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Heparan Sulfate Regulation of Progenitor Cell Fate

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Abstract Currently there is an intense effort being made to elucidate the factors that control stem and progenitor cell fate. Developments in our understanding of the FGF/FGFR pathway and its role as an effector of stem cell pluripotency have heightened expectations that a therapeutic use for stem cells will move from a possibility to a probability. Mounting evidence is revealing the molecular mechanisms by which fibroblast growth factor (FGF) signaling, together with a large number of other growth and adhesive factors, is controlled by the extracellular sugar, heparan sulfate (HS). What has resulted is a novel means of augmenting and thus regulating the growth factor control of stem and progenitor cell fate. Here, we review the numerous bioactivities of HS, and the development of strategies to implement HS-induced control of cell fate decisions. J. Cell. Biochem. 99: 1040–1051, 2006. © 2006 Wiley-Liss, Inc.

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The growth and survival of progenitor cells constitutes a vital step in the maintenance of tissue health and integrity. Control over these processes is exerted through the tissue *niche*, and particularly elements of its specialized extracellular matrix, which ultimately triggers a cascade of signals that regulate the uncommitted cells, including a plethora of cell survival and cell cycle progression events that shape developmental processes and ultimately morphogenesis [Aszodi et al., 2000; Gustafsson et al., 2003]. The coordination of this extracellular traffic requires a pericellular system that can rapidly adapt to the changing needs of tissue in their most dynamic phases.

Heparan sulfate proteoglycans (HSPGs) are abundant cell surface and extracellular matrix molecules that consist of a defining core protein (such as syndecan, glypican, or perlecan) to which are attached highly sulfated glycosaminoglycan (GAG) side-chains of heparan sulfate (HS) [Bernfield et al., 1992]. Modifications of these HS chains during biosynthesis, together with core protein variability, results in the wide structural diversity of HSPGs [Carey, 1997; Perrimon and Bernfield, 2000]. Further complexity in the HSPGs arises by virtue of their cell type-specific and developmentally regulated synthesis [Bernfield et al., 1999; Perrimon and Bernfield, 2000]. Much of what we know about the function of HS chains is derived from their actions on susceptible growth factors, such as the prototypical fibroblast growth factors (FGFs).

As they become available to cells from the extracellular matrix, FGFs first bind to HS chains with relatively low affinity, but high capacity [Ornitz, 2000]; these then catalyze their binding to high-affinity, but low capacity tyrosine kinase receptors (FGFRs) [McKeehan and Kan, 1994; McKeehan et al., 1998]. Thus, the laws of mass action, together with HS diversity, create a flow of information across their transducing receptors. Despite a limited understanding of the mechanism, it has now been well established that FGF/FGFR/HSPG complexes are necessary for optimal FGF-induced mitogenesis [Rapraeger et al., 1991; Yayon et al., 1991; Krufka et al., 1996; McKeehan et al., 1998; Chang et al., 2000]. In this manner HSPGs act as reservoirs to concentrate

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growth factors close to cells, protecting them from extracellular proteases, and then shepherd them to the cell surface and cross-bind them to their receptors [Ornitz, 2000]. Binding of HS to growth factors may also be important intracellularly, as HS chains have been shown to have a relatively longer half-life than the FGFs after endocytosis, suggesting that the protective effect of HS on FGF may extend all the way to the cell nucleus, so furthering biological function [Burgess et al., 1991; Maciag and Friesel, 1995].

In a similar manner, bone morphogenic proteins (BMPs) are thought to be brought into register by HS with their threonine–serine kinase BMP receptors (BMPRs) [Paine-Saunders et al., 2000; Irie et al., 2003; Takada et al., 2003; Fisher et al., 2006]. Although hepatic growth factor [Lyon et al., 1994] and interferon-γ [Lortat-Jacob et al., 1995] display interesting variations on this pattern, this general schema probably applies to many heparin-binding species, whereby specific protein-binding sites on the rapidly turned over HS chains orchestrate the presentation of crucial factors as cells move through successive developmental phases [Nurcombe et al., 1993; Brickman et al., 1998]. Indeed, heparanase, a matrix-degrading enzyme that cleaves HS side-chains from the core proteoglycans (PGs), has been shown to liberate HS-binding protein bioactivity, as well as contributing to extracellular matrix turnover [Joyce et al., 2005].

Further findings that cell:cell signaling molecules, such as the cellular adhesion molecules (CAMs) [Cole and Akeson, 1989], as well as almost all the extracellular matrix-resident adhesive glycoproteins [Martin et al., 1988], including laminin and fibronectin, have HS-binding sites, have similarly developed our idea of the coordinating functions of HS. The HSPGs may be acting to orientate proteins into their correct positions, whereupon liberated HS-protein complexes are released to the cell membrane to generate cell responses. Thus, the specific interactions between HSPGs and their effector proteins depend on HSPG composition, location, and 3-dimensional structure [Yanagishita and Hascall, 1992; Pye et al., 2000].

HS GLYCOSAMINOGLYCANS

PGs are composed of a core protein to which are attached sugar side-chains composed of alternating disaccharide units of uronic acid and hexosamine (Fig. 1). The overall size of these GAG chains can vary anywhere between 20 and 150 disaccharides. The variable addition of sulfate groups at irregular intervals adds a major level of complexity to their binding behaviors [Gallagher and Turnbull, 1992; Turnbull and Gallagher, 1993; Sanderson et al., 1994; Perrimon and Bernfield, 2000]. GAG synthesis begins in the Golgi after the core protein arrives from the endoplasmic reticulum [Esko and Zhang, 1996]. Once synthesized in the Golgi, PGs are either stored in intracellular organelles transported to the cell surface, or deposited out into the ECM [Carey, 1997; Prydz and Dalen, 2000].

PGs are classified into broad categories based on the type of GAG attached to the protein. HSPGs possess core proteins which position them into basement membranes, such as collagen XVIII, perlecan, and agrin, or are attached to cell surfaces, like the families of syndecans and glypicans (GPC). HS side-chains contain alternating repeating units of glucuronic or iduronic (Ido) acid and *N*-glucosamine. The growing chain, consisting of numerous GlcAβ1,4GlcNAcα1,4 disaccharide units, is modified by sulfotransferases and a GlcA C5 epimerase. HS sulfation can occur at 2-*O* of the uronic acid and 3-*O*, 6-*O*, and the free amine of the glucosamine. Each modification is incomplete, which leads to sequence variation on HS. Most of the binding sites that appear to interact with proteins are found in the NS and NA/NS domains, and critical sulfate group spacing creates a series of unique binding sites along any one HS chain, so determining the specificity of HS-protein interaction [Merry and Wilson, 2002]. The end result of this structural pattern is a number of high capacity, low-to-medium affinity binding pockets for particular combinations of signaling proteins closely adjacent to one another, and to the cell surface. Whether each protein has a unique HS-binding motif dedicated to it, or that motifs are created with more general binding capabilities, is still to be determined. In general, the binding of HS to heparan-binding growth factors seems to involve sulfated (S-) domains consisting of predominantly less-sulfated iduronic acid, and glucosamines with high levels of 6-*O*-sulfation. The presence of various *O*-sulfate groups in addition to *N*-sulfate groups in S-domains appears to be able to provide a relatively specific

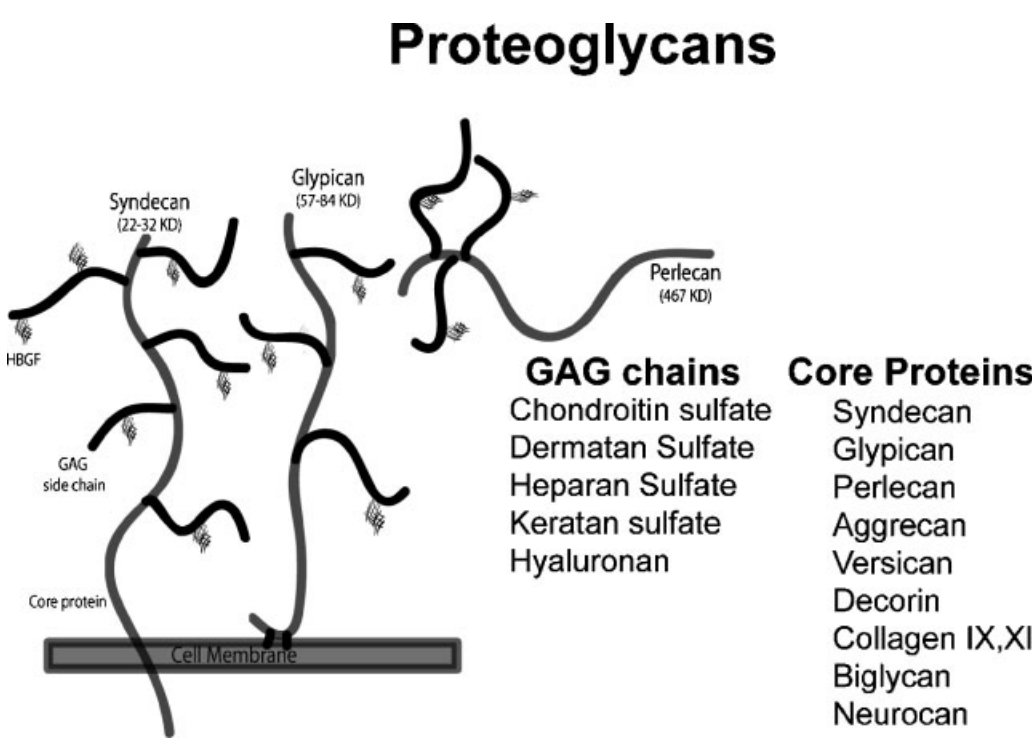

Fig. 1. Proteoglycans (PGs) are a class of heavily glycosylated proteins that have covalently linked sulfated glycosaminoglycans (GAG), (i.e., chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate). There is great complexity within the proteoglycan family, which consists of numerous GAG side-chains that can potentially bind to a range of core proteins to create a diverse set of cross-linked bioactivators. HSPGs, the most dynamic of these species, primarily consists of membrane-spanning syndecans, GPI anchored glypicans, and extracellular perlecans, with molecular weights ranging from 22 to 467 kD depending on glycosylation status. HS-GAG side-chains are responsible for binding and potentiating numerous heparan-binding growth factors (HBGFs).

affinity for different growth factors [Principalle and de Agostini, 2002]. The use of specific heparinases and sulfatases has helped with the determination that IdoA 2-*O*-sulfate and GlcN *N*-sulfate are essential to FGF-2 binding, for example, and that only HS domains of at least decasaccharide length or larger can stimulate mitogenic activity. In a similar fashion, and in contrast with FGF-2, it was found that a high GlcNS 6-*O*-sulfate content is required for specific HS interactions with FGF-1 and FGF-7 [Kreuger et al., 1999].

It has also been suggested that HS species can differ depending on the stage of development of the tissue [Nurcombe et al., 1993], as HS harvested from murine neuronal tissues at embryonic day 9 appears to preferentially bind FGF-2, while HS from embryonic day 11 favors FGF-1 binding; the temporal pattern of binding reflects the expression pattern of the ligand it activates. This suggested that developing cells might "fish" for the correct combination of growth factors needed to trigger the next phase of development using HS as "bait." If this were true, it should theoretically be possible to use the HS synthesized at different developmental stages to help determine the combination of factors needed to drive it through the next phase of maturation.

The importance of HS in mediating cell responses has also been shown with heparinase/heparitinase and sodium chlorate treatments (Fig. 2). Heparinase cleaves HS chains into inactive disaccharide and tetrasaccharide components that have been shown to inhibit FGF-2-mediated smooth muscle cell (SMC) proliferation in injured carotid arteries [Kinsella et al., 2004]. Such experiments have been part of a realization that HS domains that remain incompletely sulfated can become potent inhibitors of the interactions they would normally promote. Several in vitro studies have also cultured cells in media supplemented with chlorate to study the role of sulfated GAGs in different cell types [Rapraeger et al., 1991]. Sodium chlorate ($NaClO_3$) imparts its effects by inhibiting ATP-sulphurylase, the first enzyme in the synthesis of 3′-phosphoadenyl

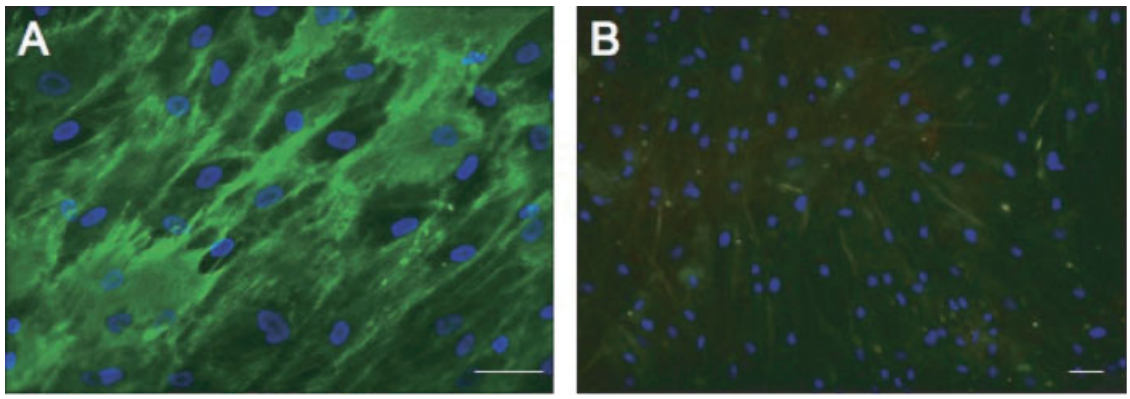

Fig. 2. Mesenchymal progenitor cell cultures express abundant heparan sulfate (HS) when expanded ex vivo, as revealed by staining with the HS-specific 10E4 antibody (the monoclonal antibody 10E4, recognizes an epitope that contains *N*-unsubstituted glucosamine, is commonly used to trace HSPGs) (**A**). This binding is lost when cultures are pre-treated with heparanase, a matrix-degrading enzyme that cleaves HS side-chains from the core protein (**B**). Cell nuclei have been stained with DAPI. Scale bar = 10 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

5′-phosphosulfate (PAPS), a high-energy sulfate donor in biological reactions [Klaassen and Boles, 1997]. The mitogenic response of cells to FGF-2 is inhibited when cells are cultured in the presence of 30 mM $NaClO_3$; *N*-sulfation of HS is unaffected by treatment with $NaClO_3$, whereas significant reductions in 2-*O* and 6-*O* sulfation are observed [Safaiyan et al., 1999]; such data have helped established the idea that HS does not only bind a ligand for delivery to its cognate receptor, but must also itself bind to the receptor, in reactions that involve 6-*O*-sulfate moieties [Guimond and Turnbull, 1999], in order for signal transduction to initiate [McKeehan et al., 1998].

Thus, although the exact roles of HS in the signaling complex have been reasonably well characterized, it is yet to be definitively established that this trimeric model extends to all the other HS-dependent systems. What is known is that defects in HS can cause the complete loss of FGF, Hedgehog, and Wingless/Wnt signaling pathways and lead to severe abnormality, including bone abnormality, during embryonic development [Paine-Saunders et al., 2000; Koziel et al., 2004; Shimo et al., 2004; Song et al., 2005; Yabe et al., 2005]. HS, of course, binds multiple species of not only growth factors, cytokines and chemokines, but also morphogens, such as Wnt and Sonic Hedgehog [Dhoot et al., 2001], most of the large glycoproteins in the ECM [Perrimon and Bernfield, 2000], let alone a suite of proteases and synthetic enzymes. The role of HS thus may be to allow extremely precise and specific molecular interactions to occur at the right times and places during maturation and regeneration. As such, PGs, and HS in particular, have been styled as catalysts of molecular encounter [Lander, 1998]. Certainly the heterogeneity seen in HS chains during development may reflect the multiple interactions being regulated at each developmental stage [Nurcombe et al., 1993]. One advantage of such tripartite systems is that a particular responsiveness (i.e., intracellular signaling) is not selected by levels of a growth factor or its receptors but by the targeting of a growth factor to a particular receptor by a specific motif on the HS chain [Chang et al., 2000].

NOVEL CONTROL OF GROWTH FACTOR SIGNALING

Among the HS-binding factors known to be important to progenitor cells are a suite of mitogens that include the PDGFs, the VEGFs, the FGFs, BMPs 2, 4, and 7, their receptors, as well as TGF-β1, collagen I, laminin, and fibronectin, together with cell–cell mediators, such as the CAMs and cadherins [Ruoslahti and Yamaguchi, 1991; San Antonio et al., 1994; Perrimon and Bernfield, 2000; Ornitz and Marie, 2002] (Table I). Among this list, members of the FGF family play a major role in maintaining human embryonic stem (hES) cell self-renewal and pluripotency [Amit et al., 2000; Xu et al., 2005]. The FGF family presently encompasses more than 20 members, of which the prototype members are FGF-1 and FGF-2 that bind to four related receptor tyrosine

TABLE I. Heparan Sulfate and Heparin-Binding Proteins (Incomplete List) [Yayon et al., 1991; Rapraeger, 1995]

Mitogens/morphogens/chemokines	FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, HGF, VEGFs, activins BMPs, TGF-βs, PDGFs, HB-GAM, pleiotropins, GM-CSF, interferon-χ. NT4/5, GDNF, Wnts Hedgehogs
Adhesive glycoproteins	Fibronectin, vitronectin, laminin, collagens, thrombospondin, tenascin, vonWillebrand factor, NCAM, *N*-cadherin
Enzymes	Lipoprotein lipase, hepatic lipase, phospholipase, apolipoprotein B, apolipoprotein E
Serine protease inhibitors	Antithrombin III, heparin cofactor II, protease nexins
Other factors	Superoxide dimustase, elastase, platelet factor 4, N-CAM, transcription factors, DNA topoisomerase, RNA polymerase, tumor necrosis factor stc

kinases (FGFR-1–4) coded by four distinct genes expressed in most cell types [Naski and Ornitz, 1998]. Over the past 10 years, we have been able to confirm seminal findings [Rapraeger et al., 1991; Yayon et al., 1991; Kan et al., 1993; Nurcombe et al., 1993; Brickman et al., 1995; Joseph et al., 1996; Boilly et al., 2000] that FGF interactions with cellular receptors are dependent on HS [Colin et al., 1999].

While not directly responsible for signal transduction, HSPGs have been shown to internalize with FGF from the cell surface, thus suggesting the bioavailability of FGF to FGFRs may be under the control of a dual role of HSPGs, those of immobilization and internalization [Colin et al., 1999]. FGF-specific HS chains have been isolated from embryonic mouse neuroepithelial cells that have potentiating effects specifically for FGF-1 and FGF-2 [Nurcombe et al., 1993, 2000], a differential mechanism that has now been confirmed in fibroblasts, macrophages, and cancer cells [Bernfield et al., 1999]. Furthermore, we used cross-linking to show that specific combination of FGF and HS preparations cause an increase in specific FGFR isoform activity [Nurcombe et al., 2000] and thus effect phenotypic change.

HS AND DEVELOPMENT

During embryonic development, the BMPs are known to exert control over crucial events including mesoderm formation, neural patterning, skeletal development, and limb formation. Tellingly, BMPs were originally isolated using heparin affinity columns, and thus the involvement of HS in the control of BMP-mediated developmental processes, including BMP signaling, has long been suspected [Sampath et al., 1990]. More recently, it has been shown in *Xenopus* embryos that HS/heparin chains are capable of binding to BMP-4 and restricting its expression pattern [Ohkawara et al., 2002]. As well as its agonistic affects on BMP, HS is also known to bind to BMP antagonists, such as noggin, a secreted polypeptide that inhibits the functions of BMPs, resulting in modification of BMP activity [Paine-Saunders et al., 2002]. In addition, mutations of the *Drosophila* gene *dally*, which encodes a *Drosophila* glypican, show abnormalities in the modulation of Decapentaplegic (Dpp), a member of the *Drosophila* TGF-β/BMP superfamily, resulting in altered morphogenesis [Jackson et al., 1997]. Recent genetic studies also support the idea that HS chains are involved in regulating multiple signaling pathways crucial to developmental processes [Nakato and Kimata, 2002]. For example, *Drosophila* mutants with defects in the genes *sulfateless* and *sugarless*, which encode for HS *N*-deacetylase/*N*-sulfotransferase and UDP-glucose dehydrogenase respectively, show abnormalities in Wingless-mediated dorsal/ventral patterning [Lin and Perrimon, 1999]. Another *Drosophila* mutant, with a defect in HS copolymerase (*tout velu*), exhibits abnormal Hedgehog diffusion [Bellaiche et al., 1998]. Furthermore, combined genetic deficiencies for GPC-3 and BMP-4 result in abnormal skeletal development [Paine-Saunders et al., 2000]. GPC-3 deficiency also abrogates BMP-2 and BMP-7-dependent ureteric bud morphogenesis in embryonic kidney explants [Grisaru et al., 2001]. Thus, HS orchestrates the molecular context of BMP bioactivity.

Antagonism between FGF and BMP signaling is a feature of development. Recently, it has been shown that introducing inhibitors of BMPs together with FGF-2 into culture medium of hES cells can facilitate the long-term maintenance of these cells in a pluripotent state [Xu et al., 2005]. Hence, the coordinated control of progenitor cell proliferation, as prescribed by the balance in FGF- and BMP-mediated signaling, imposes a primary control over subsequent

cell fate decisions. Thus, it is possible to posit that FGF-specific HS will promote the proliferation of progenitor cells, prevent their exit from the cell cycle, and contribute to maintenance of a progenitor/*naive* precursor cell identity. In contrast, it is possible to predict that BMP-specific HS should have little effect on cell proliferation, but instead would coordinate with FGF to control the initial onset of differentiation.

HS AND CELL PHENOTYPE

Until quite recently there was a consensus that the fate of adult stem cells was restricted to their tissues of origin. However, the recent spate of findings that adult stem cells could be "re-programmed" to express genes typical of differentiated cell types across all three of the mesodermal, endodermal, and ectodermal lineages when fused to cells in heterokaryons has been both informative and provocative [Brazelton et al., 2000; Mezey et al., 2000; Blau et al., 2001]. This degree of plasticity demonstrated that the differentiated state might be reversible, requiring continuous prompting to maintain the correct balance of differentiating factors needed. More recently, it has been shown that progenitor cells can assume diverse fates under physiologic conditions. Bone marrow cells can be made to yield not only all cells of the blood, but also cells bearing a liver phenotype [Petersen et al., 1999]. Muscle-derived and CNS-derived progenitor cell-like populations have also been reported that can reconstitute the blood and so rescue lethally irradiated mice [Mezey et al., 2000; Vescovi et al., 2001]. Recently, data from our lab demonstrated that adult neural progenitor-derived HS was able to apparently "transdifferentiate" primary, vimentin-expressing pre-osteoblasts into a neuronal-like phenotype that expressed MAP2 [Chipperfield et al., 2002]. When looking at progenitor cell niches, it is striking that all contain laminins, either in the form of a basal lamina (BL) in contact with the progenitor or supporting cells, or as a non-BL matrix secreted by the supporting cells, such as the bone marrow stroma [Fuchs et al., 2004]. Where laminin is expressed, HS follows.

HS and Neural Progenitors

Integrin β1-expressing neural progenitors in both embryonic and adult cerebral cortex are in intimate contact with HS-rich BL. In the embryonic cerebral cortex, stem cells are present in the periventricular niche [Cohen et al., 1986] whereas in the adult individual neural progenitor cells are contacted by extensions protruding from the blood vessels in the subventricular zones [Parati et al., 2004]. Several growth factors, including LIF, EGF, and FGF-2, are capable of triggering the proliferation of multipotent progenitor cells in vitro [Gage et al., 1995; Carpenter et al., 1999; Palmer et al., 1999]. The exact combination or cascade of growth factors necessary for this process seems to be largely determined by the stage of differentiation [Hogg et al., 2004]. FGF-2 appears to be crucial for the continued proliferation of cells that have reached the stage of being committed to a neural fate [Palmer et al., 1999] and different doses of this factor appear to trigger different phenotypes from the progenitor pool [Qian et al., 1998].

Developing neuroblasts project axons over long distances in order to reach their final synaptic targets. HS has been shown to alter the characteristics of neurite outgrowth from neurons in vitro [Bandtlow and Zimmermann, 2000]. The leading growth cone detects and responds to both the attractive and repulsive environmental cues that guide them; these include contact-mediated cues acting over short distances or secreted molecules acting over comparatively longer distances [Tessier-Lavigne and Goodman, 1996]. Axonal reactions depend on intracellular state of the growth cone, differential expression of receptor complexes, and cross-talk between intracellular signaling cascades. Axon guidance molecules can bind to GAG chains in vitro: netrins were originally purified using heparin affinity columns [Serafini et al., 1994], and the netrin receptor DCC also binds to GAG chains in vitro [Bennett et al., 1997]. The Slits, which also modulate neuritic branching and neuronal cell migration [Wang et al., 2005] are also HS-binding, although it is not known if their receptors, the Robos, are. The large family of Eph tyrosine kinases, receptors for the ephrins, are apparently modulated by HS [Wilkinson, 2001], and other signals which could potentially be modulated by HS include the semaphorins, a family of membrane-associated proteins, members of which are capable of mediating both repulsive and attractive cues [Raper, 2000].

Perhaps the most compelling evidence for HS involvement in the development of

neuroarchitecture is from amphibians. Addition of HS to the developing *Xenopus* optic pathway causes severe targeting errors [Walz et al., 1997], and inhibiting sulfation of endogenous HS with chlorate causes axons to bypass their target, the tectum [Irie et al., 2002]. Experiments with purified heparin saccharides show that bypass-inducing activity correlates with distinct structures, particularly those containing a combination of 2-*O*- and 6-*O*-sulfate groups. The results indicate that specific HS sequences, rather than gross structural composition, are critical for activity. In situ hybridization revealed differential staining patterns for HS synthetic enzymes in the region of the tectal synaptic targets, where HS 6-*O*-sulfotransferase is regionally expressed along the border of the projecting dorsal optic tract whereas 2-*O*-sulfotransferase is expressed more widely. It is now known that attractive guidance cues can be converted to repulsive ones by the extracellular matrix; thus, HS may similarly be involved in the modulation of synaptic targeting signals. Interestingly, along these lines, netrin-1 can be converted from an attractive signal for retinal axons to a repulsive one by laminin [Hopker et al., 1999].

HS and Hematopoietic Progenitors

In a similar manner, the microenvironment created by stromal cells has been used to establish cultured hematopoietic progenitors in the absence of exogenously added cytokines [Sutherland et al., 1989; Sutherland et al., 1991; Verfaillie, 1992; Verfaillie, 1993]. In these studies, up to 50% of the long-term culture initiating cells (LTC-IC) can be maintained for up to 8 weeks when cultured in the presence of media conditioned from stromal cells (stroma-derived soluble factors). Additional studies suggest that soluble PGs, but not glycoproteins, are responsible for this sustained growth [Gupta et al., 1996]. Furthermore, LTC-IC maintenance is largely retained after digestion of the PGs with proteinase K, but is completely abolished following treatment with nitrous acid, which digests an involvement of HSPG. Collectively, these studies implicate a role for HS in the maintenance of hematopoietic progenitors ex vivo. This is perhaps not surprising, considering stromal HS and hyaluronate are partly responsible for binding hematopoietic progenitors to the marrow microenvironment [Siczkowski et al., 1992; Zweegman et al., 2004]. Furthermore, HS has been shown to stimulate megakaryopoiesis [Han et al., 1996], although recent evidence suggests it may also be inhibitory by virtue of its ability to bind hematopoietic progenitors and megakaryocytopoiesis-inhibiting cytokines [Zweegman et al., 2004]. HS has also been implicated in the differentiation of the human promyelocytic leukemia cell line HL-60 [Luikart et al., 1990] through its action of modifying the activity of several growth-regulating factors, in particular the chemokines, macrophage inflammatory protein 1alpha (MIP1alpha) [Stringer et al., 2002; Stringer et al., 2003], and SDF-1 [Netelenbos et al., 2002]. In addition, loss-of-function mutations in the gene encoding the HS proteoglycan GPC-3 result in a selective impairment of the development of the common hematopoietic lineage from which monocyte/macrophages and peripheral blood mononuclear cells are derived [Viviano et al., 2005]. This was the first report of a requirement for HS, and specifically GPC-3, in the lineage-specific differentiation of these cell types in vivo.

HS and Mesenchymal Progenitors

Mesenchymal stem cells are maintained in a local, FGF-rich environment within the bone marrow; this ECM maintains the ability of the stem and progenitor cells to divide asymmetrically in times of quietude, but to rapidly mobilize the proliferation of daughter, transit-amplifying cells during times of rapid growth or tissue repair. This local environment, consisting of the sum of influences provided by support cells and its ECM, contributes to what has become known as the stem cell "niche" [Fuchs et al., 2004]. It has evolved to protect and perpetuate the self-renewing, undifferentiated state of its inhabitants, and to regulate the rate of production of committed progenitors [Marie et al., 2000]. Osteoblasts and adipocytes share a common mesenchymal precursor, whose lineage-specific differentiation is induced by members of the HS-activated BMP family [Irie et al., 2003]. The mechanisms mediating these two mutually exclusive commitment decisions appear to be directly controlled by discrete signaling pathways emanating from the differentially expressed subtypes of the BMP receptors BMPR1A and BMPR1B [Chen et al., 1998]; we consider it highly likely that these receptors are activated by different HS configurations.

Human adult mesenchymal stem cells (hMPC) are routinely expanded in tissue culture utilizing a medium rich in serum, conditions remarkably similar to those used to expand most adherent stromal cell types. Yet despite these culture similarities, the factors that mediate hMSC self-renewal to maintain these *naive* cells as progenitors capable of multilineage differentiation remains unclear. Recent evidence suggests that FGF signaling plays a pivotal role in maintaining the growth, survival, self-renewal, and pluripotency of hES cells [Xu et al., 2005]. The evolutionary conservation of key developmental pathways across germ lines strongly suggests that mechanisms controlling self-renewal and multipotentiality in hMSCs might well share essential features with hES cells.

Our group has previously shown how specific elements of the extracellular matrix within the stem cell *niche*, more particularly HS, can protect, hold, and present FGFs to cells, thus controlling intracellular signaling cascades and ultimately cell fate decisions. More recently, as detailed above, HS has also been shown to be required for BMP signaling, supporting the view that HS orchestrates the transition between proliferation and differentiation. Consequently, tissue-specific HS forms are plausible candidates for the augmention of hMSC self-renewal by potentiating the FGF signaling cascade while retarding BMP signaling. Interestingly, mutations in glycosyltransferases that function as hetero-oligomers in the Golgi to add repeating GAGs to HS chains have been shown to impair chondrocyte differentiation, and perhaps the perichondrial stem cell reserve [Hecht et al., 2005].

HS and Smooth Muscle Cells

Studies on SMCs indicate that the HS species present in the artery wall can regulate the SMC phenotype. HS extracted from the artery wall of normal non-atherosclerotic arteries not only prevents SMC phenotypic change in vitro, but inhibits myointimal thickening when applied to balloon catheter-injured arteries in a periadventitial gel [Bingley et al., 1998]. This HS is active at concentrations below that of heparin, or HS derived from other tissues. The addition of HS-degrading enzymes induces modulation of phenotype in vitro, while enzymes, such as trypsin and chondroitin ABC lyase, which degrade other matrix components, have no effect [Campbell et al., 1992]. Further to this, macrophages added to SMCs in primary culture induce changes in phenotype by degrading HS [Fitzgerald et al., 1999]. This change can be prevented by addition of HS or heparin, but not other GAGs, such as chondroitin sulfate. Such phenotypic modulation is not surprising, given that interactions between negatively charged GAG chains and growth factors are essential for the differentiation of cells during development, and for the maintenance of tissue organization [Prydz and Dalen, 2000].

CONCLUSIONS

The development of an "HS approach" for the control of progenitor phenotype has become more feasible in recent years as our understanding of HS cell biology improves. HSs are particularly attractive, as they potentiate the powerful effects of growth factors on cell recruitment, proliferation, and differentiation, and do not depend on the synthesis of other cofactors or other specific activating agents. Thus, they represent a novel approach to for the control of cell phenotype.

We would anticipate that HS, by virtue of its modulation of the biological activities of the FGFs and the BMPs, offers the possibility of intervening not just in embryonic growth, and stem and progenitor cell self-renewal, but also in stem cell-dependent wound healing and regenerative medicine. Unlike other nucleic acid-based clinical approaches (e.g., virus and siRNA), therapeutic intervention may be particularly advantaged because HSs are chemically stable, naturally occurring non-toxic sugars that can be isolated as biologically active compounds from a range of mammalian species.

Realization of the potential of HS for regenerative medicine will depend, however, on a detailed understanding of their effects on cell self-renewal and differentiation, and of how these effects are linked to downstream signaling cascades. Importantly the mechanisms by which inductive signals control the identity, proliferation, and timing of differentiation of stem and progenitor cells remains poorly understood. We hope in the future to provide evidence that HS plays a central role in the coordinated control of progenitor identity, proliferation, and differentiation by regulating FGF- and BMP-mediated signals.

REFERENCES

Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, Itskovitz-Eldor J, Thomson JA. 2000. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev Biol 227:271–278.

Aszodi A, Bateman JF, Gustafsson E, Boot-Handford R, Fassler R. 2000. Mammalian skeletogenesis and extracellular matrix: What can we learn from knockout mice? Cell Struct Funct 25:73–84.

Bandtlow CE, Zimmermann DR. 2000. Proteoglycans in the developing brain: New conceptual insights for old proteins. Physiol Rev 80:1267–1290.

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.

Bennett KL, Bradshaw J, Youngman T, Rodgers J, Greenfield B, Aruffo A, Linsley PS. 1997. Deleted in colorectal carcinoma (DCC) binds heparin via its fifth fibronectin type III domain. J Biol Chem 272:26940–26946.

Bernfield M, Kokenyesi R, Kato M, Hinkes MT, Spring J, Gallo RL, Lose EJ. 1992. Biology of the syndecans: A family of transmembrane heparan sulfate proteoglycans. Annu Rev Cell Biol 8:365–393.

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.

Bingley JA, Hayward IP, Campbell JH, Campbell GR. 1998. Arterial heparan sulfate proteoglycans inhibit vascular smooth muscle cell proliferation and phenotype change in vitro and neointimal formation in vivo. J Vasc Surg 28:308–318.

Blau HM, Brazelton TR, Weimann JM. 2001. The evolving concept of a stem cell: Entity or function? Cell 105:829–841.

Boilly B, Vercoutter-Edouarta AS, Hondermarcka H, Nurcombeb V, Le Bourhisa X. 2000. FGF signals for cell proliferation and migration through different pathways [In Process Citation]. Cytokine Growth Factor Rev 11: 295–302.

Brazelton TR, Rossi FM, Keshet GI, Blau HM. 2000. From marrow to brain: Expression of neuronal phenotypes in adult mice. Science 290:1775–1779.

Brickman YG, Ford MD, Small DH, Bartlett PF, Nurcombe V. 1995. Heparan sulfates mediate the binding of basic fibroblast growth factor to a specific receptor on neural precursor cells. J Biol Chem 270:24941–24948.

Brickman YG, Ford MD, Gallagher JT, Nurcombe V, Bartlett PF, Turnbull JE. 1998. Structural modification of fibroblast growth factor-binding heparan sulfate at a determinative stage of neural development. J Biol Chem 273:4350–4359.

Burgess WH, Shaheen AM, Hampton B, Donohue PJ, Winkles JA. 1991. Structure-function studies of heparin-binding (acidic fibroblast) growth factor-1 using site-directed mutagenesis. J Cell Biochem 45:131–138.

Campbell JH, Rennick RE, Kalevitch SG, Campbell GR. 1992. Heparan sulfate-degrading enzymes induce modulation of smooth muscle phenotype. Exp Cell Res 200: 156–167.

Carey DJ. 1997. Syndecans: Multifunctional cell-surface co-receptors. Biochem J 327 (Pt 1):1–16.

Carpenter MK, Cui X, Hu ZY, Jackson J, Sherman S, Seiger A, Wahlberg LU. 1999. In vitro expansion of a multipotent population of human neural progenitor cells. Exp Neurol 158:265–278.

Chang Z, Meyer K, Rapraeger AC, Friedl A. 2000. Differential ability of heparan sulfate proteoglycans to assemble the fibroblast growth factor receptor complex in situ. Faseb J 14:137–144.

Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, Rosen V, Mundy GR, Harris SE. 1998. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. J Cell Biol 142:295–305.

Chipperfield H, Bedi KS, Cool SM, Nurcombe V. 2002. Heparan sulfates isolated from adult neural progenitor cells can direct phenotypic maturation. Int J Dev Biol 46: 661–670.

Cohen J, Burne JF, Winter J, Bartlett P. 1986. Retinal ganglion cells lose response to laminin with maturation. Nature 322:465–467.

Cole GJ, Akeson R. 1989. Identification of a heparin binding domain of the neural cell adhesion molecule N-CAM using synthetic peptides. Neuron 2:1157–1165.

Colin S, Jeanny J-C, Mascarelli F, Vienet R, Al-Mahmood S, Courtois Y, Labarre J. 1999. In vivo involvement of heparan sulfate proteoglycan in the bioavailability, internalization, and catabolism of exogenous basic fibroblast growth factor. Mol Pharmacol 55:74–82.

Dhoot GK, Gustafsson MK, Ai X, Sun W, Standiford DM, Emerson CP, Jr. 2001. Regulation of Wnt signaling and embryo patterning by an extracellular sulfatase. Science 293:1663–1666.

Esko JD, Zhang L. 1996. Influence of core protein sequence on glycosaminoglycan assembly. Curr Opin Struct Biol 6: 663–670.

Fisher MC, Li Y, Seghatoleslami MR, Dealy CN, Kosher RA. 2006. Heparan sulfate proteoglycans including syndecan-3 modulate BMP activity during limb cartilage differentiation. Matrix Biol 25:27–39.

Fitzgerald M, Hayward IP, Thomas AC, Campbell GR, Campbell JH. 1999. Matrix metalloproteinase can facilitate the heparanase-induced promotion of phenotype change in vascular smooth muscle cells. Atherosclerosis 145:97–106.

Fuchs E, Tumbar T, Guasch G. 2004. Socializing with the neighbors: Stem cells and their niche. Cell 116:769–778.

Gage FH, Ray J, Fisher LJ. 1995. Isolation, characterization, and use of stem cells from the CNS. Annu Rev Neurosci 18:159–192.

Gallagher JT, Turnbull JE. 1992. Heparan sulphate in the binding and activation of basic fibroblast growth factor. Glycobiology 2:523–528.

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.

Guimond SE, Turnbull JE. 1999. Fibroblast growth factor receptor signalling is dictated by specific heparan sulphate saccharides. Curr Biol 9:1343–1346.

Gupta P, McCarthy JB, Verfaillie CM. 1996. Stromal fibroblast heparan sulfate is required for cytokine-mediated ex vivo maintenance of human long-term culture-initiating cells. Blood 87:3229–3236.

Gustafsson E, Aszodi A, Ortega N, Hunziker EB, Denker HW, Werb Z, Fassler R. 2003. Role of collagen type II and perlecan in skeletal development. Ann N Y Acad Sci 995: 140–150.

Han ZC, Bellucci S, Shen ZX, Maffrand JP, Pascal M, Petitou M, Lormeau J, Caen JP. 1996. Glycosaminoglycans enhance megakaryocytopoiesis by modifying the activities of hematopoietic growth regulators. J Cell Physiol 168:97–104.

Hecht JT, Hayes E, Haynes R, Cole WG, Long RJ, Farach-Carson MC, Carson DD. 2005. Differentiation-induced loss of heparan sulfate in human exostosis derived chondrocytes. Differentiation 73:212–221.

Hogg RC, Chipperfield H, Whyte KA, Stafford MR, Hansen MA, Cool SM, Nurcombe V, Adams DJ. 2004. Functional maturation of isolated neural progenitor cells from the adult rat hippocampus. Eur J Neurosci 19:2410–2420.

Hopker VH, Shewan D, Tessier-Lavigne M, Poo M, Holt C. 1999. Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1. Nature 401:69–73.

Irie A, Yates EA, Turnbull JE, Holt CE. 2002. Specific heparan sulfate structures involved in retinal axon targeting. Development 129:61–70.

Irie A, Habuchi H, Kimata K, Sanai Y. 2003. Heparan sulfate is required for bone morphogenetic protein-7 signaling. Biochem Biophys Res Commun 308:858–865.

Jackson SM, Nakato H, Sugiura M, Jannuzi A, Oakes R, Kaluza V, Golden C, Selleck SB. 1997. dally, a Drosophila glypican, controls cellular responses to the TGF-beta-related morphogen, Dpp. Development 124:4113–4120.

Joseph SJ, Ford MD, Barth C, Portbury S, Bartlett PF, Nurcombe V, Greferath U. 1996. A proteoglycan that activates fibroblast growth factors during early neuronal development is a perlecan variant. Development 122: 3443–3452.

Joyce JA, Freeman C, Meyer-Morse N, Parish CR, Hanahan D. 2005. A functional heparan sulfate mimetic implicates both heparanase and heparan sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer. Oncogene 24:4037–4051.

Kan M, Wang F, Xu J, Crabb JW, Hou J, McKeehan WL. 1993. An essential heparin-binding domain in the fibroblast growth factor receptor kinase. Science 259: 1918–1921.

Kinsella MG, Irvin C, Reidy MA, Wight TN. 2004. Removal of heparan sulfate by heparinase treatment inhibits FGF-2-dependent smooth muscle cell proliferation in injured rat carotid arteries. Atherosclerosis 175:51–57.

Klaassen CD, Boles JW. 1997. Sulfation and sulfotransferases 5: The importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. Faseb J 11:404–418.

Koziel L, Kunath M, Kelly OG, Vortkamp A. 2004. Ext1-dependent heparan sulfate regulates the range of Ihh signaling during endochondral ossification. Dev Cell 6: 801–813.

Kreuger J, Prydz K, Pettersson RF, Lindahl U, Salmivirta M. 1999. Characterization of fibroblast growth factor 1 binding heparan sulfate domain. Glycobiology 9:723–729.

Krufka A, Guimond S, Rapraeger AC. 1996. Two hierarchies of FGF-2 signaling in heparin: Mitogenic stimulation and high-affinity binding/receptor transphosphorylation. Biochemistry 35:11131–11141.

Lander AD. 1998. Proteoglycans: Master regulators of molecular encounter? Matrix Biol 17:465–472.

Lin X, Perrimon N. 1999. Dally cooperates with Drosophila Frizzled 2 to transduce Wingless signalling. Nature 400: 281–284.

Lortat-Jacob H, Turnbull JE, Grimaud JA. 1995. Molecular organization of the interferon gamma-binding domain in heparan sulphate. Biochem J 310(Pt 2):497–505.

Luikart SD, Maniglia CA, Furcht LT, McCarthy JB, Oegema TR, Jr. 1990. A heparan sulfate-containing fraction of bone marrow stroma induces maturation of HL-60 cells in vitro. Cancer Res 50:3781–3785.

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.

Maciag T, Friesel RE. 1995. Molecular mechanisms of fibroblast growth factor-1 traffick, signaling and release. Thromb Haemost 74:411–414.

Marie P, Debiais F, Cohen-Solal M, de Vernejoul MC. 2000. New factors controlling bone remodeling. Joint Bone Spine 67:150–156.

Martin GR, Timpl R, Kuhn K. 1988. Basement membrane proteins: Molecular structure and function. Adv Protein Chem 39:1–50.

McKeehan WL, Kan M. 1994. Heparan sulfate fibroblast growth factor receptor complex: Structure-function relationships. Mol Reprod Dev 39:69–81; discusison 81-82.

McKeehan WL, Wang F, Kan M. 1998. The heparan sulfate-fibroblast growth factor family: Diversity of structure and function. Prog Nucleic Acid Res Mol Biol 59:135–176.

Merry CL, Wilson VA. 2002. Role of heparan sulfate-2-O-sulfotransferase in the mouse. Biochim Biophys Acta 1573:319–327.

Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. 2000. Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow. Science 290:1779–1782.

Nakato H, Kimata K. 2002. Heparan sulfate fine structure and specificity of proteoglycan functions. Biochim Biophys Acta 1573:312–318.

Naski MC, Ornitz DM. 1998. FGF signaling in skeletal development [In Process Citation]. Front Biosci 3:D781–D794.

Netelenbos T, Zuijderduijn S, Van Den Born J, Kessler FL, Zweegman S, Huijgens PC, Drager AM. 2002. Proteoglycans guide SDF-1-induced migration of hematopoietic progenitor cells. J Leukoc Biol 72:353–362.

Nurcombe V, Ford MD, Wildschut JA, Bartlett PF. 1993. Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. Science 260: 103–106.

Nurcombe V, Smart CE, Chipperfield H, Cool SM, Boilly B, Hondermarck H. 2000. The proliferative and migratory activities of breast cancer cells can be differentially regulated by heparan sulfates. J Biol Chem 275:30009–30018.

Ohkawara B, Iemura S, ten Dijke P, Ueno N. 2002. Action range of BMP is defined by its N-terminal basic amino acid core. Curr Biol 12:205–209.

Ornitz DM. 2000. FGFs, heparan sulfate and FGFRs: Complex interactions essential for development. Bioessays 22:108–112.

Ornitz DM, Marie PJ. 2002. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. Genes Dev 16:1446–1465.

Paine-Saunders S, Viviano BL, Zupicich J, Skarnes WC, Saunders S. 2000. Glypican-3 controls cellular responses to Bmp4 in limb patterning and skeletal development. Dev Biol 225:179–187.

Paine-Saunders S, Viviano BL, Economides AN, Saunders S. 2002. Heparan sulfate proteoglycans retain Noggin at the cell surface: A potential mechanism for shaping bone morphogenetic protein gradients. J Biol Chem 277:2089–2096.

Palmer TD, Markakis EA, Willhoite AR, Safar F, Gage FH. 1999. Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. J Neurosci 19:8487–8497.

Parati EA, Pozzi S, Ottolina A, Onofrj M, Bez A, Pagano SF. 2004. Neural stem cells: An overview. J Endocrinol Invest 27:64–67.

Perrimon N, Bernfield M. 2000. Specificities of heparan sulphate proteoglycans in developmental processes. Nature 404:725–728.

Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. 1999. Bone marrow as a potential source of hepatic oval cells. Science 284:1168–1170.

Princivalle M, de Agostini A. 2002. Developmental roles of heparan sulfate proteoglycans: A comparative review in Drosophila, mouse and human. Int J Dev Biol 46:267–278.

Prydz K, Dalen KT. 2000. Synthesis and sorting of proteoglycans. J Cell Sci 113 (Pt 2):193–205.

Pye DA, Vives RR, Hyde P, Gallagher JT. 2000. Regulation of FGF-1 mitogenic activity by heparan sulfate oligosaccharides is dependent on specific structural features: Differential requirements for the modulation of FGF-1 and FGF-2. Glycobiology 10:1183–1192.

Qian X, Goderie SK, Shen Q, Stern JH, Temple S. 1998. Intrinsic programs of patterned cell lineages in isolated vertebrate CNS ventricular zone cells. Development 125: 3143–3152.

Raper JA. 2000. Semaphorins and their receptors in vertebrates and invertebrates. Curr Opin Neurobiol 10: 88–94.

Rapraeger AC. 1995. In the clutches of proteoglycans: How does heparan sulfate regulate FGF binding? Chem Biol 2: 645–649.

Rapraeger AC, Krufka A, Olwin BB. 1991. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. Science 252:1705–1708.

Ruoslahti E, Yamaguchi Y. 1991. Proteoglycans as modulators of growth factor activities. Cell 64:867–869.

Safaiyan F, Kolset SO, Prydz K, Gottfridsson E, Lindahl U, Salmivirta M. 1999. Selective effects of sodium chlorate treatment on the sulfation of heparan sulfate. J Biol Chem 274:36267–36273.

Sampath TK, Coughlin JE, Whetstone RM, Banach D, Corbett C, Ridge RJ, Ozkaynak E, Oppermann H, Rueger DC. 1990. Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily. J Biol Chem 265:13198–13205.

San Antonio JD, Lander AD, Karnovsky MJ, Slayter HS. 1994. Mapping the heparin-binding sites on type I collagen monomers and fibrils. J Cell Biol 125:1179–1188.

Sanderson RD, Turnbull JE, Gallagher JT, Lander AD. 1994. Fine structure of heparan sulfate regulates syndecan-1 function and cell behavior. J Biol Chem 269:13100–13106.

Serafini T, Kennedy TE, Galko MJ, Mirzayan C, Jessell TM, Tessier-Lavigne M. 1994. The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. Cell 78:409–424.

Shimo T, Gentili C, Iwamoto M, Wu C, Koyama E, Pacifici M. 2004. Indian Hedgehog and syndecans-3 coregulate chondrocyte proliferation and function during chick limb skeletogenesis. Dev Dyn 229:607–617.

Siczkowski M, Clarke D, Gordon MY. 1992. Binding of primitive hematopoietic progenitor cells to marrow stromal cells involves heparan sulfate. Blood 80:912–919.

Song HH, Shi W, Xiang YY, Filmus J. 2005. The loss of glypican-3 induces alterations in Wnt signaling. J Biol Chem 280:2116–2125.

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 1alpha. Blood 100:1543–1550.

Stringer SE, Nelson MS, Gupta P. 2003. Identification of an MIP-1alpha-binding heparan sulfate oligosaccharide that supports long-term in vitro maintenance of human LTC-ICs. Blood 101:2243–2245.

Sutherland HJ, Eaves CJ, Eaves AC, Dragowska W, Lansdorp PM. 1989. Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood 74:1563–1570.

Sutherland HJ, Eaves CJ, Lansdorp PM, Thacker JD, Hogge DE. 1991. Differential regulation of primitive human hematopoietic cells in long-term cultures maintained on genetically engineered murine stromal cells. Blood 78:666–672.

Takada T, Katagiri T, Ifuku M, Morimura N, Kobayashi M, Hasegawa K, Ogamo A, Kamijo R. 2003. Sulfated polysaccharides enhance the biological activities of bone morphogenetic proteins. J Biol Chem 278:43229–43235.

Tessier-Lavigne M, Goodman CS. 1996. The molecular biology of axon guidance. Science 274:1123–1133.

Turnbull JE, Gallagher JT. 1993. Heparan sulphate: Functional role as a modulator of fibroblast growth factor activity. Biochem Soc Trans 21:477–482.

Verfaillie CM. 1992. Direct contact between human primitive hematopoietic progenitors and bone marrow stroma is not required for long-term in vitro hematopoiesis. Blood 79:2821–2826.

Verfaillie CM. 1993. Soluble factor(s) produced by human bone marrow stroma increase cytokine-induced proliferation and maturation of primitive hematopoietic progenitors while preventing their terminal differentiation. Blood 82:2045–2053.

Vescovi AL, Galli R, Gritti A. 2001. The neural stem cells and their transdifferentiation capacity. Biomed Pharmacother 55:201–205.

Viviano BL, Silverstein L, Pflederer 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.

Walz A, McFarlane S, Brickman YG, Nurcombe V, Bartlett PF, Holt CE. 1997. Essential role of heparan sulfates in axon navigation and targeting in the developing visual system. Development 124:2421–2430.

Wang B, Zhang N, Qian KX, Geng JG. 2005. Conserved molecular players for axon guidance and angiogenesis. Curr Protein Pept Sci 6:473–478.

Wilkinson DG. 2001. Multiple roles of EPH receptors and ephrins in neural development. Nat Rev Neurosci 2:155–164.

Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. 2005. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. Nat Methods 2:185–190.

Yabe T, Hata T, He J, Maeda N. 2005. Developmental and regional expression of heparan sulfate sulfotransferase genes in the mouse brain. Glycobiology 15:982–993.

Yanagishita M, Hascall VC. 1992. Cell surface heparan sulfate proteoglycans. J Biol Chem 267:9451–9454.

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.

Zweegman S, Van Den Born J, Mus AM, Kessler FL, Janssen JJ, Netelenbos T, Huijgens PC, Drager AM. 2004. Bone marrow stromal proteoglycans regulate megakaryocytic differentiation of human progenitor cells. Exp Cell Res 299:383–392.