Coregulation of Fibronectin Signaling and Matrix Contraction by Tenascin-C and Syndecan-4

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 Syndecan-4 is a ubiquitously expressed heparan sulfate proteoglycan that modulates cell interactions with the extracellular matrix. It is transiently up-regulated during tissue repair by cells that mediate wound healing. Here, we report that syndecan-4 is essential for optimal fibroblast response to the three-dimensional fibrin-fibronectin provisional matrix that is deposited upon tissue injury. Interference with syndecan-4 function inhibits matrix contraction by preventing cell spreading, actin stress fiber formation, and activation of focal adhesion kinase and RhoA mediated-intracellular signaling pathways. Tenascin-C is an extracellular matrix protein that regulates cell response to fibronectin within the provisional matrix. Syndecan-4 is also required for tenascin-C action. Inhibition of syndecan-4 function suppresses tenascin-C activity and overexpression of syndecan-4 circumvents the effects of tenascin-C. In this way, tenascin-C and syndecan-4 work together to control fibroblast morphology and signaling and regulate events such as matrix contraction that are essential for efficient tissue repair.

INTRODUCTION

Tissue architecture is defined by the three-dimensional (3D) organization of the proteins and proteoglycans that comprise the extracellular matrix (ECM). Individual ECM components influence cell arrangements and activities through binding to transmembrane receptors, thus initiating intracellular signaling and altering gene expression and other downstream events. Tissues undergo continual maintenance and remodeling through controlled deposition and removal of specific ECM components. These changes in ECM composition and organization modulate cell behaviors and serve important roles throughout development and in the adult during both physiological and pathologic processes (Lukashev and Werb, 1998).

The provisional matrix that forms at sites of tissue injury undergoes extensive remodeling and turnover during wound repair. Composed predominantly of covalently cross-linked fibrin and plasma fibronectin (FN), this fibrin-FN provisional matrix coordinates the activities of cells involved in wound repair. It is remodeled over time to recapitulate normal tissue and this remodeling involves cell-mediated contraction and deposition of new FN-rich matrix (Clark, 1996; Midwood et al., 2004). FN, a major component of the matrix, is a ubiquitously expressed, multifunctional extracellular glycoprotein that promotes cell adhesion. It controls many intracellular pathways and influences a wide range of cell functions (Hynes, 1990). For example, FN within a 3D fibrin-FN provisional matrix initiates signaling via focal adhesion kinase (FAK) and RhoA in order to promote fibroblast spreading, stress fiber formation, and focal adhesion assembly (Wenk et al., 2000; Midwood and Schwarzbauer, 2002).

The provisional matrix contacts many other ECM proteins that are induced at different stages of tissue repair, including thrombospondins, SPARC, and tenascin-C. These proteins are specifically up-regulated at wound sites where they modulate cell-ECM interactions (reviewed in Midwood et al., 2004). For example, both pro- and antiadhesive properties of thrombospondin-1 and -2 act to regulate angiogenesis during tissue repair (reviewed in Bornstein, 2001). Tenascin-C also has modulatory effects on cell-ECM interactions. In cell culture, it antagonizes the adhesive effects of FN (Chiquet-Ehrismann et al., 1988; Chiquet-Ehrismann and Chiquet, 2003). Within a 3D fibrin-FN matrix, tenascin-C impacts the ability of fibroblasts to deposit and contract the matrix by affecting the morphology and signaling pathways of adherent cells (Wenk et al., 2000; Midwood and Schwarzbauer, 2002).

Along with ECM proteins, proteoglycans also communicate extracellular information to the cell. These molecules, which consist of a protein core modified with glycosaminoglycan (GAG) side chains, are found at the cell surface, inside the cell, or incorporated into the ECM (Lander, 1998). Transmembrane heparan sulfate proteoglycans (HSPGs) of the syndecan family bind to ECM proteins and cooperate with heterodimeric integrin receptors to regulate cell adhesive activities (Bloom et al., 1999; Kusano et al., 2000; Woods and Couchman, 2000). One member of this family, syndecan-4, is up-regulated during tissue repair (Gallo et al., 1996) and syndecan-4-null mice show defects in wound healing (Echtermeyer et al., 2001). Syndecan-4 binds to FN and probably to tenascin-C via its GAG side chains (Salminen et al., 1991; Woods et al., 2000). In fact, fibroblasts use both integrin...
receptors and syndecan-4 to induce cell spreading, actin cytoskeleton organization, and signaling events on FN-coated substrates (Saonella et al., 1999; Bass and Humphries, 2002). We know that fibroblasts require α5β1 integrins to bind FN within a provisional matrix and that this interaction is essential for cell adhesion, spreading, and matrix contraction (Corbett and Schwarzbauer, 1999). Here, we further determined that syndecan-4 is essential for signaling from the fibrin-FN provisional matrix and controls events such as matrix contraction. Syndecan-4 is also required for tenasin-C modulation of cell behavior. As such, syndecan-4 plays a vital role throughout tissue repair in communicating signals between the dynamic ECM and the cell.

MATERIALS AND METHODS

Protein Production
Rat plasma FN was purified by gelatin-Sepharose (Pharmacia Biotech, Airthington Heights, IL) affinity chromatography from freshly drawn plasma (Wilson and Schwarzbauer, 1992). The construction and production of the recombinant protein 70Ten has previously been described and this protein has been shown to have identical effects on cells as full-length tenasin-C (Wenk et al., 2000).

Cell Culture
NIH3T3 fibroblasts were maintained in DMEM and 10% calf serum (Hyclone Laboratories, Logan, UT). CHO K1 cells were maintained in DMEM, 2 mM glutamine, 1% nonessential amino acids, and 10% fetal calf serum (Hyclone Laboratories). CHO mutant cell lines were generously provided by Dr. Jeffrey Esko (University of California, San Diego; Esko, 1991), and grown in Ham’s F-12 medium (Life Technologies, Rockville, MD) supplemented with 7.5% fetal bovine serum (Hyclone Laboratories). Fibroblasts derived from wild-type, heterozygous, or syndecan-4–deficient mouse dermis (Echtler Meyer et al., 2001; gifts from Dr. Paul Goetinck, Massachusetts General Hospital) were cultured in DMEM plus 10% fetal bovine serum and used only up to 10 passages. Wild-type REFS2 cells and REFS2 cells stably overexpressing syndecan-4 (gifts from Dr. Greta Orend, University of Basel; Orend et al., 2003) were maintained in DMEM plus 10% fetal calf serum and used between passage numbers 5 and 9. Cells were released from tissue culture dishes using 0.2 mg/ml EDTA in PBS or brief trypsinization, washed with PBS, resuspended in serum-free DMEM, and counted before use.

Transfection
Transfections were performed using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) in accordance with the manufacturer’s guidelines. Syndecan-4–deficient mouse dermal fibroblasts were seeded at a density of 1 × 10⁶ cells per 35-mm culture dish in antibiotic-free DMEM plus 10% fetal bovine serum. After 24 h at 37°C, cells were transfected with a pcDNA3.1 expression vector containing rat syndecan-4 cDNA (kindly provided by Dr. Paul Goetinck; Wilcox-Adelman et al., 2002) plus a pCDA3-GFP expression vector (gift from Dr. Donald Winkelmann, Robert Wood Johnson Medical School) as a transfection reporter in LipofectAMINE 2000 reagent and OptiMem medium. In parallel, cells were transfected with the pcDNA3-GFP vector alone. Transfection medium was replaced with DMEM plus 10% fetal bovine serum after 5 h. Approximately 16% of cells were expressing GFP at 48 h posttransfection, at which time cells were replated on substrate-coated coverslips.

Immunofluorescence
Matrices were prepared as described previously (Midwood et al., 2002). Briefly, 600 μg/ml fibrinogen (America Diagnostica, Greenwich, CT), 30 μg/ml FN, and 15 μg/ml coagulation factor XIIIa (Calbiochem-Novabiochem, San Diego, CA) in the presence or absence of 120 μg/ml 70Ten was mixed with thrombin at 2 U/ml and the mixture was pipetted onto a glass coverslip (Fisher Scientific, Pittsburgh, PA). After overnight incubation at 4°C, the clots were aspirated from the coverslip and the substrate was blocked. Cells were plated at a density of 1.5 × 10⁵ per 35-mm dish and allowed to spread on matrices for 60 min. At the end of the incubation period, cells were washed with PBS and then lysed in 200 μl RIPA lysis buffer (Wierzbicka-Patynowski and Schwarzbauer, 2002) and the protein concentration of the supernatant was determined using the BCA Protein Assay (Pierce, Rockford, IL). Samples were run on a 6% polyacrylamide-SDS gel and transferred to nitrocellulose (Sartorius, Long Island, NY; Midwood and Schwarzbauer, 2002). Proteins were detected using an anti-FAK mAb (Transduction Laboratories, Lexington, KY) and an anti-FAK-397 polyclonal IgG (Biosource International, Camarillo, CA) at 1:5000. Primary antibodies were detected using horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibody (Pierce) diluted 1:50,000 and Supersignal chemiluminescent detection reagent (Pierce).

Contraction Assays
For culture in 3D matrices, cells were resuspended in 0.025 M HEPES, pH 7.4, 0.13 M NaCl at 1 × 10⁶/ml as described previously (Midwood and Schwarzbauer, 2002). Matrices were prepared as for immunofluorescence except cells were added to the matrix components. Immediately after the addition of thrombin, the mixture was pipetted into 48-well plates, which had been coated with 1% BSA overnight at 4°C. The cell-matrix mixture was allowed to polymerize for 30 min at 37°C. To quantitate matrix contraction, cell-matrix mixtures were carefully detached from the dishes and matrix size was visualized as described previously (Midwood and Schwarzbauer, 2002). The area of the matrix was measured over time using a ruler, subtracted from the starting area and expressed as a percentage of the starting area.

Pretreatments
Cells were incubated with the indicated concentrations of heparin (Grade I-A) or chondroitin sulfate (Sigma Chemical), for 30 min at 37°C, and with chondroitinase ABC or heparinase I and III (Sigma Chemical) for 4 h at 37°C before addition to matrix proteins. Enzymes were stopped upon the addition of electrophoresis sample buffer and heat-denatured for 5 min at 95°C and stored at −20°C. Results were compared using a Student’s t-test.

RESULTS

HSPGs Promote Cell Response to a Fibrin-FN Provisional Matrix
Within FN, binding sites for integrins lie adjacent to GAG binding domains (Hyenes, 1990; Barkalow and Schwarzbauer, 1991; Moyano et al., 1999; Mostafavi-Pour et al., 2001). We have previously shown that α5β1 integrins are required for cell adhesion to fibrin-FN matrices (Corbett and Schwarzbauer, 1999). Here, we demonstrate the additional requirement for HSPGs for optimal cell response to fibrin-FN matrices. NIH3T3 fibroblasts plated on a fibrin-FN matrix became well spread and organized their actin into stress fibers (Figure 1A). HSPG function was compromised by treating cells with heparin, which competes with HSPGs for binding to FN. Heparin prevented cell spreading and organization of actin stress fibers (Figure 1A). The removal of cell surface HS GAGs by digestion with heparinase I and III had the same effect as soluble heparin (our unpublished results). Inhibition of chondroitin sulfate proteoglycans (CSPCs) function with chondroitin sulfate or chondroitinase ABC had no effect on cell morphology or cytoskeletal organization. These data indicate that HSPGs are essential for cell spreading and stress fiber formation in response to a fibrin-FN matrix. Another measure of cell–FN interactions is the ability of cells to contract the surrounding matrix. Fibroblasts embed-
expressed as the mean. The dish and % contraction was measured after 4 h. The data are
gathered within a fibrin-FN matrix rapidly contracted it (Figure 1B). Treatment of cells with heparinase, but not chondroitin sulfate, inhibited matrix contraction in a dose-dependent manner (our unpublished results). These results indicate that HS-GAGs but not CS-GAGs are involved in mediating the contraction of a fibrin-FN matrix.

A role for HS-GAGs was clearly demonstrated using mutant Chinese hamster ovary (CHO) cell lines defective in different stages of GAG chain synthesis. Parental CHO K1 cells contracted fibrin-FN matrices to an extent comparable to fibroblasts (Figure 1C). CHO 745 cells can synthesize neither HS nor CS GAGs (Esko et al., 1985) and CHO 677 cells exhibit a reduced expression of HS GAGs and an increase in CS GAGs (Zhang and Esko, 1995). Both of these cell lines showed a significant reduction in fibrin-FN matrix contraction. In contrast, CHO 606 and CHO F17 cells with decreased sulfation (Bame and Esko, 1989; Bai and Esko, 1996) contracted fibrin-FN matrices equally as well as wild-type cells. These data show that CHO cell contractility specifically requires HS GAGs, but not CS GAGs or GAG sulfation. Furthermore, the absence of HS GAGs cannot be fully compensated for by increasing the level of expression of CSPGs. Therefore contraction of fibrin-FN matrices is a HS-GAG-dependent process.

**Cell Contractility Requires Syndecan-4 Signaling**

Syndecan-4 is a cell surface HS-GAG that functions cooperatively with α5β1 integrins in fibroblast binding to FN (Saoncella et al., 1999). Its expression by fibroblasts, among other cell types, is transiently up-regulated during tissue repair (Gallo et al., 1996). In addition, syndecan-4-null mice develop normally but show abnormal wound healing (Echtermeyer et al., 2001). For these reasons we analyzed the role of syndecan-4 in fibroblast response to the provisional matrix.

Dermal fibroblasts isolated from syndecan-4−/− deficient mice exhibited a distinctive morphology on a 3D fibrin-FN matrix (Figure 2C) that contrasted with the well-spread morphology and organized actin stress fibers of wild-type cells on this substrate (Figure 2A). Higher magnification of wild-type cells showed localization of vinculin to distinct focal adhesions at the ends of stress fibers on a fibrin-FN matrix (Figure 2B). However, syndecan-4−/− null cells, although spread, did not organize their actin into typical stress fibers. Instead these cells extended long actin-rich filopodia with vinculin localized at the base and tips (Figure 2, C and D, arrow). They also formed lamellae webbed between the filopodia that contained no detectable actin filaments but did contain punctate spots of vinculin (Figure 2D, arrowhead). Analysis of actin cytoskeleton organization over time shows that filopodia and lamellae are formed as early as 15 min and continue to extend up to 60 min (our unpublished results). On planar FN-coated substrates, both wild-type and syndecan-4−/− deficient fibroblasts became well spread and formed stress fibers and focal adhesions (our unpublished results; Ishiguro et al., 2000; Echtermeyer et al., 2001). In contrast, syndecan-4−/− null cells attached poorly and did not spread on 3D matrix composed of fibrin alone, similar to the behavior of NIH3T3 fibroblasts (Wenk et al., 2000), which indicates that the contribution of fibrin to the response is limited. Therefore, syndecan-4 plays an important role in the regulation of cell morphology, actin cytoskeleton organization, and focal adhesion formation in response to a 3D fibrin-FN matrix. Signaling was also compromised in cells lacking syndecan-4. Null cells showed a reduction in the phosphorylation of FAK compared with wild-type cells (Figure 2E), indicating that adhesive signals are reduced in the absence of syndecan-4.

Syndecan-4−/− null cells were also defective in the contraction of a fibrin-FN matrix whereas wild-type and heterozygous cells contracted matrices to an extent comparable to NIH3T3.
fibroblasts (Figure 3A). In further support of a syndecan-4 requirement, preincubation with D16 antisyndecan-4 monoclonal antibodies that have been shown to inhibit function (Kanieder et al., 2001, 2004) significantly reduced matrix contraction. However, antibodies treated with a blocking peptide had no effect on matrix contraction (Figure 3B). This function-blocking antibody also reduced the extent of cell spreading on fibrin-FN matrix (our unpublished results).

Signaling through RhoA GTPase is required for maximal fibrin-FN matrix contraction (Midwood and Schwarzbauer, 2002) and expression of syndecan-4 contributes to the activation of RhoA (Wilcox-Adelman et al., 2002). We bypassed syndecan-4 and stimulated RhoA by treatment of syndecan-4–null cells with lysophosphatidic acid (LPA). This treatment rescued matrix contraction by these cells (Figure 3A). Therefore, fibrin-FN matrix contraction is mediated by syndecan-4 signaling through RhoA pathways.

**Syndecan-4 Is Required for Tenascin-C Modulation of Cell Morphology and Contraction**

Our analyses of cell interactions with a fibrin-FN matrix have shown modulatory effects of both syndecan-4 and tenascin-C (Wenk et al., 2000; Midwood and Schwarzbauer, 2002). Indeed, the effect of compromising HSPG function mimics the effects of adding tenascin-C to the matrix, both morphologically and functionally. To determine whether tenascin-C is acting via a syndecan-4–dependent pathway, we analyzed the response of syndecan-4–null cells to fibrin-FN matrices containing 70Ten, a recombinant tenascin-C that we have previously shown to induce identical cell responses as native tenascin-C when incorporated into a fibrin-FN matrix (Wenk et al., 2000). Mouse dermal fibroblasts expressing syndecan-4 became well spread on fibrin-FN matrix (Figure 4A). With inclusion of 70Ten, these cells failed to spread (Figure 4B). In contrast, dermal fibroblasts isolated from syndecan-4–null mice were unaffected by the addition of tenascin-C to the matrix (Figure 4D) and showed a morphology that was comparable to these cells plated on a fibrin-FN matrix (Figure 4C). Syndecan-4–null cells transfected with a syndecan-4 expression construct showed a similar response to fibrin-FN + 70Ten matrix as wild-type cells. Measurements of cell areas showed that introduction of syndecan-4 caused a reduction in cell spreading (Figure 5A). Syndecan-4 transfectants, which also
expressed a GFP cotransfection marker, were smaller in size
and had fewer stress fibers than untransfected cells in the
same population (Figure 5C). In contrast to the reduced size
of wild-type fibroblasts in the presence of 70Ten, syndecan-
4–null cells transfected with the GFP expression plasmid
alone had cell areas that were not significantly different on
fibrin-FN vs. fibrin-FN
70Ten matrices (Figure 5, A and B).

These results show that tenascin-C control of cell morphol-
ogy is dependent on syndecan-4 expression.

Overexpression of syndecan-4 also rescued changes in cell
morphology and actin cytoskeleton organization caused by
tenascin-C. Rat embryo fibroblasts (REFs) plated on fi-
brin-FN
70Ten were poorly adherent and did not spread
compared with REFs on fibrin-FN matrix (Figure 6, A and
B). However, REFs overexpressing syndecan-4 did not re-
spond to tenascin-C; they spread and formed stress fibers
(Figure 6, C and D). Similarly, overexpression of syndecan-4
partially relieved the inhibition of contraction caused by
tenascin-C. Both wild-type REFs and REFs overexpressing
syndecan-4 contracted a fibrin-FN matrix equally well. Nor-
amal REF contraction was inhibited by the inclusion of 70Ten.
However, cells overexpressing syndecan-4 showed a signif-
icant increase in contraction (Figure 6E) and had higher
levels of active RhoA (our unpublished results). Thus over-
expression of syndecan-4 can circumvent the inhibitory ef-
fects of tenascin-C.

Taken together these results show that tenascin-C acts by
modulating syndecan-4 function and that syndecan-4 and
tenascin-C act cooperatively to regulate cell morphology
and matrix contraction during tissue repair.

**DISCUSSION**

We have identified a novel mechanism for the coordinated
regulation of cell behavior by multiple ECM components
and their receptors during tissue repair. Syndecan-4, to-
gether with integrins, mediates cell response to matrix FN
by initiating intracellular signaling pathways including the
activation of RhoA and FAK (Figure 7A). ECM deposition of
tenascin-C down-regulates these signals in a syndecan-4-
dependent manner (Figure 7B). Thus, syndecan-4 acts as a
regulatory receptor that monitors changes in ECM compo-
sition and organization, which it then communicates into the
cell to coordinate appropriate responses including matrix
contraction.

Studying cell responses to 3D matrices has highlighted
differences in cell behavior depending on the organization of
the ECM (Cukierman et al., 2001; Grinnell, 2003). On 3D
fibrin-FN matrices, fibroblasts showed significantly reduced
cell size and tenascin-C altered fibroblast morphology and
signaling compared with cells on 2D FN-coated substrates
(Corbett et al., 1996; Wenk et al., 2000). We show here that
syndecan-4 activities are also affected by the dimensionality
of the FN substrate. Syndecan-4–null cells formed stress
fibers and focal adhesions on planar FN-coated surfaces but
showed a distinctive cytoskeleton organization with actin-
filled filopodia on a 3D fibrin-FN matrix. These results illus-
trate the effect of substrate presentation on cell response to
ECM and suggest that there is some compensatory activity
in these cells that takes over for syndecan-4 on 2D but not 3D
substrates.

The response of syndecan-4–null cells to defined FN do-
 mains further supports a role for binding site presentation in
regulating cell activities. On a surface coated with a mixture of cell- and heparin-binding (HepII) domains from FN, stress fibers terminating at focal adhesions were formed by these fibroblasts at levels comparable to intact FN (Ishiguro et al., 2000). However, addition of HepII fragment in soluble form to cells on the cell-binding domain induced very few focal adhesions. These cells clearly have a context-dependent requirement for the heparin-binding domain in order to assemble focal adhesions.

Whatever the context, fibroblast response to FN is initiated by coordinated action of several types of cell surface receptor. Integrin α5β1 binding to the FN cell-binding domain together with HS side chains of syndecan-4 interacting with heparin-binding sites on FN are essential for cells to spread and organize their cytoskeleton (Bloom et al., 1999; Saoncella et al., 1999; Woods et al., 2000; Echtermeyer et al., 2001). Of the multiple heparin domains in FN, syndecan-4 binds to the FNIII13 module in the HepII domain (Huang et al., 2001). This interaction contributes to the activation of RhoA and FAK (Wilcox-Adelman et al., 2002), two key signaling molecules in the formation of actin stress fibers and focal adhesions. Our results show that syndecan-4 is also a critical component of a matrix contraction pathway. Its primary role appears to be activation of RhoA because the overexpression of syndecan-4 enhances RhoA activation and LPA-induced stimulation of RhoA circumvented the requirement for this receptor.

Similarities in the distribution and functional effects of tenascin-C and syndecan-4 at sites of injury suggest that these two proteins may coregulate cell activities during wound repair. Tenascin-C is highly expressed during tissue remodeling and repair (Jones and Jones, 2000; Chiquet-Ehrismann and Chiquet, 2003). Deposition of tenascin-C is markedly increased at the wound edges, particularly at the dermal-epidermal junction (Mackie et al., 1988). Its modulatory effects on cell adhesion suggest a role in promoting epidermal cell migration and proliferation. Syndecan-4 expression is also increased in response to tissue injury. In fact, as with tenascin-C, syndecan-4 expression is induced in the dermis adjacent to the injury where it has been shown to stimulate keratinocyte proliferation and migration during wound reepithelialization (Gallo et al., 1996; Echtermeyer et al., 2001). Also of significance to wound repair are the observations that syndecan-4 null mice and tenascin-C null mice are both defective in resolving tissue damage in models of nephropathy (Nakao et al., 1998; Ishiguro et al., 2001).

Our results show that tenascin-C acts in a syndecan-4-dependent manner to suppress FN-mediated signaling via RhoA and FAK. In the absence of these signals, cells are unable to carry out matrix contraction. Tenascin-C binds to the heparin-binding FNIII13 module in the HepII domain of FN allowing it to compete with HSPGs such as syndecan-4 for binding to this site (Huang et al., 2001). In our experiments with fibroblasts, addition of soluble heparin or inclusion of tenascin-C in the fibrin-FN matrix blocked stress fiber contraction.
formation indicating similar effects of these molecules on FN-syndecan-4 interactions. By masking FN-syndecan-4 interactions in this way, tenasin-C down-regulates syndecan-4-mediated signals required for matrix contraction (shown here) as well as for FN matrix assembly (K. Midwood, unpublished observations).

Like FN, tenasin-C can interact directly with the HS side chains of syndecans (Salmivirta et al., 1991) so, in addition to acting as a block to FN-HS interactions, tenasin-C can directly compete with FN for binding to HS PGs. In addition to its role in matrix contraction, tenasin-C has also been shown to act through syndecan-4 to affect cell cycle progression and proliferative capacities of normal fibroblasts and tumor cells (Huang et al., 2001; Orend et al., 2003). As such, tenasin-C may activate signaling pathways distinct from FN downstream of syndecan-4 while suppressing FN-mediated activation of RhoA and FAK.

How do these interactions regulate cell activities during the repair process? Differential patterns of expression of FN, tenasin-C, and syndecan-4 provide a mechanism for regulating cell phenotype throughout the injury (Midwood et al., 2004). At the center of the wound, the fibrin-FN provisional matrix replaces lost and damaged tissue. Fibroblasts move into this area and our previous results show that they use αβ1 integrin to attach and spread on a fibrin-FN matrix (Wenk et al., 2000). Fibroblasts also express syndecan-4, which facilitates intracellular communication from this matrix and promotes focal adhesion formation, cytoskeleton organization, and initiation of relevant signaling pathways (Figure 7A). These processes are required for deposition of new FN matrix to replace the provisional matrix and for matrix contraction to close the wound. Thus the combination of integrins and syndecan-4 binding to FN generates adhesive signaling required for replacement of damaged tissue. Furthermore, our results may help to explain one of the defects observed in the syndecan-4–null mouse, which exhibits delayed healing of dermal wounds due to insufficient matrix deposition and a failure of wound closure (Echtermeyer et al., 2001).

Tenasin-C expression is induced at the edges of the wound where it contacts the fibrin-FN provisional matrix (Mackie et al., 1988). The presence of tenasin-C in this area of the wound would serve to inhibit FN-initiated signaling events through blockade of syndecan-4 binding. The outcome would be to prevent inappropriate matrix deposition and premature contraction (Figure 7B). As such, syndecan-4 and tenasin-C coregulate the transmission of signals from a dynamic and complex extracellular environment to provide appropriate cell behaviors and efficient wound healing.

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