Proteoglycans in stem cells

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Abstract.

The remarkable promise of pluripotent and multipotent stem cells (SCs) imparts tremendous optimism for advancement of regenerative medicine, developmental biology, and drug discovery. Perhaps the greatest challenge is to finely direct, control, and command their differentiation. As those processes are managed on many levels, including genomic, transcriptomic, and epigenomic, examination of all of these components will yield powerful tools for manipulation of SCs. Carbohydrates surround all cells, including SCs as a glycocalyx. Of particular interest is the class of carbohydrates known as proteoglycans (PGs), which are a diverse group of glycoconjugates consisting of core protein with one or more glycosaminoglycan (GAG) chains attached. They are primarily located in the extracellular matrix as well as at cell surfaces, where they are bound or anchored to the membrane through their core proteins. GAG chains are linear, anionic, and highly heterogeneous carbohydrates consisting of repeating disaccharides. PGs facilitate interaction of cells with the extracellular environment by interacting with chemokines, growth factors, and other signaling molecules. Core proteins are involved in many signaling pathways, both individually, as well as through attached proteins via GAG-mediated interactions. These essential and accessible functions make PGs an excellent target for manipulating SCs and guiding their fate. Studying the role of PGs in cell development will yield valuable insight into the mechanism of SC differentiation and suggest approaches toward directing those pathways. Such studies may also help identify valuable markers for distinguishing between various cell populations during differentiation.

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Abbreviations: SC, stem cell; ESC, embryonic stem cell; PG, proteoglycan; ECM, extracellular matrix; HSC, hematopoietic stem cell; miRNA, micro RNA; Wnt, wingless-int; TGF, transforming growth factor; BMP, bone morphogenic protein; FGF, fibroblast growth factor; GAG, glycosaminoglycan; CS, chondroitin sulfate; DS, dermatan sulfate; KS, keratan sulfate; HA, hyaluronic acid; HS, heparan sulfate; HP, heparin GlcA glucuronic acid; GlcNAc, *N*-acetylglucosamine; GlcNS, *N*-sulfoglucosamine; GalNAc, *N*-acetylgalactosamine; Ext, exostose; SLRP, small leucine-rich proteoglycan; BM, basement membrane; EGF, epidermal growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; PK, protein kinase; GPI, glycosylphosphatidylinositol; NSC, neural stem cell; LeX, Lewis X antigen; SSEA, stage-specific embryonic antigen; HNK, human natural killer; FGFR, fibroblast growth factor receptor.

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1. Introduction

Stem cells (SCs) are a powerful tool in regenerative medicine. Their ability to give rise to many cell types can be harnessed to regenerate tissues that have been injured or lost in devastating diseases, including Parkinson's, diabetes, multiple sclerosis, and numerous others. Currently, SCs are isolated from embryos, as well as fetal and adult tissues, and can be differentiated into a multitude of cell types, including cardiomyocytes, neurons, hepatocytes, and insulin-producing cells [1–3].

Stem cells are also invaluable in research areas such as drug discovery and developmental biology. Cells derived from SCs can serve as models to develop drugs against specific targets in tissues of interest. Their use offers advantages over primary tissues, immortalized tumor cells, or genetically transformed cells because they can give rise to an unlimited number of uniform and genetically natural cells that can be used for

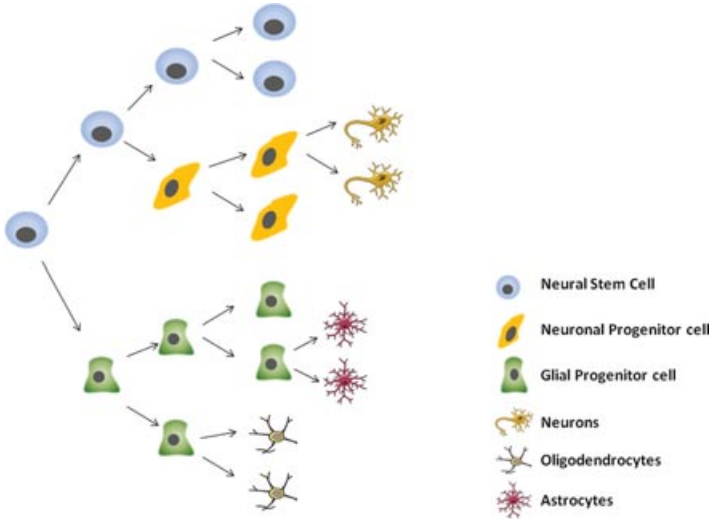

Fig. 1. Self-renewal and differentiation potential of stem cells. Stem cells are capable of symmetric (production of two stem cells) as well as asymmetric (production of a stem cell and a progenitor cell) division. On the other hand, tissue stem cells primarily differentiate through progenitor cells, which cannot undergo asymmetric division.

drug target identification [4]. Embryonic stem cells (ESCs) can be used to study the role of various developmental genes. As an example, genes of interest can be modified in the ESCs, and development can be monitored in knockout mice [5],[6]. Various effective methods for modification of the human ESC (hESC) genome have been proposed, and are in fact well suited for these purposes [5],[7].

The SC state (nondifferentiated vs. differentiated) is controlled on many levels, including genomic, proteomic, transcriptomic, and epigenomic. Although all of these levels have been studied in increasing detail, cellular glycomics, a subdivision of epigenomics, has received considerably less attention. Glycomics is the study of glycoconjugates, including proteoglycans (PGs), glycoproteins, and glycolipids.

In this review, we will summarize the established role of glycans in SC fate determination and self-renewal and detail their effect on different aspects of SC state regulation. We focus on perhaps the most prominent class of glycans, the PGs, which are primarily found on the cell surface and in the extracellular matrix (ECM). PGs are known to interact with various growth factors and are critically involved in diverse signaling pathways. Knowledge of PGs in SCs has many potential applications, some as simple as establishing markers for SC derivatives, and others as expansive as regulating and directing SC fate.

2. Stem cells

An SC can give rise to different cell types and can self-proliferate (Fig. 1). SCs can be embryonic, fetal, or adult. ESCs are isolated from the inner cell mass of preimplanted embryo and represent a pluripotent cell type that can give rise to cells from all three germ layers of the body. Embryonic germ SCs are also pluripotent and are isolated from primordial germ cells of the gonadal ridge and beget the gonads. Adult and fetal SCs are unspecialized cells located within specialized tissues and give rise to the cells of that particular tissue. They are limited in regard to the cell types to which they have the capability to differentiate into, and hence are defined as possessing multipotency. Certain adult SCs can give rise to relatively few or only one cell type of a specific tissue and are considered oligopotent and unipotent, respectively. Recently, it was shown that adult SCs from one tissue can give rise to cell types from another tissue, which can be generated from the same or a different germ layer. This property is known as plasticity [7]. Examples include hematopoietic stem cells (HSCs), which can give rise to all blood cell types in addition to liver cells [8],[9] that are also of mesodermal origin. HSCs also can give rise to neurons [10], which are ectodermal in origin. Adult tissue SCs can proliferate throughout the life of the organism. They usually give rise to progenitor cells, which are nondifferentiated cells within a given tissue. Progenitor cells are "committed" and ultimately yield terminally differentiated (primary) cells. During division, progenitor cells can produce only two progenitor cells or two specialized cells. SCs, on the contrary, can divide with the potential to yield one SC along with one progenitor cell [7] (Fig. 1). Tissue replenishment occurs primarily through progenitor cells, as tissue SCs are normally slow to proliferate in the absence of additional stimulating signals. Known sources of adult SCs are bone marrow, blood, brain, skin, lining of the gastrointestinal tract, pancreas, skeletal muscle, liver, cornea, and retina [7].

3. Mechanisms controlling SC state

Stem cells possess the extraordinary potential to self-renew or differentiate into other cell types. The state of SCs (nondifferentiated vs. differentiated) is determined by the intricate orchestration of cell-intrinsic and cell-extrinsic signals filtered through a large number of signaling pathways (Fig. 2). The primary mechanisms to control SC state have been studied in nonhuman organisms such as *Caenorhabditis elegans, Drosophila melanogaster*, and *Mus musculus*, with growing attention focused on human cell-based research.

3.1. Cell-intrinsic regulation of SC fate

Stem cell state is directed on several levels, including regulation by transcription factors [11–13], their cofactors [13–18], chromatin structure regulators [19–22], and noncoding RNAs [23–28]. Several transcription factors have been established as key players in controlling the self-renewal of pluripotent ESCs, which comprise the "core regulatory circuitry." Oct4, Nanog, and Sox2 [11] regulate the expression of many genes required for self-renewal, as well as suppression of genes that are required for fate commitment (Fig. 3). They function together to modulate their own expression in addition to the transcription of many protein-coding RNAs [11–13] and noncoding RNAs [23] involved in maintaining the pluripotency of ESCs. Oct4 has been given special attention because of the broad range of genes it oversees. For example, Oct4 positively regulates expression

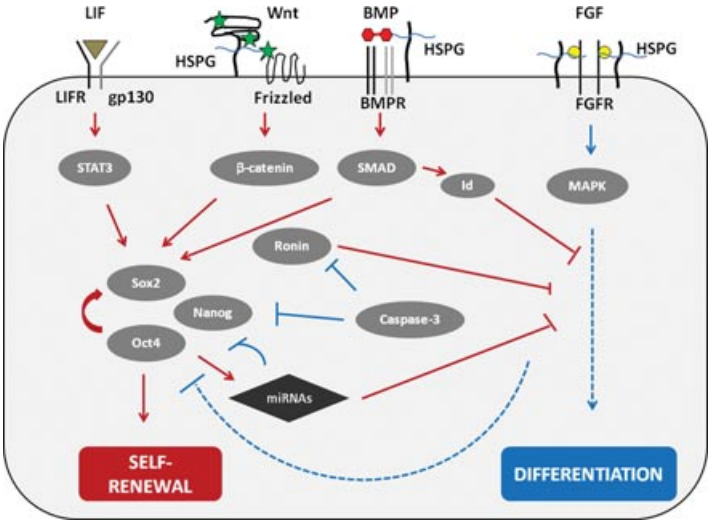

Fig. 2. Signaling pathways involved in regulation of mouse embryonic stem cells pluripotency. Stem cell fate, influenced by a complex interplay of intrinsic and extrinsic signals, is transduced through multiple interconnected pathways. LIF, Wnt, BMP, and FGF are of primary importance for mouse embryonic stem cell fate coordination. LIF, Wnt, and BMP signaling support pluripotency, whereas FGF signaling works in opposition. Wnt, BMP, and FGF signaling are mediated by heparan sulfate proteoglycans.

of histone-modifying enzymes such as Jmjd1a and Jmjd2c (histone 3 lysine 9 demethylases), and this regulation provides a mechanism to de-repress genes required for pluripotency [22]. Oct4 can also upregulate the chromatin modifier polycomb repressive complex 2 to suppress the activity of fate-commitment genes [19–21].

Core regulatory circuitry cooperates with other transcription factors such as Sall4, Tcf3, Smad1, Stat3, Esrrb, Klf4, Klf2, Klf5, E2f1, n-myc, Zfx, and Ronin to maintain ESC self-renewal [29–33]. One of the primary transcription factors that work in concert with Oct4 is the proto-oncogene c-myc [34]. The c-myc protein binds to promoter sequence and facilitates RNA elongation by stimulation of the transcriptional pause release [34],[35], thereby promoting transcription of genes activated by the core regulatory circuitry.

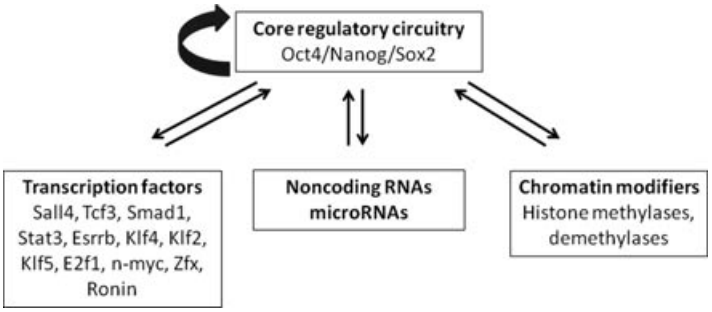

Fig. 3. Mechanisms of controlling stem cell state by core regulatory circuitry. Core regulatory circuitry, consisting of the Oct4/Nanog/Sox2 transcription factors, maintains stem cell pluripotency by activating self-renewal genes and suppressing lineage-specific genes. In addition to regulating its own expression, regulatory circuitry also exploits the noncoding RNA network and chromatin modifiers to fine-tune expression of pluripotency genes.

Many actively transcribed genes in SCs are populated with transcription cofactors, which contribute to gene expression regulation by bridging the interaction between required transcription factors without actually binding to DNA themselves [33]. ESCs are sensitive to reduced levels of cofactors such as p300 [13] and mediator [14–16]. Cohesin, in concert with mediator, physically brings together the enhancer and promoter sites of genes, a requirement for transcriptional initiation [17],[18]. DNA modifications, such as methylation, have also been shown to be important to SC state. SCs lacking DNA methylases would undoubtedly be deficient in differentiation due to an inability to suppress pluripotency genes [36],[37].

The micro RNA (miRNA) network assumes an active role in maintenance of the SC state. Oct4/Nanog/Sox2 facilitates self-renewal of SCs through miRNA in various ways. They activate genes coding for miRNAs, which in turn are involved in "fine tuning" of self-renewal genes and clearing the transcripts of fate-commitment genes [23–26]. Oct4/Nanog/Sox2 suppress the miRNAs involved in expression of lineage-specific genes, while simultaneously suppressing miRNA, which inhibit pluripotency genes [23],[27],[28]. Longer chain noncoding RNAs have been shown to recruit and stabilize the polycomb repressive complexes required to quench expression of fate-commitment genes [38–40].

Adult SC fate regulatory mechanisms are essentially the same as in pluripotent SCs. Transcriptional regulatory networks and epigenetic control ensure proper SC state. Expression of lineage-specific genes is inhibited at the transcriptional or translational level by transcription factors, RNA-binding proteins, and chromatin methylation. In *C. elegans* germ-line SCs, Fbf-1 and Fbf-2 RNA-binding proteins repress translation of factors responsible for entry into the meiotic cycle [41]. In mammalian systems, the ATF5 transcription factor [42],[43], SoxB1 family proteins [44],[45], the nuclear receptor Tailless, and the nuclear coreceptor N-coR [46],[47] are important in maintaining self-renewal of neural stem cells (NSCs) by attenuating the expression of lineage-specific genes. Differentiation toward astrocytes is also suppressed by DNA methylation of astrocyte-specific genes, such as *Gfap* [48]. In HSC lineages, specific genes can influence alternative fates. It has been proposed that mutual inhibition of these genes might be one way to prevent differentiation [49],[50].

3.2. Cell-extrinsic regulation of SC fate

Stem cells sense changes in the environment and react to it by altering their state. Most SCs reside within specialized microenvironments called niches [51]. The niche contains cells and molecules that facilitate SC maintenance and function and is involved in delivering the local and long-range signals that

regulate SC state and cell-cycle status [52]. In SCs, signals are transduced to the genome through the relevant signaling pathways. The niche also provides physical anchorage for SCs. As observed in *C. elegans* [41],[52] and *D. melanogaster* [53–55], displacement of SCs from the niche environment often leads to their differentiation as they no longer receive self-renewal signals from the niche.

In mouse ESCs, leukemia inhibitory factor, wingless-int (Wnt), and transforming growth factor-β/bone morphogenic protein (TGF-β/BMP) signaling pathways have been shown to be important for maintenance of pluripotency [56–60] (Fig. 2). Transcription factors associated with those pathways (Stat3, Tcf3, and Smad1) have been shown to contact the core regulatory circuitry directly and in this way transduce signals into cells [13],[30],[61–63]. BMP4 has also been proposed to suppress differentiation through induction of *Id* gene expression [60]. Fibroblast growth factor (FGF) signaling is associated with differentiation in mouse ESCs [64].

Human ESCs require FGF2 and the activin/nodal signaling pathways for self-renewal [65–68]. It has been shown that activin-mediated SMAD2/3 directly activates *NANOG* in hESCs to maintain the pluripotent state [69],[70]. In mammalian HSCs, the chemokine CXCL12 and glycoprotein angiopoietin-1 are required factors to maintain self-renewal [71–73] and quiescence [74], respectively. It has been proposed that the niche for HSC is created by bone-lining endosteal cells and/or perivascular cells in bone marrow. Regulator molecule release has also been associated with some of those cells, but the precise mechanism governing the way those cells regulate HSC state remains undefined [75].

To summarize, the extracellular environment, in collaboration with intracellular events, regulates SC fate. PGs, which primarily reside on the cell surface and in the ECM, interact with various growth factors, and these factors establish PGs as ideal candidates for SC niche creation and signal transduction events.

4. Proteoglycans

A PG is a core protein having one or more glycosaminoglycan (GAG) chains that are covalently attached through serine residues. They are primarily located in the ECM and on the cell surface, but are also found intracellularly (Fig. 4). PGs play important roles in cell migration and adhesion and also bind to various morphogens, cytokines, chemokines, and growth factors [76–80]. They stabilize or modulate receptors required for cell viability, development, and morphogenesis [81]. GAGs are linear, highly charged, acidic carbohydrates with a repeating disaccharide unit. On the basis of the structure of the repeating disaccharide, GAGs can be divided into following four classes: heparan sulfate/heparin (HS/HP), chondroitin sulfate/dermatan sulfate (CS/DS), keratan sulfate (KS), and hyaluronic acid (HA) [82]. In this review, we will concentrate on the role of chondroitin sulfate proteoglycans (CSPGs)/dermatan sulfate proteoglycans (DSPGs), and the heparan sulfate proteoglycans/heparin proteoglycans (HSPGs/HPPGs) in SC self-renewal and lineage commitment.

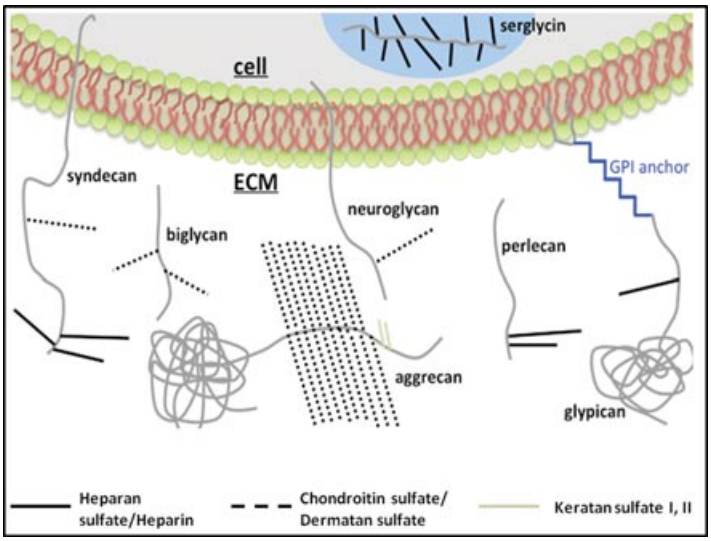

Fig. 4. Distribution of cellular proteoglycans. Proteoglycans are widely distributed on the cell surface, where they can associate via a transmembrane domain or GPI-anchoring, and inside of secretory granules. They are also abundant in the extracellular matrix. Intracellular proteoglycans primarily have storage functions, whereas extracellular and membrane-associated proteoglycans are involved in cell adhesion and migration processes as well as transduction of signals generated by growth factors, morphogens, and cytokines. Adapted from Ly et al. [82].

4.1. Structure and biosynthesis of GAGs

Chondroitin sulfate and DS GAGs consist of *N*-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA) or iduronic acid (IdoA). This basic structure can be modified through the additions of sulfo groups at positions C4 and C6 of GalNAc and C2 of GlcA/IdoA. HS/HP chains are composed of repeating disaccharide units of *N*-acetylglucosamine (GlcNAc)/*N*-sulfoglucosamine (GlcNS) and GlcA/IdoA. This structure can be modified through the addition of sulfo groups at C6 and C3 of the GlcNAc or GlcNS residue and C2 of the GlcA/IdoA residue (Fig. 5).

Chondroitin sulfate/DS and HS/HP PGs share the initial steps of biosynthesis, which start in the endoplasmic reticulum where xylose is covalently attached to the serine residue of a core protein. Next, two galactoses are added to the xylose, followed by GlcA addition. At this point, the biosynthetic pathways diverge. The exostose (Ext)-like-3 enzyme directs biosynthesis toward HS/HP, by adding GlcNAc to the chain, whereas GalNAc transferase drives biosynthesis toward CS/DS by transferring GalNAc onto the chain. HS/HP chain polymerization is catalyzed by exostose-1 and exostose-2 (Ext-1 and Ext-2), which add GlcNAc and GlcA, respectively. The growing chain receives further modification through the introduction of sulfo groups in various positions and epimerization of GlcA into IdoA. Those processes are mediated by sulfotransferases (*N*-deacetylase/*N*-sulfotransferase, 2-*O*-sulfotransferase, 6-*O*-sulfotransferase, and 3-*O*-sulfotransferase) and the GlcA

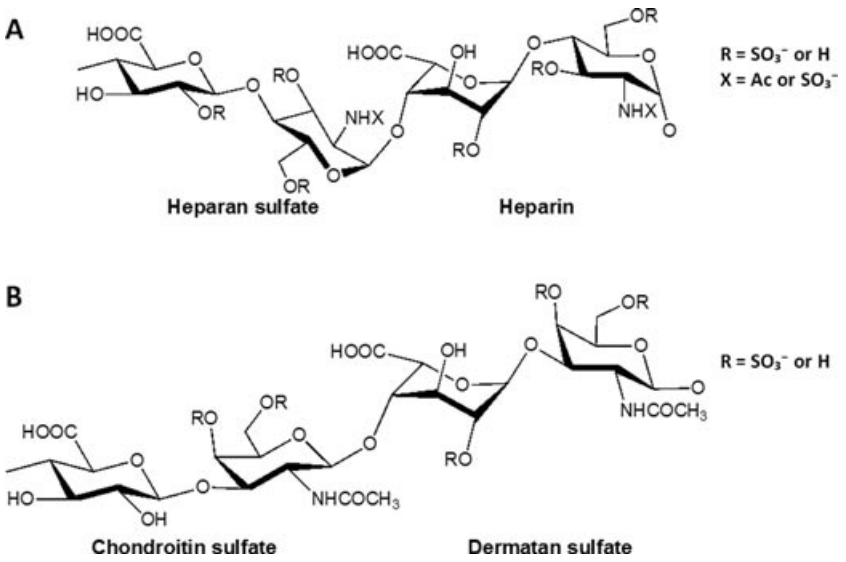

Fig. 5. Disaccharide composition of the GAG chain of heparan sulfate/heparin and chondrotin sulfate/dermatan sulfate proteoglycans. (A) Heparan sulfate/heparin disaccharide composition. Heparin chains largely consist of fully sulfated repeating disaccharides of N-sulfoglucosamine and iduronic acid. HS disaccharide primarily contains low sulfated N-acetylglucosamine and glucoronic acid. (B) Chondroitin sulfate/dermatan sulfate disaccharide composition. CS/DS chains are constructed of disaccharides of galactosamine and glucoronic acid, whereas DS consists of galactosamine and iduronic acid.

C5-epimerase, which converts GlcA to IdoA. The HS chain is highly heterogeneous because of the fact that these modifications are not always completed and result in highly modified sections of HS alternating with less highly modified sections, which creates domains within the chain. HP is a more highly sulfated version of the HS chain consisting primarily of IdoA. HS/HP biosynthetic enzymes have various isozymes, which have distinct temporal and spatial distribution. Chain elongation and modification of CS/DS is regulated by β3GlcA transferase and β4GalNac transferase, GlcA C5-epimerase, 4-*O*-sulfotransferase, 6-*O*-sulfotransferase, and 2-*O*-sulfotransferase. GlcA C5-epimerase, a distinct enzyme from the HS/HP biosynthetic pathway epimerase, is required for DS synthesis [81].

4.2. PG classes

On the basis of their distribution, PGs can be classified into ECM, membrane-bound, and intracellular PGs (Table 1). ECM PGs can be separated into small leucine-rich proteoglycans (SLRPs), aggrecan (hyalectin) family PGs, and basement membrane (BM) PGs. SLRPs, which include decorin, biglycan, lumican, and

Table 1
Distribution and properties of common proteoglycans [81]

Proteoglycan localization	Proteoglycan class	Proteoglycan name	Number and type of GAG chain attached	Core protein size (kDa)
Extracellular matrix	Small leucine-rich PGs	Decorin	1 CS	36
		Biglycan	1–2 CS	38
	Aggrecan family	Aggrecan	~100 CS	208–220
		Versican	12–15 CS	265
		Neurocan	1–2 CS	145
		Brevican	0–4 CS	96
	Basement membrane	Leprecan	1–2 CS	82
		Perlecan	1–3 HS	400
		Agrin	1–3 HS	200
		Collagen type XVIII	2–3 HS	147
Membrane bound	Transmembrane	NG2	2–3 CS	251
		CD44	1–4 CS	37
		Betaglycan	1 CS/1 HS	110
		Syndecans 1–4	1–3 CS/ 1–2 HS	31–45
	GPI anchored	Glypicans 1–6	1–3 HS	~60
Intracellular		Serglycin	10–15 Heparin/CS	10–19

fibromodulin, have leucine-rich repeats and cysteine clusters on *N*-termini, and can carry CS, DS, and KS chains. They are known to be involved in multiple signaling pathways driven by TGF-β, BMP, toll-like-receptors, receptor tyrosine kinases, and insulin-like growth factor-like receptors [83]. The aggrecan family includes four members: aggrecan, neurocan, versican, and brevican. All four can bind HA, carry CS chains, and can sometimes carry KS chains. Aggrecan, a relatively large PG with many GAG chains, represents the major cartilage PG. Neurocan, versican, and brevican affect different aspects of neuronal morphogenesis [81]. BM PGs, along with various proteins and glycoproteins, make up the complex layer of the ECM that separates the epithelium and endothelium from underlying connective tissue. Major BM PGs are collagen XVIII, perlecan, agrin, and leprecan [81],[84]. Perlecan, one of the largest single chain polypeptides in the cell with a molecular weight of $\sim$400 kDa, is a modular protein, wherein the modules interact with different growth factors and proteins involved in lipid metabolism and adhesion [85] (Table 1). Perlecan and collagen XVIII, a member of the multiplexin family [86], modulate the activity of numerous growth factors and the angiogenic processes [84], in addition to contributing to cellular homeostasis [87]. Agrin plays a role in neuromuscular junction activity by aggregating acetylcholine receptors [88] and has also been proposed to be important in renal ultrafiltration [89]. Leprecan has been shown to be involved in notochord development in tunicate *Ciona intestinalis* [90].

Membrane-bound PGs can be transmembrane (syndecans, betaglycan, NG2 CSPG, neurophilin-1, CD44, and phosphacan) or glycosylphosphatidylinositol (GPI) anchored (glypicans). Transmembrane PGs are type I proteins, which have a small cytoplasmic, single-span transmembrane domain in addition to extracellular domains with HS and/or CS chains attached [91]. Although the primary function of transmembrane PGs is associated with their GAG chains, some transmembrane PGs have functions independent of their GAG chains [91],[92]. The primary function of transmembrane PGs is linked to cell adhesion and migration processes [93], as well as mediating the function of metalloproteases [94]. They can be "full-time PGs," such as syndecans and NG2, with GAG chains continuously attached, or "part-time PGs," such as betaglycan, neurophilin-1, and CD44 [93], which can function without the GAG chain. Phosphacan is found in three alternatively spliced forms, one of which is present in the ECM, the other two of which are of the protein–tyrosine phosphatase type of transmembrane receptor [81]. Betaglycan is referred to as a "TGF-β type III receptor," as it binds various members of the TGF-β family and associates with TGF-β type I and type II receptors [95]. CD44 is a vertebrate receptor for HA and coreceptor for epidermal growth factor (EGF) and hepatocyte growth factor (HGF) [96]. NG2 CSPG carries one CS chain, is expressed in vascular mural cells and pericytes, and is a marker for oligondendrocyte progenitors. It interacts with type V and VI collagen, FGF2, and platelet-derived growth factor (PDGF)-AA [97].

Each member of the syndecan (1–4) family has a distinct temporal and spatial distribution. They have been implicated in the same pathways as different growth factors such as FGF, vascular endothelial growth factor (VEGF), TGF-β, and PDGF [91]. The extracellular domains of syndecan-1 and syndecan-3 carry HS and CS chains, whereas syndecan-2 and syndecan-4 carry only HS chains. In addition to the extracellular domain, the cytoplasmic tail of syndecans plays an important role in interactions affecting cell migration and adhesion. The cytoplasmic domain consists of three regions, each of which possess unique functionality, such as interaction with cytoskeletal proteins, activation of protein kinase Cα (PKCα), or interaction with PDZ proteins involved in intracellular targeting and trafficking. The transmembrane domain is engaged in dimerization of syndecans [98]. It has been shown that syndecan-4 activation of PKCα requires phosphatidylinositol 4,5-bisphosphate interaction with a specific region of the cytoplasmic tail [99],[100]. Cell adhesion and stress fiber formation and spreading are processes affected by this signaling. Syndecans are closely associated with metalloproteases, can act as their substrates prior to shedding [101], and can also mediate their activity [102]. Shed syndecans can compete with ligand binding to the cell surface PGs [91].

Glypicans are HSPGs that are located on the cell surface and are connected to the external side of the membrane through the GPI anchor. There are six members in this family in mammals. Unlike syndecans, with HS chains at the distal end of the protein, glypicans have HS chains that are attached to the end of the protein that is proximal to the membrane. Glypican activity has been linked to important processes in development and morphogenesis, such as the regulation of FGF, Hedgehog, Wnt, and BMP signaling [103]. Glypican-3 has a galvanizing effect on Wnt signaling by facilitating and stabilizing the ligand–receptor interaction [104]. The same mechanism is proposed for its effect in FGF signaling [105]. In Hedgehog signaling, glypicans have an inhibitory effect by binding to the Hedgehog protein and competing with the patched receptor [106]. Glypicans have also been linked to the uptake of polyamines [107]. Like syndecans, glypicans can be shed into the extracellular environment.

Serglycin is the major PG present in secretory granules of mast cells, which carry HO and oversulfated CS chains [108],[109]. Serglycin functions in granules as a storage unit for basic molecules such as proteases [110] through the interaction with serglycin's negatively charged HP chains. After release from the granules, PG partners can detach from the PG or remain associated [111]. In the latter case, the PG can present the bound partner to the HP-binding molecules [112] or can modulate the activity of bound components. This is exemplified in the interaction of HP with the antithrombin serine protease, enabling that complex to have a higher affinity to thrombin [113], which is required for the anticoagulation process. It has also been suggested that PGs might play a role in trafficking of the PG–partner complex as well as protecting partners from inhibitors [111].

5. PGs in SCs

Proteoglycans function in signal transduction and reside in prominent locations in the ECM and at the cell surface. Some SCs are anchorage dependent and their fate depends on extrinsic signals. Thus, it is easy to conclude that PGs are important in determining SC fate. In the following section, we review the

known functions of CSPGs, DSPGs, and HSPGs in ESC and tissue SCs, the majority of which were discovered from the study of nervous system SCs.

5.1. Chondroitin sulfate and DSPGs in SCs

Chondroitin sulfate and DSPGs have been found in several tissue SC types, including NSCs and skeletal muscle SCs, where they interact with various mitogens and morphogens and play an important role in SC niche creation and signal transduction. Many CSPGs have been identified in NSCs and neural progenitor cells. Neurocan, phosphacan, and neuroglycan C have been found in the rat ventricular zone of the telencephalon, with additional tenascin, aggrecan [114], and NG2 [115] in experiments with neurospheres that consist of NSC and neural progenitor cells.

Tenascin C is expressed in the mammalian embryonic central nervous system, and is important in regulating the sequential transitions of neurogenesis. NSCs deficient in tenascin C delay in transition to an EGF-responsive state. The proposed mechanism suggests that tenascin C mediates stimulatory FGF signaling and inhibitory BMP4 signaling: requirements for transitioning NSCs to the EGF-responsive state that is a prerequisite for proper neurogenesis and gliogenesis. Cells deficient in tenascin C are primarily differentiated into a neuronal fate rather than glial [116]. Tenascin C was also shown to be important in proper hematopoiesis [117]. The versican-like PG, designated DSD-1-PG, was isolated from postnatal mouse brain. It is expressed in early astrocytes and oligodendrocytes and carries CS/DS chains. It promotes neurite outgrowth on neurons at different stages of neurogenesis in rats [118].

NG2 CSPGs have been linked with progenitor cells in several tissues, such as oligodendrocyte progenitors in the central nervous system [119], as well as chondroblasts and osteoblasts in skeletal tissue [120]. In the mature central nervous system, NG2-expressing glial cells can give rise to oligondendrocytes [119]. Some NG2-expressing cells have the ability to give rise to both neurons and oligodendrocytes, and thus are considered to be multipotent NSCs [121]. This property was confirmed experimentally when NG2 was observed in neurospheres obtained from rat telencephalon [115]. NG2-positive cells from postnatal hippocampus can differentiate into neurons capable of propagating action potential [121]. NG2-positive cells from the postnatal subventricular zone possess migratory ability and can differentiate into both glial cells and neurons in the olfactory bulb, hippocampus, and striatum. NG2-positive cells from the cortex, cerebellum, and olfactory bulb have limited migratory ability and give rise to glia only in striatum and subcortical white matter [122]. Taken together, these data suggest the existence of distinct populations of NG2-expressing cells within the central nervous system with varying differentiating capacities. One possible mechanism of NG2 functionality might be through FGF2 and PDGF-AA signaling, as the NG2 core protein has been demonstrated to have high affinity for those growth factors [123]. The Olig2 transcription factor is involved in the regulation of NG2 expression, as it has been shown that mice deficient in Olig2 fail to produce NG2-positive cells at the embryonic and perinatal stages [124].

NG2 PGs have also been associated with epithelial and hair follicle SCs. NG2 PGs have marked chronological and spatial allotment during skin development. In the early stages of development, NG2 expression has been detected in all layers of the skin such as subcutis (adipose), dermis, the outer root sheath of hair follicles, and basal keratinocytes of the epidermis. Later in development, depending on the hair growth cycle, NG2 is mainly expressed in the bulge region of the hair follicle, dermal papillae, or the outer root sheath. The NG2 null mouse has a thinner epidermis and subcutis compared with wild type, which is explained by the observed reduction in keratinocyte and adipocyte proliferation [125].

Chondroitin sulfate proteoglycans have also been associated with stage-specific embryonic antigen-1 (SSEA-1), which is also called Lewis X (LeX) because the antigenic epitope contains a disaccharide fucose *N*-acetyl lactosamine structure [114]. In the nervous system, SSEA-1 can use different molecules as carriers, including CSPGs [126]. This association is affiliated with nondifferentiated and progenitor cells, such as embryonic cells and adult NSCs and neural progenitor cells [127]. However, its expression has also been reported in other mature cells such as primary sensor neurons in quail [128]. SSEA-1 function is associated with cell adhesion and compaction of the embryo at the morula stage. SSEA-1 may also be involved in Wnt and FGF8 signaling, as its expression is observed in proliferative-dense regions of the brain, which requires functionality in these signaling pathways. In the later stages of mouse cell neurogenesis, SSEA-1 is expressed in the fraction of cells that will have glial progeny [127]. In humans, SSEA-3 and SSEA-4, which have a different oligosaccharide structure from SSEA-1, are expressed [129], although little is known about their function or respective carrier molecules. Another CSPG-carried molecule in the nervous system is the human natural killer-1 (HNK-1) antigen, also known as CD57 [130]. It is expressed in avian [131] and rodent [132] neural crest cells, which migrate from the neural fold to different locations during embryonic development [133] and can give rise to various cell types, including sensory neurons, autonomic neurons, Schwann cells, and smooth muscle cells [134],[135]. HNK-1 antigen can be carried by different molecules, including aggrecan. Despite being products of the same gene locus [130], this aggrecan form is different from cartilage aggrecan in that it lacks KS chains. Experiments with mice deficient in HNK-1 antigen display defective spatial memory formation, suggesting its importance in synaptic plasticity of the hippocampus [136].

Certain SLRPs have also been shown to be important in myogenesis. Adult muscle regeneration happens as a result of the activity of satellite cells residing at the surface of the basal lamina of muscle fibers. Satellite cells are committed muscle cell progenitors. Under normal conditions they are quiescent, but when muscle tissue is injured they are activated and differentiate into myocytes to repair or revitalize muscle tissue. There are several signaling pathways, such as FGF2, TGB-β, and HGF, involved in the maintenance of satellite cell homeostasis [137],[138]. Decorin and biglycan, along with betaglycan, modulate this signaling by competing with TGF-β receptors for TGF-β [139]. Decorin and biglycan exploit the same mechanism to

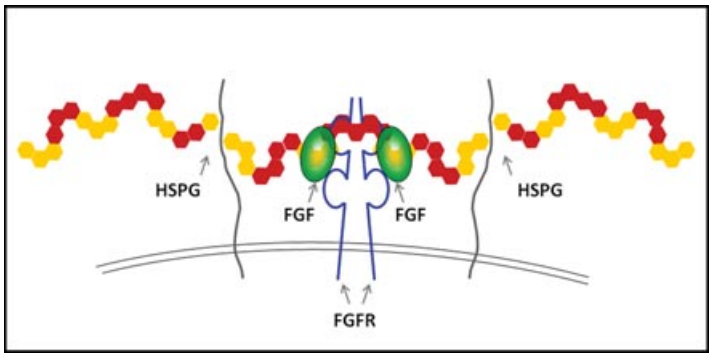

Fig. 6. Heparan sulfate mediated FGF signaling. HS is involved in many developmental processes via FGF signaling, where it mediates the interaction of the FGF receptor with its ligand. A model of FGF signaling, which requires 2 FGF molecules, 2 FGF receptors, and 2 heparan sulfate molecules (2:2:2) for proper signaling, is presented here. Adapted from Ibrahimi et al. [141].

regulate proliferation and survival of bone marrow stromal cells, which are mesenchymal SCs. Bone marrow stromal cells can give rise to different cell types, including osteoblasts, chondrocytes, and adipocytes [140].

5.2. HSPGs in stem cells

The primary effect of HSPGs stems from their requirement as cofactors and mediators for many mitokines and morphogens, including FGF, VEGF, PDGF, and BMP. One of the best characterized is the interaction of HS with FGF and FGF receptor (FGFR). It is proposed that HS mediates interaction of FGF with FGFR and the stoichiometry of this interaction is 2:2:2 [141] (Fig. 6). HSPGs have been primarily associated with neurogenesis and myogenesis. Perlecan, glypican-4, and syndecan-1, syndecan-3, and syndecan-4 are among those that have been identified and associated with SCs.

Glypican-4 and syndecan-1 are highly expressed in the developing mouse brain and localize to the ventricular regions where neural precursor cells reside. When HS synthesis is restricted, FGF2-mediated proliferation is inhibited, confirming the role of HSPG in FGF2 signaling [142]. Glypican-4 has also been shown to be upregulated in rat neural precursor cells but downregulated in more mature and terminally differentiated neurons [143].

Syndecan-3 is another HSPG important for brain development and of particular importance is its HS chain. Ext1-null mice, which are deficient in HS, do not undergo proper brain morphogenesis, which is in turn linked to FGF8 signaling. Neuronal proliferation is reduced, and as a result the cerebral cortex is thinner than in wild-type mice. Although migration of neurons is not affected, axonal guidance is altered [144]. Syndecan-3 is also involved in regulation of skeletal muscle SC differentiation. It has been shown that syndecan-3 inhibition results in aberration of myoblast fusion and altered fiber structure, although proliferation of myocytes was only slightly affected. Syndecan-3 null mice mislocalize the MyoD transcription factor, which is important for proper muscle cell differentiation. As a result, altered differentiation of myoblasts is observed [138]. Syndecan-3 was later linked to the Notch signaling pathway and was subsequently proposed to be required for proper Notch processing. Both syndecan-3 and Notch are required to maintain satellite cell self-renewal. It has also been shown that HS chains, as well as the core protein portion of syndecan-3, are important in Notch interaction [145]. Syndecan-3 also mediates Hedgehog regulation of chondrocyte proliferation [146].

Syndecan-4 has been shown to be important in neural induction in the *Xenopus laevis* embryo. Inhibition of BMP signaling is insufficient for directing ectodermal cells toward a neuronal fate. Syndecan-4 can modulate this process through two signaling pathways: FGF/ERK and PKCα. Syndecan-4 modulates FGF signaling through GAGs attached to its external domain, whereas its intracellular domain is involved in PKCα signaling [147].

In addition to ECM maintenance, BM PGs play an important role in cell survival, motility, and tissue morphogenesis through their interaction with growth factors and surface receptors [84]. Perlecan has five modules, which are involved in binding to growth factors such as FGF2, VEGF, and PDGF [148],[149]. As signaling pathways involving those growth factors are important for embryonic development and tissue morphogenesis, it was hypothesized that perlecan should play a role in those processes [84]. Indeed, one of the phenotypes of *Hspg2* (the mammalian gene coding for perlecan) mutant mice is an altered pattern of chondrocyte proliferation during cartilage development. This might be a result of an abolished interaction between perlecan and FGFs, which is required to control the amount of available FGF to interact with FGFR [84]. Mutations in the *trol* gene, which codes for perlecan and interacts with Hedgehog in *D. melanogaster*, cause cell-cycle arrest in the larval brain, and the addition of FGF2 rescues this phenotype [150],[151]. Taken together, these interactions highlight the importance of perlecan in SC proliferation and differentiation, and one mechanism is the mediation of FGF signaling.

Endostatin is an endogenous antiangiogenic protein that is formed from another BM PG, collagen XVIII, as a result of proteolytic cleavage of the NC1 fragment [84]. Endostatin inhibits endothelial cell migration and proliferation [84], and has been shown to affect the expression of STAT1 and STAT3 [152], which are important regulators of core regulatory circuitry in the SC state [153]. Endostatin, in a time- and dosage-dependent manner, suppresses expression of proteins Id1 and Id3 [152], which play important roles in SC self-renewal by inhibiting differentiation [60].

Serglycin has historically been associated with hematopoietic cells, but its expression was detected in Tal-1 null mouse ESCs and embryoid bodies, which could not produce blood cells. Treatment with retinoic acid, a neurogenesis-directing morphogen [154], elevates expression of serglycin. Interestingly, the core protein of serglycin was modified upon retinoic acid treatment. Taken together, these

data suggest that the serglycin core protein might not be involved in hematopoiesis [155], but rather in neurogenesis.

5.3. Role of the structure of the GAG chain on SCs

The majority of the work shows that PGs primarily affect SCs through their GAG chains with their core protein having only a secondary impact. The HS chain is required for SCs to exit self-renewal and commit to certain lineages; one of them was shown to be neurogenesis [156]. Null *Ext1* ESCs fail to differentiate. This effect is linked to alterations in HS-mediated FGF signaling, which in turn has a negative effect on *Nanog* [157]. Oct4 and Sox2, in addition to Nanog, are vital factors in maintaining the SC undifferentiated state [11].

The importance of the GAG chain in fact goes beyond its essential requirement. Its composition and sulfation pattern have also been shown to be indispensible for proper regulation of many signaling pathways leading to SC fate determination. ESCs express simple, *N*-sulfo HS [156]. Upon differentiation toward embryoid bodies, the total amounts of HS and CS increase in tandem with an elevated level of 6-*O*-sulfo groups [158]. Augmented levels of *N*-, 2-*O*-, and 6-*O*-sulfo groups accompany differentiation toward a neuronal fate [156]. The presence of 3-*O*-sulfo groups in the HS chain has been shown to be important in differentiation toward hemangioblasts, which are precursors for hematopoietic and endothelial cells [159],[160]. Verifying the expression level of the HS biosynthetic enzymes began the progression toward establishing the change in the level of sulfation of the HS chain [156],[158]. A striking observation is that the increased level of *N*-sulfo groups does not drastically affect the distribution of domains along the HS chain; rather, it escalates sulfation within previously sulfated domains and transition zones [156]. Taken together, these results suggest that modification of HS domains during development might be a viable mechanism for the cell to interact with various growth factors and morphogens at different stages of development. Another intriguing result is that certain configurations of HS interact with certain receptors, as was demonstrated in the case of FGFRs. HS expressed in neural precursor cells does not interact with FGFR3, whereas it does interact with FGFR1 [161].

Unlike HS, there are relatively few studies linking the structure of CS and DS to SC fate. It has been shown that the sulfation pattern of CS did not affect the transduction of extrinsic signals required for pluripotency in mouse ESCs [162]. Although expression of CS is important for FGF2-mediated neural progenitor cell proliferation, the overall structure of the CS chain does not affect proliferation [115]. Levels of 2-*O*-sulfo groups and 4-*O*-sulfo groups in the CS chain increase upon differentiation of mESCs toward embryoid bodies [158].

Analyses of PGs upon SC differentiation have been performed along a limited number of lineages, such as neurogenesis, myogenesis, and hematopoiesis. One of the growth factors mediated by PGs is HGF, which requires GAG to signal through its c-Met receptor [163]. HGF is also required for differentiation of SCs toward hepatocytes [164], which suggests a role for PGs in directing SCs to the hepatic lineage, but there is no direct evidence for this.

6. Conclusion

The ability of SCs to self-renew and differentiate into various cell types can have applications in many areas, such as regenerative medicine, developmental biology, and drug discovery. SC research has its own unique challenges, such as controlling SC state (differentiated vs. nondifferentiated), directing differentiation into target lineages, and isolating of cell populations of interest postdifferentiation, which is vital for regenerative tissue engineering. Comprehensive knowledge of the myriad mechanisms described above will yield critical tools to control and direct SC fate. Understanding the cellular glycome in the context of the genome, proteome, and transcriptome is an important step toward understanding and regulating SC fate decisions. PGs are involved in numerous signaling pathways, and in this way can mediate SC self-renewal and differentiation processes. Current information about the role of PGs in SCs comes primarily from neural and skeletal muscle SC studies. Investigating the role of PGs in other lineages can establish them as important molecules for modifying SC state and target molecules for development of therapeutic molecules to disrupt signaling pathways that are unfavorable. High-throughput analysis, coupled with modification of the SC genome and use of synthetic PGs, provides a unique opportunity to examine (even combinatorially) the impact of different intracellular and extracellular PGs on SC fate and reveal key signaling pathways underlying the differentiation process. PGs are an essential part of the SC niche, and they can be used to produce synthetic substrates to grow and manipulate SCs. PGs are located both within the ECM and on the cell surface; the latter makes them good candidates as markers to isolate various cell populations for biological and clinical studies. Future experiments should elucidate additional markers gained from the knowledge of the role of PGs in differentiating SCs toward various fates.

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