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Dually Modified Transmembrane Proteoglycans in Development and Disease

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Abstract

Aberrant cell signaling in response to secreted growth factors has been linked to the development of multiple diseases, including cancer. As such, understanding mechanisms that control growth factor availability and receptor-growth factor interaction is vital. Dually modified transmembrane proteoglycans (DMTPs), which are classified as cell surface macromolecules composed of a core protein decorated with covalently linked heparan sulfated (HS) and/or chondroitin sulfated (CS) glycosaminoglycan (GAG) chains, provide one type of regulatory mechanism. Specifically, DMTPs betaglycan and syndecan-1 (SDC1) play crucial roles in modulating key cell signaling pathways, such as Wnt, transforming growth factor- β and fibroblast growth factor signaling, to affect epithelial cell biology and cancer progression. This review outlines current and potential functions for betaglycan and SDC1, with an emphasis on comparing individual roles for HS and CS modified DMTPs. We highlight the mutual dependence of DMTPs' GAG chains and core proteins and provide comprehensive knowledge on how these DMTPs, through regulation of ligand availability and receptor internalization, control cell signaling pathways involved in development and disease.

Keywords

proteoglycan; glycosaminoglycan; cell signaling; cancer; betaglycan; syndecan

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H. Conflict of Interest

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A. Introduction

Transmembrane proteoglycans (TP) are members of the heparan sulfate proteoglycan (HSPG) family whose core domains span the cell membrane to modulate a multitude of intracellular and extracellular activities[1–10]. TP extracellular domains (ECD) contain covalently attached glycosaminoglycan (GAG) chain modifications that include heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate and/or keratan sulfate, with HS and CS chains being the most common[2]. As members of the GAG family, HS and CS chains are characterized by repeating disaccharide units containing glucuronic acid and either N-acetylgalactosamine for HS or N-acetylglucosamine for CS[1]. Of the known human transmembrane proteoglycans, only a few can present both HS and CS chains and are referred to here as dually modified transmembrane proteoglycans (DMTP). Human DMTP members include the Type III Transforming Growth Factor β Receptor/Betaglycan, Syndecans-1 and-3, CD44 and Neuropilin-1. Syndecan-3 (SDC3), similar to betaglycan and syndecan-1 (SDC1), contains both CS and HS chains but is found almost exclusively in neuronal and musculoskeletal tissue[11] with defined roles in skeletogenesis[12] and neurite growth[13]. Unlike SDC3, CD44 is expressed in many different cell types[14] and in various isoforms, which can exhibit HS and/or CS chains depending on the splice variant[2, 15–18]. In epithelial cells, the primary form of CD44, known as CD44E or CD44v8-10, lacks the variable region (exon v3) required for HS chain attachment and therefore can only express CS chains[14, 18–21]. Neuropilin-1 (NRP1), which is prominent in neuronal and endothelial cells, has clearly outlined roles in axonal growth/guidance as well as physiological and pathological angiogenesis[22–24]. Since NRP1 only contains one serine residue (Ser⁶¹²) for GAG chain attachment, it can only exist as a HS proteoglycan or a CS proteoglycan but not as a hybrid of the two[24–27]. DMTPs are present in both membrane-bound and/or shed/soluble forms with these forms able to impact cell behavior by either mimicking or opposing each other's cellular functions. Contributions from the core domain's scaffolding properties can also influence GAG chain-ligand binding[7], highlighting the importance of the HSPGs' core domain in delineating roles for GAG chains. Consequently, shed/soluble DMTPs and their attached GAG chains can affect ligand availability, downstream signaling and promote interactions between GAG chains, ligands and the extracellular environment[28].

This review will focus primarily on betaglycan and SDC1, the most ubiquitous of the DMTPs, which are chiefly expressed in epithelial cells and whose HS and CS chains have roles in regulating epithelial cell biology through alterations in growth factor signaling, receptor/growth factor internalization and proteolytic shedding. We emphasize the underappreciated mutual dependence of HSPGs' GAG chains and core protein and their impact in disease, with a focus on cancer progression. Finally, we aim to provide comprehensive information on these DMTPs for the future development of therapeutic techniques to modulate growth factor/ligand availability and thus control downstream cell signaling pathways involved in human disease.

B. DMTP Structure and Features

B1. Core Structure

Both SDC1 and betaglycan are proteoglycans containing a large ECD, a transmembrane domain and a short cytoplasmic domain (~200 amino acids or less) that lacks intrinsic kinase activity. Betaglycan's ECD contains a zona pellucida domain (ZP1 and ZP2, Figure 1), which is important for protein polymerization and protein-protein interactions[29]. Specifically, betaglycan's ZP domain contains two distinct Transforming Growth Factor β (TGF- β) binding sites: one in the membrane-distal region (ZP1)(Figure 1 and [30]) and one in the membrane-proximal ZP region (ZP2)(Figure 1 and [31]). TGF- β ligands TGF- β 1/2/3, Bone Morphogenetic Proteins (BMPs) 2/4/7 and Growth Differentiation Factor 5 (GDF-5) can bind both regions while the TGF- β member Inhibin only binds the ZP region (ZP2)[32, 33]. Unlike betaglycan, little is known about SDC1's ectodomain core independent of its GAG chain functions. SDC1's 235 amino acid[34] extracellular core, however, does support fibroblast cell adhesion[35], interacts with integrins α v β 3 and α v β 5 during angiogenesis and α v β 1 integrin during re-epithelialization of lung tissue[36–40].

The short cytoplasmic domains of DMTPs are serine/threonine rich, frequently phosphorylated and contain a PDZ (Postsynaptic Density-95/Disc large protein/Zonula occludens-1) binding motif at the extreme carboxy terminus. These cytoplasmic regions commonly interact with scaffolding proteins to control DMTP internalization and cell signaling (Figure 1, Section E and [2, 34, 41, 42]). Betaglycan's short cytoplasmic domain, through its Class I PDZ binding motif, binds the scaffolding protein GAIP-interacting protein C terminus (GIPC) to modulate TGF- β signaling (See Section D1 and [43]). Additionally, the Type II TGF- β Receptor (T β RII) can phosphorylate betaglycan's cytoplasmic domain at Thr⁸⁴¹ to promote β -arrestin2-betaglycan interactions and receptor internalization [44](See Trafficking Section E). SDC1's cytoplasmic domain is divided into two conserved regions, C1 and C2 (Figure 1), which are common regions between all syndecans, and a central variable region (V, Figure 1), which confers specific properties on each syndecan (Figure 1 and [45]). SDC1's V domain regulates cell spreading and actin cytoskeleton assembly[46] while its C1 domain, adjacent to the membrane, participates in SDC1 dimerization and intracellular protein binding[47]. The conserved C2 carboxyl-terminal tetrapeptide sequence can bind PDZ-domain-containing proteins[41, 42], which may function as membrane scaffold proteins that recruit signaling and cytoskeletal proteins to the plasma membrane. Contributions from the core domain's scaffolding properties are likely to influence GAG chain-ligand binding by either immobilizing the chains to specific sites within the plasma membrane[7] or by altering the internalization of ligands bound to GAG chains (See Trafficking Section E). Additionally, the position of the GAG chains on the core protein (plasma membrane distal or proximal) could impact GAG chain association with receptors and subsequent DMTP function.

B2. GAG Chain Structure

On the extracellular core of DMTPs are sites for linear polysaccharide GAG chain attachments (Figure 1 and [1, 2, 30, 34, 48]). Synthesis of DMTP GAG chains begins with covalent attachment of a linkage tetrasaccharide, glucuronic acid-galactose-galactose-xylose

(GlcA β 1-3Gal β 1-3Gal β 1-4Xyl β 1-O-), to a serine hydroxyl group on the core protein followed by addition of repeating disaccharide regions composed of hexosamine and hexuronic acids (Figure 1 and [48]). The repeating disaccharide units distinguish HS chains from CS chains. During initial synthesis of HS chains, a N-acetylglucosamine (GlcNAc) is attached to the linkage tetrasaccharide by α 1,4-N-acetylglucosaminyltransferase-I (α 4GlcNAcT-I). On the other hand, synthesis of the CS chains occurs when β 1,4-N-acetylgalactosaminyltransferase-I (β 4GalNAcT-I) attaches a N-acetylgalactosamine disaccharide to the linkage tetrasaccharide[48]. The first hexosamine residue thus determines which chain is made (HS or CS). After attachment of this fifth saccharide, the chondroitin synthase family (for CS chains) or exostosins (for HS chains) attach the remaining disaccharide units[48]. HS chains can then be selectively sulfated at the *N*, *3-O* and *6-O* positions of glucosamine and the *2-O* position of uronic acid residues by sulfotransferases[49] while sulfate groups on CS chains are added at *C-4* and/or *C-6* of *N*-acetyl-galactosamine and/or *C-2* of glucuronic acid[50]. In vertebrates, SDC1 is one of four syndecan family members and is the primary syndecan expressed in epithelial cells[51]. As a “full-time” proteoglycan (no GAG-less form *in vivo*), SDC1’s ECD contains three attachment sites for HS chains (Ser³⁷, Ser⁴⁵ and Ser⁴⁷) and two sites for CS chain attachment (Ser²¹⁰ and Ser²²⁰)(Figure 1 and [34, 52, 53]). While SDC1 is an obligate or “full-time” DMTP, betaglycan is commonly referred to as a “part-time” proteoglycan since it can be expressed with or without GAG chains[1, 2, 48, 54] *in vivo*. CS chains attach to betaglycan’s 100kDa protein core primarily at Ser⁵⁴⁵. CS chains can also attach at Ser⁵³⁴, but HS chains usually predominate at this site (Figure 1 and [30, 48, 55]). Both alterations in core protein expression and GAG chain density and/or composition (HS vs. CS) can impact DMTP functions.

B3. Shedding and Intracellular Domain (ICD) Cleavage

Several DMTP functions can be attributed to ectodomain shedding which leads to DMTP expression in both membrane-bound and shed/soluble forms. Both forms can impact whether GAG chains are anchored to the membrane or present as soluble GAGs in the extracellular matrix (ECM). Cleavage occurs near the transmembrane domain to release a soluble ectodomain containing GAG chains (Figure 1). This ectodomain shedding is highly regulated by the enzymatic activity of several proteases[2, 39, 56–60] including matrix metalloproteinases (MMP) and metalloprotease disintegrins. Enzymatic activity by pervanadate, a general tyrosine phosphatase inhibitor, stimulates cleavage of both SDC1[61] and betaglycan[58]. Sheddases Membrane type MMP-7[62, 63], matrilysin[64], MMP-9[65] and MMP-1[59] cleave SDC1’s ECD. Intriguingly, shedding for SDC1 has also been shown to be regulated by intracellular interaction of the small GTPase Rab5 with SDC1’s intracellular domain as Rab5’s dissociation from SDC1’s cytoplasmic domain triggers SDC1 ectodomain shedding at the cell surface[62]. It is postulated that activated GTP-Rab5 dissociates from SDC1’s cytoplasmic domain, associates with β 1 integrin and stimulates the internalization of β 1 integrin and dissociation of β 1 integrin from SDC1. This cascade of events exposes SDC1’s ECD cleavage site to sheddases[62]. Whether such intracellular control of betaglycan shedding exists has not been tested. However, the ECD of betaglycan is cleaved by MMP-1 and -3[58]. Both SDC1 and betaglycan’s transmembrane/cytoplasmic fragments can be cleaved by γ -secretase to release both DMTPs’ cytoplasmic domains

intracellularly (Figure 1 and [66, 67]). Functions for these cytoplasmic domains are reviewed in Sections D-E and also in [66, 67].

Expression of different GAG chains on membrane bound DMTPs can impact soluble DMTP (sDMTP) pools, as observed with membrane bound SDC1's HS chains, where higher membrane bound HS modified SDC1 leads to lower SDC1 shedding[68]. Altering the balance of membrane bound versus soluble DMTPs could, in effect, impact cell signaling by altering ligand interactions with sDMTPs' ECD/GAG chains (See growth factors in Section D). In the cell media, for example, increased levels of sDMTPs containing HS chains exclusively could suppress signaling as they may act as ligand traps capable of reducing ligand availability to the membrane-bound cognate receptors. Cleaved DMTP ectodomains containing CS chains could prevent ligand aggregation in the cell media, thereby facilitating ligand-receptor interaction and increasing cancer cell signaling. Given the current impact of DMTPs in regulating cell signaling, as outlined in Section D, an in-depth analysis of the GAG chain composition on membrane-bound and sDMTPs may clarify roles for DMTPs and their GAG chains in modulating growth factor signaling and cell biology.

C. Dually Modified Transmembrane Proteoglycans in Development and Disease

C1. Development

SDC1 is expressed during development to modulate tissue morphogenesis as well as during wound healing, with several of its developmental functions mediated by its GAG chains' interactions with a number of extracellular adhesion proteins and growth factors[46, 51, 69]. SDC1 interaction with laminin, for example, is completely dependent on both its HS and CS chains and leads to increased cell adhesion to the basement membrane[70]. However, SDC1's CS chains promote Slit signaling to drive axon and myotube guidance when bound to the core protein[28, 71], suggesting differential roles for the independent GAG chains. Betaglycan's significance in development is underscored by the observed embryo lethality in betaglycan knockout mice after 13.5 days[72, 73]. This lethality is partly due to significant impairment of coronary vasculogenesis in betaglycan-null mice, demonstrating a requirement for betaglycan in coronary vessel development and embryonic viability[73]. Additionally, betaglycan-specific antisera inhibits mesenchyme formation and endothelial cell migration in the atrioventricular (AV) cushion, where cardiac endothelial cells lining the lumen of the AV cushion undergo epithelial-mesenchymal transformation (EMT) to participate in valve and membranous septa formation[74–76]. Moreover, betaglycan overexpression in non-transforming ventricular endothelial cells confers transformation in response to TGF- β 2, suggesting betaglycan promotes heart cell transformation via TGF- β signaling[75]. As part of the adaptive immune response, T-cell receptors (TCRs), which are expressed on T lymphocytes, recognize antigens associated with molecules of the major histocompatibility complex[77]. In both lymphoid and stromal cells of the thymus, betaglycan has been shown to promote T-cell development by protecting thymocytes from apoptosis[78]. Individual functions for betaglycan's HS and CS chains during development are still under investigation.

C2. Disease

Over the past few decades, an increasing body of evidence suggests that HS and CS chains heavily influence cell biology and cancer progression[79]. GAG chains, which are present at the cell surface of almost all tumor cells[79], can regulate growth factor signaling, tumor cell survival, angiogenesis, growth, invasion and metastasis[79]. HSPGs can exhibit both pro-tumorigenic[3–6] and anti-tumorigenic effects depending on their GAG modifications[7–10]. In addition to cancer, changes in HSPG expression/function are also observed in other human diseases, including arthritis, inflammation[3], Alzheimer's/dementia[80], osteoporosis[81], fibrosis[82] and ischemic heart disease[83]. Furthermore, HSPGs have been shown to alter wound healing and matrix remodeling, outcomes that can impact a broad range of pathologies[3, 72, 83, 84], making our understanding of HSPG/GAG chain roles in the context of the cellular environment critical. Since much of the research emphasis has been placed on HSPG/GAG chain roles in cancer (>8% of all PubMed articles), we will focus on summarizing and analyzing DMTP GAG chain functions in cancer for the remainder of this review.

C2.1: Cancer—Both SDC1 and betaglycan have established roles in regulating tumor progression with specific roles in apoptosis, adhesion, angiogenesis, proliferation, migration, invasion and metastasis *in vitro* and *in vivo*[2, 9, 39, 51, 85–95]. The membrane-bound form of SDC1 is capable of suppressing apoptosis as SDC1 knockdown in myeloma cells induce growth arrest and apoptosis[4]. Other reports, however, indicate that recombinant human SDC1 ectodomains induce apoptosis in breast cancer cells[8]. Whether differential GAG modifications in the recombinant SDC1 ectodomains versus SDC1 in myeloma account for the differences observed is unclear. Moreover, while increased SDC1 levels correlate with a more favorable prognosis in mesothelioma, head and neck, pancreatic, lung and invasive breast cancers[2, 3, 39, 96, 97], both tumor promoting and tumor suppressing effects have been noted in colorectal and prostate cancers[39]. These seemingly contradictory functions for SDC1 emphasize the variability of DMTP roles in cancer. Similarly, in the case of betaglycan, loss or reduced expression has been demonstrated in tumors[85–87, 90, 98]. In particular, betaglycan expression has been shown to be a strong predictor of overall and recurrence-free patient-survival in certain cancers, such as breast cancer and neuroblastoma[85, 89, 99]. Although betaglycan expression is commonly lost in most tumor types, acting primarily as a tumor suppressor[2, 85–87, 89, 99], betaglycan expression has been shown to be elevated in colon cancer, a subset of triple negative breast cancers and high-grade lymphomas, indicating context dependent functions for betaglycan[5, 6, 100]. Whether or not betaglycan's differential role in cancer can be attributed to its HS and CS chain functions remains to be determined.

In addition to controlling cell signaling in an autocrine fashion, sSDC1 can also deliver growth factors in a paracrine manner to neighboring cells in the environment. sSDC1 from multiple myeloma cells, for example, transports hepatocyte growth factor (HGF) to bone marrow stromal cells to regulate gene expression[39, 101]. These HGF/sSDC1 interactions in the ECM likely cooperate with already established roles for membrane SDC1 in promoting myeloma cell growth, survival and spread through HS chains interactions with HGF[3]. Betaglycan levels in the tumor stroma may vary depending on the tumor type. For

instance, betaglycan expression is significantly higher in the stroma of early-stage, stroma-rich neuroblastomas[99], yet is down regulated in the tumor stroma of breast tumors[102]. In neuroblastoma, increased stromal expression confers a significant survival advantage as patients with increased stromal betaglycan exhibited significantly higher event-free survival due, in part, to increased Fibroblast Growth Factor (FGF) signaling and differentiation[99]. Contrastingly, in breast tumor stroma, decreased betaglycan mRNA and protein expression is linked to poorer patient outcome[102], supporting betaglycan's contextual roles in cancer. Whether stromal cells can take up sBetaglycan from tumor cells to impact gene expression, like in the case of sSDC1, has not been determined. Taken together, these data suggest that DMTPs can have important effects on cancer progression as potent regulators of tumor stromal interactions mediated partly through their GAG chains.

In the tumor environment, cancer cells can subvert the host immune system with mechanisms similar to those they use to evade immunotherapeutic strategies[103–105]. Additionally, effector cell dysfunction and an increased number of regulatory/suppressor T-cells in cancer patients may further limit the efficacy of immunotherapeutic approaches[106]. In murine and human lymphocyte subpopulations, betaglycan is significantly and preferentially expressed on naïve and central memory T-cells and is upregulated upon TCR stimulation. Treating cells with sBetaglycan decreased regulatory/suppressor FoxP3⁺ T-cell generation, in a TGF- β -dependent manner, suggesting a promoter role for betaglycan in T-cell tolerance and immune responses[77]. In both murine breast cancer and melanoma, progressive loss of betaglycan expression generates an immunotolerant tumor microenvironment whereby TGF- β signaling is enhanced and upregulates both immunoregulatory enzyme indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells (DCs) and the CCL22 chemokine in myeloid DCs[103]. This mechanism, coupled with the elevated TGF- β levels, creates an immunotolerant environment and may facilitate tumor progression and metastasis[103]. Contrary to betaglycan's ability to increase tumor sensitivity to immune defenses, SDC1 appears to aid in cancer's subversion of the host immune system. For example, myeloma cells coated with anti-SDC1 antibodies displayed enhanced cross-presentation of tumor-derived cellular antigens and generated tumor specific-killer T cells by DCs[107], suggesting an immunosuppressive role for SDC1. Additionally, anti-SDC1 immunotoxin (IT, B-B4-SO6), in combination with the chemotherapeutic drug doxorubicin, led to increased cell death in doxorubicin-sensitive multiple myeloma cells[108], further supporting SDC1's tumor promoter role. An in-depth analysis of DMTPs and their GAG chains in modulating interactions between the immune system and malignant cells is warranted as it could strongly augment the design of clinically more efficient immunotherapy strategies.

D. Cell Signaling Regulation by Dually Modified Transmembrane Proteoglycans

Tight control of growth factor/ligand availability at the cell surface is crucial to downstream signaling in both cancer and various other diseases/cell phenotypes[109–111]. Growth factors frequently utilized by cancer cells include, but are not limited to, FGF, Wingless (Wnt), BMP/GDF, Activin and TGF- β [9, 33, 86, 99, 112–124]. These growth factors can

interact with DMTPs to either promote or suppress signaling. One general mechanism for increasing signal reception is by concentrating ligands near their cognate signaling receptors to activate downstream signaling. Specifically, GAG chains on DMTPs could allosterically regulate and/or oligomerize soluble ligands, serve as guides for bringing growth factors and receptors into close proximity and/or provide proper alignment for productive binding to occur[125]. In contrast, sequestering ligands away from their signaling receptors and/or disrupting receptor-receptor interactions could promote DMTP-mediated cell signal suppression. DMTPs could also serve as growth factor hubs, able to release growth factors at specific times for utilization by a subset of cells in a context dependent manner.

By analyzing individual roles for HS and CS chains on DMTPs in growth factor signaling we can develop a framework to understand the biological impact DMTPs and their GAG chains have on ligand availability and signaling. Most importantly, this new knowledge could lead to the development of novel therapeutics to control cancer and other disease processes.

D1. TGF- β Family Signaling

To initiate signaling, TGF- β members, including BMP and Activin, interact with two types of receptors (type I and II) to transduce signals via the Smad family signal transducers[33, 124, 126, 127]. Upon ligand binding and receptor activation, the receptor complex phosphorylates the C-terminus of the receptor-regulated Smad proteins (R-Smads, Smad1, 5 and 8 for BMP signaling and Smad2 and 3 for TGF- β signaling). Activated R-Smads then interact with the common partner Smad4 and accumulate in the nucleus, where the Smad complex directly binds defined elements on DNA and regulates target gene expression[33, 124, 126, 127].

Membrane-bound Betaglycan—Independent of its GAG chains[128], betaglycan's ECD can bind all three TGF- β isoforms, BMP and Activin[33, 129–131]. Several studies suggest membrane betaglycan's primary function in TGF- β signaling is to “present” ligands, such as TGF- β 2, to the Type II TGF- β receptor to activate downstream signaling[126, 131–133]. In fibroblasts, for example, membrane betaglycan mediates TGF- β signaling by presenting ligands and trafficking the associated TGF- β receptors[126]. This coreceptor role, however, does not fully encompass betaglycan's impact on the TGF- β signaling pathway as sBetaglycan inhibits TGF- β signaling through ligand binding competition with membrane betaglycan in a variety of cell types (Figure 2 and [85, 134, 135]). Whether the impact of betaglycan in cancer is via direct TGF- β -mediated mechanisms has been poorly demonstrated/tested, as domain specific functions of betaglycan's ECD *in vivo* have not been investigated in-depth. However, upon loss of betaglycan, cancer cells do become less sensitive to TGF- β -mediated cancer growth and migration, indicating that betaglycan can impact TGF β -dependent regulation of cancer cell behavior (Figure 2 and [133]). More recently, betaglycan has been shown to tether TGF- β 1 to the surface of cancer cell exosomes, which help cancer cells grow and spread as they drive TGF- β -induced differentiation of cancer-associated fibroblasts into myofibroblasts[136].

In addition to betaglycan's ECD effects on TGF- β signaling, betaglycan's cytoplasmic domain can interact with GIPC to regulate TGF- β signaling. In breast cancer, betaglycan inhibits migration and invasion through direct inhibition of TGF- β signaling via interactions with GIPC, as supported by a strong correlation between reduced TGF- β signaling and decreased invasion and metastasis *in vitro* and *in vivo*[137]. Both effects were controlled by betaglycan's cytoplasmic domain as opposed to its ECD (Figure 2 and [137]). Whether specific GAG chain modifications impact the cytoplasmic domain interactions of betaglycan with GIPC is currently unclear. Betaglycan's cytoplasmic domain also interacts with scaffolding protein β -arrestin2 to suppress ovarian cancer cell migration. This interaction leads to activation of cell division control protein 42 homolog (Cdc42), alterations of the actin cytoskeleton and suppression of ovarian cancer migration. Although this mechanism of inhibition was found to be TGF- β ligand independent, betaglycan's ECD/GAG chains were shown to contribute to betaglycan's suppressive effects on ovarian cancer cell migration[138]. An alternate possibility could be GAG chain-dependent regulation of TGF- β co-receptor complex formation, which could lead to enhancement or suppression of TGF- β signaling. Subsequent studies in renal epithelial cells demonstrated that GAG chains on betaglycan prevent the physical association of T β RI with T β RII, which leads to suppression of TGF- β signal transduction[2, 139]. In line with betaglycan's GAG chain effects in renal epithelial cells, GAG chains on betaglycan could also inhibit proper formation of ligand receptor complexes, adding another layer of signaling regulation by betaglycan's HS and CS chains. Alternatively, impact of betaglycan on other GAG binding ligands besides TGF- β (See DMTPs and Wnt in Section D3) may be more relevant in a specific context.

To regulate BMP signaling, studies have shown that betaglycan's core domain directly binds BMP2, although with lower affinity than TGF- β [33], and BMPs' cognate receptors ALK3 and ALK6 (Figure 2 and [33]). Not only does betaglycan interact with BMP2, independent of its GAG chains[33], betaglycan's core domain also directly binds BMP4, BMP7 and GDF-5 to enhance ligand binding to ALK3 and ALK6[33]. This, in turn, promotes EMT of AV endothelial cells during development. In prostate cancer, betaglycan knockdown correlated with a significant decrease in BMP2 mediated Smad1 phosphorylation, indicating critical roles for betaglycan in facilitating BMP functions in both cancerous and non-cancerous cells[33].

In addition to betaglycan's ability to affect BMP induced cell biology through direct BMP binding, betaglycan's core domain also impinges on BMP and TGF- β /Activin signaling through high affinity core domain interactions with another TGF- β family member: Inhibin (Figure 2 and [129, 140]). Upon binding Inhibin, betaglycan promotes Inhibin interactions with ActRII, ActRIIB and/or BMPRII type II receptors[129, 141]. This Inhibin-betaglycan-type II receptor complex leads to prevention of Activin and BMP induced type I-type II receptor interactions for signaling[129, 141, 142]. Since Inhibin binds with low affinity to type II receptors in the absence of betaglycan, the core domain of betaglycan plays a central role in potentiating Inhibin antagonism of Activin and BMP signaling[141, 142].

Soluble Betaglycan—In addition to the many membrane-bound functions of DMTPs and their GAG chains in cancer, ectodomain shedding reduces the number of cell surface receptors, leading to down-regulation of signaling as soluble forms of DMTPs' ECDs

compete with their membrane-bound forms for ligands[10, 51, 99, 102, 143]. Upon cleavage from the cell membrane, for example, sBetaglycan can control tumor progression by limiting TGF- β ligand availability at the cell surface (Figure 2 and [85, 134, 135]). The remaining transmembrane/cytoplasmic betaglycan fragment is stable and targeted by γ -secretase to release the cytoplasmic domain and can enhance TGF- β 2 signaling, supporting a role for membrane bound forms of betaglycan in promoting TGF- β signaling[66]. In breast cancer cells, sBetaglycan inhibits cancer cell invasion, migration, tumor-induced angiogenesis and metastasis *in vivo* by binding and sequestering TGF- β from its cognate receptors[85, 144]. Likewise, restoring betaglycan expression in human breast cancer cell lines or treating cells with sBetaglycan inhibits BMP-induced Smad1/5/8 phosphorylation and BMP-stimulated migration and invasion (Figure 2 and [145]). Collectively, these data demonstrated the role sBetaglycan plays in suppressing TGF- β /BMP signaling. Whether all PG forms of betaglycan are shed equally (See Shedding Section B3) and contribute to sBetaglycan's TGF- β /BMP suppressor roles is currently unknown.

SDC1—Although SDC1 is the predominant HSPG in epithelial cells, information on its roles in TGF- β /BMP signaling is limited, likely due to the predominant role for betaglycan as a high affinity TGF- β /BMP HSPG. However, in the normal murine mammary gland and in human lung carcinoma epithelial cells, SDC1 cell surface expression is upregulated by TGF- β in a post-translational and Protein Kinase A (PKA) dependent manner[146]. Microarray studies from fibrosarcoma cells revealed that nuclear SDC1, promotes TGF- β signaling activity[147]. In mesothelioma cells, however, TGF- β inhibited nuclear translocation of SDC1 to reduce cell proliferation[148]. In other cancers, such as lung[149] and malignant glioma[150], SDC1 expression was unaffected by TGF- β stimulation. During cancer progression, TGF- β can induce EMT through activation of a spectrum of EMT inducing transcription factors, such as *SNAIL*. In prostate cancer, SNAIL has been shown to repress SDC1 expression, which correlates with a higher histological Gleason grade (more undifferentiated tumors) in these tissues[151], suggesting possible effects of TGF- β induced EMT via repression of SDC1. During *Xenopus* development, overexpression of SDC1 leads to suppression of BMP signaling (Figure 2 and [152]). Taken together, these data indicate a link between SDC1 and TGF- β /BMP that may have significant implications for cancer.

D2. FGF Signaling

FGF glycoproteins, via their FGF receptors, regulate cancer cell proliferation, apoptosis, angiogenesis, EMT, motility and invasion and can also contribute to chemoresistance[111, 153]. Depending on cellular context, however, both *in vitro* and *in vivo* studies have demonstrated the oncogenic potential of FGF signaling as well as contrasting tumor suppressive functions[154]. The 18 members of the FGF family initiate their signaling cascade through four highly conserved transmembrane tyrosine kinase receptors (FGFR1-4). To access these receptors, secreted FGF ligands are released from ECM HSPGs, via heparinase and protease digestions[155, 156], so that they can bind to FGFRs and cell surface HSPGs which aid in FGF receptor stabilization and downstream activation of multiple signaling pathways[154, 155]. Although direct binding of FGF to CS chains on HSPGs has not been observed in any cell type, CS chains can alter HS chain-FGF-FGFR kinetics to suppress FGF signaling during chondrocyte proliferation[157], indicating a

potential FGF signaling suppressor role for HSPG CS chains in chondrocytes. In normal murine mammary gland epithelial cells, however, CS chains on HSPG SDC1 aid in faster binding and release of FGF2 from SDC1's HS chains, indicating that CS chains on HSPGs in epithelial cells may facilitate more efficient transfer of growth factors to their cognate receptors (Figure 2 and [158, 159]). SDC1's HS chains also facilitate the FGF2-FGFR1 complex formation in lymphoma, breast and prostate cancers to promote malignant transformation[39, 160–162]. While it is well known that membrane-anchored SDC1 can regulate signaling complex formation between a number of growth factors and their receptors[56, 57], it has also been demonstrated that soluble SDC1 (sSDC1) can affect cell-signaling outcomes to control tumor progression. Similar to betaglycan, studies have identified both cooperative and opposing effects between membrane-bound SDC1 and sSDC1 in cancer[39, 51, 59, 163, 164]. In MCF-7 breast cancer cells, for instance, membrane SDC1 promotes cell proliferation and inhibits invasion by acting as a FGF2 coreceptor for mitogenic MAPK signaling. sSDC1, contrarily, inhibits cell proliferation and promotes cancer cell invasion. The proteolytic conversion of SDC1 from membrane-bound to soluble marks the switch from a proliferative to an invasive phenotype in breast cancer[51]. This switch could potentially be controlled by differential presentation of SDC1's GAG chains (HS-SDC1 vs. CS-SDC1). Since released ectodomains have the ability to compete for GAG chain binding proteins that normally interact with membrane-bound DMTPs (Figure 2), changes in cell surface SDC1 versus sSDC1/GAG chain expression could dramatically alter growth factor signaling. This has been observed in lymphoid cells where HS chains from sSDC1 antagonize FGF2 signaling to reduce mitogenicity[56, 57, 165].

Similar to SDC1, betaglycan's HS chains are required for FGF activity in osteoblasts and in neuroblastoma cells[9, 166]. In neuroblastoma, betaglycan's GAG chains bind FGF2 and FGFR1 to activate MAPK/ERK pathways and promote neuronal differentiation. This causes a significant decrease in neuroblastoma cell proliferation *in vitro* and tumor growth and metastasis *in vivo*, supporting betaglycan's tumor suppressive role in cancer[9]. Similar consequences of the membrane and sBetaglycan were observed where both forms of betaglycan upregulate the transcription factor inhibitor of DNA binding 1 (ID1), via FGFR1 and MAPK/ERK phosphorylation, to promote differentiation[9, 99]. These studies further revealed that the HS chains on sBetaglycan are primarily responsible for its tumor suppressive effects, adding to the growing body of evidence supporting regulatory functions for GAG chains on DMTPs in cancer (Figure 2). Whether the CS chains on membrane-bound or sBetaglycan act in a similar manner to SDC1's CS chains has not been determined but could reveal whether differential presentation of betaglycan's GAG chains (HS-betaglycan vs. CS-betaglycan) is responsible for the opposing and/or cooperative effects observed between membrane and sBetaglycan in cancer.

An in-depth study on individual functions for GAG chains on DMTPs, as they relate to FGF signaling and FGF-induced cancer cell biology, is warranted as it could reveal why DMTPs and sDMTPs exhibit both tumor-promoting and tumor-suppressive roles in FGF-mediated cancer progression.

D3. Wnt Signaling

The Wnt family of glycoproteins plays active roles in cancer by promoting growth, EMT and chemoresistance[114, 115, 167–178]. Wnt transduces its signals beginning with cell surface binding of Wnt to Frizzled (Fzd) and Low-density lipoprotein receptor (LDLR)-related proteins LRP5/6, resulting in phosphorylation of LRP5/6. Changes in LRP5/6 conformation, due to its phosphorylation, leads to increased interaction between Dishevelled, Fzd and Axin, resulting in the inactivation of the β -catenin destruction complex, accumulation of β -catenin in the cytoplasm and translocation to the nucleus to activate Wnt target genes[169, 170]. Wnt/ β -catenin signaling is a well-established tumor-promoting pathway in many cancers making identification of molecules regulating Wnt/ β -catenin signaling crucial to improving cancer outcomes. DMTPs can both stimulate Wnt signaling (SDC1) (Figure 2 and [179]) or suppress Wnt signaling (betaglycan) (Figure 2 and [10]). Even though Wnt glycoproteins have high affinity for GAG chains on transmembrane HSPGs[49], individual roles for HS-DMTP or CS-DMTP in Wnt signaling have been difficult to tease out due to the dichotomous roles HSPGs play in regulating Wnt signaling[117, 180].

SDC1 null mice, which develop normally, are resistant to Wnt-1 induced mammary tumor development[39, 179, 181] suggesting a role for SDC1 in Wnt-induced tumor formation. Moreover, upon crossing these tumor-resistant SDC1 null mice with mice overexpressing canonical Wnt/ β -catenin signaling effectors (gain-of-function mutations in *Wnt-1* and *β -catenin*), amplification of mammary progenitor cells as well as tumor development was reduced, pointing to a direct role for SDC1 in promoting Wnt-induced carcinogenesis[182, 183]. Supporting these studies, in triple negative breast cancer stem cells, SDC1 silencing decreased Wnt signaling through downregulation of LRP6[183, 184]. Likewise, parallel SDC1 and Wnt-1 expression were correlated with increased proliferation and invasion of intraosseous ameloblastomas[183, 185]. In multiple myeloma, SDC1's HS chains promoted cell proliferation by directly binding Wnt3a and activating paracrine Wnt-Fzd activity[186]. SDC1's HS chains also bound osteoblast-produced R-spondin, which represses Fzd degradation, supporting a promoter role for SDC1's HS chains in Wnt signaling[186]. In addition to SDC1's effects on canonical Wnt signal transduction, SDC1 can also regulate non-canonical Wnt signaling. In metastatic melanoma cells, for example, SDC1 is a critical component of the Wnt5a autocrine-signaling loop. Upon enzymatic removal of SDC1, a decrease in both cellular Wnt5a and Wnt5a signaling was observed with an associated decrease in melanoma cell invasion[117, 183]. Whether differential presentation of SDC1 (SDC1-HS v. SDC1-CS) alters SDC1-HS-Wnt interactions remains to be determined, but could offer new insight as to the significance of different GAG chain attachments in modulating growth factor availability and downstream signaling.

We recently uncovered that betaglycan's GAG chains can suppress Wnt signaling at both the signal reception level and through inhibition of β -catenin transcriptional activity (Figure 2 and [10]). In addition to the suppressive effects for membrane-bound betaglycan in the Wnt/ β -catenin pathway, sBetaglycan was able to suppress β -catenin transcriptional activity to downregulate Wnt signaling (Figure 2 and [10]), indicating sBetaglycan was sufficient to mimic membrane-anchored betaglycan's suppressive effects on Wnt signaling (Figure 2).

Interestingly, we found opposing functions for betaglycan's HS and CS chains in canonical Wnt signaling. A combination of loss and gain of function approaches suggested that betaglycan's CS chains may promote Wnt signaling while betaglycan's HS chains suppress Wnt signaling, mostly likely via direct interactions with Wnt growth factors[10, 49]. These studies suggested that the composition of GAG chains on betaglycan may significantly alter cellular response to betaglycan, supporting distinct outcomes and roles for HS and CS chains on DMTPs in regulating various growth factor signaling cascades.

E. DMTP Trafficking

DMTPs, which are ubiquitously expressed in tissues, are primarily located at the cell surface and within the ECM[2, 79]. Since many normal and pathological functions have been assigned to DMTPs, including regulation of growth factor signaling, cell migration, proliferation, differentiation, angiogenesis and cancer metastasis (Reviewed in Sections C-D), cells exercise exquisite control over DMTP cell surface expression and activity through receptor endocytosis and recycling. Receptor internalization and routing to early endosomes may lead to either recycling of the receptor back to the cell surface to propagate signaling or ubiquitination and subsequent degradation[133].

Full-length SDC1, which is usually found at the cell surface/membrane, can be internalized via clathrin-independent/caveolae/lipid raft endocytosis (Figure 3 and [39, 187]) after ligand-dependent clustering with other cell surface SDC1s[187–189]. Clustering of SDC1s' transmembrane and cytoplasmic domains leads to energy-independent movement into cholesterol-rich and detergent-insoluble membrane rafts[39, 189], a required step before endocytosis[187]. Following SDC1 localization to membrane rafts, SDC1 is internalized in two phases (Figure 3), each requiring a kinase and a corresponding cytoskeletal partner. During phase one, ligands, such as model remnant lipoproteins, can induce rapid activation of the serine/threonine-selective protein kinase ERK and localization of SDC1 into clathrin-independent vesicles[187]. ERK activation leads to SDC1 dissociation from α -tubulin, a likely anchor for SDC1 at the membrane[187]. This first phase is dependent on HS chain interactions with ligands that can trigger clustering and membrane invagination[190]. Unlike SDC1's HS chains, defined roles for SDC1's CS chains during SDC1 endocytosis have not been found. In the second phase, Src family kinases phosphorylate tyrosyl residues within SDC1's transmembrane and cytoplasmic domains [187]. Tyrosine phosphorylation of SDC1 triggers the robust recruitment of cortactin, which was found to be an essential mediator of efficient actin-dependent endocytosis[187]. A single, conserved juxtamembrane motif, MKKK, in the SDC1 cytoplasmic tail mediates the sequential activation of ERK and Src kinases to facilitate efficient endocytosis[187].

Once internalized, SDC1 can translocate to the nucleus of several cell types (Figure 3 and [39, 147]). The driving force behind localization of SDC1 in the nucleus is a nuclear localization signal in SDC1's cytoplasmic tail (RMKKK motif)[191]. In cancer, this nuclear localization is enhanced when heparanase levels are low[192], which would leave more HS chains attached to cell surface SDC1s to promote SDC1 clustering and internalization. In the nucleus, SDC1 can co-localize with FGF2 despite the fact that FGFR1, FGF's cognate receptor, is sequestered in the perinuclear area[39, 191], indicating an unknown FGF

receptor-independent function for SDC1-FGF2 interactions in the nucleus. In fibrosarcoma cells, nuclear SDC1 acts as a tumor suppressor by inhibiting cell proliferation and cell cycle progression[147]. Nuclear SDC1 also inhibits nuclear histone acetyltransferase activity and acetylation of histones. This, in turn, decreases gene expression involved in driving aggressive tumor phenotypes[193]. Differential levels of SDC1 in the nucleus could alter tumor outcomes if HS and CS chains on DMTPs have opposing roles in regulating histone acetylation. This has been noted with the shed form of SDC1, which is elevated in myeloma and localizes to the nucleus of myeloma cells. sSDC1 was also found in areas of high gene transcription, indicating a regulatory function. Nuclear sSDC1 decreases histone acetyltransferase levels (HAT) and subsequent histone acetylation in a GAG chain dependent manner. Specifically, HAT activity is decreased through sSDC1 HS chain binding to p300, a member of the HAT enzyme complex. Importantly, the GAG chains of SDC1 are required for nuclear localization of sSDC1. Heparin binding factors must be present for sSDC1 to be translocated to the nucleus (sSDC1 does not have a nuclear translocation sequence). Additionally, the HS chains of sSDC1 are required for HGF translocation to nucleus, suggesting an important role of sSDC1 and its HS chains in gene regulation within the tumor microenvironment[101].

Betaglycan is constitutively internalized, independent of TGF- β binding to its core domain, via either a clathrin-dependent mechanism or a clathrin-independent (lipid raft/caveolin-mediated) pathway (Figure 4 and [133]). Whether protein interactions with betaglycan's GAG chains impact internalization has not been determined. Betaglycan internalization via the clathrin-dependent endocytic pathway, leads to recycling back to the cell surface by way of early endosomes and has been shown to increase TGF- β /SMAD signaling (Figure 4 and [194]). Type II TGF- β receptor (T β RII)-mediated phosphorylation of betaglycan's cytoplasmic domain leads to betaglycan association with β -arrestin2 and subsequent internalization of both T β RII and betaglycan into clathrin vesicles[44]. Point mutation studies of betaglycan's cytoplasmic domain led to the discovery that Thr⁸⁴¹ was critical for interaction of betaglycan with β -arrestin2[44, 133]. In both normal epithelial and hepatocellular carcinoma cells, betaglycan has been shown to direct the Type I and II TGF- β receptors towards both clathrin independent and clathrin dependent internalization routes, suggesting either cell type specificity, or differences in the presence of other betaglycan binding ligands[195, 196]. Both clathrin dependent and independent pathways were shown to impact TGF- β signaling, with the former increasing TGF- β signaling through association with the SMAD Anchor for Receptor Activation (SARA) in early endosomes and the latter leading to degradation and reduction of TGF- β signaling (Figure 4 and [44, 133, 195, 196]).

Studies have shown that betaglycan can suppress directional migration of both normal and cancer cells through β -arrestin2-mediated direct interactions with Cdc42 and Cdc42 activation, which is GAG chain dependent but independent of TGF- β [92, 138]. Moreover, betaglycan has also been shown to co-traffic with integrin α 5 in a β -arrestin2-dependent manner for regulating epithelial cell adhesion and ECM remodeling[89]. In polarized breast cancer cells, disruption of basolateral localization of betaglycan results in global loss of cell polarity, a hallmark of EMT and cancer progression[94], implicating trafficking of betaglycan for maintaining epithelial cell polarity and suppression of breast cancer progression[94]. Although betaglycan does not contain a nuclear localization sequence in its

cytoplasmic domain[66], cleavage of the cytosolic fragment by γ -secretase may initiate trafficking of betaglycan's cytoplasmic domain to the nucleus with other nuclear proteins, as has been observed for SDC1[39, 191], in addition to its previously described roles in enhancing TGF- β signaling (Section D1 and [66]). The specific contributions of the GAG chains to betaglycan co-trafficking has not been determined and may reveal new internalization and cancer regulatory roles for betaglycan's HS and CS chains.

Since cell-surface HSPGs, including SDC1 and betaglycan, are involved in regulation of availability of multiple growth factors implicated in disease (Section D), exploring the molecular mechanisms and potential roles for HS and CS chains in SDC1- and betaglycan-mediated endocytosis will substantially advance our understanding of how cells handle these biologically and clinically targetable ligands.

F. Concluding Remarks

This review summarizes crucial roles for DMTPs and their GAG chains in pathophysiological conditions with a particular focus on cancer. We collate current knowledge and discuss potential roles for dually modified betaglycan and SDC1 in modulating cancer cell signaling via their soluble and membrane-bound forms, as well as through receptor internalization. At the cell-ECM interface, betaglycan and SDC1 control tumorigenic processes such as growth, invasion and metastasis by regulating growth factor availability through their GAG chain attachments. Further characterization of individual roles for GAG chains on DMTPs will, in the future, aid in the design of precision therapies such as specific HS-DMTP or CS-DMTP mimetics as opposed to global therapeutic approaches that are problematic. Much is known about the functions of SDC1 and betaglycan since their discoveries in 1989[34] and 1985[197] respectively, yet better definitions of the disease contexts in which the GAG chains on DMTPs are most relevant is required to improve our ability to use these DMTPs as diagnostic and prognostic markers and as biomarkers for treatment response. With the advent of new technologies, the field is well positioned to address these questions and increase our current knowledge on individual roles of HS and CS chains on DMTPs in disease. This increase in knowledge, in turn, will lead to the development of precise, context-dependent therapeutic strategies.

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Biographies

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Laura is a fourth-year Ph.D. candidate in the Department of Chemistry and Biochemistry at the University of South Carolina. Previously, she completed her Bachelor of Science in Biosystems Engineering at Clemson University and her Master of Science at Claflin University in Biotechnology. Currently, she is characterizing new roles for glycosaminoglycan chain modifications on proteoglycans in cancer and cell biology. She was recently awarded the NIH F31 Fellowship and the American Association of University Women (AAUW) Dissertation Fellowship and will complete her dissertation by May 2018. Upon her graduation, Laura will continue pursuing scientific research relevant to public health.

Ben Horst

Ben received his Bachelor of Science in Biochemistry from Furman University. As an undergraduate, he did research with Dr. Lon Knight using electron paramagnetic resonance spectroscopy to study radicals isolated in neon matrices at cryogenic temperatures. He is currently in his second year of graduate school at the University of South Carolina in the Department of Chemistry and Biochemistry. Currently, he is examining new functions for Inhibin, a TGF β family member, in tumor angiogenesis as well as its impact on signaling in endothelial cells. After finishing graduate school, Ben aspires to stay in the field of cancer research specifically in the area of drug development/pharmaceuticals.

Carly L. Lancaster

As a third-year undergraduate student, Carly is studying biochemistry and molecular biology at the University of South Carolina (USC). She attended West Ashley High School in Charleston, South Carolina where she graduated second in her class. Presently, Carly is working to characterize new roles for betaglycan's chondroitin sulfate GAG chains in Wnt signaling and has been awarded the 2017 USC Magellan Fellowship to support her during her studies. After graduating, Carly will attend graduate school and work towards her Ph.D. in Biochemistry so that she can obtain the knowledge and skills needed to successfully fulfill her goal of becoming an independent cancer biologist.

Mythreye Karthikeyan, Ph.D

received her doctoral degree from University of North Carolina at Chapel Hill in 2005. She did her postdoctoral work at Duke University and subsequently started her own laboratory at the University of South Carolina in 2013. The research group's main interest is in understanding the interplay between the TGF β family of growth factors and their receptors and how this impacts cancer progression and metastasis. The lab uses molecular, cell biological, biochemical and *in vivo* tools. Her group is a combination of undergraduate, graduate and postdoctoral trainees.

Abbreviations

ActR	activin type 2 receptor
ALK	bone morphogenetic protein receptor, type IA
AV	atrioventricular cushion
BMP	bone morphogenetic protein

BMPR	bone morphogenetic protein receptor
C1	conserved domain 1
C2	conserved domain 2
Cdc42	control protein 42 homolog
CS	chondroitin sulphate
DC	plasmacytoid dendritic cells
DMTP	dually modified transmembrane proteoglycan
ECD	extracellular domain
ECM	extracellular matrix
EMT	epithelial-mesenchymal transformation
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
Fzd	frizzled
GAG	glycosaminoglycan
GDF	growth differentiation factor
GIPC	GAIP-interacting protein C terminus
HAT	histone acetyl transferase
HGF	hepatocyte growth factor
HSPG	heparan sulfate proteoglycan
HS	heparan sulphate
ID1	inhibitor of DNA binding 1
LRP	low-density lipoprotein receptor (LDLR)-related proteins
MAPK/ERK	mitogen-activated protein kinase/extracellular signal-regulated kinase
MMP	matrix metalloproteinases
NRP-1	neuropilin-1
PDZ	postsynaptic density-95/disc large protein/zonula occludens-1
PKA	protein kinase A
pY	tyrosyl phosphorylation

R-SMAD	receptor-regulated Smad
SARA	SMAD anchor for receptor activation
sBetaglycan	soluble/shed betaglycan
SDC1	syndecan-1
SDC3	syndecan-3
sDMTP	soluble dually modified transmembrane proteoglycan
sSDC1	soluble/shed SDC1
TβR	TGF-β receptor
TCR	T-cell receptor
TGF-β	transforming growth factor β
TP	transmembrane proteoglycans
V	variable domain, Wnt, wiggless/integrated
ZP	zona pellucida,

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Highlights

- Dually modified transmembrane proteoglycans (DMTPs) betaglycan and SDC1 control growth factor availability through their GAG chain attachments and core domains *in vitro* and *in vivo*.
- DMTP ectodomain shedding affects growth factor signaling in autocrine and paracrine manners by decreasing the number of cell surface receptors and increasing the number of environmental receptors.
- DMTPs share distinct and overlapping internalization and trafficking routes that lead to either recycling of the receptor back to the cell surface to propagate signaling or degradation and subsequent cell signaling suppression.
- DMTPs have fundamental physiological roles and are also key players in pathologies such as cancer progression and metastasis.

Figure 1. Structure and features of DMTPs betaglycan and SDC1

(A) Structures of DMTP CS (top) and HS (lower) glycosaminoglycan (GAG) chains. Both GAG chains share a common linkage tetrasaccharide covalently attached to a serine hydroxyl group on the DMTP core protein. These serine residues have an adjacent glycine residue on the carboxy-terminal side and usually lie within an area rich in acidic residues. (B) Betaglycan's core protein contains two ZP domains in its ECD for ligand binding and both DMTPs contain a small cytoplasmic domain, which can be cleaved by γ -secretase to release the cytoplasmic fragment. A PDZ motif in betaglycan's cytoplasmic domain is frequently phosphorylated and regulates betaglycan cell surface expression and intracellular protein interactions. SDC1's cytoplasmic domain is divided into two conserved regions and a variable region, which are responsible for intracellular protein binding. SDC1 can also interact with proteins containing a PDZ motif. Betaglycan and SDC1 both undergo ectodomain shedding by MMPs to release (C) soluble forms of each receptor, which can both bind growth factors and modulate their activity.

Abbreviations: *C1*: Conserved Domain 1, *C2*: Conserved Domain 2, *MMP*: Matrix Metalloproteinases, *PDZ*: Postsynaptic Density-95/Disc large protein/Zonula occludens-1, *sBetaglycan*: Soluble/shed betaglycan, *sSDC1*: Soluble/shed SDC1, *V*: Variable Domain, *ZP*: Zona Pellucida, *HS*: Heparan Sulfate, *CS*: Chondroitin Sulfate

Figure 2. DMTs in growth factor signaling pathways

DMTPs betaglycan and SDC1 bind growth factors either through their GAG chains or core protein and then regulate ligand function through distinct mechanisms. Growth factors bound to membrane DMTs are localized to the cell surface and are either presented to their associated signaling receptors (→) or sequestered away to prevent signal transduction (—|). Additionally, soluble DMT forms, generated by ectodomain shedding, bind ligands and either enhance or prevent signal complex formation. Abbreviations: *BMP*: Bone Morphogenetic Protein, *BMPR*: Bone Morphogenetic Protein Receptor, *CS*: Chondroitin Sulfate, *ERK*: Extracellular Signal-regulated Kinase, *FGF*: Fibroblast Growth Factor, *FGFR*: Fibroblast Growth Factor Receptor, *FZD*: Frizzled, *GIPC*: GAIIP-interacting protein C terminus, *HS*: Heparan Sulfate, *LRP6*: LDL Receptor Related Protein 6, *MAPK*: Mitogen-activated protein kinase, *sBetaglycan*: Soluble/shed betaglycan, *sSDC1*: Soluble/shed SDC1, *TGFβ*: Transforming Growth Factor β, *TβRI*, *RII*: Transforming Growth Factor β Receptors I and II, *Wnt*: Wingless.

Figure 3. Raft-dependent SDC1 endocytosis

(A) First phase, (1) growth factor binding induces rapid (2) ERK activation with (3) SDC1 clustering leading to movement of SDC1 into membrane rafts. In the presence of cortactin and SDC1's MKKK motif, ERK is activated and (4) drives SDC1 dissociation from α-tubulin. (B) Second phase, Clustering of SDC1's MKKK motif triggers (5) Src-dependent phosphorylation of tyrosyl residues within SDC1's transmembrane and cytoplasmic domains. This phosphorylation step leads to (6) cortactin recruitment followed by (7) actin-dependent endocytosis into caveolin vesicles. Abbreviations: CS: Chondroitin Sulfate, HS: Heparan Sulfate, MK: SDC1 MKKK motif, p: phosphorylation, pY: tyrosyl phosphorylation, Src: Src kinases.

Figure 4. Betaglycan Internalization and Trafficking

Betaglycan is internalized via a (1) clathrin-independent (lipid raft/caveolin-mediated) pathway or (2) a clathrin-dependent mechanism. Clathrin dependent endocytosis increases signal transduction via (3) betaglycan association with SARA in early endosomes. This SARA-betaglycan interaction leads to recycling of betaglycan back to the cell surface to continue signal transduction. To degrade betaglycan and downregulate signaling, (4) T β R II phosphorylates betaglycan at Threonine 841, increasing (5) betaglycan association with β -arrestin2 and (6) caveolin-mediated internalization and degradation of betaglycan. Additionally, betaglycan's cytoplasmic domain can be (7) cleaved by γ -secretase to release the cytoplasmic domain and enhance growth factor signaling. Abbreviations: CS: Chondroitin Sulfate, HS: Heparan Sulfate, T841: Threonine 841, T β R II: Transforming Growth Factor β Receptors II, SARA: SMAD Anchor for Receptor Activation.