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Proteoglycan Signaling Co–receptors: Roles in Cell Adhesion, Migration and Invasion

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Abstract

Signaling co-receptors are diverse, multifunctional components of most major signaling pathways, with roles in mediating and regulating signaling in both physiological and pathophysiological circumstances. Many of these signaling co-receptors, including CD44, glypicans, neuropilins, syndecans and TβRIII/betaglycan are also proteoglycans. Like other co-receptors, these proteoglycan signaling co-receptors can bind multiple ligands, promoting the formation of receptor signaling complexes and regulating signaling at the cell surface. The proteoglycan signaling co-receptors can also function as structural molecules to regulate adhesion, cell migration, morphogenesis and differentiation. Through a balance of these signaling and structural roles, proteoglycan signaling co-receptors can have either tumor promoting or tumor suppressing functions. Defining the role and mechanism of action of these proteoglycan signaling co-receptors should enable more effective targeting of these co-receptors and their respective pathways for the treatment of human disease.

Introduction

Nearly all physiological functions are regulated through signal transduction pathways, which transmit extracellular signals into the nucleus via cell surface receptors and cytoplasmic mediators. There are two main classes of cell surface receptors: (1) signaling receptors that transduce signals either via catalytic domains or via adaptor molecules that bind signaling motifs in their cytoplasmic domains and (2) signaling co-receptors, which bind soluble ligand, regulate ligand binding and signaling through their corresponding signaling receptors but have traditionally been thought not to signal directly [1]. Signaling co-receptors are distinct from immune system co-receptors, including chemokine receptors, CD4, CD8, and CD28 (reviewed in [2]), as these co-receptors either do not bind soluble ligands or regulate signaling [1]. Signaling co-receptors are components of most major signaling pathways, including epidermal growth factor (EGF), fibroblast growth factor (FGF), Hedgehog (Hh), hepatocyte growth factor (HGF), interleukins, neurotrophins, semaphorins, transforming growth factor-β (TGF-β) superfamily ligands, vascular endothelial growth factor (VEGF) and Wnts. The signaling co-receptors are ubiquitously expressed and are structurally and functionally conserved across evolution, with essential functions from flies (*Drosophila melanogaster*) to worms (*Caenorhabditis elegans*) to humans (*Homo sapiens*). Studies in murine models have defined

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essential roles for signaling co-receptors during embryonic development, as genetic deletion of T β RIII [3] and the neuropilins [4,5] results in embryonic lethality and genetic deletion of Glypican-3 results in uniform perinatal lethality due to developmental defects [6]. In addition, the frequent mutation and altered expression of signaling co-receptors in human disease suggest important roles in human physiology/pathophysiology [1]. Signaling co-receptors generally share a number of structural and functional properties including a large, divergent extracellular domain, albeit with conserved motifs, that can be released as a soluble form and either a short, conserved cytoplasmic domain or no cytoplasmic domain (i.e. GPI-anchored) [1]. In addition, signaling co-receptors are generally promiscuous both in terms of ligand binding and in receptor complex formation, and have expanding roles in regulating the formation of morphogen gradients, cell adhesion, localization of signaling as well as signaling independent of their signaling receptor and orchestrating multiple cellular signaling pathways [1]. One feature shared by many, but not all co-receptors, is modification by glycosaminoglycan (GAG) chains to form proteoglycans (PGs). PG signaling co-receptor include CD44 (3 isoforms), glypicans (Glypican-1-6), neuropilins (NRP1, NRP2), syndecans (Syndecan-1-4) and the type III TGF- β receptor (T β RIII, or betaglycan) (Fig. 1). The GAG modifications are thought to contribute to the function of these PG signaling co-receptors, by mediating interaction with ligands, extracellular matrix proteins or other cell surface receptors. Here we review the function of the PG signaling co-receptors, focusing on their roles in regulating cell adhesion, migration and invasion. Finally, we discuss the role of these PG signaling co-receptors in the pathophysiology of human disease, with a specific focus on their emerging roles in regulating cancer progression.

I. Structural features of PG signaling co-receptors

Similar to other signaling co-receptors, PG signaling co-receptors can be either transmembrane receptors or glycosylphosphatidyl (GPI) anchored, have a large extracellular domain (ECD) with GAG attachment sites, a transmembrane domain and either no cytoplasmic domain or a short cytoplasmic domain (-200 amino acids or less) that lacks intrinsic kinase activity (Fig. 1). Different types of GAG modifications are possible including modification by heparan, heparan sulfate, chondroitin sulfate, dermatan sulfate and keratan sulfate chains (reviewed in [7]). The most commonly found modifications on the PG signaling co-receptors are heparan sulfate and chondroitin sulfate (Table 1). These modifications can occur on one or more sites on the receptor destined to reside in the extracellular space, with the type and extent of modification depending on the PG signaling co-receptor and varying in a context specific manner. Members of the glypican and syndecan families are obligate or “full-time” PGs, while T β RIII and neuropilins are referred to as “part-time” PGs since they can also be expressed without glycosaminoglycan modifications. NRP1 contains a single serine residue (S612) that can be modified, while T β RIII/betaglycan has two sites [8] and the members of the syndecan and CD44 families can be modified at three to five sites [9] (Fig 1). While glypicans are exclusively heparin sulfate PGs [10], members of the syndecan and CD44 families, NRP1 and T β RIII can be modified by heparan sulfate and/or chondroitin sulfate glycosaminoglycan chains [1] (Table I). The heparin sulfate modifications of PG signaling co-receptors can mediate interactions with FGF or extracellular matrix proteins [11,12]. PG signaling co-receptors can also bind ligands and extracellular matrix proteins through motifs in their core proteins (Fig 2). By modulating ligand binding, PG signaling co-receptors can regulate ligand function. In the simplest case these PG signaling co-receptors serve a “ligand presentation role,” facilitating ligand binding to their canonical signaling receptors (Fig. 2a). For example, the core protein of T β RIII binds TGF- β and BMP isoforms and presents these ligands to their corresponding signaling receptors, enhancing signaling ([13-17]; Table 1, Fig. 2a). However as PG signaling co-receptors can interact with multiple ligands and their respective receptors, they have the potential to regulate signaling in more complex manners. For example, T β RIII can also bind inhibin A, and promote inhibin A binding to the type II activin receptor to inhibit activin

signaling [18], while at the same time promoting inhibin A binding to the type II BMP receptor to inhibit BMP signaling [19]. In addition, the semaphorin, Sema 3A, can bind to NRP1 to decrease VEGF binding to NRP1 and antagonize VEGF signaling [20]. The GAG modifications can also regulate the interaction of the core protein with ligands. For example, the GAG modification of NRP1 can modulate responses to VEGF in smooth muscle and endothelial cells [21]. Ligands can also signal through PG signaling co-receptors in the absence of the associated signaling co-receptors, as is the case with VEGF ligands signaling through neuropilins in the absence of VEGFR1/VEGFR2 (reviewed in [22])(Fig. 2b). Such diversity in ligand binding and interactions with other proteins (see Table 1) has implications for a variety of processes including the ability of a cell to respond to environmental cues as will be discussed below (Section III).

Aside from the GPI-anchored members of the glypican family, PG signaling co-receptors have short cytoplasmic domains that do not contain intrinsic enzymatic activity. However, these cytoplasmic domains are often serine/threonine rich, are frequently phosphorylated and often have PSD-95/Dlg/ZO-1 (PDZ) binding motifs at their extreme carboxy terminus. Through these motifs, the cytoplasmic domains of PG signaling co-receptors interact with scaffolding molecules to regulate the internalization, trafficking and signaling of these co-receptors and their associated receptors as discussed below (Section V and Fig. 2). The PG signaling co-receptors are either phosphorylated in the cytoplasmic domain at specific sites (syndecans, CD44 and T β RIII) or have serine/threonine rich cytoplasmic domains that may serve as sites for phosphorylation by other signaling molecules [22], Fig. 1). For example, T β RIII is phosphorylated by T β RII, which results in the recruitment of β -arrestin2 to its cytoplasmic domain [23], while Syndecan-4 and CD44 are phosphorylated by protein kinase C (PKC) [24,25]. Essential roles for the cytoplasmic domain of T β RIII in mediating TGF- β signaling independent of the ligand presentation role [16], along with regulating cell-surface levels of T β RIII and T β RII through interactions with GAIIP-interacting protein, C terminus (GIPC) [16] and β -arrestin2 [26] have also been demonstrated. The contribution of signaling pathways regulated by the PG signaling co-receptors specifically affecting cell migration and adhesion processes are discussed in Section V.

II. Ectodomain shedding/Release of GPI-linked extracellular domains

All the transmembrane PG signaling co-receptors undergo ectodomain shedding, with proteolytic cleavage of the core protein near the transmembrane domain resulting in release of the extracellular domain from the cell surface [27]. In an analogous manner, the GPI-linked PG signaling co-receptors (i.e. the glypicans) can be released from the cell surface through the action of phospholipases or through alternative splicing of their mRNA's. Ectodomain shedding usually occurs under basal conditions and results in detectable levels of the soluble extracellular domains of PG signaling co-receptors in conditioned media from cells, as well as from biological sources, including serum and milk. The soluble extracellular domains of PG signaling co-receptors continue to carry their glycosaminoglycan modifications [28], allowing them to mediate similar interaction with their respective interacting proteins, albeit in a different context as is discussed in section V.2.(Fig. 2C). Generally speaking, the shedding of many membrane proteins can be enhanced by the phorbol ester PMA, an activator of protein kinase C and other signal transduction pathways, and the shedding of these proteins is mediated by zinc metalloproteases (for review see [27]). The disintegrin and metalloprotease ADAM 17, also known as TACE (Tumor necrosis factor -Converting Enzyme) has been implicated in mediating most protein kinase C-activated shedding events analyzed to date [27]. In contrast, the shedding of T β RIII is not stimulated by PMA suggesting alternate mechanisms of shedding [29]. The two main classes of proteins that are known to be directly involved in the cleavage process of T β RIII are the MMP's and the metalloprotease disintegrins [27, Velasco-Loyden, 2004 #17]. The ratio of soluble to membrane bound receptor can also be altered by different

physiological processes and in response to specific molecules. For instance, Syndecan-1 and Syndecan-4 shedding can be regulated by plasmin or by activation of thrombin and EGF family receptors [30], with soluble forms of these receptors accumulating following injury or inflammation [31]. In addition to regulation by extracellular signals, recently the cytoplasmic domain of Syndecan-1 has been demonstrated to regulate its shedding by binding to Rab5, a small GTPase that regulates intracellular trafficking and signaling events [32]. Many accelerated shedding events appear to involve protein tyrosine kinase activity [30]. Similarly, oncostatin M and TGF β 1 can induce the release of soluble CD44 in lung epithelial derived tumor cells [33]. However, the precise mechanisms regulating the shedding for most PG signaling co-receptors are largely unknown. Following ectodomain shedding, the remaining membrane-bound fragment of the protein, i.e. the transmembrane and cytoplasmic domain, is sometimes susceptible to cleavage within the transmembrane domain by γ -secretase-mediated regulated intramembrane proteolysis (RIP) to release the intracellular domain. In the case of Syndecan-3, this γ -secretase-mediated RIP and release of its cytoplasmic domain serves to negatively regulate the plasma membrane targeting of the transcription cofactor CASK [34]. In general, membrane T β RIII is considered a positive regulator of TGF- β signaling as it increases the affinity of the binding of TGF- β to the type II TGF- β receptor, enhancing cell responsiveness to TGF- β . In contrast, the soluble form of T β RIII, sT β RIII, has been demonstrated to bind TGF β and inhibit downstream TGF β function [15,35,36]. Thus, regulated ectodomain shedding of PG signaling co-receptors provides an important mechanism for regulating their function and associated signaling pathways. Moreover, as the soluble form of these co-receptors can regulate signaling in the cell of origin, in adjacent cells and stroma or systemically, PG signaling co-receptors have the potential to broadly mediate their functions through these regulated shedding events.

III. PG Signaling Co-receptors Mediated Regulation of Cell Adhesion, Migration and Invasion

Cancer cells can migrate and invade by multiple mechanisms including single cell migration or collective cell migration strategies. Cancer cells also respond to multiple migratory cues in response to which cells can modify their shape and stiffness to interact with surrounding tissue structures. PG signaling co-receptors are molecules that by virtue of a) being able to bind variety of ligands and ECM components, b) interacting with other receptors and c) facilitating interactions with the cellular cytoskeleton, are able to regulate cell-cell and cell-matrix adhesion, along with cell migration in a context-dependent manner (Table 1). While PG signaling co-receptors may contribute to invasion by protecting matrix degrading enzymes, or by increasing extravasation of tumor cells, (reviewed in [37]) several studies have demonstrated that PG signaling co-receptors negatively regulate the metastatic process (Section IV). The metastatic process can be broken down into different regulated steps that include altered cell-cell and cell-matrix adhesion, overall migration, and invasion through the basement membrane. The contribution of PG signaling co-receptors to these aspects of metastasis are discussed below.

1. Role of PG co-receptors in regulating adhesion

Cell-cell and cell-matrix adhesion are both important processes that directly impact physiological and pathophysiological cell migration and invasion. Cell-matrix adhesion is regulated by the formation of focal adhesions (FA), macromolecular structures through which the cell attaches to the ECM [38]. FA's mediate cell anchorage to the ECM by physically coupling the integrin family of cell surface receptors to the contractile actin cytoskeleton, and through this interaction transmit mechanical and regulatory signals [38]. PG signaling co-receptors can directly or indirectly regulate attachment of cells to the ECM via integrins and through regulation of the formation of focal adhesions (Fig. 3). The most well-characterized

PG signaling co-receptors in terms of regulating cell-matrix adhesion are the syndecans, which are necessary for the formation of stable focal adhesions on fibronectin coated substrates [39] through their interactions with integrins and fibronectin (reviewed in [40]). Syndecans interact with integrins and fibronectin through specific domains in their extracellular domain, while also mediating Rho-dependent phosphorylation of focal adhesion kinase (FAK) through their cytoplasmic domain [41] (Fig 3). Syndecans can also regulate adhesion through alternate mechanisms, including participation of syndecans with integrins in focal adhesion assembly via binding to ADAM 12 through associated proteins, syndesmos (Syn) and protein kinase C- α , leading to focal adhesion and stress fiber formation [42]. The CD44 family of PG signaling co-receptors has also been shown to facilitate cell-matrix adhesion [43]. Isoforms of CD44 that contain both heparan sulfate and chondroitin sulfate regulate adhesion via binding fibronectin through their GAG chains. CD44 has also been shown to localize to sites of cell-substrate contacts [43] and has specific domains that can bind hyaluronan (a non-sulfated glycosaminoglycan) to activate intracellular signaling cascades regulating adhesion [44]. CD44's interaction with hyaluronan stimulates interaction with ankyrin, a membrane associated cytoskeleton protein, resulting in cytoskeleton changes that are required for cell adhesion [45, Bourguignon, 2008 #313]. NRP1 has also been shown to facilitate endothelial cell adhesion to ECM proteins independent of the VEGF receptor, VEGFR2 [46]. While CD44 and NRP1 promote cell-matrix adhesion, PG signaling co-receptors can also inhibit cell-matrix adhesion possibly by interacting with anti-adhesive ECM molecules, including thrombospondin and tenascin, that destabilize cell- substrate contact sites [47]. An alternate model based on studies using soluble proteoglycans proposes that soluble forms of PG's in the ECM may prevent the cell surface receptor from interaction with its specific ligand in the ECM [48].

Cell-cell adhesion is important for maintaining an epithelial phenotype, and loss of cell-cell adhesion is associated with epithelial to mesenchymal transition (EMT), increased migration, and invasiveness. Syndecans have a well characterized role in facilitating cell-cell adhesion, mediated by the interaction of their heparan sulfate chains with adhesion molecules including N-CAM and PECAM ([49], Fig. 3b). Syndecans have been shown to localize to adherens junctions [50] and Syndecan-1 is required for maintaining an epithelial phenotype, functioning by regulating actin and E-cadherin expression [51]. As one might expect, loss of expression of PG signaling co-receptors occurs in human cancers, resulting in decreased cell-cell adhesion. Syndecan-1 expression is lost in multiple myeloma cell lines, with loss correlating with reduced cell-cell adhesion [52], which can be increased by restoring Syndecan-1 expression [53]. T β RIII expression is also lost in a number of human cancers, and in an ovarian model results in alterations in the expression of the cadherin family of proteins [54] that are essential in maintaining cell-cell junctions. In pancreatic cancer, loss of T β RIII is associated with TGF β -induced EMT, and is required for EMT associated increases in migration and invasion [55] possibly via alteration in cell-cell junctions.

2. Role of PG Signaling Co-receptors in migration

Cell movement involves the coordinated disruption and formation of adhesion sites along with changes in morphology mediated by regulation of the actin cytoskeleton. In general, molecules that cause disruption or destabilization of adhesion sites have a positive effect on motility and conversely molecules that increase adhesion cause a reduction in migration and invasion. This is exemplified by the role of syndecans in facilitating adhesion, while inhibiting invasion [56]. Efficient migration requires a balance between pro-adhesive forces (for traction) and anti-adhesive forces [57]. In addition to regulating migration via alterations in adhesion, PG signaling co-receptors can provide specific directional cues based on their environment ultimately affecting physiological processes including cell migration and cancer cell dissemination and metastasis. For example, syndecans have a specific role in regulating the directional migration of neural crest cells during development in vivo, with the Planar Cell

Polarity (PCP) pathway, a non-canonical Wnt signaling pathway, interacting with Syndecan-4 to control the direction in which cell protrusions are generated [58]. T β RIII has been shown to decrease cancer cell motility in multiple cancer models [36,55,59-63]. T β RIII's effects on suppressing cancer cell migration appear to be via disruption of directional migration by alterations in the cytoskeleton including filopodial and lamellipodial structures [54]. Some of the effects of syndecans on cell migration are mediated via heparan sulfate mediated interactions [40]. Both syndecans and T β RIII regulate migration via interactions of their cytoplasmic domain either directly with the actin cytoskeleton [49] or indirectly via proteins that regulate the actin cytoskeleton [54]. Glypican-3 has also recently been demonstrated to regulate migration via the actin cytoskeleton and modulation of the JNK pathway in a breast cancer model [64]. The role of CD44 in regulating cell migration is best characterized in inflammatory processes (reviewed in [65]). However, CD44 has also been implicated in glioma, melanoma and ovarian cancer cell motility and invasion [66]. In these cancer models effects of CD44 on motility have been attributed to its effects on directly regulating cytoskeleton structures including filopodia, lamellipodia and the adaptor protein cortactin via its interaction with Src kinase [67].

3. Role of PG signaling co-receptors in basement membrane invasion

Cancer cell invasion is a complex process involving at least two discrete events: 1) change in cell motility parameters, stiffness and shape (reviewed in [68]) as seen during EMT that allow neoplastic cells to enter lymphatic and blood vessels and disseminate, and 2) breaching the basement membrane to enter the parenchyma of the target organ where they form secondary tumors. There are two basic mechanisms by which this breach can occur: a) by secretion/regulation of matrix degrading enzymes including matrix metalloproteinases (MMP's), proteinases of the plasminogen activator systems (uPA) and heparanase, and b) by alterations in cell morphology and cytoskeleton remodeling that facilitates the invasion process. PG signaling co-receptors can regulate invasion by affecting both of these mechanisms. The primary mode of action of PG signaling co-receptors appears to be via regulating cell-cell and cell-cell matrix adhesion which indirectly impacts invasive potential as described in previous sections. In the context of basement membrane breach, CD44 is found at the leading edge of highly invasive melanoma lesions, and has been demonstrated to promote invasion by serving as a docking receptor for metalloproteinases that are key modulators of invasion (Fig. 3c, [69]). CD44 has also been shown to act as a co-receptor for hepatocyte growth factor (HGF), which also promotes motility and invasion, providing another mechanism for its action. An alternative mode by which PG signaling co-receptors can regulate invasion is through their shed extracellular domains. For example, MT1-MMP mediated shedding of Syndecan-1 to generate sSyndecan-1 has been proposed to potentiate invasion via the Wnt pathway [70]. In contrast, increasing Syndecan-1 expression in invasive hepatocellular or B lymphoid cell lines reduces their invasive potential [71,72], while antisense cDNA to Syndecan-1 increases the invasiveness of normal murine mammary gland epithelial cells [73], suggesting opposing effects of Syndecan-1 and sSyndecan-1 on invasion. In a manner somewhat analogous to that of the syndecans, T β RIII has been demonstrated to suppress cancer cell invasion in multiple models [36,61-63] and in at least one system, T β RIII has been shown to regulate the levels of MMP's [62] suggesting that this anti-invasive effect may be via an MMP dependent mechanism. In a reciprocal manner siRNA-mediated silencing of T β RIII expression increases invasiveness of normal ovarian surface epithelial cells [54] and pancreatic cancer cells [55]. In the case of ovarian cells, siRNA-mediated silencing of T β RIII expression is also associated with induction of some EMT markers [54]. Glypicans also inhibit invasion, as increasing Glypican-3 expression reduces the invasiveness of breast cancer cells in an MMP dependent mechanism, resulting in fewer metastases in vivo [74], while Glypican-1 has a limited role in regulating invasiveness [75].

IV. Effect of PG signaling co-receptors on physiological processes

Due to the multifaceted functional effects of PG signaling co-receptors, they can affect cell migration in many physiological and pathophysiological contexts including early development, wound healing and cancer metastasis. As the molecular program of normal wound healing might play an important role in the process of cancer metastasis [76,77], here we will examine the contribution of the PG signaling co-receptors to wound healing and cancer progression to illustrate the diverse function of PG signaling co-receptors in regulating cell adhesion, migration and invasion.

1. Cancer Progression

The significance of PG signaling co-receptors in mediating complex physiological processes is exemplified by their frequent mutation or altered expression in human disease including cancer [1]. The contribution of PG signaling co-receptors to cancer progression is complex as their effects are highly context dependent as already illustrated. Indeed, PG signaling co-receptors have been demonstrated to have either positive or negative effects on cancer progression based on either primarily correlative clinical data or experimental manipulations in model systems (summarized in Table 2).

In terms of clinical data, Syndecan-1 expression is decreased in several human cancers, including hepatocellular carcinoma [78], laryngeal cancer [79], head and neck carcinoma [80], colorectal carcinoma [81], mesothelioma [82] and non-small-cell lung cancer [83]. In the case of squamous cell carcinomas, decreased Syndecan-1 expression also correlates with histological differentiation grade with the lowest expression levels in poorly differentiated tumors [80]. In addition, increased Syndecan-1 expression correlates with a more favorable prognosis for patients with head and neck carcinomas [80]. In contrast, Syndecan-1 expression is increased in pancreatic cancer specimens [84]. The prognostic significance of syndecan expression in breast cancer has been somewhat controversial with evidence demonstrating that loss of Syndecan-1 epithelial expression is of strong prognostic value in breast carcinomas [85] and evidence suggesting that high Syndecan 1 expression in breast carcinoma correlates with a poorer prognosis [86]. Glypican-1 is also up regulated in pancreatic cancer [87], while Glypican-3 is overexpressed in a number of human cancer including certain subsets of gastric cancer [88], hepatocellular carcinoma [89], malignant melanoma, ovarian cancer [90], testicular yolk sac tumor [91], follicular and papillary thyroid cancer [92]. T β RIII expression is decreased in multiple cancers, including breast cancer [36,59], kidney cancer [60], lung cancer [61], ovarian cancer [62], pancreatic cancer [55] and prostate cancer [63,93], with loss of T β RIII expression associated with disease progression, advanced stage or grade and/or a poorer prognosis for patients. While both NRP1 and NRP2 are expressed on several tumor types (Table 2), expression of NRP1 correlates with tumor growth and invasiveness in prostate, colorectal, lung, breast and bladder cancer (reviewed in [22,94]) and the expression of both NRP1 and NRP2 are increased in non-small cell lung cancer relative to non-neoplastic tissues [95]. Finally, increased expression of CD44 and its splicing variant Cd44v6 are associated with poor prognosis for cancer patients [96]. Indeed, CD44 has a well characterized role in enhancing cancer progression [97].

While these clinical correlations are suggestive of a role of PG signaling co-receptors in human cancers, investigating the effects of altering expression of these co-receptors in cancer models has provided additional information both supportive and conflicting with clinical evidence. Consistent with loss of T β RIII expression during cancer progression, restoring T β RIII expression decreased tumorigenicity and cancer progression in breast [36,59], lung [61] and prostate [63] cancer models in vivo and cell migration and invasion in ovarian [62] and pancreatic [55] cancer models in vitro. Similarly, Syndecan-1 has also been demonstrated to be required for maintaining a differentiated epithelial phenotype, as suppression of Syndecan-1

expression caused alteration to a mesenchymal phenotype including loss of E-cadherin and alteration in cell morphology [73]. However, in many cases experimental findings in model systems have not been congruous with the clinical observations in humans. For example, while NRP's are upregulated in a variety of cancers, NRP1 was shown to suppress tumorigenic properties in a human pancreatic adenocarcinoma cell line [98], knockdown studies of NRP1 caused an increase in tumor growth and in gastrointestinal carcinoid tumors, and loss of NRP-2 expression correlated with tumor progression [99]. While Glypican-3 expression is often increased in human cancers, increasing Glypican-3 expression in a murine mammary carcinogenesis model decreased local invasiveness and metastatic potential [74]. Several hypotheses for these discrepancies have been put forth. One model suggests that in the initial steps in tumorigenesis, PG signaling co-receptor expression is lost [81], promoting migration, followed by subsequent accumulation of PG signaling co-receptor expression in the stromal compartment associated with enhanced invasiveness of the malignant cells [100]. Additional models propose that these differences in expression and function could be due to alterations in GAG modification of the PG signaling co-receptors (see section IV.5) between the early and later stages of cancer progression or that an increase of proteases in the tumor microenvironment could result in low levels of full length membrane anchored PG signaling co-receptors. Further studies will allow us to distinguish these possibilities for the individual PG signaling co-receptors.

2. Wound healing

Normal wound healing and tissue repair after damage involves the formation of granulation tissue and alteration of the stromal microenvironment. The latter has been shown to effect cancer progression [76] possibly by altering the levels of cytokines and growth factors that are capable of binding PG signaling co-receptors. The best studied wound healing model is the skin. The role of neuropilins in wound angiogenesis has been well characterized, with NRP1 having an essential role in the formation of new vasculature [101,102]. Similarly, CD44 has been shown to have a positive role in fibroblast invasion into wounded areas during dermal wound closure [103]. In this case it has been proposed that the co-receptor function of CD44 in binding either MMP9, EGF family erbB1-3 and/or HGF is important (reviewed in [104]). The contribution of syndecans is highly context dependent, as dermal wound repair induced by incision injury to adult or neonatal skin results in transient induction of Syndecan-1 and Syndecan-4 expression in keratinocytes at the wound edge [41], while wound closure is delayed in both Syndecan-4 heterozygous and Syndecan-4 null mice compared to the wild type mice [105]. However, keratinocytes migrating into wounded skin areas appear to lose their cell surface associated Syndecan-1 with a corresponding increase in sSyndecan-1 and sSyndecan-4 into wound fluids (see section IV). While several studies support a role for Syndecan-1 and Syndecan-4 during cutaneous wound healing, the molecular basis remains unknown. A detailed description of the role of syndecans during wound healing is reviewed elsewhere [106]. Despite redundancy in function between the syndecans and the glypicans, there is limited evidence on the role of glypicans in wound healing functions [106]. The role of T β RIII in affecting wound healing has been derived primarily from studies on primary human skin cell types: dermal fibroblasts and keratinocytes. T β RIII is primarily expressed in dermal fibroblasts that are responsive to TGF β 3's anti-migratory effects, but not in keratinocytes, which are TGF β 3 resistant. However, expression of T β RIII in keratinocytes confers sensitivity to serum-mediated inhibition of migration and TGF β 3-mediated inhibition of TGF α -driven migration [107].

V. Factors regulating the function of PG signaling co-receptors in adhesion, migration and invasion

PG signaling co-receptors differ from other signaling receptors in that they can bind a diversity of ligands, albeit with different affinities [1]. Although PG signaling co-receptors have traditionally not been thought to signal independently in part due to short cytoplasmic tails that do not contain enzymatic activity, recent studies suggest they may well signal independently of their respective signaling receptors and influence signaling that in turn affects migration and adhesion. The role of PG signaling co-receptors in migration, adhesion and invasion is determined by multiple factors including their signaling ability, localization, presence of modifying enzymes, presence of the GAG modifications and molecules that interact with PG signaling co-receptors. How these distinct features contribute to the effects of PG signaling co-receptors on adhesion, migration and invasion is discussed below.

1. Signaling function

PG signaling co-receptors can interact with ligands both through their GAG modifications and through their core proteins. The most extensively studied interaction of a PG signaling co-receptor through its GAG modification is that of FGF with syndecans, where it has been demonstrated that binding of FGF to its signaling receptor requires prior binding either to the heparan sulfate side chains of the PG signaling co-receptor or to free heparan sulfate chains [108]. Syndecan-4 has been shown to mediate FGF-2's effects on migration via its cytoplasmic domain [109]. Hence both the HS chains and the core protein of syndecans are important for potentiating FGF's effects. In addition, Syndecan-4 activates PKC α in complex with PIP₂ [110] which in turn regulates Syndecan-4 incorporation into sites of focal adhesions [111]. Syndecans are crucial components in relaying signals to regulate adhesion via integrins to fibronectin resulting in alterations in cytoskeleton assembly either due to colocalization of the syndecans with actin filaments [52] or possibly via other interacting molecules such as integrins [56].

In contrast to FGF, most ligands bind to T β RIII via two ligand binding domains on its core protein as discussed above in Section I. Recent studies have suggested essential, non-redundant roles for T β RIII in regulating signaling independently of its respective TGF β signaling receptors, T β RII and T β RI [112,113], including β -arrestin2 dependent activation of Cdc42 to regulate epithelial and cancer cell migration [54]. In the case of NRP, which can bind VEGF and SEMA 3A, the effects of NRP on migratory processes in several systems appear to be governed by the ratio of SEMA3A/VEGF. SEMA3A and VEGF have opposing effects on migration, with SEMA3A mediating anti-migratory and VEGF mediating pro- migratory effects [114]. VEGF has been shown to promote membrane ruffling in breast cancer cells via its interaction with NRP, and this VEGF function can be blocked by SEMA3F, which competes with VEGF for NRP binding [115]. In addition SEMA3A has been shown to inhibit the migration of breast cancer cell lines through an NRP-1/plexin-1A pathway [114]. Most recently NRP-1, through its cytoplasmic domain and independently of VEGF and SEMA3A, has been demonstrated to promote α 5 β 1-integrin-mediated EC adhesion to fibronectin by directly interacting with α 5 β 1 at adhesion sites [116]. Further, this interaction has been shown to be crucial for vascular development. In the case of CD44, hyaluronan binding to the extracellular domain of CD44 has been shown to signal via ankyrin and the Rho GTPases to generate specific signaling cascades that increase migration and adhesion responses [117].

2. Contribution of ectodomain shedding

The soluble extracellular domains of PG signaling co-receptors generated by ectodomain shedding (see Ectodomain shedding, section II) have been demonstrated to regulate cell adhesion and migration [118]. The soluble extracellular domains of these PG signaling co-

receptors can either have opposing effects from the membrane bound form, or could mimic the effects of the membrane bound form in a non-cell autonomous fashion. For instance, Syndecan-1 and Syndecan-4 shedding have been reported in many studies of wound healing. Specifically, in keratinocytes migrating into the wound the shed ectodomain of Syndecan-1 and Syndecan-4 are increased in the wound fluids (section IV.2). Cleavage of the ECD of Syndecan-1 by MT1-MMP has been demonstrated to stimulate cell movement [119], suggesting that the shed form may serve to convey specific motility signals distinct from the cell surface bound form. The isolated ectodomain, free of glycosaminoglycan, has been shown to promote adhesion of human fibroblasts, by binding to an as-yet-unknown cell-surface 'receptor' with high affinity [120]. In the case of T β RIII, the soluble form (sT β RIII) is able to fully recapitulate the inhibitory effect of membrane bound T β RIII on invasion in a breast cancer model [36]. sT β RIII could be functioning either by sequestering T β RIII-binding ligands or by binding to extracellular matrix proteins and blocking their ability to interact with T β RIII on the cell surface. NRP1 also exists in a soluble form that functions as an antagonist of VEGF signaling [20] and VEGF's tumor promoting activities, including its pro-migratory and pro-invasive function [22]. In the case of CD44, blockage of the cleavage of membrane bound CD44 has been shown to suppress lung cancer cell migration in a substrate specific manner [121].

3. Role of scaffolding proteins/accessory factors

PG signaling co-receptors can also regulate adhesive and migratory processes through interactions of their cytoplasmic domains with scaffolding proteins including members of the PDZ family of proteins and the Arrestins. T β RIII, neuropilins and syndecans all contain a PDZ binding domains at their carboxy terminus that can bind the PDZ domain containing proteins including GIPC[16], NIP [122] and CASK [123].

PDZ domain containing proteins are implicated in organizing the localization of signaling complexes at distinct membrane locations, including cell-cell junctions and at the leading edge of migrating cells. Several PDZ domain containing proteins have been implicated in regulating PG signaling co-receptor mediated migration [124], acting as molecular scaffolds than can link a number of binding partners through PDZ domains and other protein-binding motifs [125] (Fig 3). Syndecans can interact with three PDZ domain proteins: syntenin, CASK and synectin/GIPC [124,126,127]. These interactions may regulate trafficking and/or aid in establishing networks of submembranous signaling complexes [126] that in turn affect syndecans primary roles in adhesion and migration. In the case of the syndecan-syntenin interaction, syntenin interacts with unphosphorylated Syndecan-4, and following syndecan's phosphorylation, this interaction is abolished [128], possibly affecting the incorporation of syntenin into sites of adhesion [126]. CASK probably serves as a scaffolding complex for the syndecans that binds to other transmembrane receptors and cell-cell junctional adhesion molecules in epithelial cells [129]. The Synectin-Syndecan4 interaction has also been shown to be required for cell polarization and migration through a Rac1 dependent pathway [130]. In contrast, the synectin-syndecan interaction was shown to be not required for syndecans effects on adhesion [124]. Hence, different PDZ proteins may aid in facilitating the different roles of syndecans in migration and adhesion. NRP1 has been shown to interact with GIPC through its C-terminus, and this interaction has been shown to be essential for NRP1 mediated migration [131]. T β RIII has also been shown to interact with GIPC via its c-terminus, and this interaction has been shown to modulate TGF β signaling [16]. However, no specific contribution of this interaction to migratory and adhesion related mechanisms has yet been defined. CD44 is somewhat distinct from the others in that it lacks a PDZ binding domain but can interact with protein such as LARG (leukemia-associated Rho guanine nucleotide exchange factor) through its PDZ domain to regulate cytoskeleton function in a head and neck squamous cell carcinoma model [132]. A

specific effect of the PDZ domain on migration and adhesion in the case of the other PG signaling co-receptors has not yet been clearly defined.

The second class of scaffold molecules that PG signaling co-receptors interact with are the arrestins that were originally characterized as structural adaptor proteins that modulate the trafficking and desensitization of seven-membrane-spanning receptors. β -arrestin2 has been demonstrated to have an analogous trafficking and desensitization role for T β RIII [23]. T β RIII's interaction with β arrestin2 is triggered by phosphorylation on its cytoplasmic domain at Thr 841. Recent findings demonstrate that the interaction between T β RIII and β arrestin2 is required for T β RIII's effects on cytoskeleton reorganization, migration and activation of Cdc42 in a breast and ovarian cancer models [54]. The arrestins have recently emerged as potential regulators of directional migration [133] either via desensitization and recycling of receptors essential for maintaining cell polarity, or as a signaling scaffold to localize molecules involved in cytoskeleton reorganization (Fig 3c), much like the PDZ family of proteins. The receptor ligand complexes function at the cell surface; however, internalization of these complexes as part of normal recycling followed by uptake by lysosomes is not only required for receptor clearance and degradation of heparan sulfate chains [134], but also for regulating both duration and magnitude of a specific signal [135]. Other PG-signaling co-receptors have not yet been shown to interact with the arrestins. Further studies are required to elucidate the contribution of arrestins to the role PG signaling co-receptors in migration and adhesion processes.

4. Localization

Cell migration is a dynamic process and requires a leading edge in the direction of migration and the formation of specific adhesion points from which tension is generated to propel the cell body forward. Localization of either the PG signaling co-receptor itself or the signaling molecules they bind at strategic locations on the plasma membrane serves to regulate cell substrate contacts and cytoskeleton rearrangements, polarize proteins required for making the contacts or invasion front and for sensing of chemical gradients.

PG signaling co-receptors can localize to specific 'domains' that may serve to concentrate signal and/or activate specific cytoskeleton rearrangements. For example, Syndecan-4 has a well characterized role in regulating focal adhesions, localizing to focal adhesions as well as clustering at the cell surface of endothelial cells thereby causing activation of Rac1, induction of cell polarization and stimulation of cell migration [130]. Similarly, in polarized, cultured B-lymphoid cells, Syndecan-1 is localized to a membrane domain — the uropod — in a heparan-sulfate-dependent manner [136]. The localization pattern can also result in interaction with molecules such as integrins in a competitive fashion that could cause alterations in pivotal integrin-matrix interactions, thereby affecting migration and adhesion. In addition to the syndecans, CD44 has been shown to localize to leading edge of cells, specifically to lamellipodial structures [137-139], providing support for the cytoskeletal anchoring of CD44 in the regulation of hyaluronan (HA) mediated adhesion. In addition, the localization of CD44 to the cell surface along MMP's, specifically MT1-MMP and MMP-9, serves as a mechanism by which CD44 facilitates tumor cell migration and invasion [69,140] (Fig 3c). In terms of localization of signaling, all signaling activities of CD44 in lymphocytes and tumor cells are influenced by CD44's localization to glycolipid enriched membrane microdomains that serve as a platform for signaling molecules [141,142]. T β RIII internalizes via lipid rafts [143] and NRP localizes to the apical surface of polarized cells [144]. However, much less is known about the consequences of the localization of T β RIII and NRP relative to regulating adhesion, migration and invasion.

5. Contribution of the GAG modifications

Although the PG signaling co-receptors can have both heparan sulfate (HS) and chondroitin sulfate modifications, the contribution of the HS chains has been more extensively studied. Several reviews have focused on the contribution of the fine structure of the HS on proteoglycan function [40,66,145,146]. Furthermore, since the PG signaling co-receptors bind multiple ligands through their GAG chains in addition to core protein interactions, these distinct ligands require distinct HS sequences for binding. Changes in the fine structure during physiological processes will influence the ability of the PG signaling co-receptors to bind ligand. The fine structure of HS modification of Syndecan-1 has been demonstrated to control cell-matrix adhesion. For instance, HS modifications on syndecans from different murine B lymphoid cells reveal that despite having the same amount of sulfation, the type of sulfation (reviewed elsewhere [7]) regulates the ability of cells to bind to collagen [147]. Additionally, the heparan sulfate chains on syndecan have been shown to be important for its localization to cell-cell junctions and the leading edge of polarized cells [53,136]. In the case of NRP1, VEGF was able to induce the motility of vascular smooth muscle cells expressing a non-GAG modified NRP1 to a greater extent than SMC's expressing wild type NRP1 [21] due to the specific ability of the non-GAG modified NRP1 to up regulate VEGFR2 expression [21]. In the case of T β RIII, it has been reported that in some cell lines the nature of the glycosaminoglycan attached to membrane T β RIII core protein may sterically prevent its association with the TGF- β type II receptor, turning T β RIII into an inhibitor of TGF- β signaling [148] and alter tumor cell responsiveness to TGF β . More recently, expression of T β RIII lacking its GAG modifications in ovarian surface epithelial cell lines diminished the ability of T β RIII to suppress motility [54]. An in depth characterization of the extent and type of GAG modifications on these PG signaling co-receptors under different conditions including during cancer progression, wound healing and in other disease states will give further insight into the extent of regulation of these modifications and how they may impact the ultimate role of these PG signaling co-receptors in human disease.

VI. Summary and Future directions

PG signaling co-receptors have functions beyond their ability to bind and present ligands to their respective signaling receptors, including regulating signaling in a cell autonomous and non-cell autonomous manner through regulated ectodomain shedding, localizing signaling, orchestrating signaling and signaling independently. Through a combination of these functions, PG signaling co-receptors mediate essential roles in regulating physiological and pathophysiological processes in a tissue and context dependent manner, including the effects on adhesion, migration and invasion as outlined here. While the current body of literature supports complex roles for PG signaling co-receptors in mediating their effects, many questions remain in terms of how these receptors function under physiological circumstances and during disease progression. For example, as PG signaling co-receptors bind multiple ligands and other proteins both through their extracellular and cytoplasmic domains, and via their core protein and GAG modification, defining the precise nature and relationship of these interactions and their functional consequences remains a challenge. Here, more careful structure/function studies and the ability to knockdown endogenous PG signaling co-receptor expression with RNAi technology and rescue with defined mutants should generate significant insight. The relationship between membrane bound and soluble forms of PG signaling co-receptors and how this ratio is regulated by ectodomain shedding remains incompletely understood. Thus, defining the mechanisms regulating shedding, the enzymes involved and the sites of proteolytic cleavage to generate shedding resistant forms will be quite useful. Efforts to define the function of PG signaling co-receptors in regulating cell migration have also proven cumbersome due to the difficulty in isolating effects on cell movement from the effects on proliferation and other intracellular signaling pathways that indirectly affect cell movement. Specific focus on the

multiple facets of motility including the change from a non-motile to motile phenotype, cytoskeleton-cell membrane interactions, cell-cell and cell-matrix interactions that regulate collective cell motility and factors governing directionality will give additional insights into the role of these PG signaling co-receptors in migration and adhesion events. The advent of more accurate and quantitative cellular assays and the availability of sophisticated imaging technologies *in vitro* and *in vivo* will help clarify these roles. Cancer treatment strategies designed to target either adhesion receptors or proteases have not been effective in clinical trials possibly due to the ability of cancer cells to adapt/alter their mode of migration in response to different environments. Hence, an increased understanding of the mechanics of cancer cell deformability and its interactions with the environment coupled with insight into the role of these PG signaling co-receptors in defining the mechanical properties of cells promises to provide novel therapeutic targets in human disease. Additional studies involving more genetically tractable vertebrate models, including *Xenopus* and Zebrafish, would also be useful in this regard. Finally, while much attention has been focused on some of the PG signaling co-receptors, including the syndecans and CD44, investigation of other PG signaling co-receptors, including T β RIII and the neuropilins, is just emerging. Insight into the role and mechanism of action of PG signaling co-receptors in signaling, biology and human disease should advance our ability to target these co-receptors and their respective signaling pathways in human diseases.

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Figure 1.

Structural Features of the Proteoglycan Signaling Co-receptors. The PG signaling co-receptors can be either GPI (Glycosylphosphatidylinositol) anchored (the glypican family) or transmembrane receptors. Sites of GAG modification are indicated by the dashed lines. These coreceptors all undergo ectodomain shedding at the site indicated by an arrow to release the soluble form of the receptor that can both bind ligand and modulate activity. p -The cytoplasmic domains are frequently phosphorylated in their carboxyl terminus.

Abbreviations: a1/a2- complement binding domains, b1/b2-coagulation factor V homology domain, c- homology with complement components C1r and C1s, C1-conserved domain 1, C2-conserved domain 2, MAM-mephrin domain, PDZ domain-post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1), TβRIII-type III TGFβ receptor, V-Variable domain, ZP1- Zona Pellucida.

Figure 2.

Function of Proteoglycan Signaling Co-receptors on the Cell Surface. PG signaling co-receptors bind ligand (blue circles) either via their GAG modifications (dashed lines) or via the core protein and then regulate ligand function through distinct mechanisms. a) The bound ligands are immobilized on the cell surface, facilitating presentation of ligand to the associated signaling receptors or in recruitment of the associated signaling receptors to form functional signaling complexes. b) The PG signaling co-receptor can also bind to ligand in the absence of the signaling receptor c) The soluble form of the PG signaling co-receptor generated by ectodomain shedding can function as in model (a) or (b). In addition, these soluble forms can sequester ligand from PG signaling co-receptors and signaling receptors on the cell surface.

Figure 3.

Mechanisms by which Proteoglycan Signaling Co-receptors Regulate Adhesion, Migration and Invasion. a) Cells bind to extracellular-matrix proteins (i.e. fibronectin) either directly or via integrins. The strength of cell-matrix adhesion is enhanced by clustering of integrins resulting in direct association of the PG signaling co-receptor with the cytoskeleton or via integrins to regulate cell spreading and focal adhesion formation. b) Cell-cell adhesion can be regulated by PG signaling co-receptor interactions via the heparan sulfate chains to specific adhesion receptors (i.e. N-CAM, light blue ovals) to facilitate cell-cell adhesion. c) PG signaling co-receptors bound to ligand or ECM components can alter migration via their cytoplasmic domain binding effector proteins, including β -arrestins, PDZ domain containing

proteins, and PKC, which in turn interact with actin association factors, including such GEF's (GTP exchange factors) to facilitate cytoskeleton remodeling events. PG signaling co-receptors can also serve as docking sites for MMP's to regulate invasion

Table 1
Functional Traits of Proteoglycan Signaling Co-receptors-

Co-Receptor	Ligands	GAG Modification	Effect on Motility	Effect on Signal Transduction	Cell surface interactions	Cytoplasmic domain effectors
Neuropilin family	VEGF, PlGF, PDGFB, Semaphorins, FGF2, FGF4, TGFβ, heparin binding protein	CS/HS	Inhibits	+	VEGFR1, VEGFR2 Plexins (1-10), MET, L1CAM	NRP-1 interacting protein, PSD/ ZO-1
TβRIII/betaglycan	TGFβ1, TGFβ2, TGFβ3, inhibin, BMP2, BMP4, BMP7, FGF2, GDF-5	CS/HS	Inhibits	+/-	TβRI, TβRII ActRII BMPRII, ALK-3, ALK-6	GIPC, β-arrestin2
Glypican family	Wnt, Dpp/TGFβ, Hh, FGF, Cripto1, VEGF, BMP, Midkine, IGF	HS	Inhibits	+/-		NA
Syndecan family	FN, FGF2, Hh, HGF, VEGF, Midkine, VN, Laminin	CS/HS	Inhibits	+	Integrins: αβ5, αβ3, α5β1, α2, β1α6 β4	PKCα, Syntenin, CASK Src/Fyn, Rab5, Syndesmos tactinin, DynaminII
CD44	Hyaluronan, osteopontin, collagen, Laminin, FN	CS/HS	Inhibits	+	MMP	N-WASP, Ankyrin

Abbreviations: ALK-Activin like kinase, BMP-bone morphogenetic protein, CASK-calmodulin-associated serine/threonine kinase, GIPC- Gα interacting protein-interacting protein Cterminus, CS-chondroitin sulfate, FN-Fibronectin, Hh-Hedgehog, HS-Heparan sulfate, MMP-matrix metalloprotease, TGF-Transforming growth factor, FGF-fibroblast growth factor, VEGF-vascular endothelial growth factor, PlGF-Placental growth factor, PDGF-Platelet derived growth factor, IGF- Insulin growth factor, HGF-Hepatocyte growth factor, Hh-Hedgehog, MET-hepatocyte growth factor tyrosine kinase receptor, L1CAM-L1 cell adhesion molecule, MMP-Matrix metalloprotease,

Table 2
Proteoglycan Signaling Co-receptor Expression in Human Cancers

Co-receptor	Expression in cancer		References
	Reduced	Elevated	
Neuropilin1-2		Brain, lung, prostate ovary, non-small cell lung	[95] Reviewed in [22] [94] [36,59], [60], [61], [62], [55] [63,93] [10,87,149],[88], [89], [90], [92], [91] [78,81,150], [79,80], [83] [82], [84,151] [152-155]
TßRIII	Breast, kidney, lung, ovary, pancreas, prostate		
Glypican1-3	Breast, mesothelioma	Ovary, gastric, colon, HCC, thyroid, pancreas	
Syndecan 1-6	Cervical, colon, HCC, lung, laryngeal, head and neck, non-small-cell lung cancer mesothelioma	Pancreas, breast, endometrium, ovary	
CD44		Ovary, colon, squamous cell carcinoma, head and neck, breast, bladder	

Abbreviations: HCC-hepatocellular carcinoma