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Review

Chondroitin sulfate-cell membrane effectors as regulators of growth factor-mediated vascular and cancer cell migration☆

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ABSTRACT

Background: Glycosylation is a multi-step post-translational enzymatic process which enhances the functional diversity of secreted or membrane proteins and is implicated in physiological and pathological conditions. Chondroitin sulfate (CS) chains are glycosaminoglycan chains, consisting of disaccharide units of glucuronic acid and *N*-acetylgalactosamine, attached to proteins as part of proteoglycans.
Scope of Review: The existing knowledge on glycosylation by CS (CS glycanation) of cell membrane proteins and receptors, such as syndecans, chondroitin sulfate proteoglycan 4, betaglycan, neuropilin-1, integrins and receptor protein tyrosine phosphatase β/ζ, is summarized and the importance of CS glycanation in growth factor-induced migration, angiogenesis and tumor growth and invasion is described.
Major Conclusions: Identification of glycosylation so far used to be a means of further characterizing and categorizing proteins and receptors. Although there is a significant amount of information regarding the interaction of growth factors with CS chains, very little information exists on the core proteins involved. It is now evident that there is more than meets the eye regarding the addition of glycans.
General Significance: Future effort should focus on characterizing CS glycanation of membrane proteins and receptors of interest in an attempt to elucidate its contribution in fine-tuning growth factor-induced signaling. This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

1. Introduction

Glycosylation is increasingly recognized as a common and biologically significant site-specific post-translational protein modification, usually associated with secreted or membrane proteins. It is a complex, multi-step enzymatic process carried out by glycosyltransferases that use activated sugar donors and are found in almost every living organism. Protein glycosylation is a feature that enhances the functional diversity of proteins and impacts on a plethora of functions, such as protein folding, intracellular trafficking, protein localization, degradation, signaling, and cell–cell interactions [1–4]. More than 30 genetic disorders of glycosylation have been described and have been associated with forms of mental disabilities [5], muscular dystrophies [6] and many other pathologies [4,7–9], strengthening the notion that protein glycosylation in humans is important for physiological functions and involved in pathologies. In line with this, modifications of protein glycosylation are a fundamental characteristic of tumorigenesis and are being considered as biomarkers for cancer detection [10–14].

The distinctive chemical properties of carbohydrates are ideal for generating compact units carrying specific information. Variability is achieved by both the glycan primary structure and their ability of branching and twisting. Heterogeneity is inherent to carbohydrates and crucial for their diverse biological roles, and it is interesting to note that between 0.5% and 1% of the transcribed human genome is dedicated to the generation of proteins involved in the biosynthesis, degradation and function of glycoconjugates [15]. Glycans are found attached to proteins as in glycoproteins and proteoglycans, and can be classified as *N*- and *O*-linked glycoproteins, glycosaminoglycans (GAGs), glycosylphosphatidylinositol-anchored proteins and glycolipids.

Chondroitin sulfate (CS) chains are complex, linear polymers consisting of disaccharide units of glucuronic acid and *N*-acetylgalactosamine, synthesized and attached to proteins/scaffolds as GAG chains in order to form proteoglycans [16]. CS chains vary in structure, e.g. length, disaccharide unit arrangement, sulfation pattern, and are classified mainly as motifs O, A, B, C, D and E [16] (Table 1). CS-A and CS-B are common chains in mammalian tissues, whereas CS-D and CS-E are considered rare motifs, although they seem to over-express in pathological conditions, such as cancer growth and metastasis. In recent years, CSs have attracted attention due to their roles in cell adhesion and growth factor mediated-signaling (see below).

In this review existing data on the importance of glycosylation are summarized, with an emphasis on the role of glycosylation by CS (CS glycanation) of cell membrane proteins and receptors in cellular functions related to growth factor-induced cell migration, angiogenesis, cancer progression and cancer cell invasion.

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Table 1
Disaccharide units found in CS chains in vertebrates, classified according to their sulfation patterns. 2S, 4S and 6S represent the 2-O, 4-O and 6-O sulfate groups respectively [16].

Motif	Sequence
O	GlcA-GalNAc
A	GlcA-GalNAc(4S)
B	GlcA(2S)-GalNAc(4S)
C	GlcA-GalNAc(6S)
D	GlcA(2S)-GalNAc(6S)
E	GlcA-GalNAc(4S,6S)

2. Growth factors, glycans and cell migration

The most important angiogenic growth factor is vascular endothelial growth factor (VEGF). VEGF acts through its specific tyrosine kinase receptors VEGFR-1 and VEGFR-2 and promotes both embryonic angiogenesis and postnatal neovascularization. Moreover, VEGF plays a significant role in angiogenesis associated with tumor growth, age-related macular degeneration, diabetic retinopathy and many other angiogenesis-related conditions [17]. VEGFR-2 is synthesized as a 150 kDa protein and rapidly becomes glycosylated to a 200 kDa intermediate form, and then further glycosylated to a mature 230 kDa protein expressed on the plasma membrane [18]. The type of VEGFR-2 glycosylation is largely unknown and the sole information up to date is that VEGFR-2 contains β1,6-*N*-acetylglucosaminyltransferase 5 (Mgat5)-modified *N*-glycans. These are the sites of interaction with galectin-3, a member of the galectin family of β-galactoside binding proteins which preferentially interacts with glycoproteins modified by Mgat5 [19]. The biological significance of VEGFR-2 glycosylation is not clear; it can modulate binding or signaling of VEGF, although conclusive evidence for either possibility is missing. Heparin or heparin-like molecules modulate the binding of VEGF to VEGFRs [20] and decreased VEGFR-2 glycosylation by the antifungal drug itraconazole results in blockade of VEGF binding [21], supporting the notion that glycosylation is playing a role in VEGF binding. Similarly to VEGFR-2, galectin-3 interacts with the epidermal growth factor (EGF) and transforming growth factor β (TGFβ) receptors [22] and another possibility is that glycosylation of growth factor cell surface receptors contributes to their retention on the plasma membrane, thus enhancing intracellular signaling and consequently, cell migration and tumor metastasis [22].

Other growth factor receptors whose extracellular domains contain potential *N*-glycosylation sites and whose *N*-glycans can modulate ligand binding and ligand-mediating functions are EGF, insulin-like growth factor, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and to a lesser extent, TGF receptors [23]. Notch receptors are also covered with a variety of glycans [24] and elucidation of the role of the addition of particular sugars at specific locations on Notch is a challenge for the future.

A large number of recent reports show enhanced expression of the multi-functional protein nucleolin (NCL) on the surface of numerous cells, among which activated endothelial and a wide range of cancer cells. Cell surface NCL acts as a receptor for several extracellular ligands, such as P-selectin, endostatin, tumor necrosis factor α, midkine (MK), pleiotrophin (PTN) and VEGF [25–30], contributing to their effects on cell migration, tumor growth and angiogenesis [26,27,31]. NCL is composed of three structural domains: an N-terminal domain with long stretches of acidic residues, a central domain with 4 RNA recognition motifs, and a C-terminal domain containing repeats of Arg and Gly. Cell surface NCL has been shown to contain 2 lactosaminic *N*-glycans in residues Asn317 and Asn492 of the RNA-binding domain [32] and may also contain two sialylated *O*-glycans [33] among 5 potential sites in the N-terminal domain [32], although the latter has not been proven experimentally. Glycosylation is assumed to be responsible for the cell surface localization of the protein [25,34,35], and use of the *N*-glycosylation inhibitor tunicamycin resulted in the reduction of solely the cell surface and not of the nuclear NCL [34]. It has been also suggested that *N*-glycosylation enhances NCL self-interactions [36] and we have preliminary unpublished observation that it may also affect binding of several angiogenic growth factors. Glycosylated cell surface NCL expression has been correlated with the glioma malignancy grade and is a property which can be used as a marker for the diagnosis and treatment of cancer ([37] and references therein).

3. CS glycanation of cell membrane proteins that affect growth factor actions

3.1. Syndecans

Syndecans are a family of cell surface proteoglycans that consists of 4 members (syndecan-1 to 4) in vertebrates. Each syndecan has a short cytoplasmic domain, a single transmembrane domain, and an extracellular domain with attachment sites for 3 to 5 heparan sulfate (HS) or CS chains. HS attachment sites are located near the N-terminal, while the CS attachment sites are located near the transmembrane region. Syndecans can interact with numerous ligands that contain a heparin-binding motif, and are suggested to act as both adhesion and docking receptors, mediating signal transduction from the extracellular to the intracellular environment or regulating extracellular events, respectively [38,39]. Syndecans therefore play diverse roles in cellular signaling, adhesion, migration, angiogenesis and tumorigenesis [40–42]. Variations in the degree and the type of glycosylation of the extracellular domain of syndecans alter the binding properties of the receptor, a feature which further supports the versatility of this family [43].

It has been long recognized that various combinations of enzymes involved in post-translational HS chain modifications produce unique binding motifs that selectively recognize different proteins. HS chains interact with a large number of proteins, including the heparin-binding growth factors bFGF, VEGF, TGFβ, and PDGF. Furthermore, HS chains facilitate interactions with various extracellular matrix proteins, including fibronectin and plasma membrane proteins, such as antithrombin-1 [40].

The structure and functional role of syndecan CS chains are less clear. Syndecan-1 is a hybrid-type proteoglycan composed of both HS and CS chains [44]. The extent of the CS chain sulfation of syndecan-1 has been shown to be higher than that of the HS chains. The CS chains were mainly composed of CS-A and -C motifs, but also contained a small but appreciable proportion of the CS-E motif [45]. The CS chains of syndecan-4 have been shown to have a higher degree of sulfation compared with syndecan-1 [46]. MK and PTN, which belong to the same, distinct family of heparin-binding growth factors [47,48], but not bFGF, bind to the CS chains of syndecan-4 with higher affinity than to those of syndecan-1 [46], in line with data showing that CS-E has high binding affinity for both MK [49–51] and PTN [49,51,52]. Adhesion of cells to laminin involves binding CS chains of syndecan-1 [53] and more recently, it was shown that the membrane-anchored CS-modified extracellular domain of *Drosophila* syndecan is both necessary and sufficient to mediate proper Slit signaling-dependent axon and myotube guidance during central nervous system development and muscle pattern formation, possibly by concentrating and presenting the ligand (Slit) to the Robo receptors [54]. However, any specific functions the CS chains of syndecans may exhibit in different biological systems or under different circumstances still remain to be clarified.

3.2. Chondroitin sulfate proteoglycan 4

CS proteoglycan 4 (CSPG4), also known as melanoma-associated CS proteoglycan or neuron–glial antigen 2, is a CS proteoglycan consisting of a very large extracellular domain, a transmembrane domain and a relatively short cytoplasmic domain. Although CSPG4 was originally described in the central nervous system, it is expressed by many types of cells in several other tissues, among which the developing vasculature,

where its expression is restricted to vascular mural cells (smooth muscle cells and pericytes) and is absent from the endothelium. CSPG4 is involved in migration, angiogenesis, wound repair and cancer progression. CSPG4 is a cell surface ligand for type VI collagen in glioma cells mediating cell migration [55] and interacts with a galectin-3/$\alpha_3\beta_1$ integrin complex on the endothelial cell surface, resulting in enhanced β_1 integrin signaling, greater endothelial cell motility, enhanced endothelial tube formation in vitro, and dramatically increased blood vessel development in vivo [56,57]. CSPG4 and $\alpha_3\beta_1$ are co-expressed and form a physical complex on the surface of several tumor cell types and CSPG4 has been implicated as a co-receptor for β_1 integrin ligands [58]. CSPG4 knockdown has been shown to reduce melanoma proliferation and increase apoptosis and necrosis, suggesting that CSPG4 may represent a target for cancer therapy [59]. In CSPG4 null mice, corneal angiogenesis induced by ectopic FGF, but not VEGF, implantation is strongly impaired. Similarly, responsiveness of CSPG4-deficient pericytes to paracrine and autocrine stimulation by several FGFs was attenuated, while proliferation induced by other growth factors was not affected. CSPG4 was found to directly bind bFGF in a GAG-independent, core protein-mediated manner and to retain bFGF on the cell surface for subsequent receptor presentation [60].

3.3. Betaglycan

Betaglycan, also known as the TGFβ type III receptor by its ability to bind various members of the TGFβ family, is a HS/CS proteoglycan of >300 kDa. The 92-kDa core protein consists of an extracellular region containing potential sites for GAG attachment and *N*-linked glycosylation, a membrane-spanning region, and a short cytoplasmic tail. Various forms of betaglycan include HS forms, CS forms, forms lacking GAG chains, and the soluble form of betaglycan. It has been shown that TGFβ binds to the core protein of betaglycan, but the GAG chains are not involved in TGFβ binding or in the betaglycan expression at the cell surface [61]. More recently, other TGFβ family members, such as inhibins and several bone morphogenetic proteins, have been also shown to bind to the core protein of betaglycan [61,62], while the HS chains of betaglycan are the sites of bFGF binding [63]. Up to date, there is no information on the functional role of CS chains of betaglycan.

3.4. Neuropilin-1

Neuropilin-1 (NRP1) is a 130 kDa non-tyrosine kinase receptor whose basic structure comprises five domains: 3 extracellular domains, a transmembrane domain, and a short cytoplasmic domain [64]. It was originally discovered as a co-receptor for semaphorin-3A [65] and was later identified as a co-receptor for VEGF [66]. It was recently shown in vitro that NRP1 but not NRP2 can be proteoglycan-modified with either HS or CS on a single conserved Ser residue, suggesting that endogenous NRP1 exists as either an HS or CS proteoglycan, but not as a hybrid proteoglycan [67]. Interestingly, both the degree and length of GAG modification and the predominant side chain added differ between endothelial and smooth muscle cells; GAG addition to NRP1 in endothelial cells enhanced VEGF signaling through VEGFR-2, while in smooth muscle cells it negatively affected VEGF activity. It is noteworthy that the major form of NRP1 in smooth muscle cells is CS-modified and although the functional significance of this modification has not been addressed, it was hypothesized that it may contribute to the function of NRP1 as a decoy receptor, rather than a co-receptor [67]. This hypothesis is also supported by data showing that the highly invasive nature of gliomas correlates, in part, by reduced expression of CS-modified NRP1, suggesting that the balance between CS-modified and unmodified NRP1 might be important for determining invasive potential [68]. Similarly to endothelial and smooth muscle cells, two populations of NRP1 have been detected in diverse human tumor cell lines: one that comprises an *N*-linked glycosylated core protein, and the other that is a high molecular weight species modified by the addition of CS to a single conserved Ser residue [68]. NRP1 also plays a significant role in the stimulation of human vascular smooth muscle cell migration by PDGF-AA and PDGF-BB and in this case, CS modification of NRP1 seems to be required for the migratory effect of PDGF without affecting PDGF signaling [69]. Interestingly, semaphorin-3A was recently found to bind to CS-E units [70], although the protein core(s) involved have not been identified. Further studies are required and will be of interest to identify the in vivo composition of NRP1 GAG chains under several pathophysiological circumstances and to elucidate their possible functional significance.

3.5. Integrins

Integrins constitute a major family of cell–cell and cell–extracellular matrix adhesion proteins involved in intracellular signaling and regulation of cytoskeletal formation [71]. Integrin expression is up-regulated in migratory cells [72] and their activities have been linked to pathological processes such as tumor metastasis [73–75]. Up to date there are 24 identified integrins, each consisting of non-covalently linked α and β subunits. Each subunit is composed of an extracellular domain, a single transmembrane domain, and a short cytoplasmic tail.

Integrin glycosylation is known to be critical for integrin activity by regulating the specificity and affinity of ligand binding to integrins [1]. Although the type of glycosylation among the various integrins playing roles in cancer cell functions has not been elucidated in most of the cases, $\alpha_5\beta_1$ integrin has been characterized in several cell lines as a hybrid-type proteoglycan, containing both HS and CS covalently linked chains in both α and β subunits [76] that plays an essential role in the control of the haptotactic motility of both normal and malignant cells on fibronectin. The proportion of the sulfated subunits varies among the different cell lines [77] and the functional significance of such variation remains to be elucidated.

Integrins are also known to associate with several different proteoglycans, such as syndecans (see also above). For example, in mammary carcinoma and endothelial cells during angiogenesis and tumorigenesis in vitro and in vivo, $\alpha_v\beta_3$ integrin activity is dependent on syndecan-1 [78,79], and syndecan-4 interacts with $\alpha_v\beta_3$ to regulate neuronal Thy-1-induced astrocyte adhesion [80]. However, the involvement of syndecans' HS and/or CS chains in these interactions/functions has not been addressed.

4. Receptor protein tyrosine phosphatases

Receptor protein tyrosine phosphatases (RPTPs) are involved in developmental processes, signal transduction and a number of pathological conditions including cancer. RPTPs consist of one or two highly conserved intracellular phosphatase domains and a variable extracellular domain across subtypes. From the total of 22 RPTPs, which are grouped into 8 subfamilies, only few are subject to glycosylation [81]. Variable glycosylation influences the size, shape and charge of RPTPs and ultimately ligand binding and RPTP-mediated signaling [82]. Leukocyte common antigen-related protein (LAR, type IIa subfamily), RPTP-κ (type IIb subfamily), RPTP-η (type III subfamily), RPTP-γ (type V subfamily), RPTP-ε (type IV subfamily) and RPTP-α (type IV subfamily) contain multiple *N*-linked glycosylation sites, with the latter also containing *O*-linked sites [81,83–85]. Leukocyte common antigen 45 (type I subfamily) is also heavily *N*- and *O*-glycosylated [81,86]. RPTP-β/ζ is the sole RPTP proposed to be expressed as a CS proteoglycan [87].

4.1. Receptor protein tyrosine phosphatases that bind CS

Interestingly, binding of RPTPs to HS or CS can confer opposing results; interaction of RPTP-σ (type IIa subfamily) with HS can mediate axonal growth, while interaction with CS inhibits axonal growth [88–90] and may mediate incomplete remyelination following central

Table 2
Cell membrane CS proteoglycans, type of the CS repeating units, binding molecules and functions attributed to the CS chain.

Protein	CS motif	Molecules binding through CS chains	Function	Function (or postulated function) related to CS
Syndecans	CS-A, CS-C, CS-E [45,46]	PTN [46], MK [46], laminin [53], Slit [54]	Cell adhesion, migration, angiogenesis and tumorigenesis [40]; neurite outgrowth [41]; osteoblast migration [42]	Presenting Slit to its receptors [54]
CSPG4	Unknown	Unknown	Migration, angiogenesis and cancer progression [55–57,59]; binding and presentation of bFGF to its receptors [60]; co-receptor for β1 integrin ligands [58]	Unknown
Betaglycan	Unknown	Unknown	TGFβ presentation [61]; suppression of cancer progression and metastasis [62]; binds inhibin and suppresses activin signaling [62]	Unknown
NRP1	Unknown	Unknown	Co-receptor for semaphorin-3 [65] and VEGF [66]	Negatively affects VEGF signaling in vascular smooth muscle cells, acting as a decoy receptor [67]; inhibitory effect on glioma invasion [68]; required for the migratory effect of PDGF in human vascular smooth muscle cells [69]
$\alpha_5\beta_1$	Unknown	Unknown	Angiogenesis and tumor progression [76,77]	Haptotactic motility of both normal and malignant cells on fibronectin [77]
RPTP-β/ζ	CS-A, CS-C, CS-D and CS-E [49,52,98,113]	PTN [49,51,52,113,118,119], MK [49–51,132,133], VEGF [27], IL-34 [117]	Neurite outgrowth, cell adhesion, migration, angiogenesis, tumorigenesis [48,81]	PTN- and MK-induced neuronal migration [49,52,113]; PTN-induced endothelial cell migration and tube formation on matrigel [103,106]; VEGF-induced endothelial cell migration [27]; IL-34-induced decrease of human glioma U251 cells proliferation, clonogenicity and motility in vitro [117]

nervous system injury [91]. Specific binding of RPTP-σ has been shown for neurocan and aggrecan [92]. It was recently identified that the CS-E motif interacts directly with RPTP-σ and activates signaling pathways involved in inhibiting axon growth [93]. Because the first two Ig domains of LAR and RPTP-δ are close structural homologs of those of RPTP-σ and contain an essentially identical GAG-binding site, it has been speculated that they also bind to both HS and CS proteoglycans during neurogenesis. More recent data support this hypothesis by showing that similarly to RPTP-σ, LAR also plays an important role in regulating inhibition of axonal elongation by functioning as a high affinity receptor for CS proteoglycans. Functional blockade of LAR reverses neurite growth inhibition induced by CS proteoglycans [94]. Besides HS and CS, RPTP-σ also interacts with the glycosylated cell surface NCL in skeletal muscle tissue [95], although the functional significance of this interaction remains unknown.

4.2. Receptor protein tyrosine phosphatase β/ζ

RPTP-β/ζ (type V subfamily) is composed of an extracellular region containing a carbonic anhydrase-like domain, a single fibronectin type III domain and a Ser, Gly-rich domain for CS attachment, a transmembrane region, and an intracellular region that contains two tandemly repeated phosphatase domains, of which only the membrane proximal is catalytically active, and a PDZ-binding motif [47,81]. RPTP-β/ζ exists in 4 isoforms, two transmembrane and two secreted, as a result of alternative splicing. The transmembrane isoforms differ in their extracellular domain: the long isoform is a heavily glycosylated CS proteoglycan, while the short transmembrane isoform is a glycoprotein which lacks 859 residues from the extracellular domain where the sites of glycosylation are. The secreted isoforms, phosphacan and a short isoform of phosphacan, correspond to the extracellular portions of the long and short transmembrane isoforms, respectively [87,96]. Both transmembrane RPTP-β/ζ isoforms and phosphacan have been implicated in several functions [48], while there is no information on the short isoform of phosphacan. The full length form of RPTP-β/ζ has been also detected in a non-proteoglycan form in some tissues [97].

Most of the efforts to identify CS glycanation of RPTP-β/ζ have used its secreted proteoglycan isoform. Phosphacan is composed of CS chains of varying structures [98,99], which seem to be differentially changed during the developmental stages of the brain [16,52,100]. Phosphacan is predominantly composed of CS-C in the early developmental stages, while later it mainly has CS-A chains [52,98]. More recently, RPTP-β/ζ has been presented as a new substrate for *O*-mannosyl glycosylation. An *N*-acetylglucosaminyltransferase has been shown to alter RPTP-β/ζ glycosylation by promoting the addition of the *O*-mannosyl-linked HNK-1 modification in neuroblastoma cells. This was suggested to be mediated by RPTP-β/ζ binding to galectin-1, which then inhibits RPTP-β/ζ phosphatase activity, increases phosphorylation of β-catenin, retains RPTP-β/ζ on the plasma membrane and results in decreased cell adhesion and increased migration [101]. Furthermore, RPTP-β/ζ/phosphacan has been suggested to be hypoglycosylated in a model of muscle–eye–brain disease lacking *O*-mannose β-1,2-*N*-acetylglucosaminyltransferase, a glycotransferase involved in *O*-mannosyl glycosylation [102].

RPTP-β/ζ is abundantly expressed in the brain and numerous cell lines including endothelial and tumor cells [103–106] and is involved in neurite outgrowth, cell adhesion, migration, angiogenesis and tumorigenesis [81]. RPTP-β/ζ is the first RPTP for which a heterophilic ligand, contactin, was reported [107]. Since then, a number of ligands which bind the extracellular region of RPTP-β/ζ have been extensively studied including cell adhesion molecules (e.g. contactin and TAG-1/Axonin-1) and components of the extracellular matrix (e.g. tenascin) [82,108,109]. RPTP-β/ζ has been also proposed as a putative F3 receptor on Schwann cells [110] and as a receptor for VacA, the toxin of the *Helicobacter pylori* [97]. RPTP-β/ζ is a functional receptor for the soluble ligand PTN and was initially found to be involved in

PTN-induced neuronal migration [52], as well as endothelial [103] and several types of cancer cell migration [105,106,111,112]. RPTP-β/ζ is also a receptor for MK-induced migration, survival and tumorigenesis [49,113–116]. More recently, RPTP-β/ζ was identified as an interleukin-34 (IL-34) receptor in human glioma U251 cells (K_d ~ 0.1 μM), mediating the suppressing effect of IL-34 on U251 cells proliferation, clonogenicity and motility in vitro [117].

RPTP-β/ζ is the best characterized PTN receptor to date. It has been shown that binding of PTN to phosphacan involves low (K_d = 3 nM) and high (K_d = 0.25 nM) affinity binding sites [52], which most likely represent sites with different developmentally regulated CS structures which affect phosphacan's affinity for PTN [100] and possibly other ligands. Binding of PTN on phosphacan/RPTP-β/ζ has been found to be strongly inhibited by CS-D and -E, and moderately inhibited by CS-B and -C with subsequent inhibition of the PTN-induced neuronal migration, while CS-A only had a poor effect [49,52,113]. Similarly, CS-C, -D, and -E have been shown to inhibit PTN binding to RPTP-β/ζ and therefore affect the morphogenesis of Purkinje cell dendrites, while CS-A had no effect suggesting that oversulfation of CSs is essential for PTN affinity and PTN-mediated functions [118]. PTN has been reported to bind the extracellular domain of RPTP-β/ζ via CS-E also in glioblastoma cells [119], while CS-C inhibits RPTP-β/ζ-mediated PTN-induced human endothelial cell migration and tube formation in vitro [103].

Binding of PTN to RPTP-β/ζ decreases the tyrosine phosphatase activity of the receptor in glioblastoma cells, thus increasing the tyrosine phosphorylation of β-catenin, its dissociation from E-cadherin and its accumulation in the cytoplasm [119]. PTN-induced oligomerization of RPTP-β/ζ increases tyrosine phosphorylation of the RPTP-β/ζ substrates, G protein-coupled receptor kinase-interactor 1 and membrane associated guanylate kinase, WW and PDZ domain containing 1 [120]. Other downstream targets of the PTN/RPTP-β/ζ signaling are β-adducin [121] and a member of the c-Src family, Fyn [122], all affecting cytoskeletal integrity, adhesion and cell migration. On the other hand, it has been reported that PTN binding to RPTP-β/ζ leads to dephosphorylation and activation of c-Src kinase, focal adhesion kinase, phosphatidylinositol 3-kinase (PI3K), and Erk1/2 [103,123–128]. More recently, it was shown that binding of PTN to RPTP-β/ζ and the subsequent activation of c-Src, lead to β_3 integrin Tyr773 phosphorylation, which is required for PTN-induced cell migration [106]. RPTP-β/ζ interacts with $\alpha_v\beta_3$ integrin on the plasma membrane of endothelial cells and this interaction is required for the RPTP-β/ζ-mediated stimulatory effects of PTN on endothelial and glioma cell migration [106]. In cells expressing RPTP-β/ζ but lacking $\alpha_v\beta_3$, PTN inhibits cell migration [106]; however, the role of RPTP-β/ζ in this inhibitory effect has not been determined. More recently, it has been shown that RPTP-β/ζ-induced c-Src-mediated β_3 Tyr773 phosphorylation is required for PTN- and VEGF-induced endothelial cell surface NCL localization [27,129], which as has been described above is also a prerequisite for the stimulatory effect of both growth factors on cell migration. Interestingly, through RPTP-β/ζ, VEGF induces interaction of VEGFR-2 with $\alpha_v\beta_3$ and affects endothelial cell migration. Binding of VEGF to RPTP-β/ζ is inhibited by PTN [27] and is in line with data showing binding of VEGF to CS chains [130,131].

Similar to PTN, binding of MK on phosphacan/RPTP-β/ζ has been found to be strongly inhibited by CS-D and -E, and moderately inhibited by CS-B and -C with subsequent inhibition of MK-induced neuronal migration, while CS-A had only a poor effect [49]. Arg78 in MK has been identified as the residue that interacts with the CS moiety on RPTP-β/ζ and the oversulfated CSs [49]. MK binding to the extracellular domain of RPTP-β/ζ via CS-E has been suggested to decrease the receptor's tyrosine phosphatase activity [132]. In addition, CS-B and -E, but not -A, -C and -D, have been shown to inhibit MK-induced osteoblast-like cell migration [133]. Migration has been further demonstrated to be mediated by RPTPβ/ζ, which activates c-Src and PI3K [133]. CS-E has been also reported to inhibit MK-induced neuronal cell adhesion [50].

The RPTP-β/ζ CS chains have been recently shown to also affect IL-34 binding. Treatment of solubilized human glioma U251 cell membranes with chondroitinase ABC removed the RPTP-β/ζ-dependent IL-34 binding sites, while preincubation of IL-34 with cartilage CS but not CS-C blocked IL-34 inhibition of IL-34 binding to RPTP-β/ζ. PTN failed to compete for the binding of IL-34 to RPTP-β/ζ, suggesting that IL-34 binding could involve a different CS GAG moiety not recognized by PTN [117]. Binding of IL-34 to RPTP-β/ζ increased tyrosine phosphorylation of focal adhesion kinase, paxillin and G protein-coupled receptor kinase interactor 1/Cool-associated, tyrosine-phosphorylated 1 in U251 cells, similarly to the corresponding effect of PTN [117].

5. Conclusions and perspectives

Many growth factors and cytokines such as PTN, MK, VEGF, FGF, hepatocyte growth factor, monokine-induced by interferon-γ, stromal cell derived factor-1β, heparin-binding epidermal growth factor-like growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor interact with CS chains [130,134]. Among all CS chains, evidence is accumulating on the role of CS-E in cancer growth and invasiveness. CS-E chains and/or *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST) play a crucial role in pulmonary metastasis of Lewis lung carcinoma cells [134] and expression of GalNAc4S-6ST by astrocytic tumor cells is associated with poor patient prognosis, maybe by enhancing CS-E-mediated tumor cell motility in the presence of PTN and/or MK [135]. Moreover, CS-E is found at a

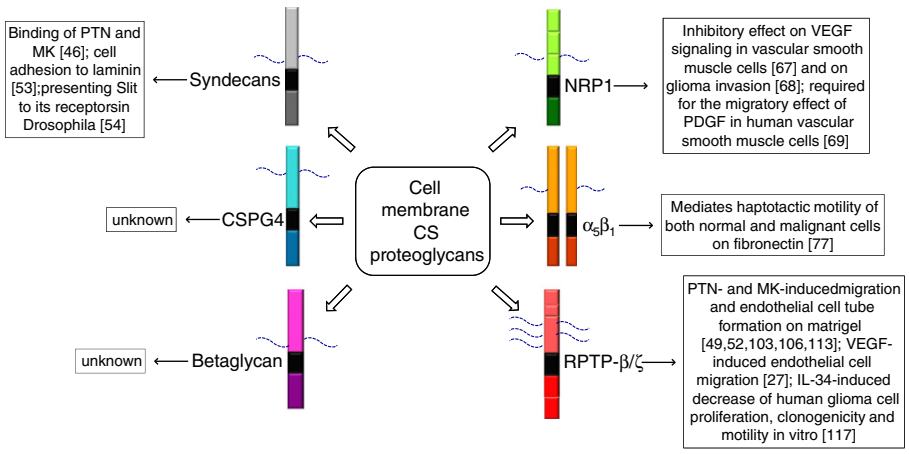

Fig. 1. Schematic representation of the cell membrane proteins that have been found to contain CS chains and the up to date known functions specifically attributed to the CS chain. In most cases, the type of the CS motif, as well as the site(s) of CS glycanation on the cell surface molecules, are unknown (for more details, see text). In all proteins, the transmembrane domain is shown in black.

higher proportion on the surface of the murine osteosarcoma cell line LM8G7 and seems to participate in the invasive ability of LM8G7 cells in vitro and tumor focal formation of LM8G7 cells in the liver in mice [136].

Despite accumulating evidence on the potential role of CS chains in growth factor-mediated cell migration, with implications in both the nervous system, as well as tumor growth, angiogenesis and invasiveness, there are almost no data as to the proteins that carry these functional CS chains and how they co-operate to orchestrate these functions (Table 2, Fig. 1). Most of the existing evidence refers to RPTP-β/ζ, while there are some preliminary data on the existence of CS chains on other proteins, such as NRP1, integrins and syndecans. Moreover, although several cell membrane proteins seem to be glycosylated and glycosylation is implicated in CS-mediated growth factor binding and function (our preliminary observations), no data exist on the type of glycosylation and/or the CS motif involved, as well as on the role it plays in regulating signaling and function. Future effort should therefore focus on identifying CS glycanation of specific cell membrane proteins and receptors and elucidating how it affects growth factor-induced signaling that leads to cell migration, angiogenesis or/and tumor growth and metastasis. The challenge will still be to differentiate between the role of the core proteins and that of their covalently linked CS chains in different physiological or pathological situations.

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