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Syndecans Shed Their Reputation as Inert Molecules

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Abstract

The syndecan transmembrane proteoglycans synergize with receptors for extracellular matrix molecules and growth factors to initiate cytoplasmic signals in response to a range of extracellular stimuli. Syndecans influence a wide range of physiological processes, but their contribution is most apparent during wound repair. Aspects of syndecan biology that have attracted research interest include extracellular matrix binding, outside-to-inside plasma membrane signal propagation, activation of cytoplasmic signals, and shedding of the syndecan extracellular domain, but the mechanisms by which syndecan cytoplasmic signals modulate extracellular function remain largely unresolved. Hayashida *et al.* have now discovered that association between an endocytic regulator, Rab5, and the syndecan-1 cytoplasmic domain controlled the shedding of the syndecan-1 extracellular domain. The work describes a mechanistic investigation into inside-to-outside syndecan signaling and highlights several gaps in our understanding of the relation between cell-surface receptors and proteases. In this Perspective, we summarize the current understanding of receptor interplay and identify the challenges that face investigators of adhesion- and growth factor–dependent signaling.

The syndecan transmembrane proteoglycans play critical regulatory roles in many biological processes, including wound healing, inflammation, neural patterning, and angiogenesis (1, 2). The mammalian syndecan family comprises four members, each with large heparan sulfate and chondroitin sulfate chains covalently attached to the extracellular domain (3) and short cytoplasmic tails that interact with a number of signaling adaptors and enzymes (1). Syndecan-1 is predominantly present on epithelial cells, whereas syndecan-4 is found ubiquitously but most notably on fibroblasts (3), and these two family members exhibit the closest functional similarities. Disruption of either of the genes that encode these proteins is nonlethal, but results in distinct wound-healing defects in mice (4, 5). In recent years, the study of the molecular mechanisms regulating syndecan function has gained momentum as the library of possible ligands and biological roles has expanded. Proteoglycans are not the primary receptors of extracellular matrix molecules, growth factors, or chemokines, but they cooperate with the prototypic receptors through simultaneous ligand engagement. Historically, the issues of ligand recognition and cytoplasmic signaling by syndecans have been addressed independently and, although there is strong evidence for signal transduction across the membrane (6), there is surprisingly little known about the mechanism by which intra- and extracellular domain functions are integrated.

Extracellular domain shedding is believed to play a key role in regulating the link between syndecan-ligand interactions and intracellular signaling. Proteolytic cleavage of the syndecan extracellular domain at a membrane-proximal site causes accumulation of shed ectodomains that compete with intact syndecans for extracellular ligands. The consequences

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of ectodomain competition are twofold, as both the signaling capabilities of the intact syndecan and the associated prototypic receptors are compromised (7, 8) (Fig. 1). Tissue fluids surrounding a wound contain an abundance of shed syndecan-1 and -4 ectodomains (9), which are thought to regulate inflammation and protect against tissue damage by modulating chemokine bioavailability. The syndecan ectodomains are cleaved by various secreted and membrane-associated matrix metalloproteinases (MMPs) that include MMP-7 (10), MMP-9 (11), and membrane type 1 (MT1)–MMP (MMP-14) (12). Syndecans are shed through both constitutive and inducible pathways, and shedding agonists include epidermal growth factor, thrombin, chemokines, and several bacterial virulence factors (9, 11, 13). The critical question, however, is whether ectodomain shedding is regulated indirectly by induced expression of metalloproteinases (11) or MMP inhibitors [e.g., tissue inhibitor of metalloproteinase 3 (TIMP-3) (14)], or whether the syndecan itself plays an active role in the process.

Hayashida *et al.* have now provided the first report of syndecan-regulated syndecan shedding. The authors identified an association between the syndecan-1 cytoplasmic domain and the endocytic regulator, Rab5, which influenced ectodomain shedding, but not surface expression, of syndecan-1 (15). Syndecan-1 bound exclusively to the inactive, guanosine diphosphate (GDP)–bound form of Rab5, and it was postulated that the release of sequestered Rab5 might allow Rab5 activation (Fig. 1). It was further speculated that Rab5-dependent endocytosis of transmembrane receptors that shield syndecan-1 would expose the syndecan-1 extracellular domain to MMPs and would explain the phenomenon of regulated shedding. Alternatively, Rab5 could regulate the trafficking of membrane-associated MMPs or their inhibitors, and thus could control their ability to cleave syndecan-1 at the cell surface. Both of these potential mechanisms, although requiring further investigation, are intriguing and not necessarily mutually exclusive. In relation to the shielding concept, integrins would be ideal candidates for the associated receptors. Integrins and syndecans work synergistically during extracellular matrix engagement, with syndecan-1 cooperating with $\alpha_v\beta_3$ integrin (16), and syndecan-4 cooperating with $\alpha_5\beta_1$ integrin (17). Endocytosis of various integrins is regulated by Rab5 (18, 19), and syndecans and integrins accumulate in the same endocytic vesicles (20). Furthermore, the protein cores of syndecan extracellular domains can act as integrin ligands, such that syndecan ectodomains can support integrin-mediated cell spreading (16, 21). The potential for direct interactions between the extracellular domains of these two families of adhesion receptors has major implications for the regulation of both integrin activity and shedding of the syndecan ectodomain. There is strong evidence linking integrin function with the expression, distribution, and activity of MMPs (both membrane-bound and soluble) and TIMPs (22-24), and many MMPs are regulated by endocytosis (25-27). Collectively, these reports of the mutual interdependence of syndecans, integrins, and MMPs for endocytosis indicate a robust, cyclic feedback loop that would hold in balance the abundance of cooperating receptors on the cell surface.

Apart from its role in the regulation of inflammatory responses, little is known about the biological significance of syndecan shedding. It is clear that extracellular cleavage of syndecan-1 by MT1-MMP promotes cell migration (28) and that the trafficking and cell surface distribution of MT1-MMP is polarized in migrating cells (29). Although this is thought to be associated primarily with spatially restricted extracellular matrix degradation, it would be interesting to know whether, in a migrating cell, syndecan-1 shedding occurs in a polarized manner. The spatial regulation of syndecan-extracellular matrix engagement through shedding is a concept worthy of attention. There is a precedent for syndecan-mediated regulation of directional cell migration, as extracellular engagement of syndecan-4 promotes directional persistence in a protein kinase C (PKC) α– and Rac1-dependent manner (6, 30), and the syndecan-1 cytoplasmic tail regulates migration toward laminin (31).

In summary, it is now becoming clear that syndecans fulfill dual roles: First, syndecans function as coreceptors, facilitating interactions between extracellular ligands and receptors; second, syndecans have signaling capabilities and propagate intracellular signals in response to extracellular matrix engagement. Therefore, the most important role of syndecans is in the integration of these two apparently distinct processes. Some remaining questions are as follows: What are the signaling consequences of syndecan ectodomain shedding? What role does syndecan shedding play in directional cell migration? Do syndecans regulate recycling of other cell surface receptors? If so, can this function be regulated by syndecan shedding or syndecan-dependent regulation of Rab5? And, precisely what effect does syndecan shedding have on growth factor and chemokine receptor signaling? As we move toward a greater understanding of the complexity of signaling networks, through proteomic analyses and systems biology combined with precise molecular manipulation of syndecan function both in vitro and in vivo, it is likely that many of these issues will be resolved.

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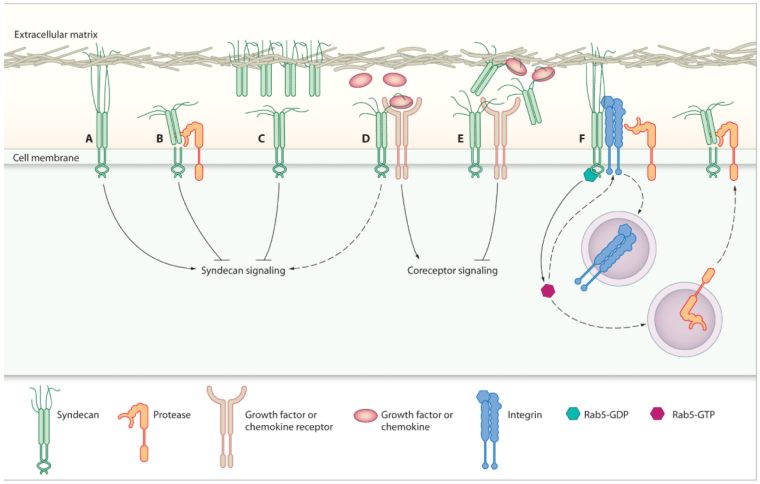

Fig. 1.

The proposed functions of syndecan extracellular domain shedding. Extracellular matrix engagement of syndecans (**A**) elicits syndecan-specific intracellular signals, which are (**B**) terminated by proteolytic cleavage of the syndecan extracellular domain. (**C**) Cleaved syndecan ectodomains further disrupt syndecan signaling by competing with intact syndecan for matrix engagement. (**D**) The extracellular domain of membrane-bound syndecan promotes prototypic growth factor and chemokine receptor signaling by facilitating association with their ligands in a heparan sulfate–dependent manner, and (**E**) it is believed that shed syndecan ectodomains disrupt this signaling by sequestering the ligands away from their primary receptors. (**F**) Syndecan-1 proteolytic cleavage is regulated by a cytoplasmic domain interaction with Rab5-GDP. It was proposed that release of Rab5 from syndecan-1 permits Rab5 activation and modulates trafficking of integrins, which could serve to shield syndecans from proteolysis (15). Alternatively, regulation of Rab5 activity could mediate shedding by coordinating protease recycling.

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