Identification of an Invasion Regulatory Domain within the Core Protein of Syndecan-1*

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Among the four members of the syndecan family there exists a high level of divergence in the ectodomain core protein sequence. This has led to speculation that these core proteins bear important functional domains. However, there is little information regarding these functions, and thus far, the biological activity of syndecans has been attributed largely to their heparan sulfate chains. We have previously demonstrated that cell surface syndecan-1 inhibits invasion of tumor cells into three-dimensional gels composed of type I collagen. Inhibition of invasion is dependent on the syndecan heparan sulfate chains, but a role for the syndecan-1 ectodomain core protein was also indicated. To more closely examine this possibility and to map the regions of the ectodomain essential for syndecan-1-mediated inhibition of invasion, a panel of syndecan-1 mutational constructs was generated, and each construct was transfected individually into myeloma tumor cells. The anti-invasive effect of syndecan-1 is dramatically reduced by deletion of an ectodomain region close to the plasma membrane.

Further mutational analysis identified a stretch of 5 hydrophobic amino acids, AVAAV (amino acids 222–226), critical for syndecan-1-mediated inhibition of cell invasion. This invasion regulatory domain is 26 amino acids from the start of the transmembrane domain. Importantly, this domain is functionally specific because its mutation does not affect syndecan-1-mediated cell binding to collagen, syndecan-1-mediated cell spreading, or targeting of syndecan-1 to specific cell surface domains. This invasion regulatory domain may play an important role in inhibiting tumor cell invasion, thus explaining the observed loss of syndecan-1 in some highly invasive cancers.

The syndecan family of heparan sulfate proteoglycans is composed of four vertebrate members (syndecans 1–4) that have distinct cell, tissue, and developmental patterns of expression. Differences in function among members of the family can be ascribed to these differential patterns of expression and to differences among the members in the variable region of their cytoplasmic domains (1). Importantly, the syndecans also have highly divergent extracellular domain (ectodomain) core protein sequences, suggesting that this may dictate differing functions. However, differences in ectodomain core protein structure among syndecans apparently do not exist to dictate heparan sulfate structural features because different syndecan core proteins on the same cell surface bear heparan sulfate chains very similar in structure (2). This leaves the more likely possibility that divergent regions within the core proteins have distinct functions that are either independent of heparan sulfate activity or coordinated with heparan sulfate activity.

Loss of syndecan-1 expression has been shown to correlate with a poor prognosis in several tumor types including head and neck carcinoma (3), squamous cell lung carcinoma (4), and gastric cancer (5). However, although much is known regarding the functional significance of this molecule, the mechanisms by which syndecan-1 exerts its effects remain unclear.

It has been speculated that loss of syndecan-1 expression leads to weakened cell adhesion resulting in increased tumor invasiveness in vivo (6). This idea is supported by our previous in vitro finding demonstrating that cell surface syndecan-1 inhibits invasion of tumor cells within type I collagen gels (7). The anti-invasive activity of syndecan-1 is dependent on its heparan sulfate chains but does not require the cytoplasmic or transmembrane domains of the core protein (8). However, the same studies also revealed that adhesion of tumor cells to collagen via heparan sulfate is not sufficient to inhibit cell invasion. This suggested the possibility that a domain within the syndecan-1 core protein is critical for inhibition of cell invasion.

In the present study, expression of syndecan-1 having specific deletions or mutations of the ectodomain core protein was used to further explore the function of the core protein in inhibiting tumor cell invasion. Using this strategy we have identified an invasion regulatory domain within a region close to the transmembrane domain of syndecan-1. Together our findings indicate that syndecan-1-mediated inhibition of cell invasion requires a specific core protein domain as well as heparan sulfate chains.

MATERIALS AND METHODS

Cell Culture and Mutagenesis—A cDNA for murine syndecan-1 (9) in the pCDNA3.1 vector was subjected to oligonucleotide-directed mutagenesis (Transformer™ site-directed mutagenesis kit; Clontech, Palo Alto, CA) to yield constructs having defined segments of the syndecan-1 ectodomain either removed or mutated (Fig. 1). Each mutated cDNA encodes syndecan-1, which retains all three of its heparan sulfate attachment sites, the epitope for monoclonal antibody 281.2 (10), and unmodified transmembrane and cytoplasmic domains. Each construct was verified by DNA sequencing and separately transfected into the human B lymphoid cell line ARH-77. The deletion of 165 amino acids (del 88–252; Phe-88—Glu-252) was accomplished by two separate mutagenesis reactions. The first reaction introduced an Nhel site 3’ of the codons for Glu-252, the final amino acid of the syndecan-1 ectodomain either removed or mutated (Fig. 1). Each mutated cDNA encodes syndecan-1, which retains all three of its heparan sulfate attachment sites, the epitope for monoclonal antibody 281.2 (10), and unmodified transmembrane and cytoplasmic domains. Each construct was verified by DNA sequencing and separately transfected into the human B lymphoid cell line ARH-77. The deletion of 165 amino acids (del 88–252; Phe-88—Glu-252) was accomplished by two separate mutagenesis reactions. The first reaction introduced an Nhel site 3’ of the codons for Glu-252, the final amino acid of the ectodomain that is adjacent to the beginning of the hydrophobic sequence of the transmembrane domain. The second reaction generated a separate syndecan-1 cDNA an Nhel site just 5’ of the codons for Phe-88. The region coding for the amino-terminal fragment of syndecan-1 was excised from the mutagenic vector (with BamHI and Nhel) as was the region coding for the...
carboxyl terminal fragment (i.e., the transmembrane and cytoplasmic domains), which was removed from the second mutagenic vector (with Nhel and HindIII). Both fragments were ligated together and placed into the multiple cloning site of pDN A 3.1 (between the BstHI and HindIII restriction sites). This resulted in a syndecan-1 cDNA lacking the codons for amino acids Phe-88–Gl u-252. A similar strategy was employed to generate deletions that code for proteins having smaller regions of the ectodomain core protein deleted while leaving the transmembrane and cytoplasmic domains intact (del 121–252, del 147–252, del 202–252 and del 222–252). The deletion of the 16 amino acids (del 222–237; Asp-222 → Asp-237) was accomplished by using a 51-bp oligonucleotide primer (5′-CATCTGGGGAAAGACACAGGAGGACCA-
TAGG-3′) that “bridged” the region of the syndecan-1 cDNA to be deleted. This resulted in a hairpin loop of the unwanted bases that were eliminated from the copied construct during the process of mutagenesis.

Mutations were also prepared by oligonucleotide-directed mutagenesis. Mut 222–226 was generated using the primer 5′-TCTGCGGG-
GAAACCCAGTCAGGGTCAGGGGAGCCCGCTGGC-3′, changing amino acids 222–226 (AAVAAWHPHMGPMQ-GL, changing amino acids 147–162 (QAAVTSHPHGGMQPGL)
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Affinity Co-electrophoresis—Syndecan-1 was isolated by extracting cells with 20 mMTris-HCl, pH 7.4, containing 1% Triton X-100, 0.15 M NaCl, 5 mM N-ethylmaleimide, 5 mM benzimidazole, and 1 mM phenylmethylsulfon fluoride on ice for 30 min. After centrifugation, supernatants were brought to a final concentration of 8 M urea and 50 mM sodium acetate. Syndecan-1 was isolated by ion exchange and affinity chromatography as described previously (12). Equivalent aliquots of proteoglycan, still labeled and treated with chondroitinase ABC as described above under “Western blotting”, were digested twice with chondroitinase ABC (0.5 munits/ml) (Seikagaku, Tokyo, Japan), then eluted and analyzed on a 4–12% acrylamide, Tris/glycine gel (Invitrogen) and transferred to cationic membranes (Zeta probe membrane; Bio-Rad). Blots were probed with 125I-labeled 281.2 antibody and visualized on a PhosphorImager (Amer sham Biosciences).

Gel Filtration Chromatography—35SO4-labeled syndecan-1 was isolated and treated with chondroitin ABC as described above except with the following modifications (12). After centrifugation, supernatants were brought to a final concentration of 8 M urea and 50 mM sodium acetate. Syndecan-1 was isolated by ion exchange and affinity chromatography as described previously (12). Equivalent aliquots of proteoglycan, still labeled and treated with chondroitinase ABC (0.5 munits/ml) (Seikagaku, Tokyo, Japan), then eluted and analyzed on a 4–12% acrylamide, Tris/glycine gel (Invitrogen) and transferred to cationic membranes (Zeta probe membrane; Bio-Rad). Blots were probed with 125I-labeled 281.2 antibody and visualized on a PhosphorImager (Amer sham Biosciences).

Affinity Co-electrophoresis—The affinity of syndecan-1 for collagen was determined by affinity co-electrophoresis as described previously (13). Briefly, 35SO4-labeled syndecan-1 was isolated by extracting cells as described above. After affinity isolation and buffer exchange into running buffer (0.1 M sodium acetate, 50 mM sodium MOPS* (Fluka Biochemika, Ronkonkoma, NY), pH 7.0, 0.02% sodium azide) and analyzed on a 0.5 × 46-cm column of Affinigel-1B (Amer sham Biosciences).

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*The abbreviations used are: MOPS, 3-(N-morpholino)-2-hydroxypropanesulfonic acid; BSA, bovine serum albumin; PBS, phosphate-buffered saline; TDM, triple deletion mutant.

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RESULTS

A Membrane-proximal Region of the Syndecan-1 Ectodomain Is Required for Inhibition of Cell Invasion—Syndecan-1-mediated inhibition of cell invasion into collagen gels requires the heparan sulfate chains of core protein, and previous work indicates that regions of the core protein may also be required (89). To examine this possibility cells expressing a syndecan-1 deletion mutant lacking amino acids 88–252 (del 88–252) of the core protein were engineered. This form of syndecan-1 retains the cytoplasmic and transmembrane domains as well as the aminoterminal region of the core protein that includes the heparan sulfate attachment sites (Fig. 1). In contrast to cells expressing wild-type syndecan-1, which fail to invade collagen gels, the cells expressing the del 88–252 form of the proteoglycan readily invade (Fig. 2A). The depth of invasion of these cells is ~90% that of control cells that express a form of syndecan-1
lacking heparan sulfate chains (TDM). These TDM-expressing cells invade to an extent similar to that of cells lacking syndecan-1 expression (13). In addition, the number of invading cells is also greatly enhanced by deletion of this large region of the ectodomain (Fig. 2B).

To confirm a role for syndecan-1 in inhibiting invasion and to map the region within the core protein that is required for the anti-invasive effect of syndecan-1, a series of transfected cells expressing core proteins with deletions or mutations was generated. Each construct included the region of the core protein that contains the heparan sulfate attachment sites as well as the intact transmembrane and extracellular domains (Fig. 1). Testing of cells expressing the other deletions (del 122–252, del 147–252, del 202–252, and del 222–252) indicated that the location of the region active in regulating cell invasion was within the region containing amino acids 222–252 (not shown). A further deletion narrowed this down to a stretch of 16 amino acids (222–237) (Fig. 2). Mutational analysis using relatively conservative amino acid substitutions narrowed the active region down to a stretch of 5 amino acids (amino acids 222–226) (Fig. 2). To confirm this result and ensure that it was not due to a transfection artifact, a second transfection was performed, and the cells were sorted and examined in the invasion assay. As before, cells bearing syndecan-1 with the 222–226 mutation invaded collagen gels (data not shown). As a control a mutation in a region more distal from the membrane (amino acids 147–162) was constructed. Cells expressing this construct failed to invade gels, thus mimicking the behavior of cells expressing wild-type syndecan-1 (Fig. 2). Also tested were cells expressing a syndecan-1/CD4 chimera. This chimeric proteoglycan is composed of the syndecan-1 cytoplasmic and transmembrane domains and most of the ectodomain except 15 amino acids of the wild-type syndecan-1 juxtamembrane domain (amino acids 238–252) which are replaced by the corresponding amino acid sequence of the human CD4 antigen (17). These cells also failed to invade gels, indicating that the juxtamembrane domain is not required for inhibition of invasion (data not shown). Thus, the core protein region of syndecan-1 spanning amino acids 222–226 comprises at least in part an invasive regulatory domain that can dramatically influence the behavior of invading cells.

Characterization of the Mutated Syndecans—Characterization of the cells and purified syndecans was undertaken to ensure that the mutated syndecans were expressed at the cell surface and bore heparan sulfate attachment sites similar to wild-type syndecan-1. Western blotting shows that the wild-type syndecan-1 and del 222–237 are virtually identical in size (Fig. 3A). This is expected because the del 222–237 only varies by 16 amino acids from the wild-type molecule. The broad smear present after chondroitinase ABC digestion indicates that the core proteins of the two molecules are similar in heparan sulfate content. This is confirmed by demonstration that syndec-
can-1 isolated from cells expressing either wild-type or del 222–237 syndecan have virtually identical affinities for type I collagen (Fig. 3B). In addition, analysis of isolated heparan sulfate chains reveals that even in syndecans lacking a large region of the ectodomain (amino acids 88–252), the heparan sulfate chains are similar in size to those present on wild-type syndecan-1 (Fig. 3C). Analysis by flow cytometry of immunostained syndecan-1 revealed that all of the mutants express relatively high levels of the proteoglycan on the cell surface (mean fluorescence intensity levels of 123, 139, 110, and 194 for del 88–252, del 222–237, mut 222–226, and mut 147–162, respectively).

To exert its anti-invasive effect, syndecan-1 must be retained at the cell surface (7). Thus, the invasive behavior of cells expressing syndecan-1 with ectodomain deletions or mutations could result from enhanced ectodomain shedding from the cell surface. To examine this possibility, immunohistochemical examination of the cells invading within the collagen gels was performed (Fig. 4). The highly invasive control cells that express the triple deletion mutant of syndecan-1 (TDM), which lacks heparan sulfate chains, clearly has syndecan-1-positive cells that are deeply invasive (Fig. 4A). Similarly, cells bearing mut 222–226 also retained syndecan-1 expression on invading cells (Fig. 4B). Thus, enhanced invasion of the cells bearing mutated syndecan-1 is not due to the absence of syndecan-1 on the cell surface of the invading cells.

As a final characterization step, the structure within and adjacent to the invasion regulatory domain of the wild-type syndecan-1 core protein (amino acids 222–226) was assessed for its structure using a conformational family approach. The secondary structure prediction method, NnPredict (18), indicates that the invasion regulatory domain is within a short α-helical segment that is flanked on either side by an expanse of random loop structure (Fig. 5). This analysis also reveals that the helical structure present in this region of the wild-type core protein is maintained in the mut 222–226 syndecan-1. Thus, loss of the ability of mut 222–226 to inhibit invasion is not due to a dramatic difference in protein structural conformation between the wild-type and mutant core proteins.

Elimination of the Invasion Regulatory Domain Does Not Alter Syndecan-1-mediated Cell Adhesion, Cell Spreading, or Membrane Targeting—In the ARH-77 cell model used for these studies, we have previously demonstrated that expression of syndecan-1 mediates cell adhesion to type I collagen-coated wells (7). Thus, to determine whether the mutation in the ectodomain core protein of syndecan-1 that regulates the invasion of these cells in collagen gels has an effect on cell adhesion, we tested them in a cell binding assay. In this assay cells are added to collagen-coated wells of a 96-well plate. After a short incubation period, the plate is subjected to low speed centrifugation. Adhesive cells resist the applied centrifugal force leaving them evenly coated in the well. Non-adhesive cells form a pellet in the bottom of the well. Using this assay, we find that all ectodomain mutants tested have the ability to anchor cells to collagen (Fig. 6A).

Because changes in cell morphology are required for active cell migration, we examined cells expressing the syndecan-1 ectodomain mutants for their ability to mediate changes in cell morphology (i.e. cell spreading). We reasoned that because cells bearing mutant syndecans are more invasive than controls, they may have a spreading phenotype distinct from controls. However, this is not the case, as cells bearing mutant synde-
parameters designed to classify protein structure according to training sets that limit structures to all-protein shown (amino acids 212–248) was subjected to secondary structure analysis using a computational neural network approach that contains targeting of the molecule that could affect its function (Fig. 6).

Together, these findings demonstrate that mutation of the invasion regulatory domain does not alter syndecan-1-mediated cell adhesion to collagen, cell spreading, or targeting of syndecan-1 to uropods. Thus, the invasion regulatory domain appears to specifically promote the anti-invasive function while not impacting other known functions of syndecan-1.

**DISCUSSION**

Using a panel of cell lines bearing various mutations in the syndecan-1 ectodomain core protein, we identify an "invasion regulatory domain" composed of five amino acids relatively close to the cell membrane. This domain is required for mediating the anti-invasive effect of syndecan-1. Deletion of the region containing this domain or mutation of this domain renders the otherwise intact syndecan-1 unable to inhibit cell invasion into type I collagen gels. Taken together with our previous studies (13), we conclude that there are at least two requirements for syndecan-1 to mediate its anti-invasive effect; that is, the presence of heparan sulfate chains and the invasion regulatory domain within the ectodomain core protein. We envision that the heparan sulfate chains engage the extracellular matrix or adjacent cell surface receptors, and the invasion regulatory domain either independently or in cooperation with the heparan sulfate chains interacts with receptors at the cell surface to generate signals that block invasion of the cell.

Although it is possible that manipulation of the syndecan-1 ectodomain core protein sequences has caused a conformational change that leads to the loss of biological activity, several lines of data suggest otherwise. First, although mutation of the invasion regulatory domain abolishes the syndecan-1-mediated inhibition of cell invasion, all other biological functions of syndecan-1 examined (adhesion to collagen, cell spreading, and localization on the cell surface) are unaffected. Second, mutation of a 16-amino acid region distant from the cell surface (mut 147–162) does not alter syndecan-1-mediated inhibition of cell invasion. Third, prediction of the secondary structure of the core protein of wild-type syndecan-1 and that of the most restricted mutation (mut 222–226) suggests that the physical structure of the core protein is not substantially altered in this mutation (Fig. 5).

Another possibility is that changes in the invasive capacity of cells can be dictated by signals generated via the formation of syndecan-1 homo-oligomers and that mutations in the core protein can block these oligomers from forming. For example, syndecan-3 forms multimers upon extraction and examination on SDS-PAGE (21). The region that appears to mediate this association lies within the transmembrane domain and a small portion of the juxtamembrane ectodomain of syndecan-3. This is within a highly conserved area and is shared between all members of the syndecan family. However, this conserved region that mediates oligomerization does not extend far enough to encompass the invasion regulatory domain that we have identified in the present study. Furthermore, work with the syndecan/glypican-1 chimeras suggests that oligomerization is not required to inhibit invasion. This is based on the finding that an intact syndecan-1 ectodomain linked to the cell surface via the glypicann-1 glycosylphosphatidylinositol tail inhibits cell invasion as effectively as wild-type syndecan-1 (8). This chimera contains the invasion regulatory domain but not the syndecan-1 transmembrane domain regions critical for oligomer formation.

To our knowledge there have not been any previous reports of a function for the AVAAV stretch of amino acids that encompass the invasion regulatory domain. Importantly, this domain of syndecan-1 is highly conserved among species, including murine (AVAAV) and human (AVVAV) (9, 22), further supporting its role in a critical function. The domain does not appear in murine syndecan-2 and syndecan-4 but is present in murine syndecan-3, although the sequence has a glutamic acid residue in place of the initial alanine (EVVAV). Syndecan-1 and syndecan-3 also differ in that the domain lies 26 amino acids from the start of the transmembrane domain in syndecan-1 but is 48 amino acids from the start of the transmembrane domain in syndecan-3. The function of syndecan-3 and relevance of the invasion regulatory domain has not been tested in our model systems.

In addition to our finding regarding an invasion regulatory
domain, there is mounting evidence that other specific domains within syndecan core proteins can participate in regulating signaling and cell behaviors. By screening for cell attachment using recombinant syndecan ectodomains, a stretch of 54 amino acids proximal to the GAG attachment sites in the syndecan-4 ectodomain was shown to mediate adhesion of several cell types (23, 24). Weak binding of Swiss 3T3 cells to syndecan-1 ectodomain was also noted using this approach, but the adhesion has not been mapped to a specific ectodomain region. Similarly, recombinant syndecan-2 ectodomain (which lacks heparan sulfate chains) was shown to participate in adhesion and regulation of cytoskeletal organization of colon carcinoma cells (25). In separate studies, an important functional link between the syndecan-1 ectodomain core protein and activation of $\alpha_v\beta_3$ integrin has recently been discovered. Breast cancer cell lines will spread on plates coated with antibody to the syndecan-1 core protein, and this cell spreading requires signaling via the $\alpha_v\beta_3$ integrin (26). Soluble syndecan-1 ectodomain core protein, but not syndecan-4 ectodomain, can inhibit cell spreading. Thus, there is a specific region within the syndecan-1 core protein that either directly or indirectly interacts with $\alpha_v\beta_3$ to promote cell spreading of breast carcinoma cell lines. However, the region of the syndecan-1 core protein mediating spreading of the breast carcinoma cells is distinct from that mediating inhibition of invasion of myeloma cells because the spreading activity maps to the region containing amino acids 87–121 (27).

Similar to the interaction of the syndecan-1 ectodomain with $\alpha_v\beta_3$, that promotes cell spreading of breast carcinoma cells, we speculate that syndecan-1-mediated inhibition of cell invasion occurs via an interaction between the invasion regulatory domain and an as yet unidentified signaling molecule (e.g. integrin) or signaling complex. Although the evidence supporting this is circumstantial, what is known does fit this model. First, when expressed in ARH-77 cells, a chimeric proteoglycan composed of the syndecan-1 ectodomain anchored to the cell surface via the glycosylphosphatidylinositol tail of glypican-1 mediates the inhibition of invasion, adhesion to collagen, and cell spreading on 281.2 (8). With no direct association with the cytoplasmic compartment, signals from syndecan-1 alone, leading to such profound changes in cell behavior as inhibition of cell invasion, would be highly unlikely. Although it is possible that the glypican-1 tail of the chimeric proteoglycan may in some fashion be capable of signaling, this does not seem to be the case in these cells. This is supported by the finding that wild-type glypican-1, although it bears heparan sulfate chains that have the capacity to bind collagen, is not capable of inhibiting cell invasion into collagen gels (8). Glypican-1 is also incapable of anchoring ARH-77 cells to collagen with sufficient support to withstand the centrifugal force in the cell adhesion assay. This suggests that there is no connection between the glypican-1 tail and the cytoskeleton. Taken together, at least in the ARH-77 cells, it is highly unlikely that the glypican-1 tail functions in any manner other than to hold the proteoglycan ectodomain on the cell surface. Second, when the glypican-1 ectodomain is displayed on the cell via the syndecan-1 transmembrane and cytoplasmic domains, it gains the stability to tightly adhere cells to collagen; yet this syndecan-1/glypican-1 chimera does not inhibit cell invasion into collagen gels (8). This further supports the finding that adhesion to collagen is not in itself sufficient to inhibit cell invasion. Moreover, in the syndecan/glypican chimera, the failure to inhibit invasion likely occurs because glypican-1 lacks the invasion regulatory domain and, therefore, lacks the ability to generate proper signals that inhibit cell invasion. Last, in the present work we show that mutation of the invasive regulatory domain results in the loss of anti-invasive cell behavior even though secondary protein structure is maintained (Fig. 5). This supports the notion that a co-receptor molecule or complex of molecules interacts with this domain with specificity and that specificity is lost when the domain is mutated even when the mutation consists of amino acid substitutions that are relatively conservative.

Whether syndecan-1 functions as a molecular facilitator, as has been suggested for molecules such as the tetraspanins (28), or is an individual partner organized by other molecules re-

![Fig. 6. Deletion or mutation of the invasion regulatory domain does not alter syndecan-1-mediated adhesion to collagen, cell spreading, or localization of syndecan-1 to uropods.](http://www.jbc.org/)

*Fig. 6. Deletion or mutation of the invasion regulatory domain does not alter syndecan-1-mediated adhesion to collagen, cell spreading, or localization of syndecan-1 to uropods.* A, binding of cells to type I collagen-coated microwell plates. After centrifugation, cells binding to collagen form a uniform coat over the well surface; cells not binding form a pellet. Cells do not bind control wells coated with BSA. ARH-77 cells bearing wild-type syndecan-1 or any of the ectodomain deletions or mutations bind to type I collagen (lane 1, wild-type syndecan-1; lane 2, mut 147–162; lane 3, mut 222–226; lane 4, del 222–237; lane 5, del 88–252). Parental ARH-77 cells that lack syndecan-1 expression fail to bind to collagen (lane 6). B, cells expressing either wild-type, deleted, or mutated forms of syndecan-1 were assessed for their morphology after a 30-min incubation at 37 °C on coverslips that were coated with monoclonal antibody to syndecan-1 (clone 281.2). C, cells expressing wild-type or mut 222–226 syndecan-1 were fixed immediately after their removal from cell culture, stained with monoclonal antibody 281.2, and analyzed for localization of syndecan-1 (arrows point to uropods).
mains to be determined. It is clear, however, that although the heparan sulfate chains are critical for anchorage to extracellular matrix components (i.e. collagen), the ectodomain core protein of syndecan-1 is also required for inhibition of cell invasion. Thus, the loss or reduction in the levels of the syndecan-1 core protein that is seen in many human tumors (3–5) could directly contribute to enhanced tumor invasion and metastasis. Therefore, treatments designed to preserve or enhance syndecan-1 on the surface of tumor cells represents a potentially important therapeutic strategy.

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