Extracellular Matrix-dependent Activation of Syndecan-1 Expression in Keratinocyte Growth Factor-treated Keratinocytes*

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 Syndecan-1 is a major heparan sulfate proteoglycan of the epidermis. Its expression is strongly induced in migrating and proliferating keratinocytes during wound healing and, on the other hand, diminished or lost in invasive squamous cell carcinoma. We have recently found in the syndecan-1 gene an enhancer (fibroblast growth factor-inducible response element (FiRE)) that activates gene expression in wound edge keratinocytes (Jaakkola, P., Kontusari, S., Kauppi, T., Määttä, A., and Jalkanen, M. (1998) FASEB J. 12, 959–969). Now, we demonstrate that the activation of this enhancer by keratinocyte growth factor (KGF) is modulated by the components of the extracellular matrix (ECM). MCA-3D mouse immortal keratinocytes growing on fibrillar collagen failed to activate FiRE and subsequently to induce syndecan-1 in response to KGF. The same cells growing on fibronectin or laminin, however, increased FiRE-dependent reporter gene expression upon KGF treatment. The inhibition of the KGF induction by collagen appears to be specific for signaling to FiRE, as the increase in cell proliferation by KGF was not affected. The effect was selective to KGF, as EGF-induction was independent on ECM composition. Changes in the transcription factor binding were not involved in the differential activation of FiRE, as the levels and composition of the AP-1 complexes were unchanged. However, application of anisomycin, an activator of Jun amino-terminal kinase, resulted in a lower response in cells growing on collagen compared with fibronectin. These results indicate that the composition of ECM and availability of growth factors can play a role in the epidermal regulation of syndecan-1 expression and that FiRE is a novel target for gene regulation by the extracellular matrix.

Heparan sulfate proteoglycans have been shown to have a regulatory role in a number of cellular processes. This versatility is due to the ability of glycosaminoglycan side chains to specifically bind proteins such as growth factors, matrix components, or enzymes (1, 2). For example, signaling by bFGF (3, 4) and wingless (5) is strongly augmented by the presence of cell surface heparan sulfate (3, 4). This is possibly related to findings that mutations in the members of glypican family of heparan sulfate proteoglycans lead to delayed progression of cell cycle in Drosophila (6), and to an overgrowth syndrome in man (7). Finally, the enzymatic activity of proteases present in acute wound fluids is regulated by heparan sulfate (8).

Syndecan-1 is an integral membrane proteoglycan that bears both heparan and chondroitin sulfate side chains. In adult tissues, it is almost exclusively found in simple and stratified epithelia. In simple epithelia, it has been proposed to participate in the maintenance of the epithelial phenotype (9, 10). In the epidermis, syndecan-1 expression is strongest in the suprabasal cell layers although undetectable in the cornified layer (11). In cultured keratinocytes, syndecan-1 mRNA levels increase upon differentiation of the cells by exposure to high Ca²⁺ concentrations (12). Additional regulation takes place at the posttranslational level: the glycosaminoglycan chains of syndecan-1 are longer in stratified than in undifferentiated keratocytes (13). Remarkably, both syndecan-1 protein and mRNA levels are strongly induced in the wound edge keratinocytes during wound healing (14). This elevated expression in keratinocytes appears to be specific for syndecan-1, as no changes have been detected for the other syndecans, although a strong induction of syndecan-4 occurs in the dermis (15). The opposite situation is found during the malignant transformation of keratinocytes. Syndecan-1 is lost in invasive carcinomas and reduced in poorly differentiated epidermal dysplasias (12, 16). Likewise, syndecan-1 levels are very low in a cell line derived from a carcinoma induced by chemical carcinogenesis but normal in epithelial cells, such as immortal MCA-3D keratinocytes (17).

In an effort to understand both the induction of syndecan-1 during development and repair processes and, on the other hand, the suppression of its expression in malignancies, we have cloned the syndecan-1 gene (18) and analyzed the key regulatory regions for the basal and induced expression (19, 20). Finally, we have found that an enhancer for syndecan-1 gene (named FiRE for FGF-inducible response element) is activated in vivo in wound edge keratinocytes and that cultured keratinocytes, FiRE can be activated by KGF and EGF (21, 22). Interestingly, this enhancer element is not sufficient for the default expression in suprabasal cells: the only epidermal expression detected in the FiRE-LacZ mice occurs at the wound edge (22). In this study, we addressed whether different matrix molecules can modulate the syndecan-1 levels. We were interested in that aspect of the regulation of syndecan-1 gene for the following reasons. First, the activation of the syndecan-1 in the migrating epidermal sheet of the wound edge is not

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‡ The abbreviations used are: bFGF, basic fibroblast growth factor; FGF, fibroblast growth factor; FiRE, FGF-inducible response element; CAT, chloramphenicol acetyltransferase; ECM, extracellular matrix; EGF, epidermal growth factor; JNK, Jun amino-terminal kinase; KGF, keratinocyte growth factor; TGF, transforming growth factor; USF, upstream stimulatory factor.

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uniform, as the merging frontiers of the two sheets are syndecan-1-negative (14). Second, the extracellular matrix is a strong candidate to modify the gene expression of wound keratinocytes that both encounter a new matrix and produce and remodel it, as shown by the collagen-induced changes in the expression of the interstitial collagenase (23). In this paper, we demonstrate that fibrillar collagen but not fibronectin suppresses the activation of syndecan-1 by KGF. EGF and TGF-α, on the other hand, were able to activate FiRE regardless the matrix. This regulation takes place at transcriptional level and establishes FiRE as a target for both ECM and growth factor receptor derived signals.

**Experimental Procedures**

**Cell Culture and Growth Factor Treatments—**MCA-3D is a keratinocyte cell line derived from carcinogen-treated mouse skin (24). It is not, however, tumorigenic in nude mice (17). MCA-3D cells were grown in Ham’s F-12 medium supplemented with 10% fetal calf serum, 1-glutamine, streptomycin, and penicillin. The effects of various growth factors were studied in cells starved for 24 or 48 h in Ham’s medium without serum or in Ham’s medium supplemented in 1% carboxymethyl-Sephadex-eluted fetal calf serum. Growth factors were purchased from Peprotech and used at a concentration of 10 ng/ml. To study the effects of ECM molecules, tissue culture plates were coated overnight at +4 °C with 50 μg/ml fibrillar collagen (Vitrogen-100, Collagen Corp.), fibronectin (Sigma), laminin-1 (EHS-laminin), or native bovine serum albumin (Sigma). Cell proliferation was determined as the incorporation of [125I]dUTP (Amersham Pharmacia Biotech). To this end, the extract (30) was performed as in Ref. 19. The multiclonal cell lines selected for for expression of the interstitial collagenase (23). In this paper, we investigated whether the activation of FiRE is controlled by combined effects of growth factors and extracellular matrix.

We plated polyclonal MCA-3D cell lines harboring stably integrated FiRE-CAT constructs onto either collagen, laminin, or fibronectin matrices and measured CAT activities in response to growth factor treatment. A consistent induction in the CAT activity was observed in the cells lines harboring the constructs with the FiRE enhancer when cells were grown on fibronectin and stimulated by KGF for 6–8 h (Fig. 1, A–C). The enhancer was placed with the minimal promoter of syndecan-1 gene (p271-FiRE) or a longer, 1.1-kilobase pair portion of syndecan promoter (pSynProm-FiRE). The effect of the enhancer on the response to KGF was similar with both of these promoters. No induction could be seen with the construct having the promoter alone (pSynProm).

Contrary to results with fibronectin, FiRE transfected cells grown on collagen displayed no induction of CAT activity after the application of KGF (Fig. 1, A–C). This suppression was targeted to the FiRE only as the basal CAT activity of the promoter constructs was similar on either matrix. When the KGF treatment was prolonged to 12 or 24 h, a modest induction could be seen on collagen-coated plates, as well (Fig. 1B). However, the measured CAT activities in cells on collagen were always remarkably lower than in cells grown on fibronectin coating. This suppression of the response to KGF was detected in several serial passages of the cells, was independent on the plating density of the cells, and was seen in both transient and stable transfections of the reporter gene constructs (data not shown).

Induction by KGF in cells growing on laminin-1 was over 3-fold. Thus, laminin-1 could not inhibit the activation of FiRE similarly to collagen but acted in a manner similar to fibronectin (Fig. 1). The activation of the enhancer by EGF was independent on the matrix (Fig. 2). Equal CAT activity was seen on all the matrix molecules after EGF (or TGF-α) treatment. Similarly to the KGF treatment, the promoter lacking an enhancer was not activated by EGF on any of the matrix molecules (Fig. 2).

Silencing of FiRE by Collagen Leads to Loss of Increase of Syndecan-1 RNA and Core Protein—To study whether the different abilities of KGF and EGF to activate FiRE levels in cells on fibrillar collagen are recapitulated at the mRNA level, we performed Northern blotting analyses on combined effect of extracellular matrix and the growth factor action. Cells grown on fibronectin showed increased syndecan-1 mRNA levels after treatment with KGF for 4 h (Fig. 3A). Interestingly, the KGF-dependent induction of syndecan-1 mRNA was inhibited in cells grown on fibrillar collagen (Fig. 3A). In the case of treatments with EGF, induction of syndecan-1 expression appeared unaffected by the choice of matrix used (Fig. 3B). The induction caused by EGF was usually higher than by KGF. Typically, EGF increased syndecan-1 steady-state mRNA levels 6–8-fold.
whereas KGF resulted in an about 3–4-fold induction (Fig. 3, A and B). Thus, a similar combined effect of KGF and collagen was seen in mRNA levels as in reporter gene activation by FiRE enhancer.

Syndecan-1 protein levels were measured from MCA-3D keratinocytes grown on different matrix molecules or on native bovine serum albumin as a nonspecific adhesive substrate. The concentration of shed syndecan-1 ectodomain in the conditioned medium of the keratinocytes was found to be directly proportional to the level of the core protein synthesis (not shown). Thus, the effect of the growth factors on the syndecan-1 protein levels were measured from conditioned medium collected during the last 4 h of a 24-h growth factor treatment. At a concentration of 10 ng/ml, KGF, EGF, and TGF-α were the most effective inducers of the syndecan-1 (Fig. 4). Both EGF and TGF-α resulted in a marked (over 10-fold) increase in the syndecan-1 ectodomain levels regardless of the matrix used as a substratum for cell attachment (Fig. 4). Intriguingly, how-

![Graph](image1)

**Fig. 1. Activation of the enhancer for syndecan-1 gene (FiRE) by KGF.** Stably transfected polyclonal MCA-3D cell lines carrying syndecan-1 enhancer/promoter-CAT constructs were plated on fibronectin, laminin, collagen, or native bovine serum albumin (BSA), serum-starved, and treated for 6 h or overnight with 10 ng/ml KGF. A, KGF induces CAT activity in 6 h from the pPst-FiRE construct with the enhancer and a 0.1-kilobase pair promoter in cells on fibronectin (FN, gray columns), laminin (striped columns), BSA (white columns) but not in cells on fibrillar collagen (COL, black columns). B, CAT activities from the pPst-FiRE construct accumulated in 6 or 12 h in cells on fibronectin (open symbols) or collagen (filled symbols) indicate a delayed response to KGF on collagen. C, CAT activity in a polyclonal cell line carrying a construct in which the FiRE enhancer is in the context of a longer (1.1-kilobase pair) promoter. D, CAT activity of the 1.1-kilobase pair promoter only construct. The results shown represent mean and range (error bars) of two replicate wells in a representative experiment out of at least three independent ones.

![Graph](image2)

**Fig. 2. Induction of the FiRE activity by EGF.** The same cell lines as in Fig. 3 were plated on the indicated matrix molecules, serum-starved, and treated with 10 ng/ml of EGF for 6 h. For all the plasmids, control values without the growth factor represent 1.0 after the correction for total protein levels.
ever, the production of syndecan-1 protein in response to cell treatments with KGF appeared to be dependent on the matrix. MCA-3D cells grown on fibronectin responded to KGF by an increase in syndecan-1 production. On the other hand, cells grown on fibrillar collagen failed to increase syndecan-1 levels in response to KGF exposure (Fig. 4). Thus, the loss of activation seen in the reporter gene assays and at the mRNA steady state levels was finally reflected in the synthesis of syndecan-1 core protein. Quantitation of syndecan production after growth factor treatment was subsequently used to screen possible interactions of ECM signaling and additional growth factors. A modest enhancement was seen in cells grown on all matrix molecules and treated with either bFGF or acidic FGF (Fig. 4). No effects on the syndecan-1 levels were seen by TGF-β or platelet-derived growth factor (data not shown). This result emphasizes further the selectivity of KGF-response of FiRE and syndecan-1 mRNA levels in keratinocytes grown on collagen or fibronectin were treated with 10 ng/ml of KGF (A) or EGF (B) for 4 h. Total RNA was isolated, and 10-μg aliquots were electrophoresed in formaldehyde agarose gels and sequentially blotted for syndecan-1 (top) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a loading control (middle). Quantitation of the hybridizations (bottom) (syndecan/glyceraldehyde-3-phosphate dehydrogenase) was performed with an image analyzer.

**FIG. 3.** Northern blot analysis of the syndecan-1 mRNA levels in keratinocytes grown on collagen or fibronectin. Serum-starved cells on either collagen or fibronectin were treated with 10 ng/ml of KGF (A) or EGF (B) for 4 h. Total RNA was isolated, and 10-μg aliquots were electrophoresed in formaldehyde agarose gels and sequentially blotted for syndecan-1 (top) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a loading control (middle). Quantitation of the hybridizations (bottom) (syndecan/glyceraldehyde-3-phosphate dehydrogenase) was performed with an image analyzer.

KGF Is Able to Promote the Proliferation of MCA-3D Cells Plated on Collagen—We were interested to see whether collagenous matrix can suppress all of the end points of KGF signaling in MCA-3D cells. The ability of KGF to promote the proliferation of MCA-3D cells was assayed by the means of iodo-deoxyuridine (Idu) incorporation into newly synthesized DNA. A similar induction of DNA synthesis was seen in cells on all ECM molecules (Fig. 5). Thus, all aspects of KGF signaling are not compromised in the cells growing on collagen. This is supported by Western blot analysis of FGFR-2, which revealed similar amounts of the KGF-receptor in cells plated on either fibronectin or collagen (not shown).

**Fibrillar Collagen Does Not Change the Binding of Transcription Factors to FiRE—**Next, we investigated whether matrix composition can cause changes in the pattern of transcription factors that bind FiRE and enable syndecan-1 induction. We have previously characterized the enhancer by DNaseI footprinting and gel mobility shift assays (20, 21). An overview of the enhancer is presented in the Fig. 6A. In 3T3 fibroblasts, the enhancer can be bound by both transcription factors that are induced by bFGF and by factors constitutively present in the cells. The enhancer harbors binding sites for two growth factor-inducible AP-1 complexes and for one inducible, as yet uncharacterized transcription factor (20). In MCA-3D keratinocytes, the on other hand, transcription factor binding is not affected by growth factors that activate FiRE (21). However, there remained a possibility that ECM can cause the induction or suppression of transcription factor binding and thus modulate the growth factor effects on the cell.

In order to investigate the effect of ECM on transcription factor binding to FiRE, we used gel mobility shift assays. The most 3′-motif (motif 1) of the enhancer binds an uncharacterized 46-kDa protein constitutively expressed in 3T3 cells (21). The DNA binding activity of this protein was equally present in treated and control MCA-3D cells and was unchanged by extracellular matrix molecules (data not shown). Motif 2 contains an E-box that is bound by USF-1 in both cell lines (20, 21). Fig. 6B shows equal binding activity of USF in both control and growth factor-treated cells on fibronectin and in KGF-treated cells grown on collagen. An antibody against USF abolished the bound specific complex, whereas antibodies against other bHLH factors (anti-Max shown) did not affect the complex formation (Fig. 6B). Motif 3, which binds an bFGF-inducible unidentified factor in 3T3 cells does not bind that protein in growth factor-treated MCA-3D cells (21). Neither fibronectin nor collagen alone or in combination with growth factors induced specific transcription factor binding to this site (Fig. 6C). Motifs 4 and 5 are occupied by the members of AP-1 (Fos/Jun) family (20, 21). The AP-1 binding activity was unchanged by the extracellular matrix (Fig. 7A). We furthermore investigated whether the composition of AP-1 complexes is affected by matrix. Again, the same complexes were formed in cells growing on collagen as in those on fibronectin. Antibodies against c-Fos or JunD abrogated or supershifted the complexes with motif 4 (Fig. 7B). The same complex was observed in cells growing on all tested ECM molecules and treated with either KGF or EGF (data not shown). In summary, changes in transcription factor binding activities did not explain the diminished activation in cells grown on collagen. This suggested that matrix-dependent
regulation of syndecan-1 induction during growth factor treatment occurred at the posttranslational modifications of transcription factors.

As AP-1 sites were necessary for the FiRE activation in vitro (20, 21), we examined several pathways that converge AP-1 activation. Anisomycin, a known inducer of Jun amino-terminal kinase and p38 stress-activated mitogen-activated protein kinase (JNK) activity (31), caused a reduction in FiRE activation in cells growing on collagen compared with fibronectin (Fig. 8A). Thus, it appears that the activation of JNK could, in turn, activate FiRE in keratinocytes in a manner that can be inhibited by the ECM. The activity of the pSynProm construct without the enhancer sequences was unchanged by anisomycin (Fig. 8B). This indicates that the induction seen with the enhancer construct is not due to nonspecific effect of the anisomycin on the translation or stability of the CAT protein. Interestingly, KGF and anisomycin had a synergistic effect, as the activation of the enhancer was higher when they were administered together (Fig. 8A). This synergistic activation was, as well, lower on collagen than on fibronectin (Fig. 8A). The finding suggests that parallel signaling pathways act in concert to regulate FiRE. Evidence supporting multipathway regulation came from our recent experiments with FiRE-LacZ, in which anisomycin and a growth factor mixture were needed together to activate FiRE in an organ culture of unwounded skin (22). Additionally, a wound healing assay performed on the FiRE-LacZ mice determined that PD 098059, an inhibitor of extracellular matrix regulated kinases 1 and 2, can inhibit reporter gene activity (22). Likewise, we found that inhibition of extracellular matrix regulated kinase activity by PD098059 inhibits the reporter gene activity in cells growing on fibronectin (data not shown), which is further evidence for an involvement of several parallel signaling pathways in the regulation of FiRE activity.

and syndecan-1 levels were measured by monoclonal antibody 281-2 using enhanced chemiluminescence. Syndecan-1 levels were quantitated by an image analyzer (Molecular Imaging) and normalized by the cell number. Columns and bars represent the mean and S.E. of the values from four replicate wells in a representative experiment.
syndecan-1 gene, an increase in syndecan-1 steady state mRNA levels, and a rise in the concentration of syndecan-1 protein in the conditioned medium of the cells (Ref. 21 and this study). We have shown that a treatment of mouse MCA-3D keratinocytes by KGF or members of the EGF family results in the activation of an enhancer for syndecan-1 gene, an increase in syndecan-1 steady state mRNA levels, and a rise in the concentration of syndecan-1 protein in the conditioned medium of the cells (Ref. 21 and this study). Taken together with our previous findings that syndecan-1 levels are induced severalfold in keratinocytes at the leading edge of the wound (14) and that FiRE directs reporter gene expression to the wound edge in transgenic mice (22), these data suggest that certain growth factors can be the key activators of syndecan-1 gene in keratinocytes.

KGF is a known to increase re-epithelialization in experimental wound healing studies (32, 33). Accordingly, the overexpression of a dominant-negative form of the KGF-receptor variant of the FGFR-2 retards healing in transgenic mice (34), although wounds in KGF knock-out mice appear to heal normally (35). Our study establishes that KGF signaling to FiRE is regulated by ECM. Most notably, KGF failed to rapidly activate syndecan-1 expression in cells plated on fibrillar collagen, whereas there was a severalfold induction in cells growing on fibronectin, nonspecific substrata, or the ambient matrix laid on uncoated plastic dishes. This indicates that extracellular matrix is selectively able to modulate the regulation of syndecan-1 expression.

Fibronectin is a major component of the provisional matrix, the first extracellular matrix that is deposited in the wound space. It supports keratinocyte adhesion and migration (36, 37) and, by acting through different integrin receptors, modulates gene expression (38, 39). In this study, fibronectin supported an induction of syndecan-1 expression by KGF. Collagen as well promotes keratinocyte adhesion and migration (38). It is present in the provisional matrix but is deposited in the granulation tissue later than fibronectin (40). Thus, the local composition of the matrix and the availability of growth factors may determine the activation status of the syndecan-1 gene in keratinocytes. Hence, it is possible that interactions with the provisional matrix could mediate the differential expression of syndecan-1 in wound keratinocytes. The role of fibrillar collagen has previously been implicated in the regulation of other events critical to wound healing, such as the induction of interstitial collagenase in migrating keratinocytes (23). In this case, the role is inductive, as the collagenase expression is detected only in primary keratinocytes growing on collagen but not on gelatin (23). This, in turn, corresponds to the in vivo findings that collagenase expression is detected in keratinocytes that during wound healing encounter the dermal matrix. It appears that the default signaling pathway activated by KGF leads to syndecan-1 expression in keratinocytes and that this regulation is specifically blocked by a collagenous matrix. The KGF-induced pathway is likely to converge with EGF signaling, because the FiRE enhancer binds the same transcription factors upon the treatment by either growth factor.

Interestingly, there was no difference between the cells grown on either fibronectin or collagen in the binding activities of the transcription factors recognizing the enhancer element, suggesting that both the effect of growth factors and the modulation by collagenous matrix occur at the level of the modification of pre-existing factors. Alternatively, levels of modulator(s) of the transcriptional activity that mediate the interaction of enhancer-bound complexes to the basal transcriptional machinery or function as co-activators are regulated in these cells. For example, JAB1, a co-activator of c-Jun and JunD, does not affect the association rate of AP-1 to its binding site but increases the stability and transcriptional activity of the complexes (41).

The potential ability of the matrix to regulate the capacity for transcriptional activity of the factors binding to the enhancer is suggested by the results obtained with anisomycin, an activator of JNK (stress-activated mitogen-activated protein kinase) (31, 42). JNK can phosphorylate the amino-terminal activation domain of transcription factor c-Jun (42). Anisomycin elicited a stronger response in CAT activity of in cells growing on fibronectin than on collagen. This response was
specific for the enhancer constructs, whereas the CAT activity with the promoter alone remained unaffected. This might indicate that FiRE can be activated via the JNK pathway. Moreover, regulation of JNK kinase activity could be a target for matrix induced signals. Interestingly, anisomycin had a synergistic effect on reporter gene activation when administered together with growth factors. Additionally, an inhibitor to extracellular matrix regulated kinase activity, PD098059 was able to block the LacZ expression in wounds of transgenic mice (22) and largely to suppress increase in CAT activity in the case of both growth factors regardless of the matrix. Thus, there is evidence for existence of parallel signaling pathways that activate FiRE in keratinocytes. It is likely that during wound healing, several independent signals are needed for high level syndecan-1 expression. Furthermore, it is possible that anisomycin activates a different signaling cascade to KGF but that

FIG. 7. Transcription factors binding to the AP-1 sites of the FiRE in MCA-3D cells growing on fibronectin or collagen. A, growth factors do not increase the amount of AP-1 complexes binding to FiRE either on collagen or on fibronectin. The radiolabeled motifs 4 (lanes 1–6) and 5 (lanes 7–12) were used to detect the AP-1-binding proteins from cells treated with growth factors on either collagen or fibronectin. B, supershifts demonstrate the presence of c-Fos and JunD in the complexes. The gel was run for a longer time than other presented. A competition with a 100-fold excess of unlabeled motif 4 oligonucleotide (lane 2) compared with a nonspecific competitor (lane 3, 100-fold excess of an Sp1 binding site) shows the specific binding. An antibody against c-Fos abolishes the binding to motif 4 (lane 4). To assess the Fos family members in the complexes, antibodies against FosB (lane 5), Fra-1 and Fra-2 and a pan-Fos antibody were used (not shown). In the Jun family, the predominant protein participating the complexes is JunD (lane 8; the supershift is marked by an asterisk), whereas antibodies against c-Jun (lane 7) or JunB (not shown) did not compromise the interaction. For both the Jun and Fos families, all the extracts from different growth factor/matrix combinations resulted in the similar results in the supershift assays (not shown).

FIG. 8. Anisomycin induces a higher CAT activity in cells growing on fibronectin than on collagen. A, the stably transfected cells harboring p-271-FiRE CAT-construct were treated with 30 \( \mu \)g/ml Anisomycin for 30 min before the addition of 10 ng/ml KGF or EGF for overnight or treated with anisomycin alone (labeled A). Columns and bars represent the combined mean and S.E. from three independent experiments. Note that the difference in the EGF treatment between cells on collagen or fibronectin is not statistically significant. B, anisomycin treatment of the pSynProm-CAT cells does not cause any changes in the CAT activity compared with untreated controls.
they both are independently controlled by collagenous matrix.

Several studies indicate how changes in the expression of proteoglycans can have profound biological consequences. In the case of the syndecan family of cell surface proteoglycans, the studies have focused on the effect of syndecan-1 on the regulation and maintenance of the simple polarized epithelial cell phenotype (9, 10) or on the ability of the cells to bind and respond to bFGF (43, 44). As a forced expression of syndecan-1 in fibroblasts leads to a loss of response to bFGF, it has been postulated that syndecans, unlike some other proteoglycans, such as perlecain (45), could be inhibitory for the FGF signaling (44). In wound fluids, syndecan-1 ectodomain acts as a dual regulator of bFGF activity. The intact ectodomain inhibits growth factor action, but degradation of the glycosaminoglycan chains by platelet heparanase liberates heparan sulfate fragments that activate bFGF (46). So far it remains to be studied how elevated syndecan-1 levels participate in re-epithelialization of wounds. We have recently started transgenic approaches to look for syndecan-1 functions in mice. We found that KGF signaling is modulated by the extracellular matrix. For example, cell adhesion modulates signaling from platelet-derived growth factor or EGF receptors at the level of receptor phosphorylation (47, 48). Moreover, integrin signaling can modify events that occur downstream at the levels of Raf or MEK activation (49). In this study, we found that KGF signaling is modulated by the extracellular matrix. This co-ordinated regulation is likely to play a role in the specific spatial and temporal expression of syndecan-1 during wound healing. Further studies are needed to characterize the integrins and signaling pathways involved. One point, perhaps worth of further investigation, is what events modulate KGF regulation of proliferation distinctly from FIRE activation.

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