 1994; van den Hoven et al. 2006; Wijnhoven et al. 2006). However, confusion arose when subsequent *in vivo* studies failed to substantiate that removal of GBM HS with heparinase can result in acute proteinuria, and in fact enzyme digestion actually prevented proteinuria induced by removal of sialic acids (Wijnhoven et al. 2007a,b). Furthermore, tissue-specific deletion of the major GBM HSPGs in mice does not cause proteinuria (Rossi et al. 2003; Harvey et al. 2007; Goldberg et al. 2009), nor does complete ablation of HS biosynthesis in mouse podocytes until 8 months of age when proximal tubule abnormalities become prevalent (Chen et al. 2008). Taken together, it appears that the actual function of HS in the glomerulus is associated with its control of podocyte behavior and not as an ultrafiltration barrier.

Although HSPGs do not appear to play a substantial role in permselectivity in the kidney, cell surface HSPGs of the syndecan family have been shown to play a major role in maintaining the barrier integrity of the intestinal epithelium (Bode et al. 2006, 2008). The loss of syndecan-1 or its GAG chains from the intestinal epithelium has been shown to correlate with an efflux of plasma proteins into the intestinal lumen,

causing a potentially lethal condition known as protein-losing enteropathy (PLE) (Murch et al. 1993, 1996; Westphal et al. 2000). The relationship between loss of syndecan-1 and PLE appears to be because of the ability of syndecan-1 to down-regulate inflammatory cytokines, such as IFN- γ and TNF- α , which work together to disrupt interepithelial integrity. Thus, it is thought that loss of syndecan-1 or its HS chains in the intestine exposes the epithelium to cytokine insult, resulting in the disruption of cell–cell interactions and PLE (Bode et al. 2008). Syndecan-1 may play a direct role in sealing the gaps between intestinal epithelial cells, acting as a physical barrier to prevent protein leakage (Bode et al. 2008). Importantly, the administration of nonanticoagulant heparin to syndecan-1-deficient mice as well as to one patient with PLE has proven effective at correcting protein leakage (Bode et al. 2008; Liem et al. 2008), suggesting that patients suffering from barrier dysfunction diseases associated with HSPG deficiency could be treated similarly.

MORPHOGEN AND CHEMOKINE GRADIENTS

Morphogens are signaling molecules that are expressed in restricted regions of tissue and can form gradients that specify cellular differentiation and patterning during development. Studies of morphogen diffusion in the *Drosophila* wing disk have shown that some morphogens, such as wingless (Wg), hedgehog (Hh) and Dpp, require HS for effective diffusion and will not cross cellular regions deficient in HS (Jackson et al. 1997; The et al. 1999; Tsuda et al. 1999; Baeg et al. 2001; Bornemann et al. 2004). Similar studies in the *Drosophila* wing disc revealed that glypicans are essential for morphogen diffusion (Belenkaya et al. 2004; Han et al. 2005; Yan and Lin 2009). These findings suggest a model of morphogen mobility known as restricted diffusion, where morphogens are transferred from one HSPG to the next at the cell surface, moving from regions of high concentration to regions of low concentration along a path that is defined by the

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interacting ligand (Yan and Lin 2009). Although this model might describe the mechanism by which HSPGs regulate short-range morphogen gradients, other modes of morphogen transmission are thought to exist. For example, in studies of morphogen diffusion in the *Drosophila* wing disc, Eaton and colleagues have described extracellular vesicles (argosomes) and lipoprotein particles (Lipophorin) that can mediate the transmission of morphogens over long distances (Greco et al. 2001; Eugster et al. 2007). Interestingly, the packaging of morphogens, such as Wg into argosomes, is HS-dependent and membrane-associated glypicans can recruit Lipophorin containing lipid-modified forms of Hh and Wg to disc tissue. Lander has discussed in detail the complexity of factors that can affect the shape of morphogen gradients and other ways that HSPGs participate in this process (Lander et al. 2002; Lander 2007).

Glypicans may play an important role in morphogen gradient formation because of their mode of attachment to the cell surface. Unlike other proteoglycans, glypicans are bound to the cell membrane via a GPI anchor, allowing diffusion to occur in the outer leaflet of the plasma membrane and affiliation with specific membrane structures such as lipid rafts (Taylor et al. 2009; Gutierrez and Brandan 2010). The ability of glypicans to localize into lipid rafts may allow them to associate more directly with a number of morphogens, such as Hh and Wg, which are themselves lipidated (Rietveld et al. 1999; Zhai et al. 2004). It is also interesting to note that the GPI anchor of glypicans can be cleaved from the cell surface by the hydrolase Notum (Kirkpatrick et al. 2004; Kreuger et al. 2004), a process that may impact the ability of these proteoglycans to regulate morphogen gradients. In support of this idea, overexpression of a secreted form of glypican that lacks a GPI anchor dramatically expands the range of the Hh activity in the *Drosophila* wing disc (Takeo et al. 2005). The reason for this expansion is unclear, but may be caused by a stabilizing effect of this secreted proteoglycan on Hh as it diffuses from its source. Alternatively, secreted glypican may interfere with Hh posttranslational processing

events, such as cholesterol modification. HSPGs can modulate morphogen mobility by promoting their association with modifier enzymes such as ADAMs (a disintegrin and metalloproteinases) and transglutaminases (Dierker et al. 2009a,b). Whether the ability of HSPGs to mediate restricted diffusion and morphogen modification represents distinct mechanisms for the control of gradients is currently unclear.

One should also keep in mind that many other factors diffuse through the ECM en route to their final destinations and therefore would encounter HSPGs on the surfaces of cells, in the interstitial matrix or in a basement membrane. For example, lipoprotein lipase is expressed by adipocytes and skeletal and cardiac myocytes, but its site of action is on the luminal side of the capillary endothelium in resident blood vessels. A recent study has shown that deletion of collagen XVIII results in chylomicronemia caused by decreased presentation of the lipase in the vasculature (Bishop et al. 2010). Because deficiency of collagen XVIII causes thickening of basement membranes (Utriainen et al. 2004), the decreased presentation of the lipase might be caused by delayed diffusion through the basement membrane underlying capillaries in tissues involved in lipolysis. Plasma lipids are normal in perlecan mutants lacking the HS attachment sites (Tran-Lundmark et al. 2008; Bishop et al. 2010), indicating specificity might exist in the interaction of the lipase with HSPGs in the matrix.

STEM CELL NICHE

The generation, maintenance and repair of different tissues during development is regulated by stem cell populations that reside in defined cellular microenvironments known as stem cell niches. These niches are essential for determining the ability of stem cells to retain a self-perpetuating pluripotent state or to differentiate into committed tissue specific progenitors (Nurcombe and Cool 2007). Interestingly, many of the signaling molecules involved in stem cell maintenance, such as Wnts and FGFs, are regulated by HSPGs (Sato et al. 2004; Xu et al. 2005a,c). Furthermore, embryonic

stem cells change the structure of their HS as they differentiate into specific lineages (Johnson et al. 2007; Baldwin et al. 2008).

To directly address the role of HSPGs in stem cell differentiation, mouse embryonic stem cells with mutations in HS biosynthesis have been studied. Embryonic stem cells that lack HS because of *Ext1* gene deficiency are incapable of differentiation on removal of leukemia inhibitory factor, apparently caused by a defective response to FGF (Kraushaar et al. 2010). These findings were corroborated by studies in embryonic stem cells lacking *Ndst1/2*, which also cannot differentiate in response to FGF because of reduced sulfation (Lanner et al. 2010). In addition, mouse embryonic stem cells deficient in *Ndst1/2* were found to be unable to respond to VEGF, preventing their differentiation into blood capillary structures (Jakobsson et al. 2006). Taken together, these studies substantiate the importance of HS in stem cell differentiation at least *ex vivo*.

To address how HSPGs might regulate stem cells in their native cellular environments, Nakato and colleagues examined whether glypican participated in the maintenance of stem cells in the *Drosophila* germline stem cell niche. Interestingly, this function appears to be related to the ability of glypicans to restrict the localization and activity of the morphogen Dpp to the outer boundary of the niche (Hayashi et al. 2009). Stem cells directly adjacent to this Dpp-rich pocket were shown to be activated *in trans* by this morphogen and remained pluripotent. Daughter cells that were not able to physically associate with this region remained resistant to Dpp signaling and subsequently underwent differentiation. These findings will likely have important implications for stem-cell-based treatments of disease and for the design of synthetic matrices for stem-cell-based tissue engineering.

CONCLUDING REMARKS

The purpose of this article was to provide an overview of HSPGs and their biological roles in the ECM. As described above, HSPGs bind many ligands, modulate numerous cellular

activities, and aid in tissue architecture and physiology. The examples selected for presentation represent only a subset of activities associated with HSPGs. However, it is striking that so many essential activities appear to be regulated by such a small family of macromolecules. Understanding how cells regulate the expression and composition of HSPGs to achieve these diverse activities in a coordinated fashion is a major biological problem to solve. The problem may be as complex as unraveling the genetic code, given the enormous complexity of heparan sulfate.

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REFERENCES

- Abrink M, Grujic M, Pejler G. 2004. Serglycin is essential for maturation of mast cell secretory granule. *J Biol Chem* **279**: 40897–40905.
- Adhikari N, Basi DL, Townsend D, Rusch M, Mariash A, Mullegama S, Watson A, Larson J, Tan S, Lerman B, et al. 2010. Heparan sulfate *Ndst1* regulates vascular smooth muscle cell proliferation, vessel size and vascular remodeling. *J Mol Cell Cardiol* **49**: 287–293.
- Ai X, Do AT, Lozynska O, Kusche-Gullberg M, Lindahl U, Emerson CP Jr. 2003. QSulf1 remodels the 6-O sulfation states of cell surface heparan sulfate proteoglycans to promote Wnt signaling. *J Cell Biol* **162**: 341–351.
- Ai X, Kitazawa T, Do AT, Kusche-Gullberg M, Labosky PA, Emerson CP Jr. 2007. SULF1 and SULF2 regulate heparan sulfate-mediated GDNF signaling for esophageal innervation. *Development* **134**: 3327–3338.
- Aikawa T, Whipple CA, Lopez ME, Gunn J, Young A, Lander AD, Krc M. 2008. Glypican-1 modulates the angiogenic and metastatic potential of human and mouse cancer cells. *J Clin Invest* **118**: 89–99.
- Arikawa-Hirasawa E, Watanabe H, Takami H, Hassell JR, Yamada Y. 1999. Perlecan is essential for cartilage and cephalic development. *Nat Genet* **23**: 354–358.
- Arikawa-Hirasawa E, Wilcox WR, Le AH, Silverman N, Govindraj P, Hassell JR, Yamada Y. 2001. Dyssegmental dysplasia, Silverman–Handmaker type, is caused by functional null mutations of the perlecan gene. *Nat Genet* **27**: 431–434.
- Arikawa-Hirasawa E, Rossi SG, Rotundo RL, Yamada Y. 2002. Absence of acetylcholinesterase at the neuromuscular

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- junctions of perlecan-null mice. *Nature Neurosci* **5**: 119–123.
- Arrington CB, Yost HJ. 2009. Extra-embryonic syndecan 2 regulates organ primordia migration and fibrillogenesis throughout the zebrafish embryo. *Development* **136**: 3143–3152.
- Ashikari S, Habuchi H, Kimata K. 1995. Characterization of heparan sulfate oligosaccharides that bind to hepatocyte growth factor. *J Biol Chem* **270**: 29586–29593.
- Ashikari-Hada S, Habuchi H, Kariya Y, Kimata K. 2005. Heparin regulates vascular endothelial growth factor 165-dependent mitogenic activity, tube formation, and its receptor phosphorylation of human endothelial cells. Comparison of the effects of heparin and modified heparins. *J Biol Chem* **280**: 31508–31515.
- Atha DH, Lormeau JC, Petitou M, Rosenberg RD, Choay J. 1985. Contribution of monosaccharide residues in heparin binding to antithrombin III. *Biochemistry* **24**: 6723–6729.
- Baeg GH, Lin X, Khare N, Baumgartner S, Perrimon N. 2001. Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development* **128**: 87–94.
- Baldwin RJ, ten Dam GB, van Kuppevelt TH, Lacaud G, Gallagher JT, Kouskoff V, Merry CL. 2008. A developmentally regulated heparan sulfate epitope defines a subpopulation with increased blood potential during mesodermal differentiation. *Stem Cells* **26**: 3108–3118.
- Bao X, Moseman EA, Saito H, Petryanik B, Thiriot A, Hatakeyama S, Ito Y, Kawashima H, Yamaguchi Y, Lowe JB, et al. 2010. Endothelial heparan sulfate controls chemokine presentation in recruitment of lymphocytes and dendritic cells to lymph nodes. *Immunity* **33**: 817–829.
- Bass MD, Roach KA, Morgan MR, Mostafavi-Pour Z, Schoen T, Muramatsu T, Mayer U, Ballestrem C, Spatz JP, Humphries MJ. 2007. Syndecan-4-dependent Rac1 regulation determines directional migration in response to the extracellular matrix. *J Cell Biol* **177**: 527–538.
- Beauvais DM, Burbach BJ, Rapraeger AC. 2004. The syndecan-1 ectodomain regulates $\alpha_3\beta_3$ integrin activity in human mammary carcinoma cells. *J Cell Biol* **167**: 171–181.
- Beauvais DM, Ell BJ, McWhorter AR, Rapraeger AC. 2009. Syndecan-1 regulates $\alpha_3\beta_3$ and $\alpha_5\beta_3$ integrin activation during angiogenesis and is blocked by synstatin, a novel peptide inhibitor. *J Exp Med* **206**: 691–705.
- Beiting DP, Park PW, Appleton JA. 2006. Synthesis of syndecan-1 by skeletal muscle cells is an early response to infection with *Trichinella spiralis* but is not essential for nurse cell development. *Infect Immun* **74**: 1941–1943.
- Belenkaya TY, Han C, Yan D, Opoka RJ, Khodoun M, Liu H, Lin X. 2004. *Drosophila* Dpp morphogen movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. *Cell* **119**: 231–244.
- Bellaiche Y, The I, Perrimon N. 1998. *Tout-velu* is a *Drosophila* homologue of the putative tumour suppressor EXT-1 and is needed for Hh diffusion. *Nature* **394**: 85–88.
- Belting M. 2003. Heparan sulfate proteoglycan as a plasma membrane carrier. *Trends Biochem Sci* **28**: 145–151.
- Bernfield M, Gotte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. 1999. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* **68**: 729–777.
- Bezakova G, Ruegg MA. 2003. New insights into the roles of agrin. *Nat Rev Mol Cell Biol* **4**: 295–308.
- Bhanot P. 2002. *Plasmodium yoelii* sporozoites infect syndecan-1 deficient mice. *Molec Biochem Parasitol* **123**: 143–144.
- Bishop JR, Schuksz M, Esko JD. 2007. Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature* **446**: 1030–1037.
- Bishop JR, Passos-Bueno MR, Fong L, Stanford KI, Gonzales JC, Yeh E, Young SG, Bensadoun A, Witztum JL, Esko JD, et al. 2010. Deletion of the basement membrane heparan sulfate proteoglycan type XVIII collagen causes hypertriglyceridemia in mice and humans. *PLoS ONE* **5**: e13919.
- Bix G, Iozzo RV. 2008. Novel interactions of perlecan: Unraveling perlecan's role in angiogenesis. *Microsc Res Tech* **71**: 339–348.
- Bode L, Murch S, Freeze HH. 2006. Heparan sulfate plays a central role in a dynamic in vitro model of protein-losing enteropathy. *J Biol Chem* **281**: 7809–7815.
- Bode L, Salvestrini C, Park PW, Li JP, Esko JD, Yamaguchi Y, Murch S, Freeze HH. 2008. Heparan sulfate and syndecan-1 are essential in maintaining murine and human intestinal epithelial barrier function. *J Clin Invest* **118**: 229–238.
- Bornemann DJ, Duncan JE, Staatz W, Selleck S, Warrior R. 2004. Abrogation of heparan sulfate synthesis in *Drosophila* disrupts the Wingless, Hedgehog and Decapentaplegic signaling pathways. *Development* **131**: 1927–1938.
- Boyanovsky BB, Shridas P, Simons M, van der Westhuyzen DR, Webb NR. 2009. Syndecan-4 mediates macrophage uptake of group V secretory phospholipase A2-modified LDL. *J Lipid Res* **50**: 641–650.
- Brule S, Charnaux N, Sutton A, Ledoux D, Chaigneau T, Safar L, Gattegno L. 2006. The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9. *Glycobiology* **16**: 488–501.
- Bullock SL, Fletcher JM, Beddington RS, Wilson VA. 1998. Renal agenesis in mice homozygous for a gene trap mutation in the gene encoding heparan sulfate 2-sulfo-transferase. *Genes Dev* **12**: 1894–1906.
- Bulow HE, Hobert O. 2006. The molecular diversity of glycosaminoglycans shapes animal development. *Annu Rev Cell Dev Biol* **22**: 375–407.
- Campbell ID, Humphries MJ. 2011. Integrin structure, activation, and interactions. *Cold Spring Harb Perspect Biol* **3**: a004994.
- Campos-Xavier AB, Martinet D, Bateman J, Belluoccio D, Rowley L, Tan TY, Baxova A, Gustavson KH, Borochowitz ZU, Innes AM, et al. 2009. Mutations in the heparan-sulfate proteoglycan glypican 6 (GPC6) impair endochondral ossification and cause recessive omodysplasia. *Am J Hum Genet* **84**: 760–770.
- Cano-Gauci DE, Song HH, Yang H, McKerlie C, Choo B, Shi W, Pullano R, Piscione TD, Grisaru S, Soon S, et al.

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1999. Glypican-3-deficient mice exhibit developmental overgrowth and some of the abnormalities typical of Simpson–Golabi–Behmel syndrome. *J Cell Biol* **146**: 255–264.
- Capila I, Linhardt RJ. 2002. Heparin–protein interactions. *Angew Chem Int Edit* **41**: 391–412.
- Capurro MI, Xu P, Shi W, Li F, Jia A, Filmus J. 2008. Glypican-3 inhibits Hedgehog signaling during development by competing with patched for Hedgehog binding. *Dev Cell* **14**: 700–711.
- Cardin AD, Weintraub HJ. 1989. Molecular modeling of protein–glycosaminoglycan interactions. *Arteriosclerosis* **9**: 21–32.
- Celie JW, Rutjes NW, Keuning ED, Soininen R, Heljasvaara R, Pihlajaniemi T, Drager AM, Zweegman S, Kessler FL, Beelen RH, et al. 2007. Subendothelial heparan sulfate proteoglycans become major L-selectin and monocyte chemoattractant protein-1 ligands upon renal ischemia/reperfusion. *Am J Pathol* **170**: 1865–1878.
- Celie JW, Beelen RH, van den Born J. 2009. Heparan sulfate proteoglycans in extravasation: assisting leukocyte guidance. *Front Biosci* **14**: 4932–4949.
- Cevikbas F, Schaefer L, Uhlig P, Robenek H, Theilmeier G, Echtermeyer F, Bruckner P. 2008. Unilateral nephrectomy leads to up-regulation of syndecan-2- and TGF- β -mediated glomerulosclerosis in syndecan-4 deficient male mice. *Matrix Biol* **27**: 42–52.
- Chen E, Stringer SE, Rusch MA, Selleck SB, Ekker SC. 2005. A unique role for 6-O sulfation modification in zebrafish vascular development. *Dev Biol* **284**: 364–376.
- Chen S, Wassenhove-McCarthy DJ, Yamaguchi Y, Holzman LB, van Kuppevelt TH, Jenniskens GJ, Wijnhoven TJ, Woods AC, McCarthy KJ. 2008. Loss of heparan sulfate glycosaminoglycan assembly in podocytes does not lead to proteinuria. *Kidney Int* **74**: 289–299.
- Chiao E, Fisher P, Crisponi L, Deiana M, Dragatsis I, Schlesinger D, Pilia G, Efstratiadis A. 2002. Overgrowth of a mouse model of the Simpson–Golabi–Behmel syndrome is independent of IGF signaling. *Dev Biol* **243**: 185–206.
- Condac E, Silasi-Mansat R, Kosanke S, Schoeb T, Townner R, Lupu F, Cummings RD, Hinsdale ME. 2007. Polycystic disease caused by deficiency in xylosyltransferase 2, an initiating enzyme of glycosaminoglycan biosynthesis. *Proc Natl Acad Sci* **104**: 9416–9421.
- Conrad HE. 1998. *Heparin-binding proteins* Academic Press, San Diego.
- Cornelison DDW, Wilcox-Adelman SA, Goetinck PF, Rauvala H, Rapraeger AC, Olwin BB. 2004. Essential and separable roles for Syndecan-3 and Syndecan-4 in skeletal muscle development and regeneration. *Genes Dev* **18**: 2231–2236.
- Costell M, Carmona R, Gustafsson E, González-Iriarte M, Fässler R, Muñoz-Chápuli R. 2002. Hyperplastic conotruncal endocardial cushions and transposition of great arteries in perlecan-null mice. *Circ Res* **91**: 158–164.
- Couchman JR. 2010. Transmembrane signaling proteoglycans. *Annu Rev Cell Dev Biol* **26**: 89–114.
- Cox BD, Natarajan M, Stettner MR, Gladson CL. 2006. New concepts regarding focal adhesion kinase promotion of cell migration and proliferation. *J Cell Biochem* **99**: 35–52.
- Crawford BE, Garner OB, Bishop JR, Zhang DY, Bush KT, Nigam SK, Esko JD. 2010. Loss of the heparan sulfate sulfotransferase, Ndst1, in mammary epithelial cells selectively blocks lobuloalveolar development in mice. *PLoS ONE* **5**: e10691.
- Danielson KG, Martinez-Hernandez A, Hassell JR, Iozzo RV. 1992. Establishment of a cell line from the EHS tumor: Biosynthesis of basement membrane constituents and characterization of a hybrid proteoglycan containing heparan and chondroitin sulfate chains. *Matrix* **11**: 22–35.
- Dierker T, Dreier R, Migone M, Hamer S, Grobe K. 2009a. Heparan sulfate and transglutaminase activity are required for the formation of covalently cross-linked hedgehog oligomers. *J Biol Chem* **284**: 32562–32571.
- Dierker T, Dreier R, Petersen A, Bordych C, Grobe K. 2009b. Heparan sulfate–modulated, metalloprotease-mediated sonic hedgehog release from producing cells. *J Biol Chem* **284**: 8013–8022.
- Ding K, Lopez-Burks M, Sanchez-Duran JA, Korc M, Lander AD. 2005. Growth factor–induced shedding of syndecan-1 confers glypican-1 dependence on mitogenic responses of cancer cells. *J Cell Biol* **171**: 729–738.
- Echtermeyer F, Streit M, Wilcox-Adelman S, Saoncella S, Denhez F, Detmar M, Goetinck P. 2001. Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. *J Clin Invest* **107**: 9–14.
- Echtermeyer F, Bertrand J, Dreier R, Meinecke I, Neugebauer K, Fuerst M, Lee YJ, Song YW, Herzog C, Theilmeier G, et al. 2009. Syndecan-4 regulates ADAMTS-5 activation and cartilage breakdown in osteoarthritis. *Nat Med* **15**: 1072–1076.
- Edovitsky E, Lerner I, Zcharia E, Peretz T, Vlodavsky I, Elkin M. 2006. Role of endothelial heparanase in delayed-type hypersensitivity. *Blood* **107**: 3609–3616.
- Elson-Schwab L, Garner OB, Schuksz M, Crawford BE, Esko JD, Tor Y. 2007. Guanidinylated neomycin delivers large, bioactive cargo into cells through a heparan sulfate-dependent pathway. *J Biol Chem* **282**: 13585–13591.
- Endo K, Takino T, Miyamori H, Kinsen H, Yoshizaki T, Furukawa M, Sato H. 2003. Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. *J Biol Chem* **278**: 40764–40770.
- Esko JD, Kimata K, Lindahl U. 2009. Proteoglycans and Sulfated Glycosaminoglycans. In *Essentials of glycobiology* (ed. A Varki, et al.), pp. 229–248. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Esko JD, Linhardt RJ. 2009. Proteins that bind sulfated glycosaminoglycans. In *Essentials of glycobiology* (ed. A Varki, et al.), pp. 501–512. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Esko JD, Selleck SB. 2002. Order out of chaos: Assembly of ligand binding sites in heparan sulfate. *Annu Rev Biochem* **71**: 435–471.
- Esko JD, Stewart TE, Taylor WH. 1985. Animal cell mutants defective in glycosaminoglycan biosynthesis. *Proc Natl Acad Sci* **82**: 3197–3201.

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- Eugster C, Panakova D, Mahmoud A, Eaton S. 2007. Lipo-protein–heparan sulfate interactions in the Hh pathway. *Dev Cell* **13**: 57–71.
- Fan G, Xiao L, Cheng L, Wang X, Sun B, Hu G. 2000. Targeted disruption of *NDST-1* gene leads to pulmonary hypoplasia and neonatal respiratory distress in mice. *FEBS Lett* **467**: 7–11.
- Feyzi E, Lustig F, Fager G, Spillmann D, Lindahl U, Salmivirta M. 1997. Characterization of heparin and heparan sulfate domains binding to the long splice variant of platelet-derived growth factor A chain. *J Biol Chem* **272**: 5518–5524.
- Filmus J, Capurro M, Rast J. 2008. Glypicans. *Genome Biol* **9**: 224.
- Fitzgerald ML, Wang ZH, Park PW, Murphy G, Bernfield M. 2000. Shedding of syndecan-1 and-4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase. *J Cell Biol* **148**: 811–824.
- Floer M, Gotte M, Wild MK, Heidemann J, Gassar ES, Domschke W, Kiesel L, Luegering A, Kucharzik T. 2010. Enoxaparin improves the course of dextran sodium sulfate-induced colitis in syndecan-1-deficient mice. *Am J Pathol* **176**: 146–157.
- Forsberg E, Pejler G, Ringvall M, Lunderius C, Tomasini-Johansson B, Kusche-Gullberg M, Eriksson I, Ledin J, Hellman L, Kjellén L. 1999. Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. *Nature* **400**: 773–776.
- Frankel AD, Pabo CO. 1988. Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* **55**: 1189–1193.
- Fransson LÅ, Edgren G, Havsmark B, Schmidtchen A. 1995. Recycling of a glycosylphosphatidylinositol-anchored heparan sulphate proteoglycan (glypican) in skin fibroblasts. *Glycobiology* **5**: 407–415.
- Fuki IV, Meyer ME, Williams KJ. 2000. Transmembrane and cytoplasmic domains of syndecan mediate a multi-step endocytic pathway involving detergent-insoluble membrane rafts. *Biochem J* **351**: 607–612.
- Fukai N, Eklund L, Marneros AG, Oh SP, Keene DR, Tamarin L, Niemelä M, Ilves M, Li E, Pihlajaniemi T, et al. 2002. Lack of collagen XVIII/endostatin results in eye abnormalities. *EMBO J* **21**: 1535–1544.
- Fukai N, Kenagy RD, Chen L, Gao L, Daum G, Clowes AW. 2009. Syndecan-1: An inhibitor of arterial smooth muscle cell growth and intimal hyperplasia. *Arterioscler Thromb Vasc Biol* **29**: 1356–1362.
- Fuki IV, Kuhn KM, Lomazov IR, Rothman VL, Tuszynski GP, Iozzo RV, Swenson TL, Fisher EA, Williams KJ. 1997. The syndecan family of proteoglycans. Novel receptors mediating internalization of atherogenic lipoproteins in vitro. *J Clin Invest* **100**: 1611–1622.
- Fuster MM, Wang L, Castagnola J, Sikora L, Reddi K, Lee PHA, Radek K, Schuksz M, Bishop JR, Gallo RL, et al. 2007. Genetic alteration of endothelial heparan sulfate selectively inhibits tumor angiogenesis. *J Cell Biol* **177**: 539–549.
- Garner OB, Yamaguchi Y, Esko JD, Videm V. 2008. Small changes in lymphocyte development and activation in mice through tissue-specific alteration of heparan sulphate. *Immunology* **125**: 420–429.
- Garner OB, Bush KT, Nigam KB, Yamaguchi Y, Xu D, Esko JD, Nigam SK. 2011. Stage-dependent regulation of mammary ductal branching by heparan sulfate and HGF-cMet signaling. *Dev Biol* (in press).
- Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie JP, Sanes JR. 1996. Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. *Cell* **85**: 525–535.
- Gautam M, DeChiara TM, Glass DJ, Yancopoulos GD, Sanes JR. 1999. Distinct phenotypes of mutant mice lacking agrin, MuSK, or rapsyn. *Brain Res Dev Brain Res* **114**: 171–178.
- Geiger B, Yamada KM. 2011. Molecular architecture and function of matrix adhesions. *Cold Spring Harb Perspect Biol* **3**: a005033.
- Gingras J, Rassadi S, Cooper E, Ferns M. 2007. Synaptic transmission is impaired at neuronal autonomic synapses in agrin-null mice. *Dev Neurobiol* **67**: 521–534.
- Girós A, Morante J, Gil-Sanz C, Fairén A, Costell M. 2007. Perlecan controls neurogenesis in the developing telencephalon. *BMC Dev Biol* **7**: 29.
- Goldberg S, Harvey SJ, Cunningham J, Tryggvason K, Miner JH. 2009. Glomerular filtration is normal in the absence of both agrin and perlecan-heparan sulfate from the glomerular basement membrane. *Nephrol Dial Transplant* **24**: 2044–2051.
- Gopal S, Bober A, Whiteford JR, Multhaupt HA, Yoneda A, Couchman JR. 2010. Heparan sulfate chain valency controls syndecan-4 function in cell adhesion. *J Biol Chem* **285**: 14247–14258.
- Gotte M, Kresse H. 2005. Defective glycosaminoglycan substitution of decorin in a patient with progeroid syndrome is a direct consequence of two point mutations in the galactosyltransferase I (β 4GalT-7) gene. *Biochem Genet* **43**: 65–77.
- Gotte M, Jousen AM, Klein C, Andre P, Wagner DD, Hinkes MT, Kirchhof B, Adamis AP, Bernfield M. 2002. Role of syndecan-1 in leukocyte-endothelial interactions in the ocular vasculature. *Invest Ophthalmol Vis Sci* **43**: 1135–1141.
- Gotte M, Bernfield M, Jousen AM. 2005. Increased leukocyte-endothelial interactions in syndecan-1-deficient mice involve heparan sulfate-dependent and -independent steps. *Curr Eye Res* **30**: 417–422.
- Govindraj P, West L, Koob TJ, Neame P, Doege K, Hassell JR. 2002. Isolation and identification of the major heparan sulfate proteoglycans in the developing bovine rib growth plate. *J Biol Chem* **277**: 19461–19469.
- Greco V, Hannus M, Eaton S. 2001. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell* **106**: 633–645.
- Green M, Loewenstein PM. 1988. Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. *Cell* **55**: 1179–1188.
- Grisaru S, Cano-Gauci D, Tee J, Filmus J, Rosenblum ND. 2001. Glypican-3 modulates BMP- and FGF-mediated effects during renal branching morphogenesis. *Dev Biol* **231**: 31–46.
- Grobe K, Inatani M, Pallerla SR, Castagnola J, Yamaguchi Y, Esko JD. 2005. Cerebral hypoplasia and craniofacial

- defects in mice lacking heparan sulfate *Ndst1* gene function. *Development* **132**: 3777–3786.
- Grujic M, Braga T, Lukinius A, Eloranta ML, Knight SD, Pejler G, Abrink M. 2005. Serglycin-deficient cytotoxic T lymphocytes display defective secretory granule maturation and granzyme B storage. *J Biol Chem* **280**: 33411–33418.
- Grujic M, Christensen JP, Sørensen MR, Abrink M, Pejler G, Thomsen AR. 2008. Delayed contraction of the CD8⁺ T cell response toward lymphocytic choriomeningitis virus infection in mice lacking serglycin. *J Immunol* **181**: 1043–1051.
- Grunewald FS, Prota AE, Giese A, Ballmer-Hofer K. 2010. Structure-function analysis of VEGF receptor activation and the role of coreceptors in angiogenic signaling. *Biochim Biophys Acta* **1804**: 567–580.
- Guimond S, Maccarana M, Olwin BB, Lindahl U, Rapraeger AC. 1993. Activating and inhibitory heparin sequences for FGF-2 (basic FGF). Distinct requirements for FGF-1, FGF-2, and FGF-4. *J Biol Chem* **268**: 23906–23914.
- Gutierrez J, Brandan E. 2010. A novel mechanism of sequestering fibroblast growth factor 2 by glypican in lipid rafts, allowing skeletal muscle differentiation. *Mol Cell Biol* **30**: 1634–1649.
- Habuchi H, Nagai N, Sugaya N, Atsumi F, Stevens RL, Kimata K. 2007. Mice deficient in heparan sulfate 6-O-sulfotransferase-1 exhibit defective heparan sulfate biosynthesis, abnormal placentation, and late embryonic lethality. *J Biol Chem* **282**: 15578–15588.
- Häcker U, Lin XH, Perrimon N. 1997. The *Drosophila* *sugarless* gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis. *Development* **124**: 3565–3573.
- HajMohammadi S, Enjyoji K, Princivalle M, Christi P, Lech M, Beeler D, Rayburn H, Schwartz JJ, Barzegar S, De Agostini AI, et al. 2003. Normal levels of anticoagulant heparan sulfate are not essential for normal hemostasis. *J Clin Invest* **111**: 989–999.
- Hamano Y, Okude T, Shirai R, Sato I, Kimura R, Ogawa M, Ueda Y, Yokosuka O, Kalluri R, Ueda S. 2010. Lack of collagen XVIII/endostatin exacerbates immune-mediated glomerulonephritis. *J Am Soc Nephrol* **21**: 1445–1455.
- Han C, Yan D, Belenkaya TY, Lin X. 2005. *Drosophila* glypicans Dally and Dally-like shape the extracellular Wingless morphogen gradient in the wing disc. *Development* **132**: 667–679.
- Handel TM, Johnson Z, Crown SE, Lau EK, Proudfoot AE. 2005. Regulation of protein function by glycosaminoglycans—As exemplified by chemokines. *Annu Rev Biochem* **74**: 385–410.
- Harvey SJ, Jarad G, Cunningham J, Rops AL, van der Vlag J, Berden JH, Moeller MJ, Holzman LB, Burgess RW, Miner JH. 2007. Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. *Am J Pathol* **171**: 139–152.
- Hasegawa H, Wang F. 2008. Visualizing mechanosensory endings of TrkC-expressing neurons in HS3ST-2-hPLAP mice. *J Comp Neurol* **511**: 543–556.
- Hayashi Y, Kobayashi S, Nakato H. 2009. *Drosophila* glypicans regulate the germline stem cell niche. *J Cell Biol* **187**: 473–480.
- Hayashida K, Stahl PD, Park PW. 2008. Syndecan-1 ectodomain shedding is regulated by the small GTPase Rab5. *J Biol Chem* **283**: 35435–35444.
- Hayashida A, Bartlett AH, Foster TJ, Park PW. 2009a. *Staphylococcus aureus* β -toxin induces lung injury through syndecan-1. *Am J Pathol* **174**: 509–518.
- Hayashida K, Parks WC, Park PW. 2009b. Syndecan-1 shedding facilitates the resolution of neutrophilic inflammation by removing sequestered CXC chemokines. *Blood* **114**: 3033–3043.
- Haynes A, Ruda F, Oliver J, Hamood AN, Griswold JA, Park PW, Rumbaugh KP. 2005. Syndecan 1 shedding contributes to *Pseudomonas aeruginosa* sepsis. *Infect Immun* **73**: 7914–7921.
- Henningsson F, Hergeth S, Cortelius R, Abrink M, Pejler G. 2006. A role for serglycin proteoglycan in granular retention and processing of mast cell secretory granule components. *FEBS J* **273**: 4901–4912.
- Hienola A, Tumova S, Kulleskiy E, Rauvala H. 2006. N-syndecan deficiency impairs neural migration in brain. *J Cell Biol* **174**: 569–580.
- Hileman RE, Fromm JR, Weiler JM, Linhardt RJ. 1998. Glycosaminoglycan–protein interactions: Definition of consensus sites in glycosaminoglycan binding proteins. *BioEssays* **20**: 156–167.
- Hilgenberg LGW, Ho KD, Lee D, O'Dowd DK, Smith MA. 2002. Agrin regulates neuronal responses to excitatory neurotransmitters in vitro and in vivo. *Molec Cell Neurosci* **19**: 97–110.
- Holst CR, Bou-Reslan H, Gore BB, Wong K, Grant D, Chalasani S, Carano RA, Frantz GD, Tessier-Lavigne M, Bolon B, et al. 2007. Secreted sulfatases Sulfi and Sulf2 have overlapping yet essential roles in mouse neonatal survival. *PLoS ONE* **2**: e575.
- Humphries DE, Wong GW, Friend DS, Gurish MF, Qiu WT, Huang CF, Sharpe AH, Stevens RL. 1999. Heparin is essential for the storage of specific granule proteases in mast cells. *Nature* **400**: 769–772.
- Hurskainen M, Eklund L, Hägg PO, Fruttiger M, Sormunen R, Ilves M, Pihlajaniemi T. 2005. Abnormal maturation of the retinal vasculature in type XVIII collagen/endostatin deficient mice and changes in retinal glial cells due to lack of collagen types XV and XVIII. *FASEB J* **19**: 1564–1566.
- Ilan N, Elkin M, Vlodavsky I. 2006. Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. *Int J Biochem Cell Biol* **38**: 2018–2039.
- Inatani M, Irie F, Plump AS, Tessier-Lavigne M, Yamaguchi Y. 2003. Mammalian brain morphogenesis and midline axon guidance require heparan sulfate. *Science* **302**: 1044–1046.
- Iozzo RV, Zoeller JJ, Nystrom A. 2009. Basement membrane proteoglycans: Modulators par excellence of cancer growth and angiogenesis. *Mol Cells* **27**: 503–513.
- Ishiguro K, Kadomatsu K, Kojima T, Muramatsu H, Nakamura E, Ito M, Nagasaka T, Kobayashi H, Kusugami K, Saito H, et al. 2000. Syndecan-4 deficiency impairs the

S. Sarrazin et al.

- fetal vessels in the placental labyrinth. *Dev Dyn* **219**: 539–544.
- Ishiguro K, Kadomatsu K, Kojima T, Muramatsu H, Iwase M, Yoshikai Y, Yanada M, Yamamoto K, Matsushita T, Nishimura M, et al. 2001. Syndecan-4 deficiency leads to high mortality of lipopolysaccharide-injected mice. *J Biol Chem* **276**: 47483–47488.
- Ishiguro K, Kojima T, Muramatsu T. 2002. Syndecan-4 as a molecule involved in defense mechanisms. *Glycoconj J* **19**: 315–318.
- Iwabuchi T, Goetinck PF. 2006. Syndecan-4 dependent FGF stimulation of mouse vibrissae growth. *Mech Dev* **123**: 831–841.
- Izumikawa T, Kanagawa N, Watamoto Y, Okada M, Saeki M, Sakano M, Sugahara K, Sugihara K, Asano M, Kitagawa H. 2010. Impairment of embryonic cell division and glycosaminoglycan biosynthesis in glucuronyltransferase-1-deficient mice. *J Biol Chem* **285**: 12190–12196.
- Izvolzsky KI, Lu J, Martin G, Albrecht KH, Cardoso WV. 2008. Systemic inactivation of Hs6st1 in mice is associated with late postnatal mortality without major defects in organogenesis. *Genesis* **46**: 8–18.
- Jackson SM, Nakato H, Sugiura M, Jannuzzi A, Oakes R, Kaluza V, Golden C, Selleck SB. 1997. dally, a *Drosophila* glypican, controls cellular responses to the TGF- β -related morphogen, Dpp. *Development* **124**: 4113–4120.
- Jakobsson L, Kreuger J, Holmborn K, Lundin L, Eriksson I, Kjellen L, Claesson-Welsh L. 2006. Heparan sulfate in trans potentiates VEGFR-mediated angiogenesis. *Dev Cell* **10**: 625–634.
- Jen YH, Musacchio M, Lander AD. 2009. Glypican-1 controls brain size through regulation of fibroblast growth factor signaling in early neurogenesis. *Neural Dev* **4**: 33.
- Jiang D, Liang J, Campanella GS, Guo R, Yu S, Xie T, Liu N, Jung Y, Homer R, Meltzer EB, et al. 2010. Inhibition of pulmonary fibrosis in mice by CXCL10 requires glycosaminoglycan binding and syndecan-4. *J Clin Invest* **120**: 2049–2057.
- Johnson CE, Crawford BE, Stavridis M, Ten Dam G, Wat AL, Rushton G, Ward CM, Wilson V, van Kuppevelt TH, Esko JD, et al. 2007. Essential alterations of heparan sulfate during the differentiation of embryonic stem cells to Sox1-enhanced green fluorescent protein-expressing neural progenitor cells. *Stem Cells* **25**: 1913–1923.
- Jones KB, Piombo V, Searby C, Kurriker G, Yang B, Grabellus F, Roughley PJ, Morcuende JA, Buckwalter JA, Capocchi MR, et al. 2010. A mouse model of osteochondromagenesis from clonal inactivation of Ext1 in chondrocytes. *Proc Natl Acad Sci* **107**: 2054–2059.
- Kaksonen M, Pavlov I, Vöikar V, Lauri SE, Hienola A, Riekkilä R, Lakso M, Taira T, Rauvala H. 2002. Syndecan-3-deficient mice exhibit enhanced LTP and impaired hippocampus-dependent memory. *Molec Cell Neurosci* **21**: 158–172.
- Kamimura K, Koyama T, Habuchi H, Ueda R, Masu M, Kimata K, Nakato H. 2006. Specific and flexible roles of heparan sulfate modifications in *Drosophila* FGF signaling. *J Cell Biol* **174**: 773–778.
- Kanwar YS, Linker A, Farquhar MG. 1980. Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion. *J Cell Biol* **86**: 688–693.
- Kato M, Wang H, Bernfield M, Gallagher JT, Turnbull JE. 1994. Cell surface syndecan-1 on distinct cell types differs in fine structure and ligand binding of its heparan sulfate chains. *J Biol Chem* **269**: 18881–18890.
- Kato M, Wang H, Kainulainen V, Fitzgerald ML, Ledbetter S, Ornitz DM, Bernfield M. 1998. Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. *Nat Med* **4**: 691–697.
- Kelly T, Suva LJ, Nicks KM, MacLeod V, Sanderson RD. 2010. Tumor-derived syndecan-1 mediates distal cross-talk with bone that enhances osteoclastogenesis. *J Bone Miner Res* **25**: 1295–1304.
- Kirkpatrick CA, Dimitroff BD, Rawson JM, Selleck SB. 2004. Spatial regulation of Wingless morphogen distribution and signaling by Dally-like protein. *Dev Cell* **7**: 513–523.
- Kirkpatrick CA, Knox SM, Staatz WD, Fox B, Lercher DM, Selleck SB. 2006. The function of a *Drosophila* glypican does not depend entirely on heparan sulfate modification. *Dev Biol* **300**: 570–582.
- Koenig A, Norgard-Sumnicht K, Linhardt R, Varki A. 1998. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J Clin Invest* **101**: 877–889.
- Kon S, Ikesue M, Kimura C, Aoki M, Nakayama Y, Saito Y, Kurotaki D, Diao H, Matsui Y, Segawa T, et al. 2008. Syndecan-4 protects against osteopontin-mediated acute hepatic injury by masking functional domains of osteopontin. *J Exp Med* **205**: 25–33.
- Kramer KL, Yost HJ. 2002. Ectodermal syndecan-2 mediates left–right axis formation in migrating mesoderm as a cell-nonautonomous Vg1 cofactor. *Dev Cell* **2**: 115–124.
- Kraushaar DC, Yamaguchi Y, Wang L. 2010. Heparan sulfate is required for embryonic stem cells to exit from self-renewal. *J Biol Chem* **285**: 5907–5916.
- Kreuger J, Salmivirta M, Sturiale L, Giménez-Gallego G, Lindahl U. 2001. Sequence analysis of heparan sulfate epitopes with graded affinities for fibroblast growth factors 1 and 2. *J Biol Chem* **276**: 30744–30752.
- Kreuger J, Perez L, Giraldez AJ, Cohen SM. 2004. Opposing activities of Dally-like glypican at high and low levels of Wingless morphogen activity. *Dev Cell* **7**: 503–512.
- Ksiazek I, Burkhardt C, Lin S, Seddik R, Maj M, Bezakova G, Jucker M, Arber S, Caroni P, Sanes JR, et al. 2007. Synapse loss in cortex of agrin-deficient mice after genetic rescue of perinatal death. *J Neurosci* **27**: 7183–7195.
- Lamanna WC, Baldwin RJ, Padva M, Kalus I, Ten Dam G, van Kuppevelt TH, Gallagher JT, von Figura K, Dierks T, Merry CL. 2006. Heparan sulfate 6-O-endosulfatases: discrete in vivo activities and functional co-operativity. *Biochem J* **400**: 63–73.
- Lamanna WC, Kalus I, Padva M, Baldwin RJ, Merry CL, Dierks T. 2007. The heparanome—The enigma of encoding and decoding heparan sulfate sulfation. *J Biotechnol* **129**: 290–307.
- Lamanna WC, Frese MA, Balleininger M, Dierks T. 2008. Sulf loss influences N-, 2-O-, and 6-O-sulfation of multiple heparan sulfate proteoglycans and modulates fibroblast growth factor signaling. *J Biol Chem* **283**: 27724–27735.

Heparan Sulfate Proteoglycans

- Lander AD. 2007. Morpheus unbound: Reimagining the morphogen gradient. *Cell* **128**: 245–256.
- Lander AD, Nie Q, Wan FY. 2002. Do morphogen gradients arise by diffusion? *Dev Cell* **2**: 785–796.
- Lanner F, Lee KL, Sohl M, Holmborn K, Yang H, Wilbertz J, Poellinger L, Rossant J, Farnebo E. 2010. Heparan sulfation-dependent fibroblast growth factor signaling maintains embryonic stem cells primed for differentiation in a heterogeneous state. *Stem Cells* **28**: 191–200.
- Laremore TN, Zhang F, Dordick JS, Liu J, Linhardt RJ. 2009. Recent progress and applications in glycosaminoglycan and heparin research. *Curr Opin Chem Biol* **13**: 633–640.
- Lawrence R, Olson SK, Steele RE, Wang L, Warrior R, Cummings RD, Esko JD. 2008. Evolutionary differences in glycosaminoglycan fine structure detected by quantitative glycan reductive isotope labeling. *J Biol Chem* **283**: 33674–33684.
- LeClair EE, Mui SR, Huang A, Topczewska JM, Topczewski J. 2009. Craniofacial skeletal defects of adult zebrafish *Glypican 4* (*knypek*) mutants. *Dev Dyn* **238**: 2550–2563.
- Li Q, Olsen BR. 2004. Increased angiogenic response in aortic explants of collagen XVIII/endostatin-null mice. *Am J Pathol* **165**: 415–424.
- Li JP, Vlodavsky I. 2009. Heparin, heparan sulfate and heparanase in inflammatory reactions. *Thromb Haemost* **102**: 823–828.
- Li Q, Park PW, Wilson CL, Parks WC. 2002. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* **111**: 635–646.
- Li JP, Gong F, Hagner-McWhirter A, Forsberg E, Abrink M, Kisilevsky R, Zhang X, Lindahl U. 2003. Targeted disruption of a murine glucuronyl C5-epimerase gene results in heparan sulfate lacking L-iduronic acid and in neonatal lethality. *J Biol Chem* **278**: 28363–28366.
- Li JP, Galvis ML, Gong F, Zhang X, Zcharia E, Metzger S, Vlodavsky I, Kisilevsky R, Lindahl U. 2005. In vivo fragmentation of heparan sulfate by heparanase overexpression renders mice resistant to amyloid protein A amyloidosis. *Proc Natl Acad Sci* **102**: 6473–6477.
- Liem YS, Bode L, Freeze HH, Leebeek FW, Zandbergen AA, Paul Wilson J. 2008. Using heparin therapy to reverse protein-losing enteropathy in a patient with CDG-Ib. *Nat Clin Pract Gastroenterol Hepatol* **5**: 220–224.
- Lin XH, Buff EM, Perrimon N, Michelson AM. 1999. Heparan sulfate proteoglycans are essential for FGF receptor signaling during *Drosophila* embryonic development. *Development* **126**: 3715–3723.
- Lin X, Wei G, Shi ZZ, Dryer L, Esko JD, Wells DE, Matzuk MM. 2000. Disruption of gastrulation and heparan sulfate biosynthesis in EXT1-deficient mice. *Dev Biol* **224**: 299–311.
- Lindahl U, Li JP. 2009. Interactions between heparan sulfate and proteins—Design and functional implications. *Int Rev Cell Mol Biol* **276**: 105–159.
- Lindahl B, Lindahl U. 1997. Amyloid-specific heparan sulfate from human liver and spleen. *J Biol Chem* **272**: 26091–26094.
- Lindahl U, Backstrom G, Thunberg L, Leder IG. 1980. Evidence for a 3-O-sulfated D-glucosamine residue in the antithrombin-binding sequence of heparin. *Proc Natl Acad Sci* **77**: 6551–6555.
- Longley RL, Woods A, Fleetwood A, Cowling GJ, Gallagher JT, Couchman JR. 1999. Control of morphology, cytoskeleton and migration by syndecan-4. *J Cell Sci* **112**: 3421–3431.
- Lortat-Jacob H. 2009. The molecular basis and functional implications of chemokine interactions with heparan sulphate. *Curr Opin Struct Biol* **19**: 543–548.
- Lortat-Jacob H, Turnbull JE, Grimaud JA. 1995. Molecular organization of the interferon γ -binding domain in heparan sulphate. *Biochem J* **310**: 497–505.
- Lortat-Jacob H, Grosdidier A, Imberty A. 2002. Structural diversity of heparan sulfate binding domains in chemokines. *Proc Natl Acad Sci* **99**: 1229–1234.
- Lum DH, Tan J, Rosen SD, Werb Z. 2007. Gene trap disruption of the mouse heparan sulfate 6-O-endosulfatase gene, *Sulf2*. *Mol Cell Biol* **27**: 678–688.
- Lyon M, Deakin JA, Mizuno K, Nakamura T, Gallagher JT. 1994. Interaction of hepatocyte growth factor with heparan sulfate. Elucidation of the major heparan sulfate structural determinants. *J Biol Chem* **269**: 11216–11223.
- MacArthur JM, Bishop JR, Wang L, Stanford KI, Bensadoun A, Witztum JL, Esko JD. 2007. Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J Clin Invest* **117**: 153–164.
- Maccarana M, Lindahl U. 1993. Mode of interaction between platelet factor 4 and heparin. *Glycobiology* **3**: 271–277.
- Maccarana M, Casu B, Lindahl U. 1993. Minimal sequence in heparin/heparan sulfate required for binding of basic fibroblast growth factor. *J Biol Chem* **268**: 23898–23905.
- Makino H, Ikeda S, Haramoto T, Ota Z. 1992. Heparan sulfate proteoglycans are lost in patients with diabetic nephropathy. *Nephron* **61**: 415–421.
- Manon-Jensen T, Itoh Y, Couchman JR. 2010. Proteoglycans in health and disease: The multiple roles of syndecan shedding. *FEBS J* **277**: 3876–3889.
- Marneros AG, Olsen BR. 2001. The role of collagen-derived proteolytic fragments in angiogenesis. *Matrix Biol* **20**: 337–345.
- Marneros AG, Olsen BR. 2003. Age-dependent iris abnormalities in collagen XVIII/endostatin deficient mice with similarities to human pigment dispersion syndrome. *Investig Ophthalmol Visual Sci* **44**: 2367–2372.
- Marneros AG, She H, Zambarakji H, Hashizume H, Connolly EJ, Kim I, Gragoudas ES, Miller JW, Olsen BR. 2007. Endogenous endostatin inhibits choroidal neovascularization. *FASEB J* **21**: 3809–3818.
- Marois E, Mahmoud A, Eaton S. 2006. The endocytic pathway and formation of the Wingless morphogen gradient. *Development* **133**: 307–317.
- Masouleh BK, Ten Dam GB, Wild MK, Seelige R, van der Vlag J, Rops AL, Echtermeyer FG, Vestweber D, van Kuppevelt TH, Kiesel L, et al. 2009. Role of the heparan sulfate proteoglycan syndecan-1 (CD138) in delayed-type hypersensitivity. *J Immunol* **182**: 4985–4993.
- Matsumoto K, Irie F, Mackem S, Yamaguchi Y. 2010. A mouse model of chondrocyte-specific somatic mutation reveals a role for Ext1 loss of heterozygosity in multiple

S. Sarrazin et al.

- hereditary exostoses. *Proc Natl Acad Sci* **107**: 10932–10937.
- Matsumoto-Miyai K, Sokolowska E, Zurlinden A, Gee CE, Luscher D, Hettwer S, Wolfel J, Ladner AP, Ster J, Gerber U, et al. 2009. Coincident pre- and postsynaptic activation induces dendritic filopodia via neurotrypsin-dependent agrin cleavage. *Cell* **136**: 1161–1171.
- Matthews HK, Marchant L, Carmona-Fontaine C, Kuriyama S, Larrain J, Holt MR, Parsons M, Mayor R. 2008. Directional migration of neural crest cells in vivo is regulated by Syndecan-4/Rac1 and non-canonical Wnt signaling/RhoA. *Development* **135**: 1771–1780.
- McDermott SP, Ranheim EA, Leatherberry VS, Khwaja SS, Klos KS, Alexander CM. 2007. Juvenile syndecan-1 null mice are protected from carcinogen-induced tumor development. *Oncogene* **26**: 1407–1416.
- McKeehan WL, Wu XC, Kan M. 1999. Requirement for anticoagulant heparan sulfate in the fibroblast growth factor receptor complex. *J Biol Chem* **274**: 21511–21514.
- McLaughlin D, Karlsson F, Tian N, Pratt T, Bullock SL, Wilson VA, Price DJ, Mason JO. 2003. Specific modification of heparan sulphate is required for normal cerebral cortical development. *Mech Dev* **120**: 1481–1488.
- McQuade KJ, Beauvais DM, Burbach BJ, Rapraeger AC. 2006. Syndecan-1 regulates $\alpha_v\beta_3$ integrin activity in B82L fibroblasts. *J Cell Sci* **119**: 2445–2456.
- Merry CLR, Bullock SL, Swan DC, Backen AC, Lyon M, Beddington RSP, Wilson VA, Gallagher JT. 2001. The molecular phenotype of heparan sulfate in the *Hs2st*^{-/-} mutant mouse. *J Biol Chem* **276**: 35429–35434.
- Miner JH. 1999. Renal basement membrane components. *Kidney Int* **56**: 2016–2024.
- Mitsi M, Hong Z, Costello CE, Nugent MA. 2006. Heparin-mediated conformational changes in fibronectin expose vascular endothelial growth factor binding sites. *Biochemistry* **45**: 10319–10328.
- Mitsi M, Forsten-Williams K, Gopalakrishnan M, Nugent MA. 2008. A catalytic role of heparin within the extracellular matrix. *J Biol Chem* **283**: 34796–34807.
- Morgan MR, Humphries MJ, Bass MD. 2007. Synergistic control of cell adhesion by integrins and syndecans. *Nat Rev Mol Cell Biol* **8**: 957–969.
- Morita H, Yoshimura A, Inui K, Ideura T, Watanabe H, Wang L, Soininen R, Tryggvason K. 2005. Heparan sulfate of perlecan is involved in glomerular filtration. *J Am Soc Nephrol* **16**: 1703–1710.
- Moulton KS, Olsen BR, Sonn S, Fukai N, Zurakowski D, Zeng X. 2004. Loss of collagen XVIII enhances neovascularization and vascular permeability in atherosclerosis. *Circulation* **110**: 1330–1336.
- Mulloy B, Linhardt RJ. 2001. Order out of complexity—Protein structures that interact with heparin. *Curr Opin Struct Biol* **11**: 623–628.
- Munoz R, Moreno M, Oliva C, Orbenes C, Larrain J. 2006. Syndecan-4 regulates non-canonical Wnt signalling and is essential for convergent and extension movements in *Xenopus* embryos. *Nat Cell Biol* **8**: 492–500.
- Murakami M, Horowitz A, Tang S, Ware JA, Simons M. 2002. Protein kinase C (PKC) δ regulates PKC α activity in a syndecan-4-dependent manner. *J Biol Chem* **277**: 20367–20371.
- Murch SH, MacDonald TT, Walker-Smith JA, Levin M, Lionetti P, Klein NJ. 1993. Disruption of sulphated glycosaminoglycans in intestinal inflammation. *Lancet* **341**: 711–714.
- Murch SH, Winyard PJD, Koletzko S, Wehner B, Cheema HA, Risdon RA, Phillips AD, Meadows N, Klein NJ, Walker-Smith JA. 1996. Congenital enterocyte heparan sulphate deficiency with massive albumin loss, secretory diarrhoea, and malnutrition. *Lancet* **347**: 1299–1301.
- Nakato H, Futch TA, Selleck SB. 1995. The *division abnormally delayed* (*dally*) gene: A putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in *Drosophila*. *Development* **121**: 3687–3702.
- Ng A, Wong M, Viviano B, Erlich JM, Alba G, Pflederer C, Jay PY, Saunders S. 2009. Loss of glypican-3 function causes growth factor-dependent defects in cardiac and coronary vascular development. *Dev Biol* **335**: 208–215.
- Nicole S. 2000. Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz–Jampel syndrome (chondrodystrophic myotonia). *Nature Genetics* **26**: 480–483.
- Niemann CU, Abrink M, Pejler G, Fischer RL, Christensen EI, Knight SD, Borregaard N. 2007. Neutrophil elastase depends on seryglycin proteoglycan for localization in granules. *Blood* **109**: 4478–4486.
- Noguer O, Villena J, Lorita J, Vilaró S, Reina M. 2009. Syndecan-2 downregulation impairs angiogenesis in human microvascular endothelial cells. *Exp Cell Res* **315**: 795–808.
- Norgard-Sumnicht KE, Varki A. 1995. Endothelial heparan sulfate proteoglycans that bind to L-selectin have glucosamine residues with unsubstituted amino groups. *J Biol Chem* **270**: 12012–12024.
- Nurcombe V, Cool SM. 2007. Heparan sulfate control of proliferation and differentiation in the stem cell niche. *Crit Rev Eukaryot Gene Expr* **17**: 159–171.
- Oh ES, Couchman JR. 2004. Syndecans-2 and -4; close cousins, but not identical twins. *Mol Cells* **17**: 181–187.
- Oh ES, Couchman JR, Woods A. 1997a. Serine phosphorylation of syndecan-2 proteoglycan cytoplasmic domain. *Arch Biochem Biophys* **344**: 67–74.
- Oh ES, Woods A, Couchman JR. 1997b. Multimerization of the cytoplasmic domain of syndecan-4 is required for its ability to activate protein kinase C. *J Biol Chem* **272**: 11805–11811.
- Oh ES, Woods A, Couchman JR. 1997c. Syndecan-4 proteoglycan regulates the distribution and activity of protein kinase C. *J Biol Chem* **272**: 8133–8136.
- Okina E, Manon-Jensen T, Whiteford JR, Couchman JR. 2009. Syndecan proteoglycan contributions to cytoskeletal organization and contractility. *Scand J Med Sci Sports* **19**: 479–489.
- Ono K, Hattori H, Takeshita S, Kurita A, Ishihara M. 1999. Structural features in heparin that interact with VEGF₁₆₅ and modulate its biological activity. *Glycobiology* **9**: 705–711.
- Ori A, Wilkinson MC, Fernig DG. 2008. The heparanome and regulation of cell function: Structures, functions and challenges. *Front Biosci* **13**: 4309–4338.

Heparan Sulfate Proteoglycans

- Otsuki S, Hanson SR, Miyaki S, Grogan SP, Kinoshita M, Asahara H, Wong CH, Lotz MK. 2010. Extracellular sulfatases support cartilage homeostasis by regulating BMP and FGF signaling pathways. *Proc Natl Acad Sci* **107**: 10202–10207.
- Pallerla SR, Lawrence R, Lewejohann L, Pan Y, Fischer T, Schlomann U, Zhang X, Esko JD, Grobe K. 2008. Altered heparan sulfate structure in mice with deleted NDST3 gene function. *J Biol Chem* **283**: 16885–16894.
- Pan Y, Woodbury A, Esko JD, Grobe K, Zhang X. 2006. Heparan sulfate biosynthetic gene *Ndst1* is required for FGF signaling in early lens development. *Development* **133**: 4933–4944.
- Pan Y, Carbe C, Powers A, Zhang EE, Esko JD, Grobe K, Feng GS, Zhang X. 2008. Bud specific N-sulfation of heparan sulfate regulates Shp2-dependent FGF signaling during lacrimal gland induction. *Development* **135**: 301–310.
- Park PW, Pier GB, Preston MJ, Goldberger O, Fitzgerald ML, Bernfield M. 2000a. Syndecan-1 shedding is enhanced by LasA, a secreted virulence factor of *Pseudomonas aeruginosa*. *J Biol Chem* **275**: 3057–3064.
- Park PW, Reizes O, Bernfield M. 2000b. Cell surface heparan sulfate proteoglycans: Selective regulators of ligand-receptor encounters. *J Biol Chem* **275**: 29923–29926.
- Park PW, Pier GB, Hinkes MT, Bernfield M. 2001. Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence. *Nature* **411**: 98–102.
- Parthasarathy N, Goldberg IJ, Sivaram P, Mulloy B, Flory DM, Wagner WD. 1994. Oligosaccharide sequences of endothelial cell surface heparan sulfate proteoglycan with affinity for lipoprotein lipase. *J Biol Chem* **269**: 22391–22396.
- Partovian C, Ju R, Zhuang ZW, Martin KA, Simons M. 2008. Syndecan-4 regulates subcellular localization of mTOR complex2 and Akt activation in a PKC α -dependent manner in endothelial cells. *Molec Cell* **32**: 140–149.
- Patterson AM, Gardner L, Shaw J, David G, Loreau E, Aguilar L, Ashton BA, Middleton J. 2005. Induction of a CXCL8 binding site on endothelial syndecan-3 in rheumatoid synovium. *Arthritis Rheum* **52**: 2331–2342.
- Pilia G, Hughes-Benzie RM, MacKenzie A, Baybayan P, Chen EY, Huber R, Neri G, Cao A, Forabosco A, Schlesinger D. 1996. Mutations in *GPC3*, a glypican gene, cause the Simpson–Golabi–Behmel overgrowth syndrome [see comments]. *Nat Genet* **12**: 241–247.
- Poon GM, Gariepy J. 2007. Cell-surface proteoglycans as molecular portals for cationic peptide and polymer entry into cells. *Biochem Soc Trans* **35**: 788–793.
- Pratt T, Conway CD, Tian NM, Price DJ, Mason JO. 2006. Heparan sulphation patterns generated by specific heparan sulfotransferase enzymes direct distinct aspects of retinal axon guidance at the optic chiasm. *J Neurosci* **26**: 6911–6923.
- Proudfoot AEI, Handel TM, Johnson Z, Lau EK, LiWang P, Clark-Lewis I, Borlat F, Wells TNC, Kosco-Vilbois MH. 2003. Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. *Proc Natl Acad Sci* **100**: 1885–1890.
- Pruessmeyer J, Martin C, Hess FM, Schwarz N, Schmidt S, Kogel T, Hoettecke N, Schmidt B, Sechi A, Uhlig S, et al. 2010. A disintegrin and metalloproteinase 17 (ADAM17) mediates inflammation-induced shedding of syndecan-1 and -4 by lung epithelial cells. *J Biol Chem* **285**: 555–564.
- Qu X, Carbe C, Tao C, Powers A, Lawrence R, van Kuppevelt TH, Cardoso WV, Grobe K, Esko JD, Zhang X. 2011. Lacrimal gland development and Fgf10-Fgfr2b signaling are controlled by 2-O- and 6-O-sulfated heparan sulfate. *J Biol Chem* **286**: 14435–14444.
- Raats CJI, Van den Born J, Berden JHM. 2000. Glomerular heparan sulfate alterations: Mechanisms and relevance for proteinuria. *Kidney Int* **57**: 385–400.
- Rapraeger AC, Krufka A, Olwin BB. 1991. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* **252**: 1705–1708.
- Ratzka A, Kalus I, Moser M, Dierks T, Mundlos S, Vortkamp A. 2008. Redundant function of the heparan sulfate 6-O-endosulfatases Sulf1 and Sulf2 during skeletal development. *Dev Dyn* **237**: 339–353.
- Reizes O, Benoit SC, Strader AD, Clegg DJ, Akunuru S, Seeley RJ. 2003. Syndecan-3 modulates food intake by interacting with the melanocortin/AgRP pathway. *Ann NY Acad Sci* **994**: 66–73.
- Ren Y, Kirkpatrick CA, Rawson JM, Sun M, Selleck SB. 2009. Cell type-specific requirements for heparan sulfate biosynthesis at the *Drosophila* neuromuscular junction: Effects on synapse function, membrane trafficking, and mitochondrial localization. *J Neurosci* **29**: 8539–8550.
- Richardson GD, Fantauzzo KA, Bazzi H, Määttä A, Jahoda CAB. 2009. Dynamic expression of Syndecan-1 during hair follicle morphogenesis. *Gene Express Patterns* **9**: 454–460.
- Rietveld A, Neutz S, Simons K, Eaton S. 1999. Association of sterol- and glycosylphosphatidylinositol-linked proteins with *Drosophila* raft lipid microdomains. *J Biol Chem* **274**: 12049–12054.
- Ringvall M, Ledin J, Holmborn K, Van Kuppevelt T, Ellin F, Eriksson I, Olofsson AM, Kjellén L, Forsberg E. 2000. Defective heparan sulfate biosynthesis and neonatal lethality in mice lacking *N*-deacetylase/*N*-sulfotransferase-1. *J Biol Chem* **275**: 25926–25930.
- Robinson CJ, Mulloy B, Gallagher JT, Stringer SE. 2006. VEGF165-binding sites within heparan sulfate encompass two highly sulfated domains and can be liberated by K5 lyase. *J Biol Chem* **281**: 1731–1740.
- Rodgers KD, San Antonio JD, Jacenko O. 2008. Heparan sulfate proteoglycans: A GAGgle of skeletal-hematopoietic regulators. *Dev Dyn* **237**: 2622–2642.
- Rogalski TM, Williams BD, Mullen GP, Moerman DG. 1993. Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes* **7**: 1471–1484.
- Rops AL, Götte M, Baselmans MH, van den Hoven MJ, Steenbergen EJ, Lensen JF, Wijnhoven TJ, Cevikbas F, van den Heuvel LP, van Kuppevelt TH, et al. 2007. Syndecan-1 deficiency aggravates anti-glomerular basement membrane nephritis. *Kidney Int* **72**: 1204–1215.
- Rossi M, Morita H, Sormunen R, Airene S, Kreivi M, Wang L, Fukai N, Olsen BR, Tryggvason K, Soininen R. 2003. Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. *EMBO J* **22**: 236–245.

S. Sarrazin et al.

- Sadir R, Imberty A, Baleux F, Lortat-Jacob H. 2004. Heparan sulfate/heparin oligosaccharides protect stromal cell-derived factor-1 (SDF-1)/CXCL12 against proteolysis induced by CD26/dipeptidyl peptidase IV. *J Biol Chem* **279**: 43854–43860.
- Sanderson RD, Yang Y. 2008. Syndecan-1: A dynamic regulator of the myeloma microenvironment. *Clin Exp Metastasis* **25**: 149–159.
- Saoncella S, Echtermeyer F, Denhez F, Nowlen JK, Mosher DE, Robinson SD, Hynes RO, Goetinck PF. 1999. Syndecan-4 signals cooperatively with integrins in a Rho-dependent manner in the assembly of focal adhesions and actin stress fibers. *Proc Natl Acad Sci* **96**: 2805–2810.
- Sarraj MA, Escalona RM, Umbers A, Chua HK, Small C, Griswold M, Loveland K, Findlay JK, Stenvers KL. 2010. Fetal testis dysgenesis and compromised Leydig cell function in *Tgfb3* (betaglycan) knockout mice. *Biol Reprod* **82**: 153–162.
- Sarrazin S, Wilson B, Sly WS, Tor Y, Esko JD. 2010. Guanidylated neomycin mediates heparan sulfate-dependent transport of active enzymes to lysosomes. *Mol Ther* **18**: 1268–1274.
- Sasisekharan R, Raman R, Prabhakar V. 2006. Glycomics approach to structure–function relationships of glycosaminoglycans. *Annu Rev Biomed Eng* **8**: 181–231.
- Sasse P, Malan D, Fleischmann M, Roell W, Gustafsson E, Bostani T, Fan Y, Kolbe T, Breitbach M, Addicks K, et al. 2008. Perlecan is critical for heart stability. *Cardiovasc Res* **80**: 435–444.
- Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. 2004. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med* **10**: 55–63.
- Schellings MWM, Vanhoutte D, van Almen GC, Swinnen M, Leenders JJG, Kubben N, van Leeuwen REW, Hofstra L, Heymans S, Pinto YM. 2010. Syndecan-1 amplifies angiotensin II-induced cardiac fibrosis. *Hypertension* **55**: 249–256.
- Schwartz MA. 2010. Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harb Perspect Biol* **2**: a005066.
- Seidler DG, Faiyaz-Ul-Haque M, Hansen U, Yip GW, Zaidi SH, Teebi AS, Kiesel L, Gotte M. 2006. Defective glycosylation of decorin and biglycan, altered collagen structure, and abnormal phenotype of the skin fibroblasts of an Ehlers–Danlos syndrome patient carrying the novel Arg270Cys substitution in galactosyltransferase I (β GalT-7). *J Mol Med* **84**: 583–594.
- Seppinen L, Sormunen R, Soini Y, Elamaa H, Heljasvaara R, Pihlajaniemi T. 2008. Lack of collagen XVIII accelerates cutaneous wound healing, while overexpression of its endostatin domain leads to delayed healing. *Matrix Biol* **27**: 535–546.
- Serpinskaya AS, Feng G, Sanes JR, Craig AM. 1999. Synapse formation by hippocampal neurons from agrin-deficient mice. *Dev Biol* **205**: 65–78.
- Sertie AL, Sossi V, Camargo AA, Zatz M, Brahe C, Passos-Bueno MR. 2000. Collagen XVIII, containing an endogenous inhibitor of angiogenesis and tumor growth, plays a critical role in the maintenance of retinal structure and in neural tube closure (Knobloch syndrome). *Hum Mol Genet* **9**: 2051–2058.
- Shukla D, Liu J, Blaiklock P, Shworak NW, Bai XM, Esko JD, Cohen GH, Eisenberg RJ, Rosenberg RD, Spear PG. 1999. A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* **99**: 13–22.
- Shworak NW, Kojima T, Rosenberg RD. 1993. Isolation and characterization of ryudocan and syndecan heparan sulfate proteoglycans, core proteins, and cDNAs from a rat endothelial cell line. *Haemostasis* **23**: 161–176.
- Shworak NW, HajMohammadi S, DeAgostini AI, Rosenberg RD. 2002. Mice deficient in heparan sulfate 3-O-sulfotransferase-1: Normal hemostasis with unexpected perinatal phenotypes. *Glycoconj J* **19**: 355–361.
- Smith EM, Mitsi M, Nugent MA, Symes K. 2009. PDGF-A interactions with fibronectin reveal a critical role for heparan sulfate in directed cell migration during *Xenopus* gastrulation. *Proc Natl Acad Sci* **106**: 21683–21688.
- Smits NC, Kurup S, Rops AL, Ten Dam GB, Massuger LF, Hafmans T, Turnbull JE, Spillmann D, Li JP, Kennel SJ, et al. 2010. The heparan sulfate motif (GlcNAc6S-IdoA2S)3, common in heparin, has a strict topography and is involved in cell behavior and disease. *J Biol Chem* **285**: 41143–41151.
- Soker S, Goldstaub D, Svahn CM, Vlodavsky I, Levi B-Z, Neufeld G. 1994. Variations in the size and sulfation of heparin modulate the effect of heparin on the binding of VEGF₁₆₅ to its receptors. *Biochem Biophys Res Commun* **203**: 1339–1347.
- Song HH, Shi W, Xiang Y-Y, Filmus J. 2005. The loss of glypican-3 induces alterations in Wnt signaling. *J Biol Chem* **280**: 2116–2125.
- Spillmann D, Lookene A, Olivecrona G. 2006. Isolation and characterization of low sulfated heparan sulfate sequences with affinity for lipoprotein lipase. *J Biol Chem* **281**: 23405–23413.
- Stanford KI, Bishop JR, Foley EM, Gonzales JC, Niesman IR, Witztum JL, Esko JD. 2009. Syndecan-1 is the primary heparan sulfate proteoglycan mediating hepatic clearance of triglyceride-rich lipoproteins in mice. *J Clin Invest* **119**: 3236–3245.
- Stanford KI, Wang L, Castagnola J, Song D, Bishop JR, Brown JR, Lawrence R, Bai X, Habuchi H, Tanaka M, et al. 2010. Heparan sulfate 2-O-sulfotransferase is required for triglyceride-rich lipoprotein clearance. *J Biol Chem* **285**: 286–294.
- Stenvers KL, Tursky ML, Harder KW, Kountouri N, Amatayakul-Chantler S, Grail D, Small C, Weinberg RA, Sizeland AM, Zhu HJ. 2003. Heart and liver defects and reduced transforming growth factor β 2 sensitivity in transforming growth factor β type III receptor–deficient embryos. *Mol Cell Biol* **23**: 4371–4385.
- Stepp MA, Gibson HE, Gala PH, Iglesia DDS, Pajoohesh-Ganji A, Pal-Ghosh S, Brown M, Aquino C, Schwartz AM, Goldberger O, et al. 2002. Defects in keratinocyte activation during wound healing in the syndecan-1-deficient mouse. *J Cell Sci* **115**: 4517–4531.
- Stevenson JL, Varki A, Borsig L. 2007. Heparin attenuates metastasis mainly due to inhibition of P- and L-selectin, but non-anticoagulant heparins can have additional effects. *Thromb Res* **120**: S107–S111.

Heparan Sulfate Proteoglycans

- Stickens D, Zak BM, Rougier N, Esko JD, Werb Z. 2005. Mice deficient in Ext2 lack heparan sulfate and develop exostoses. *Development* **132**: 5055–5068.
- Strader AD, Reizes O, Woods SC, Benoit SC, Seeley RJ. 2004. Mice lacking the syndecan-3 gene are resistant to diet-induced obesity. *J Clin Invest* **114**: 1354–1360.
- Stringer SE, Gallagher JT. 1997. Specific binding of the chemokine platelet factor 4 to heparan sulfate. *J Biol Chem* **272**: 20508–20514.
- Stringer SE, Forster MJ, Mulloy B, Bishop CR, Graham GJ, Gallagher JT. 2002. Characterization of the binding site on heparan sulfate for macrophage inflammatory protein 1 α . *Blood* **100**: 1543–1550.
- Stringer SE, Nelson MS, Gupta P. 2003. Identification of an MIP-1 α -binding heparan sulfate oligosaccharide that supports long-term in vitro maintenance of human LTC-ICs. *Blood* **101**: 2243–2245.
- Sugahara K, Kitagawa H. 2002. Heparin and heparan sulfate biosynthesis. *IUBMB Life* **54**: 163–175.
- Sugaya N, Habuchi H, Nagai N, Ashikari-Hada S, Kimata K. 2008. 6-O-sulfation of heparan sulfate differentially regulates various fibroblast growth factor-dependent signalings in culture. *J Biol Chem* **283**: 10366–10376.
- Symes K, Smith EM, Mitsi M, Nugent MA. 2010. Sweet cues: How heparan sulfate modification of fibronectin enables growth factor guided migration of embryonic cells. *Cell Adh Migr* **4**: 507–510.
- Takahashi I, Noguchi N, Nata K, Yamada S, Kaneiwa T, Mizumoto S, Ikeda T, Sugihara K, Asano M, Yoshikawa T, et al. 2009. Important role of heparan sulfate in postnatal islet growth and insulin secretion. *Biochem Biophys Res Commun* **383**: 113–118.
- Takeo S, Akiyama T, Firkus C, Aigaki T, Nakato H. 2005. Expression of a secreted form of Dally, a *Drosophila* glypican, induces overgrowth phenotype by affecting action range of Hedgehog. *Dev Biol* **284**: 204–218.
- Tamsma JT, van den Born J, Bruijn JA, Assmann KJ, Weening JJ, Berden JH, Wieslander J, Schrama E, Hermans J, Veerkamp JH, et al. 1994. Expression of glomerular extracellular matrix components in human diabetic nephropathy: Decrease of heparan sulphate in the glomerular basement membrane. *Diabetologia* **37**: 313–320.
- Taylor DR, Whitehouse IJ, Hooper NM. 2009. Glypican-1 mediates both prion protein lipid raft association and disease isoform formation. *PLoS Pathog* **5**: e1000666.
- Telci D, Wang Z, Li X, Verderio EAM, Humphries MJ, Baccharini M, Basaga H, Griffin M. 2008. Fibronectin-tissue transglutaminase matrix rescues RGD-impaired cell adhesion through syndecan-4 and β 1 integrin co-signaling. *J Biol Chem* **283**: 20937–20947.
- The I, Bellaiche Y, Perrimon N. 1999. Hedgehog movement is regulated through *tout velu*-dependent synthesis of a heparan sulfate proteoglycan. *Mol Cell* **4**: 633–639.
- Thompson WR, Modla S, Grindel BJ, Czymbek KJ, Kirn-Safran CB, Wang L, Duncan RL, Farach-Carson MC. 2011. Perlecan/Hspg2 deficiency alters the pericellular space of the lacuno-canalicular system surrounding osteocytic processes in cortical bone. *J Bone Mineral Res* **26**: 618–629.
- Tkachenko E, Lutgens E, Stan RV, Simons M. 2004. Fibroblast growth factor 2 endocytosis in endothelial cells proceed via syndecan-4-dependent activation of Rac1 and a Cdc42-dependent macropinocytic pathway. *J Cell Sci* **117**: 3189–3199.
- Tkachenko E, Rhodes JM, Simons M. 2005. Syndecans: New kids on the signaling block. *Circ Res* **96**: 488–500.
- Topczewski J, Sepich DS, Myers DC, Walker C, Armores A, Lele Z, Hammerschmidt M, Postlethwait J, Solnica-Krezel L. 2001. The zebrafish glypican knypek controls cell polarity during gastrulation movements of convergent extension. *Dev Cell* **1**: 251–264.
- Tran-Lundmark K, Tran PK, Paulsson-Berne G, Friden V, Soininen R, Tryggvason K, Wight TN, Kinsella MG, Boren J, et al. 2008. Heparan sulfate in perlecan promotes mouse atherosclerosis: Roles in lipid permeability, lipid retention, and smooth muscle cell proliferation. *Circ Res* **103**: 43–52.
- Tsuda M, Kamimura K, Nakato H, Archer M, Staatz W, Fox B, Humphrey M, Olson S, Futch T, Kaluza V, et al. 1999. The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*. *Nature* **400**: 276–280.
- Tveit H, Dick G, Skibeli V, Prydz K. 2005. A proteoglycan undergoes different modifications en route to the apical and basolateral surfaces of Madin–Darby canine kidney cells. *J Biol Chem* **280**: 29596–29603.
- Utriainen A, Sormunen R, Kettunen M, Carvalhaes LS, Sajanti E, Eklund L, Kauppinen R, Kitten GT, Pihlajaniemi T. 2004. Structurally altered basement membranes and hydrocephalus in a type XVIII collagen deficient mouse line. *Hum Mol Genet* **13**: 2089–2099.
- van den Hoven MJ, Rops AL, Bakker MA, Aten J, Rutjes N, Roestenberg P, Goldschmeding R, Zcharia E, Vlodavsky I, van der Vlag J, et al. 2006. Increased expression of heparanase in overt diabetic nephropathy. *Kidney Int* **70**: 2100–2108.
- Vanpouille C, Deligny A, Delehedde M, Denys A, Melchior A, Lienard X, Lyon M, Mazurier J, Fernig DG, Allain F. 2007. The heparin/heparan sulfate sequence that interacts with cyclophilin B contains a 3-O-sulfated N-unsubstituted glucosamine residue. *J Biol Chem* **282**: 24416–24429.
- Varki A, Etzler ME, Cummings RD, Esko JD. 2009. Discovery and classification of glycan-binding proteins. In *Essentials of glycobiology* (ed. A Varki, et al.), pp. 375–386. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Viviano BL, Silverstein L, Pfleiderer C, Paine-Saunders S, Mills K, Saunders S. 2005. Altered hematopoiesis in glypican-3-deficient mice results in decreased osteoclast differentiation and a delay in endochondral ossification. *Dev Biol* **282**: 152–162.
- Vogl-Willis CA, Edwards IJ. 2004. High glucose-induced alterations in subendothelial matrix perlecan leads to increased monocyte binding. *Arterioscler Thromb Vasc Biol* **24**: 858–863.
- Vreys V, David G. 2007. Mammalian heparanase: What is the message? *J Cell Mol Med* **11**: 427–452.
- Waern I, Jia J, Pejler G, Zcharia E, Vlodavsky I, Li JP, Wernersson S. 2010. Accumulation of Ym1 and formation of intracellular crystalline bodies in alveolar macrophages lacking heparanase. *Mol Immunol* **47**: 1467–1475.
- Wang LC, Brown JR, Varki A, Esko JD. 2002. Heparin's anti-inflammatory effects require glucosamine 6-O-sulfation

S. Sarrazin et al.

- and are mediated by blockade of L- and P-selectins. *J Clin Invest* **110**: 127–136.
- Wang L, Fuster M, Sriramarao P, Esko JD. 2005. Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nat Immunol* **6**: 902–910.
- Watt FM, Fujiwara H. 2011. Cell–extracellular matrix interactions in normal and diseased skin. *Cold Spring Harb Perspect Biol* **3**: a005124.
- Wernersson S, Braga T, Sawesi O, Waern I, Nilsson KE, Pejler G, Abrink M. 2009. Age-related enlargement of lymphoid tissue and altered leukocyte composition in serglycin-deficient mice. *J Leuk Biol* **85**: 401–408.
- West L, Govindraj P, Koob TJ, Hassell JR. 2006. Changes in perlecan during chondrocyte differentiation in the fetal bovine rib growth plate. *J Orthop Res* **24**: 1317–1326.
- Westphal V, Murch S, Kim S, Srikrishna G, Winchester B, Day R, Freeze HH. 2000. Reduced heparan sulfate accumulation in enterocytes contributes to protein-losing enteropathy in a congenital disorder of glycosylation. *Am J Pathol* **157**: 1917–1925.
- Wijnhoven TJ, Lensen JF, Rops AL, van der Vlag J, Kolset SO, Bangstad HJ, Pfeffer P, van den Hoven MJ, Berden JH, van den Heuvel LP, et al. 2006. Aberrant heparan sulfate profile in the human diabetic kidney offers new clues for therapeutic glycomimetics. *Am J Kidney Dis* **48**: 250–261.
- Wijnhoven TJ, Lensen JF, Wismans RG, Lamrani M, Monnens LA, Wevers RA, Rops AL, van der Vlag J, Berden JH, van den Heuvel LP, et al. 2007a. In vivo degradation of heparan sulfates in the glomerular basement membrane does not result in proteinuria. *J Am Soc Nephrol* **18**: 823–832.
- Wijnhoven TJ, Lensen JF, Wismans RG, Lefeber DJ, Rops AL, van der Vlag J, Berden JH, van den Heuvel LP, van Kuppevelt TH. 2007b. Removal of heparan sulfate from the glomerular basement membrane blocks protein passage. *J Am Soc Nephrol* **18**: 3119–3127.
- Wilcox-Adelman SA, Denhez F, Goetinck PE. 2002. Syndecan-4 modulates focal adhesion kinase phosphorylation. *J Biol Chem* **277**: 32970–32977.
- Williams KJ, Fuki IV. 1997. Cell-surface heparan sulfate proteoglycans: Dynamic molecules mediating ligand catabolism. *Curr Opin Lipidol* **8**: 253–262.
- Wittrup A, Zhang SH, Svensson KJ, Kucharszewska P, Johansson MC, Morgelin M, Belting M. 2010. Magnetic nanoparticle-based isolation of endocytic vesicles reveals a role of the heat shock protein GRP75 in macromolecular delivery. *Proc Natl Acad Sci* **107**: 13342–13347.
- Woods A, Longley RL, Tumova S, Couchman JR. 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.
- Woulfe DS, Lillendahl JK, August S, Rauova L, Kowalska MA, Abrink M, Pejler G, White JG, Schick BP. 2008. Serglycin proteoglycan deletion induces defects in platelet aggregation and thrombus formation in mice. *Blood* **111**: 3458–3467.
- Xu D, Fuster MM, Lawrence R, Esko JD. 2010a. Heparan sulfate regulates VEGF165 and VEGF121-mediated vascular hyperpermeability. *J Biol Chem* **286**: 734–745.
- Xu Z, Ichikawa N, Kosaki K, Yamada Y, Sasaki T, Sakai LY, Kurosawa H, Hattori N, Arikawa-Hirasawa E. 2010b. Perlecan deficiency causes muscle hypertrophy, a decrease in myostatin expression, and changes in muscle fiber composition. *Matrix Biol* **29**: 461–470.
- Xu C, Rosler E, Jiang J, Lebikowski JS, Gold JD, O'Sullivan C, Delavan-Boorsma K, Mok M, Bronstein A, Carpenter MK. 2005a. Basic fibroblast growth factor supports undifferentiated human embryonic stem cell growth without conditioned medium. *Stem Cells* **23**: 315–323.
- Xu J, Park PW, Kheradmand F, Corry DB. 2005b. Endogenous attenuation of allergic lung inflammation by syndecan-1. *J Immunol* **174**: 5758–5765.
- Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. 2005c. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat Methods* **2**: 185–190.
- Yamada S, Morimoto H, Fujisawa T, Sugahara K. 2007. Glycosaminoglycans in *Hydra magnipapillata* (Hydrozoa, Cnidaria): Demonstration of chondroitin in the developing nematocyst, sting organelle, and structural characterization of glycosaminoglycans. *Glycobiology* **17**: 886–894.
- Yamaguchi Y, Inatani M, Matsumoto Y, Ogawa J, Irie F. 2010. Roles of heparan sulfate in mammalian brain development: current views based on the findings from Ext1 conditional knockout studies. *Prog Mol Biol Transl Sci* **93**: 133–152.
- Yan D, Lin X. 2009. Shaping morphogen gradients by proteoglycans. *Cold Spring Harb Perspect Biol* **1**: a002493.
- Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. 1991. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* **64**: 841–848.
- Ye S, Luo YD, Lu WQ, Jones RB, Linhardt RJ, Capila I, Toida T, Kan M, Pelletier H, McKeenhan WL. 2001. Structural basis for interaction of FGF-1, FGF-2, and FGF-7 with different heparan sulfate motifs. *Biochemistry* **40**: 14429–14439.
- Zak SM, Schuksz M, Koyama E, Mundy C, Wells DE, Yamaguchi Y, Pacifici M, Esko JD. 2011. Compound heterozygous loss of *Ext1* and *Ext2* is sufficient for formation of multiple exostoses in mouse ribs and long bones. *Bone* **48**: 979–987.
- Zcharia E, Zilka R, Yaar A, Yacoby-Zeevi O, Zetser A, Metzger S, Sarid R, Naggi A, Casu B, Ilan N, et al. 2005. Heparanase accelerates wound angiogenesis and wound healing in mouse and rat models. *FASEB J* **19**: 211–221.
- Zcharia E, Jia J, Zhang X, Baraz L, Lindahl U, Peretz T, Vlodavsky I, Li JP. 2009. Newly generated heparanase knockout mice unravel co-regulation of heparanase and matrix metalloproteinases. *PLoS ONE* **4**: e5181.
- Zernichow L, Abrink M, Hallgren J, Grujic M, Pejler G, Kolset SO. 2006. Serglycin is the major secreted proteoglycan in macrophages and has a role in the regulation of macrophage tumor necrosis factor- α secretion in response to lipopolysaccharide. *J Biol Chem* **281**: 26792–26801.
- Zhai L, Chaturvedi D, Cumberledge S. 2004. *Drosophila* wnt-1 undergoes a hydrophobic modification and is targeted to lipid rafts, a process that requires porcupine. *J Biol Chem* **279**: 33220–33227.

Heparan Sulfate Proteoglycans

- Zhang L, Lawrence R, Frazier BA, Esko JD. 2006. CHO glycosylation mutants: Proteoglycans. *Methods Enzymol* **416**: 205–221.
- Zimmermann P, Zhang Z, Degeest G, Mortier E, Leenaerts I, Coomans C, Schulz J, N’Kuli F, Courtoy PJ, David G. 2005. Syndecan recycling [corrected] is controlled by syntenin-PIP2 interaction and Arf6. *Dev Cell* **9**: 377–388.
- Zoeller JJ, Whitelock JM, Iozzo RV. 2009. Perlecan regulates developmental angiogenesis by modulating the VEGF-VEGFR2 axis. *Matrix Biol* **28**: 284–291.
- Zuberi RI, Ge XN, Jiang S, Bahaie NS, Kang BN, Hosseinkhani RM, Frenzel EM, Fuster MM, Esko JD, Rao SP, et al. 2009. Deficiency of endothelial heparan sulfates attenuates allergic airway inflammation. *J Immunol* **183**: 3971–3979.

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