Syndecan-4 signaling at a glance

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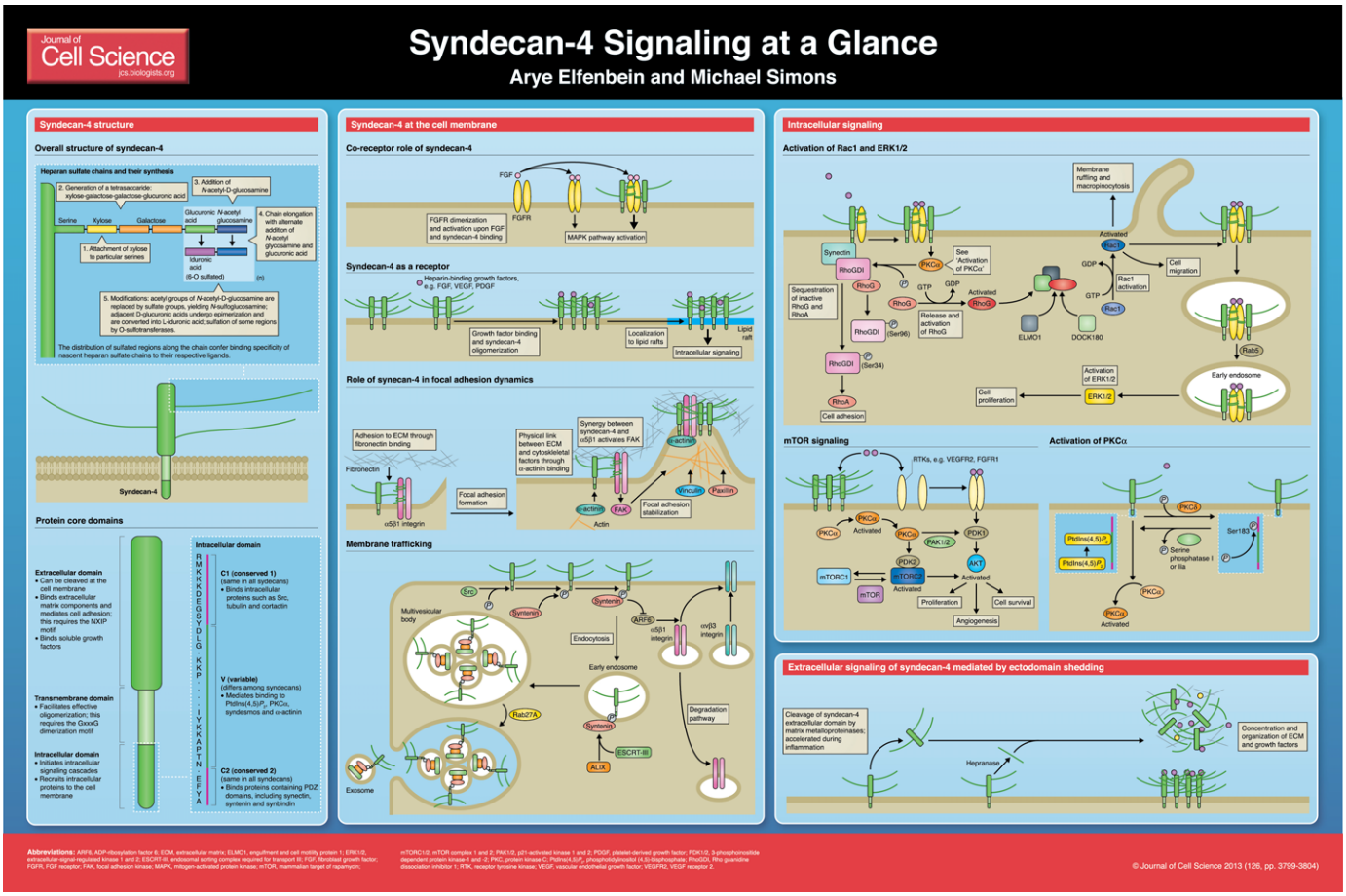
Summary

Syndecan-4, a ubiquitous cell surface proteoglycan, mediates numerous cellular processes through signaling pathways that affect cellular proliferation, migration, mechanotransduction and endocytosis. These effects are achieved through syndecan-4 functioning as both a co-receptor for the fibroblast growth factor receptors (FGFR1–FGFR4) and its ability to independently activate signaling pathways upon ligand binding. As an FGFR co-receptor, syndecan-4 strengthens the duration and intensity of downstream signaling upon ligand binding; this is particularly evident with regard to mitogen-activated protein kinase (MAPK) signaling. In contrast, syndecan-4 also functions as an independent receptor for heparin-binding growth factors, such as fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs) and platelet-derived growth factors (PDGFs). These signaling cascades affect canonical signaling components, such as the mammalian target of rapamycin (mTOR), AKT1 and the Rho family of GTPases. In combination with the integrin family of proteins, syndecan-4 is also able to form physical connections between the extracellular matrix (ECM) and cytoskeletal signaling proteins, and it has a key role in regulation of integrin turnover. This unique versatility of the interactions of syndecan-4 is characterized in this Cell Science at a Glance article and illustrated in the accompanying poster.

Introduction

Syndecan-4, a proteoglycan receptor, is a central mediator of cell adhesion, migration, proliferation, endocytosis and mechanotransduction. The broad effects of this molecule are exemplified by its unique versatility in extracellular, cell membrane and intracellular interactions.

Like all other proteoglycans, syndecan-4 contains a protein core to which linear chains of polysaccharides are covalently linked. Known as glycosaminoglycans, these sugar chains are attached to the extracellular domain of syndecan-4 and mediate its extracellular interactions (see the ‘Syndecan-4 structure’ panel in the accompanying poster). The membrane-spanning region of syndecan-4 is a single-pass domain that is highly conserved between each of the four members of the syndecan family. In contrast, the intracellular domain contains a variable region that uniquely defines the signaling pathways that are initiated by this molecule. This variable region is flanked by two domains that are conserved across



(See poster insert)

all syndecans, which further expand their signaling capabilities.

The intracellular domains of syndecans endow them with the ability to interact with numerous binding partners and initiate a wide range of signaling processes. Diverse physiological processes are initiated by the networks of signal transduction downstream of syndecan-4, including wound healing (Kainulainen et al., 1998; Bass et al., 2011), arterial development (Chittenden et al., 2006; Lanahan et al., 2010), blood pressure regulation (Partovian et al., 2008), immunosuppression (Chung et al., 2013) and protection from endotoxic shock (Ishiguro et al., 2001). The roles of syndecan-4 in these physiological processes stem from its ability to function in various signaling pathways, which are described and illustrated here. This Cell Science at a Glance and accompanying poster will examine syndecan-4 biology in terms of its extracellular, membrane-based and intracellular signaling pathways.

Extracellular signaling

The extracellular-binding partners of syndecan-4 can be generally classified into heparin-binding growth factors, which are involved in modulating the effects of various extracellular signaling proteins, and cell adhesion molecules, which are responsible for establishing, stabilizing and dismantling extracellular sites of attachment.

As a proteoglycan with extracellular heparan sulfate chains, syndecan-4 interacts with numerous heparin-binding growth factors. These include the fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs) and platelet-derived growth factors (PDGFs) among others (reviewed by Tkachenko et al., 2005). Through the binding of these growth factors, syndecan-4 is able to organize their distribution in the extracellular space. Interestingly, the arrangement and concentration of these proteoglycans in the extracellular space has a greater influence on signal transduction than proteoglycan structure, which implies that there is a considerable overlap in ligand-binding affinities and significant redundancy in proteoglycan signaling (Kreuger et al., 2006). In this way, syndecan-4 and other heparan sulfate proteoglycans generate variable spatial distributions of not only growth factors but also of other extracellular matrix (ECM) components, such as proteases and protease inhibitors (Kainulainen et al., 1998). The physiological significance of this function remains unclear at this time.

One mechanism through which syndecan-4 mediates its extracellular signaling is the cleavage and shedding of its extracellular domain (see poster). Proteolytic cleavage of the ectodomain occurs constitutively and is accelerated under certain physiological conditions, such as inflammation (Kainulainen et al., 1998; Subramanian et al., 1997). The cleaved soluble syndecan-4 ectodomain fragments are released into the ECM with intact glycosaminoglycan chains, which preserves their ability to bind growth factors, such as fibroblast growth factor 2 (FGF2) (Elenius et al., 1992). In the ECM, syndecan-4, in concert with the glycoprotein tenascin-C, has been implicated in matrix contraction, which is an indispensable stage of wound healing (Midwood et al., 2004). Although it remains unclear what exact roles the soluble syndecan-4 ectodomain fulfills (i.e. whether it mediates growth factor signaling, facilitates ECM contraction, or sequesters extracellular proteases under physiological conditions), it is likely that these functions contribute to the regulation of wound healing and inflammation, during which syndecan-4 is expressed at increased levels (Alexopoulou et al., 2007).

The shed syndecan-4 ectodomain also mediates cellular adhesion to the surrounding matrix and is capable of mediating the direct contact of cells with ECM proteins, such as fibronectin (Tumova et al., 2000). These interactions form external points of cell attachment and affect the directionality of cellular migration (Bass et al., 2007a). In some cases, these extracellular attachment sites develop into focal adhesions, localized membrane regions with characteristically increased tensile strength, specialized signaling and an enrichment of cytoskeletal proteins that include vinculin, paxillin and actin (Woods and Couchman, 2001). An extracellular NXIP motif, which is present in syndecan-4, has been specifically implicated in interactions that mediate adhesion to the ECM, although the underlying molecular details are not fully understood (Whiteford and Couchman, 2006).

Although syndecan-4 is a crucial mediator of focal adhesion formation, it achieves this in concert with the integrins, another family of transmembrane receptors. Named for their ability to integrate extracellular signals towards the cytoplasm, integrins signal as a pair of α- and β-glycoprotein-subunits that bind to specific ECM components. At least 18 α- and 8 β-subunits have been identified to date, and each α–β combination (of which 24 have been characterized), binds with high affinity to specific ECM components (Hood and Cheresh, 2002). Sites of attachment between α5β1 integrin and extracellular fibronectin can mature into functional signaling units, and this process depends on the ability of syndecan-4 to bind and activate protein kinase Cα (PKCα) (Mostafavi-Pour et al., 2003). The details of syndecan-4-mediated activation of PKCα are described below, and PKCα controls the endocytosis of β1 integrin and thus regulates signaling at focal adhesions (Ng et al., 1999).

Syndecan-4 also activates ADP-ribosylation factor 6 (ARF6), a Ras superfamily GTPase involved in membrane trafficking, actin cytoskeletal remodeling and cell motility, which affects the trafficking of α5β1 integrin (Brooks et al., 2012). More specifically, the phosphorylation of syndecan-4 by Src has been shown to promote the binding of syntenin, a PDZ (postsynaptic density protein, disks large, zona occludens)-domain-containing cytoplasmic protein that regulates syndecan recycling (Zimmermann et al., 2005) and the subsequent inhibition of ARF6; this results in a preferential endocytosis and degradation of α5β1 integrin and an upregulation of αvβ3 integrin at the cell surface (Morgan et al., 2013) (see poster). In this way, syndecan-4 influences the assembly and disassembly of integrin complexes at focal adhesion sites, and this results in the preferential enrichment of different combinations of heterodimeric integrins. Similarly, syndecan-4 binding to fibronectin is necessary for the activation of focal adhesion kinase (FAK), a tyrosine kinase that regulates focal adhesions (Wilcox-Adelman et al., 2002). Beyond the role in facilitating the formation of focal adhesions, the heparan sulfate chains of syndecan-4 have been suggested to function as sensors of extracellular stress that are capable of transmitting mechanical force into signaling events (Florian et al., 2003; Moon et al., 2005). This occurs in the absence of integrin engagement and involves downstream intracellular signaling through the mitogen-activated protein kinase (MAPK) pathway (Bellin et al., 2009).

Recently, besides acting as an extracellular receptor, syndecan-4 has also been reported to act as a ligand. This function has been primarily characterized within the context of the immune system; here, the extracellular domain of syndecan-4 functions as a ligand for DC-HIL (also known as GPNMB), an inhibitory type I transmembrane receptor that is expressed on the surface of antigen-presenting cells. In the absence of syndecan-4, the inhibitory effect of the DC-HIL receptor is lost in transplanted immunologically active (allo-reactive) T cells, which results in increased mortality in animal models of graft-versus-host disease (Chung et al., 2012).

With these studies that characterized new extracellular roles for syndecan-4, it has become apparent that the functions of syndecan-4 by far surpass its initially postulated role as a low-affinity site for growth factor binding. The mechanisms of the extracellular interactions described above represent indispensable means for cells to interact with their environments; however, they encompass only a few of the numerous physiological functions of syndecan-4. We will next concentrate on interactions of syndecan-4 with other proteins at the cell membrane and its regulation of intracellular signaling cascades.

Syndecan-4 signaling at the cell membrane

At the cell membrane, syndecan-4 fulfills three signaling functions. First, syndecan-4 non-covalently clusters into SDS-resistant oligomers that directly activate signaling cascades. This occurs upon the localized concentration of syndecan-4 that is induced by ligand binding (e.g. growth factor binding at focal adhesion sites, or by it forming a complex with FGFRs) (Oh et al., 1997; Tkachenko and Simons, 2002) and is dependent on the GxxxG dimerization motif in the syndecan-4 transmembrane domain (Dews and Mackenzie, 2007). Syndecan-4 multimers have been detected in cholesterol- and sphingolipid-rich regions of the cell membrane known as lipid rafts, cellular microdomains that can initiate numerous downstream signaling events (Fuki et al., 2000; Tkachenko and Simons, 2002; Tkachenko et al., 2004). These microdomains serve to recruit scaffolding and signaling molecules that facilitate effective intracellular signaling, and the localization of syndecan-4 into lipid rafts is essential for its signaling functions.

Second, syndecan-4 serves to stabilize the interaction between growth factors and other cell membrane receptors (see poster). This aspect has perhaps been best studied for the FGFs, a family of 23 growth factors that primarily signal through four tyrosine kinase cell membrane receptors (FGFR1–FGFR4) (Jastrebova et al., 2006; Rahmoune et al., 1998). Although FGFs are able to bind to FGFRs with high affinity, this interaction and the subsequent signaling events are amplified by the presence of heparan sulfate chains (Nugent and Edelman, 1992; Sperinde and Nugent, 2000; Yayon et al., 1991), probably through a heparin-binding domain present in FGFRs (Kan et al., 1993). This leads to the formation of the ligand–heparin–receptor complex with a 2:2:2 stoichiometry predicted by the crystal structure (Schlessinger et al., 2000). This facilitates prolonged high-affinity ligand–receptor interactions and effectively allows the activation of FGFRs with lower absolute concentrations of ligand (Forsten-Williams et al., 2005).

Enhanced FGF signaling in the presence of heparin has been demonstrated for several canonical FGF–FGFR signaling pathways, including MAPK signaling, and is primarily mediated by the extracellular glycosaminoglycan chains of syndecan-4 (Nikitovic et al., 2007). In addition to the extracellular domains of syndecan-4 mediating growth factor binding, its cytoplasmic domains can also initiate FGF-induced signaling independently of FGFRs (Volk et al., 1999). This has been demonstrated in the context of cell migration through the activation of Rac1 (illustrated on the poster) (Horowitz et al., 2002; Tkachenko et al., 2006), as well as for the *in vivo* roles of syndecan-4 as an effector of nitric-oxide-mediated vasodilation (Zhang et al., 2003). The molecular details of syndecan-4-mediated signal transduction are discussed below.

The third function of syndecan-4 at the cell membrane encompasses its ability to serve as a direct link between the ECM and intracellular signaling proteins. Syndecan-4 has been shown to simultaneously bind to extracellular fibronectin and the intracellular actin-associated protein α-actinin, thus directly linking the actin cytoskeleton to the ECM (Greene et al., 2003). Similarly, syndecan-4 is able to recruit other proteins to the sites of focal adhesion, including the cytoplasmic signaling protein syndesmos (Denhez et al., 2002). This protein, in turn, binds to paxillin, a protein that is crucial in maintaining focal adhesion functionality (Turner, 2000). These interactions are physiologically significant, as cells deficient in syndecan-4 have been shown to have abnormal cell morphologies and deficiencies in migratory potential (Gopal et al., 2010; Elfenbein et al., 2009).

Syndecan-4 establishes another extracellular–intracellular connection at the cell membrane through the recruitment of PKCα to sites where focal adhesions form and by mediating its subsequent activation (Lim et al., 2003). The ability of syndecan-4 to activate PKCα depends on its binding to cytoplasmic phosphotidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], which is inhibited by phosphorylation of the intracellular domain of syndecan-4 at Ser183 (Horowitz and Simons, 1998b; Horowitz et al., 1999) (see poster). Structural data have likewise revealed that phosphorylation at Ser183 inhibits syndecan-4 oligomerization, whereas PtdIns(4,5)P_2 promotes it (Koo et al., 2006). Phosphorylation of Ser183, which is mediated by PKCδ (Murakami et al., 2002), inhibits the binding of PtdIns(4,5)P_2 and thus prevents PKCα activation. Dephosphorylation of Ser183, which is accomplished by a protein phosphatase of the IIa class, promotes PtdIns(4,5)P_2 binding, in turn leading to activation of PKCα signaling (Horowitz and Simons, 1998a).

Upon binding to PtdIns(4,5)P_2, syndecan-4 is able to mediate the recruitment of other proteins to the cell membrane, most notably that of syntenin (Zimmermann et al., 2001), a protein that has been shown to mediate the membrane recycling of other syndecans (Zimmermann et al., 2005) and the trafficking of different integrin heterodimers to and from the cell membrane (described above). The localization of syntenin to the cell membrane is mediated by the binding of PtdIns(4,5)P_2 to its PDZ domain, to which syndecans also bind directly (Zimmermann et al., 2002). As is the case for its activation of PKCα, the phosphorylation of syndecan-4 at Ser183 abrogates its ability to bind to the PDZ domain of syntenin (Koo et al., 2006). Beyond its role in integrin recycling at the cell membrane, it has also been demonstrated that syntenin is involved in the formation of secretory vesicles (see below).

Overall, the functions of syndecan-4 at the cell membrane include the stabilizing of growth-factor–receptor interactions, its clustering into oligomers that are able to signal independently of other receptors, and to create a physical interface between cytoplasmic proteins and the ECM. Next, we discuss the underlying mechanisms that facilitate the regulation of multiple downstream intracellular signaling pathways.

Intracellular signaling

The wide-ranging signaling effects of syndecan are largely due to its diverse intracellular binding partners. As mentioned above, one of its major binding partners is synectin, to which it binds through the PDZ-binding domain that is conserved among all syndecans (Gao et al., 2000). In the absence of ligand binding and syndecan-4 activation, the interaction between syndecan-4 and synectin facilitates the binding of Rho guanidine dissociation inhibitor 1 (RhoGDI1; also known as ARHGDIA and RhoGDI-α) (see poster) and serves to sequester and suppress the activity of Rho family GTPases that are incorporated into the syndecan-4–synectin–RhoGDI1 complex at the cell membrane (Elfenbein et al., 2009). GTPases act as molecular switches, alternating between an inactive GDP- and an active GTP-bound form, and specific Rho GTPases, including RhoG, Rac1 and RhoA, orchestrate the remodeling of the actin cytoskeleton at the cell membrane, thus regulating cell motility (Burridge and Wennerberg, 2004). By sequestering and suppressing the activity of Rho GTPases, such as RhoG and Rac1, syndecan-4 ensures a low rate of cell migration in the absence of growth factor stimulation. In this basal state, the related Rho GTPase RhoA exhibits high activity, which is diminished upon stimulation of syndecan-4 (Brooks et al., 2012). The mechanism by which RhoG, Rac1 and RhoA are regulated involves PKCα-mediated phosphorylation of RhoGDI1, as described below.

Although syndecan-4 maintains low levels of RhoG and Rac1 activity in the absence of growth factor stimulation, its oligomerization by growth factors or other ligands, such as fibronectin, triggers the reversal of this suppression through its ability to bind and activate PKCα. PKCα in turn phosphorylates RhoGDI1 at Ser96, which allows the release of sequestered RhoG and Rac1. RhoG forms a trimeric complex with ELMO1 and DOCK180, forming a functional guanine exchange factor (GEF) that subsequently activates Rac1 (Katoh and Negishi, 2003). This, in turn, leads to membrane ruffling, the formation of cellular protrusions and enhanced migration (Elfenbein et al., 2009). RhoA activation is similarly regulated by PKCα-mediated phosphorylation of RhoGDI1, although at a different site (Ser34) (Dovas et al., 2010). In this way, syndecan-4 controls both the suppression and activation of Rho GTPases through distinct mechanisms that involve different binding partners.

Equally as important, syndecan-4 has been implicated in the establishment of cell polarity (i.e. determining which parts of a migrating cell lead and which trail behind). In the absence of syndecan-4, the suppression of Rho GTPases under normal unstimulated conditions is lost, and cells exhibit constitutively high levels of RhoG and Rac1 activity. Although high levels of Rac1 activity are required for cell migration, cells that are devoid of syndecan-4 paradoxically show diminished migration; this is because the spatial distribution of activated syndecan-4 also determines the locations at which RhoG and Rac1 are activated. This spatial control of Rac1 activation by syndecan-4 also helps to establish appropriate cell polarity and ensures that only pools of Rac1 at a specific location are activated during directional cell migration (Bass et al., 2007b; Elfenbein et al., 2009; Pankov et al., 2005). Syndecan-4 has been shown to regulate the migration of neural crest cells in a similar manner, through localized inhibition of Rac1 (Matthews et al., 2008).

The activation of RhoG and Rac1 by syndecan-4 not only affects cell polarity, actin polymerization and cell migration, but also controls the form of endocytic uptake known as macropinocytosis. This mechanism of internalization involves relatively large membrane regions that are internalized after RhoG- and Rac1-mediated membrane ruffling. Through its control of RhoG and Rac1 activity, syndecan-4 regulates both the rate of macropinocytic uptake and the signaling events that result from the subsequent internalization of cell surface receptors. The best-studied example of this is the role of syndecan-4 in regulating the kinetics of FGFR1-induced extracellular-signal-regulated (ERK)1/2 activation, which determines the duration and intensity of ERK1/2 phosphorylation activity after activation of FGFR1 (Elfenbein et al., 2012). This presumably occurs by syndecan-4 regulating the quantity and rate of internalized vesicles that contain activated FGFR1. Non-macropinocytotic uptake of FGFR1 has also been reported (Haugsten et al., 2008; Jean et al., 2010), although its physiological significance is uncertain.

In addition to macropinocytosis, syndecan-4 has also been implicated in the regulation of caveolin- and dynamin-dependent internalization of β1 integrins within the context of wound healing (Bass et al., 2011). It is therefore likely that syndecan-4 can affect multiple endocytic pathways, depending on the upstream signal (e.g. fibronectin, FGF or other heparin-binding growth factors). The syndecans, especially syndecan-1 and -4, have also recently been implicated in the process of forming exosomes, or secreted vesicles that affect intercellular signaling. This occurs when the syndecan-binding partner syntenin recruits another cytoplasmic protein, ALIX (also known as PDCD6IP), to the sites of cytoplasmic vesicles. ALIX, in turn, recruits a specialized multi-protein complex known as the endosomal-sorting complex required for transport III (ESCRT-III) to these vesicles, which facilitates the formation of cytoplasmic vesicular aggregations known as multi-vesicular bodies (Baietti et al., 2012). These intracellular cargo-containing vesicles eventually fuse with the cell membrane, releasing their contents into the extracellular space and subsequently initiating a diverse range of intercellular signaling processes (see poster) (Simons and Raposo, 2009).

Syndecan-4 also mediates multiple intracellular signaling pathways that act in parallel through the activation of effectors that are common to these pathways. This is perhaps best exemplified by its activation of PKCα, which leads to RhoG and Rac1 activation (as noted above). However, syndecan-4-dependent activation of PKCα also has a crucial role in regulation of assembly of the mammalian target of rapamycin (mTOR) complex 2 (mTORC2) and activation of PDK1. Both of these enzymes, in turn, control the activation of the serine/threonine kinase AKT (see poster), which requires two phosphorylation events: Thr308 phosphorylation accomplished PDK1, and Ser473 phosphorylation by mTORC2 (also called PDK2 in this context). In the absence of syndecan-4, PKCα activation is reduced, leading to impaired assembly of mTORC2 (Partovian et al., 2008) and diminished activation of PDK1, which it controls

through the p21-activated kinases 1 and 2 (PAK1/2) (Ju and Simons, 2013). Thus AKT activity is reduced, which leads to an increase in blood pressure; a phenotype that can be rescued by introducing a constitutively active PKCα construct (Partovian et al., 2008).

In this way, diverse intracellular binding partners of syndecan-4 facilitate its ability to initiate several parallel signaling pathways and regulate endosomal trafficking. These downstream effects collectively influence cellular processes that include cell migration, establishment of cellular polarity, endocytosis, vesicular secretion of intracellular proteins and cellular homeostasis involving the mTOR signaling pathways.

Perspectives

Initially characterized as a ubiquitous low-affinity co-receptor for heparin-binding growth factors, syndecan-4 is now understood to also independently control a myriad of extracellular and intracellular signaling processes. This broad functionality is partially on account of the promiscuity of syndecan-4 with regard to its ligand-binding capabilities and, partially, owing to its ability to interact with numerous intracellular signaling partners. The molecular mechanisms underlying syndecan-4 function are also varied; this proteoglycan serves not only as a co-receptor for tyrosine kinase signaling, but can also initiate independent signaling cascades upon its oligomerization and activation. Syndecan-4 furthermore modulates these signaling responses through a complex crosstalk with other cellular processes, including receptor endocytosis. Therefore, the unique signaling capabilities of syndecan-4 have defied the conventional notions of receptor-mediated signal transduction that encompass the initiation, modulation and termination of signaling pathways.

The molecular mechanisms underlying many syndecan-4-mediated processes remain incompletely understood, and their elucidation is likely to yield further insight into the versatility of syndecan-4 signaling. This, in turn, is likely to enhance our understanding of biological processes as diverse as wound healing, blood pressure control, inflammation and atherosclerosis.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.124636/-/DC1

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