Activation of extracellular signal-regulated protein kinase1,2 results in down-regulation of decorin expression in fibroblasts

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Decorin is a small leucine-rich extracellular matrix proteoglycan, the expression of which is down-regulated in proliferating and malignantly transformed cells. In the present study we show that the expression of decorin in fibroblasts is suppressed by epidermal growth factor (EGF) and PMA, and that the effect of both is potently inhibited by blocking the extracellular signal-regulated protein kinase (ERK)1,2 signalling pathway (Raf/MEK1,2/ERK1,2) with the specific MAPK/ERK kinase (MEK)1,2 inhibitor, PD98059. In addition, specific activation of ERK1,2 by adenovirus-mediated expression of constitutively active MEK1 in dermal fibroblasts results in marked reduction in decorin mRNA abundance and production. Co-transfection of NIH-3T3 fibroblasts with human decorin promoter/chloramphenicol acetyltransferase (CAT) construct (pDEC-879/CAT) in com-

bination with the expression vectors for constitutively active Raf-1 and MEK1 markedly suppressed decorin promoter activity. Co-transfections of human decorin promoter 5'-deletion constructs with constitutively active MEK1 expression vector identified the region -278 to -188 as essential for ERK1,2 mediated down-regulation of decorin promoter activity. These results show that activation of the ERK1,2 signalling pathway by a mitogenic growth factor, a tumour promoter or transformation suppresses decorin gene expression in fibroblasts, which in turn may promote proliferation and migration of normal and malignant cells.

Key words: adenovirus, biglycan, EGF, proteoglycan, mitogen-activated protein kinase.

INTRODUCTION

Decorin is a chondroitin/dermatan sulphate proteoglycan, which belongs to the gene family of small leucine-rich proteoglycans that also includes biglycan, fibromodulin and lumican [1,2]. Decorin is an abundant component of the extracellular matrix (ECM) of skin and bone and it associates with type I, II and VI collagen fibrils, and fibronectin *in vivo* [1,2]. In addition, decorin can inhibit the biological activity of transforming growth factor- β (TGF- β) by binding it with the core protein [3].

Decorin is abundantly expressed by normal fibroblasts in which its expression is enhanced by interleukin-1 [4,5] and dexamethasone [6], and inhibited by TGF- β [7], tumour necrosis factor- α (TNF- α) [8] and retinoic acid [6]. The expression of decorin in fibroblasts is cell cycle-dependent; it is low in proliferating cells and high in quiescent cells [8]. Decorin inhibits proliferation of A431 squamous carcinoma cells by binding to epidermal growth factor (EGF) receptors (EGFRs), activating EGFR signalling, including the extracellular signal-regulated protein kinase (ERK)1,2 pathway, and expression of p21 [9–11]. Furthermore, overexpression of decorin in fibroblasts inhibits their proliferation (L. Häkkinen, S. Strassburger, F. Scott, V.-M. Kähäri, K. G. Danielsson, R. V. Iozzo and H. Larjava, unpublished work). These findings are in accordance with previous observations showing that malignant transformation of cells results in loss of decorin expression and that restoration of

decorin expression causes growth arrest in malignant cells [12–14]. In addition, viral transformation of human fibroblasts by the *v-src* oncogene results in marked reduction of decorin expression [15].

In the present study we show that in fibroblasts, suppression of decorin expression by EGF and tumour promoter PMA is mediated by the ERK1,2 signalling cascade, and that activation of ERK1,2 by constitutively active MEK1 potently suppresses decorin expression at the transcriptional level. These results show that activation of the ERK1,2 pathway by mitogenic growth factors, tumour promoters or transformation suppresses decorin expression in fibroblasts, which may in turn promote proliferation and migration of normal and malignant cells and play a role in malignant transformation.

MATERIALS AND METHODS

Materials

MEK1,2 inhibitor PD98059 (2'-amino-3'-methoxyflavone) [16], p38 inhibitor SB203580 [4-(4-fluorophenyl)-2-(4-methylsulphanylphenyl)-5-(4-pyridyl)-1H-imidazole] [17] and human recombinant EGF were obtained from Calbiochem (San Diego, CA, U.S.A.). PMA was obtained from Sigma (St. Louis, MO, U.S.A.).

Abbreviations used: CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; CS, calf serum; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular signal-regulated protein kinase; FBS, fetal bovine serum; FCS, fetal CS; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MKK, MAPK kinase; MOI, multiplicity of infection; pfu, plaque-forming units; RSV, Rous sarcoma virus; TGF- β , transforming growth factor- β ; TNF- α , tumour necrosis factor- α .

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Cell culture

Normal human skin fibroblasts established by explantation from punch biopsy were obtained from a healthy male volunteer (age 23), and human gingival fibroblasts were derived from healthy gingiva of a 25-year old female. The fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM glutamine, 100 units/ml penicillin-G and 100 µg/ml streptomycin. Mouse NIH-3T3 fibroblasts (from A.T.C.C.; Rockville, MD, U.S.A.) were cultured in DMEM supplemented with 10% (v/v) calf serum (CS), 2 mM glutamine, 100 units/ml penicillin-G and 100 µg/ml streptomycin. For experiments, human skin and gingival fibroblasts were maintained in culture medium supplemented with 0.5% fetal CS (FCS) for 18 h. EGF or PMA was added and incubations were continued for 24 h. In experiments with mitogen-activated protein kinase (MAPK) inhibitors, these were added 1 h prior to the addition of EGF or PMA.

Assay of MAPK activation

The activation of ERK1,2 and p38 MAPK was determined by Western-blotting using antibodies specific for phosphorylated, activated, forms of the corresponding MAPKs (New England Biolabs, Beverly, MA, U.S.A.). Fibroblasts were treated with EGF and PMA in DMEM supplemented with 0.5% FCS for different periods of time, and lysed in 100 µl of Laemmli sample buffer. The samples were then sonicated, separated by SDS/PAGE (10% polyacrylamide) and transferred to Hybond ECL[®] membrane (Amersham, Little Chalfont, Bucks., U.K.). Western-blotting was performed as described previously [18], with phospho-specific antibodies against ERK1,2 and p38 at 1:1000 dilution, using ECL[®] for detection (Amersham). As loading controls Western-blots were also performed using antibodies against total p38 (New England Biolabs) at 1:1000 dilution.

Infection of fibroblasts with recombinant adenoviruses

Recombinant replication-deficient adenovirus RAdLacZ (RAd35) [19], which contains the *Escherichia coli* β-galactosidase gene (*LacZ*) driven by the cytomegalovirus (CMV) IE promoter was kindly provided by Dr Gavin W. G. Wilkinson (University of Cardiff, Cardiff, Wales). Construction and characterization of recombinant adenovirus RAdMEK1CA, coding for constitutively active MEK1, driven by the CMV IE promoter, has been described previously [20]. Recombinant adenoviruses RAdMKK3bE [21] and RAdMKK6bE [21], coding for constitutively active MKK3b and MKK6b (where MKK is MAPK kinase), respectively, were kindly provided by Dr Jiahui Han (Scripps Research Institute, La Jolla, CA, U.S.A.).

Human skin and gingival fibroblasts (5×10^5) in suspension were infected with recombinant adenoviruses RAdLacZ, RAdMEK1CA, RAdMKK3bE or RAdMKK6bE at a multiplicity of infection (MOI) of 500 plaque-forming units (pfu)/cell, plated and incubated for 18 h, as described in [18,22]. Culture medium (DMEM supplemented with 1% FCS) was changed and conditioned media was collected after 24 h for assay of decorin production. The cell layers were harvested for extraction of total RNA or determination of activation of ERK1,2 and p38 MAPK, as described above.

RNA analysis

Total cellular RNA was isolated from cells using the single-step method [23]. Northern-blot hybridizations were performed as described previously [6,7], using a 1.8 kb human decorin cDNA [24] (kindly provided by Dr Tom Krusius, Finnish Red Cross,

Helsinki, Finland), a 1.6 kb human biglycan cDNA [25] (kindly provided by Dr Larry Fisher, NIDR, Bethesda, MD, U.S.A.), and a 1.3 kb rat cDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [26]. The [³²P]cDNA-mRNA hybrids were visualized by autoradiography. Decorin and biglycan mRNA levels were quantified by scanning densitometry, and corrected for the levels of GAPDH mRNA in the same samples.

Assay of decorin production

Equal aliquots of conditioned media were analysed by Western-blotting, as described previously [7] with rabbit antiserum against human decorin (kindly provided by Dr Tom Krusius) at 1:500 dilution. Specific binding of antibodies was detected by horseradish peroxidase-conjugated secondary antibodies, followed by ECL[®] (Amersham). The levels of immunoreactive decorin were quantified by densitometric scanning of the X-ray films.

Transient transfections and chloramphenicol acetyltransferase (CAT) assays

Confluent NIH-3T3 fibroblast cultures were transiently transfected with 5 µg of human decorin promoter/CAT 5'-deletion constructs (pDEC-879/CAT, pDEC-480/CAT, pDEC-326/CAT, pDEC-278/CAT, pDEC-222/CAT, pDEC-188/CAT or pDEC-140/CAT) [8,27]. In co-transfections the cells were transfected with pDEC-879/CAT in combination with 5 µg of expression vectors for wild-type MEK1, constitutively active MEK1 [28] (kindly provided by Dr Natalie Ahn, University of Colorado, Boulder, CO, U.S.A.), wild-type ERK1 and ERK2 [29] (kindly provided by Dr Melanie Cobb, Southwestern Medical Center, Dallas, TX, U.S.A.), or constitutively active Raf-1 [30] (kindly provided by Dr Ulf Rapp, University of Würzburg, Germany). Control cultures were co-transfected in parallel with the respective empty expression vectors.

Transfections were performed using the calcium phosphate/DNA co-precipitation method, followed by a 2 min glycerol shock [31]. The cells were maintained in DMEM and 1% FBS for 48 h, and CAT activity was measured as described previously [31]. The transfection efficiency was monitored by co-transfecting the cells with 2 µg of Rous sarcoma virus (RSV)/β-galactosidase construct and correcting the CAT activities for β-galactosidase activity [31].

RESULTS

Suppression of decorin expression by EGF and PMA in fibroblasts is mediated by ERK1,2

To examine the regulation of fibroblast decorin gene expression by mitogenic stimuli, we first studied the effect of EGF and protein kinase C activator, phorbol ester PMA, on the expression of decorin mRNA levels by Northern-blot hybridizations. Treatment of dermal fibroblasts with EGF (25 ng/ml) resulted in rapid activation of ERK1,2 and this activation was potently inhibited by blocking the ERK1,2 pathway (Raf/MEK1,2/ERK1,2) by a specific MEK1,2 inhibitor, PD98059 (20 µM) (Figure 1, left-hand upper panel). EGF also rapidly and transiently activated p38 MAPK in fibroblasts, but this activation was not altered by co-treatment with PD98059. As shown in the left-hand middle and lower panels of Figure 1, EGF treatment of cells resulted in the suppression (60%) of decorin mRNA expression. Interestingly, co-treatment of cells with MEK1,2 inhibitor PD98059 (40 µM) prevented the inhibitory effect of EGF on decorin mRNA expression (left-hand middle and lower panels of Figure 1). In contrast, inhibition of p38 MAPK activity

Figure 1 Suppression of decorin expression by EGF and TPA is mediated by ERK1,2

Upper panels: Confluent cultures of human skin fibroblasts were incubated with (left panel) EGF (25 ng/ml) or (right panel) PMA (60 ng/ml) alone or in combination with MEK1,2 inhibitor PD98059 (20 μ M) in DMEM supplemented with 0.5% FCS for different periods of time, as indicated. The levels of activated ERK1 and ERK2 (p-ERK1, p-ERK2), and p38 (p-p38) were determined by Western-blot analysis using phosphospecific antibodies for the corresponding MAPKs. Middle and lower panels: Confluent cultures of human skin fibroblasts in DMEM supplemented with 0.5% FCS were incubated for 24 h with (left panel) EGF (25 ng/ml) or (right panel) PMA (60 ng/ml) alone or in combination with PD98059 (40 μ M with EGF and 20 μ M with PMA), or with SB203580 (20 μ M with EGF and 10 μ M with PMA), a selective p38 MAPK inhibitor. The expression of decorin, biglycan and GAPDH mRNAs was analysed by Northern-blot hybridizations of total RNA (12 μ g). Densitometric quantifications of decorin and biglycan mRNA levels corrected for GAPDH mRNA levels are shown relative to the levels in untreated control cells (100) (histograms). TPA $\equiv$ PMA.

by SB203580 (20 μ M) slightly augmented the down-regulatory effect of EGF on decorin mRNA expression (left-hand middle and lower panels of Figure 1). In the same cultures biglycan mRNA levels were also decreased (by 43%) by EGF and this reduction was inhibited by PD98059 (left-hand middle and lower panels of Figure 1). EGF treatment also inhibited decorin mRNA expression by 78% in human gingival fibroblasts, and the inhibition was in part prevented by PD98059 (results not shown).

Treatment of dermal fibroblasts with PMA (60 ng/ml) also resulted in rapid activation of ERK1,2, and p38 MAPK in dermal fibroblasts, and the activation of ERK1,2 was potently

inhibited by PD98059 (right-hand upper panel of Figure 1). PMA also rapidly and transiently activated p38 MAPK in these cells (right-hand upper panel of Figure 1). Treatment of dermal fibroblasts with PMA also resulted in the suppression of the abundance of decorin mRNA (by 52%) and the down-regulation was entirely prevented by PD98059 (20 μ M), but not by SB203580 (10 μ M) (right-hand middle and lower panels of Figure 1). Similarly, PMA treatment also inhibited decorin mRNA expression (by 78%) in human gingival fibroblasts, and the inhibition was in part prevented by PD98059 (results not shown). The inhibitory effect of PMA on biglycan expression was minimal

Figure 2 Activation of ERK1,2 results in suppression of decorin expression by dermal fibroblasts

Confluent human skin fibroblast cultures were transduced at a MOI of 500 pfu/ml with recombinant adenoviruses harbouring β -galactosidase (RAdLacZ), constitutively active MEK1 (RAdMEK1CA), MKK3b (RAdMCK3bE) or MKK6b (RAdMCK6bE), and incubated for 18 h. The media were changed and the incubations continued for another 24 h. Left panel: the levels of activated ERK1 and ERK2 (p-ERK1, p-ERK2), and p38 (p-p38) in cell lysates were determined by Western-blot analysis using phosphospecific antibodies for the corresponding MAPKs. As loading control, the levels of total p38 MAPK were determined in the same samples using a specific antibody. Middle panel: the levels of decorin, biglycan and GAPDH mRNAs were measured by Northern-blot hybridizations in aliquots (15 μ g) of total RNA from uninfected (control) and adenovirally infected cells. Decorin and biglycan mRNA levels were quantified by scanning densitometry, corrected for the levels of GAPDH mRNA, and shown relative to the levels in untreated control cells (100) (histogram). Right panel: aliquots of conditioned media of uninfected dermal fibroblasts (control) or cells infected with RAdLacZ or RAdMEK1CA were assayed for the levels of decorin by Western-blot analysis using a decorin antiserum. The migration positions of molecular-mass markers ($M_r \times 10^{-3}$) are shown on the left. The values below the panel represent the levels of decorin relative to the uninfected control cultures (1.0) determined by densitometric scanning of the X-ray films.

(17%), but was augmented to 50% by SB203580 (right-hand middle and lower panels of Figure 1). Together these results show that inhibition of decorin expression in fibroblasts by both EGF and PMA is mediated via ERK1,2, whereas blocking the p38 pathway does not prevent down-regulation of decorin expression. In addition, the suppression of biglycan expression by EGF is mediated by the ERK1,2 signalling cascade.

Activation of ERK1,2 inhibits decorin expression in fibroblasts

To directly examine the role of ERK1,2 and p38 MAPK signalling pathways in the regulation of decorin gene expression we infected fibroblasts with recombinant adenoviruses; RAdMEK1CA, coding for constitutively active MEK1 (the activator of ERK1,2), RAdMCK3bE or RAdMCK6bE, harbouring constitutively active p38 activators MKK3b and MKK6b, respectively. Cells were infected at a MOI of 500 pfu/cell, which results in nearly 100% transduction efficiency of human primary fibroblasts [18]. As shown in the left panel of Figure 2, infection of fibroblasts

with adenovirus for constitutively active MEK1 (RAdMEK1CA) resulted in activation of ERK1,2, but not p38 MAPK. In parallel, adenovirus-mediated expression of constitutively active MKK3b (RAdMCK3bE) or MKK6b (RAdMCK6bE) resulted in activation of p38, but not ERK1,2 (left panel of Figure 2). Expression of constitutively active MEK1, MKK3b or MKK6b had no effect on the total cellular levels of p38, determined as loading control (left panel of Figure 2). Infection of cells with control virus RAdLacZ did not result in sustained activation of ERK1,2 but slightly activated p38 (left panel of Figure 2).

As shown in the middle panel of Figure 2, adenovirus-mediated expression of constitutively active MEK1 resulted in a marked (61%) reduction in decorin mRNA abundance, as compared with cells infected with the control virus (RAdLacZ). In contrast, infection of parallel cultures with adenoviruses for constitutively active MKK3b or MKK6b had no marked effect on decorin mRNA levels (middle panel of Figure 2). Adenovirus-mediated expression of constitutively active MEK1 also suppressed biglycan mRNA levels (by 57%), and infection with

Figure 3 Activation of ERK1,2 results in down-regulation of decorin promoter activity in fibroblasts

Upper panel: confluent NIH-3T3 fibroblast cultures were transiently transfected with 5 μ g of human decorin promoter/CAT construct (pDEC-879/CAT) in combination with 5 μ g of expression vectors for wild-type MEK1 (MKK1wt) alone or together with expression vectors for constitutively active Raf-1 (BXB). Middle panel: NIH-3T3 fibroblast cultures were co-transfected with pDEC-879/CAT in combination with the expression vector for constitutively active MEK1 (MKK1RF4) alone or together with the expression vectors for wild-type ERK1 and ERK2 (wtERK1,2) (2.5 μ g each). Lower panel: NIH-3T3 fibroblasts were transfected with different decorin promoter/CAT 5'-deletion constructs (5 μ g) in combination with the expression vector for constitutively active MEK1 (MKK1RF4) (5 μ g). For all experiments shown in this figure control cultures were co-transfected with 5 μ g of the respective empty expression vectors

RadMKK3bE or RadMKK6bE up-regulated biglycan mRNA levels by 33% and 22%, respectively, compared to RadLacZ infected cells (middle panel of Figure 2). Transduction of fibroblasts with control virus RadLacZ also down-regulated decorin mRNA levels (by 36%) and up-regulated biglycan mRNA expression (by 39%), as compared to uninfected cells (middle panel of Figure 2). The effect of RadLacZ on decorin expression may be due to rapid and transient activation of the ERK1,2 pathway by adenoviral infection [32]. Transduction of human gingival fibroblasts with RadMEK1ca also suppressed decorin mRNA abundance (by 77%), in comparison to RadLacZ infected cells (results not shown).

Assay of the levels of immunoreactive decorin in the conditioned media of human dermal fibroblasts showed that infection of these cells with RadMEK1ca also resulted in 67% reduction in decorin production, as compared to RadLacZ infected cells (right panel of Figure 2). Infection of fibroblasts with RadLacZ also reduced decorin production by 40% (right panel of Figure 2), again possibly due to transient activation of ERK1,2 by adenovirus infection [32]. Together these results clearly show that activation of ERK1,2 by constitutively active MEK1 results in potent suppression in decorin gene expression by primary fibroblasts.

Activation of ERK1,2 results in down-regulation of decorin promoter activity in fibroblasts

To examine whether activation of ERK1,2 resulted in the suppression of decorin promoter activity we transiently transfected NIH-3T3 fibroblasts with the human decorin promoter/CAT construct, pDEC-879/CAT, in combination with the expression vector for constitutively active Raf-1. As shown in the upper panel of Figure 3 co-expression of constitutively active Raf-1 with its substrate, wild-type MEK1, resulted in potent (67%) inhibition of decorin promoter activity, as compared to cells co-transfected with the empty expression vectors. Co-transfection of pDEC-879/CAT with wild-type MEK1 alone also inhibited decorin promoter activity by 41%, possibly due to auto-activation of the wild-type MEK1 (upper panel of Figure 3). Similarly, co-transfection of pDEC-879/CAT with the expression vector for constitutively active MEK1 suppressed decorin promoter activity by 53% (middle panel of Figure 3). In addition, simultaneous expression of constitutively active MEK1 with its substrates, wild-type ERK1 and ERK2 resulted in even more potent (71%) suppression of the activity of pDEC-879/CAT (middle panel of Figure 3). These transfections show that activation of the ERK1,2 signalling cascade at the level of Raf-1 or MEK1 results in potent inhibition of decorin promoter activity in fibroblasts.

Next, we co-transfected NIH-3T3 fibroblasts with different human decorin promoter/CAT 5'-deletion constructs spanning the region from -879 to -140 in combination with the expression vector for constitutively active MEK1. Elimination of the human decorin promoter region between -278 and -188 resulted in loss of down-regulation of promoter activity by

(pCMV5, pKRSPA or CEP4L). After the glycerol shock the cultures were maintained for 48 h in DMEM containing 1% FBS, the cells were harvested, and CAT activity was measured as an indicator of decorin promoter activity. Transfection efficiency was monitored by co-transfecting the cells with 2 μ g of RSV/ β -galactosidase construct. The values represent the means of two or three independent transfections each performed in duplicate and are shown relative to the activity of pDEC-879/CAT co-transfected with the empty expression vectors (100). Values are means $\pm$ S.E.M. (where S.E.M. is less than 16% for each transfection; except 35% for pDEC-140/CAT).

constitutively active MEK1 (lower panel of Figure 3). These observations show that down-regulation of decorin expression as a result of ERK1,2 activation is mediated by *cis*-elements distinct from those mediating the inhibition of decorin gene transcription by TNF- α , which involves *cis*-elements in the region -188 to -140 and in the 5'-untranslated region of exon Ib [8].

DISCUSSION

It has been shown that decorin expression is suppressed in proliferating and malignant transformed cells [8,12–14] and that decorin itself can activate EGFR signalling, leading to activation of ERK1,2 [9–11,13]. The aim of the present study was to elucidate the role of MAPK signalling pathways in mediating the inhibition of decorin expression in proliferating fibroblasts. We demonstrate that decorin expression in primary human fibroblasts is inhibited by a mitogenic growth factor, EGF, and by tumour promoter, PMA, via the ERK1,2 pathway. In addition, our results show that activation of the ERK1,2 signalling cascade results in suppression of decorin production by human skin and gingival fibroblasts, and that the inhibition takes place at the transcriptional level.

It has been shown previously that the expression of constitutively active MEK1 results in transformation of fibroblasts [28]. Using adenovirus-mediated gene delivery, we show in the present study that the expression of constitutively active MEK1 also results in inhibition of decorin expression in normal human fibroblasts. Our recent observations show that adenovirus-mediated expression of constitutively active MEK1 in gingival fibroblasts results in the activation of c-Jun N-terminal kinase (JNK)1, as detected by Western-blot analysis using a phospho-JNK specific antibody [18]. It is therefore possible that in the experiments with constitutively active MEK1 the inhibition of decorin expression also involves activation of JNK1, which may play a role in transformation of fibroblasts by constitutively active MEK1 together with ERK1,2.

As decorin can serve as a ligand for the EGFR and activate its downstream signalling pathways, including the ERK1,2 cascade [10,11], the results of the present study suggest that decorin inhibits its own expression in fibroblasts via the ERK1,2 pathway. This suggests that the expression of decorin in normal cells is under strict control by contact with the surrounding ECM. Furthermore, these observations showing that activation of the ERK1,2 cascade in fibroblasts by mitogenic growth factors, tumour promoters or transformation potentially inhibits decorin expression, provide an explanation why decorin does not belong to the cluster of genes expressed by proliferating cells. Interestingly, it was shown recently that decorin can also inhibit cell migration, an important aspect of cell invasion [33]. It is therefore probable that inhibition of decorin production, as a result of ERK1,2 activation, promotes cell proliferation and favours cell migration.

Although the inhibition of decorin expression, obtained by activation of ERK1,2, was consistent from one experiment to another, it should be noted that the maximal suppression of decorin gene expression was approximately 70%. This is in accordance with the level of suppression obtained with other inhibitors of decorin expression [6–8], and provides further evidence that decorin gene expression in normal fibroblasts is under strict control. It was also interesting to note that activation of the ERK1,2 signalling cascade suppressed expression of biglycan by dermal fibroblasts. However, biglycan does not induce phosphorylation of the EGFR [10]. In addition, the 5'-regulatory regions of human decorin and biglycan genes are quite different [27,34,35], reflecting the differential expression

patterns and regulation of these two proteoglycans [1,2,4–8]. In conclusion, these observations identify a novel mechanism for suppression of decorin expression in proliferating and transformed cells and offer a new therapeutic approach for modulating decorin expression in fibroblasts.

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