

# Response of Hepatic Stellate Cells to TGF $\beta$ 1 Differs from the Response of Myofibroblasts. Decorin Protects against the Action of Growth Factor

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**Abstract** Regardless to the exact nature of damage, hepatic stellate cells (HSCs) and other non-parenchymal liver cells transform to activated myofibroblasts, synthesizing the accumulating extracellular matrix (ECM) proteins, and transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) plays a crucial role in this process. Later it was discovered that decorin, member of the small leucine rich proteoglycan family is able to inhibit this action of TGF- $\beta$ 1. The aim of our present study was to clarify whether HSCs and activated myofibroblasts of portal region exert identical or different response to TGF- $\beta$ 1 exposure, and the inhibitory action of decorin against the growth factor is a generalized phenomenon on myofibroblast of different origin? To this end we measured mRNA expression and production of major collagen components (collagen type I, III and IV) of the liver after stimulation and co-stimulation with TGF- $\beta$ 1 and decorin in primary cell cultures of HSCs and myofibroblasts (MFs). Production of matrix proteins, decorin and members of the TGF- $\beta$ 1 signaling pathways were assessed on Western blots. Messenger RNA expression of collagens and *TIEG* was quantified by real-time RT-PCR. HSCs and MFs responded

differently to TGF- $\beta$ 1 exposure. In contrast to HSCs in which TGF- $\beta$ 1 stimulated the synthesis of collagen type I, type III, and type IV, only the increase of collagen type IV was detected in portal MFs. However, in a combined treatment, decorin seemed to interfere with TGF- $\beta$ 1 and its stimulatory effect was abolished. The different mode of TGF- $\beta$ 1 action is mirrored by the different activation of signaling pathways in activated HSCs and portal fibroblasts. In HSCs the activation of pSMAD2 whereas in myofibroblasts the activation of MAPK pathway was detected. The inhibitory effect of decorin was neither related to the Smad-dependent nor to the Smad-independent signaling pathways.

**Keywords** Hepatic fibrosis · HSC · Myofibroblast · Decorin · TGF- $\beta$  signaling

## Introduction

Hepatic stellate cells (HSCs, Ito cells, lipocytes) store the majority of the vitamin A content of the human body in intracytoplasmic lipid vacuoles [1]. In addition to the role of Ito cells in vitamin A metabolism, synthetic activity of the HSCs have been identified as a principal source of extracellular matrix (ECM) in the liver [2–4]. In liver injury, HSCs transform from quiescent to activated form. In this process, HSCs lose their lipid droplets; simultaneously, contractile elements such as smooth muscle actin ( $\alpha$ -SMA) appear in their cytoplasm, and the cells acquire a “myofibroblast-like” phenotype (MFs). Excessive deposition of ECM proteins leads to fibrosis, which may eventually progress into cirrhosis of the liver [5, 6]. Liver fibrosis is associated with major alterations in both the quantity and composition of ECM. Comparing to the normal liver in

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advanced stages, the cirrhotic liver contains approximately six times more ECM, as a result of both increased synthesis and decreased degradation [7]. TGF- $\beta$ 1 has not only been proven to have an outstanding role in stimulating fibrogenesis but surprisingly was also found to significantly inhibit the CD95 agonistic antibody-induced apoptosis of HSCs [8–12].

However, activation confers MFs phenotype not only for HSCs, but for portal fibroblasts and vascular MFs, as well [13]. Recently it was demonstrated, that bone-marrow stem cells are also capable to differentiate to liver myofibroblast [14–16].

Decorin was described to act as a natural regulator of TGF- $\beta$ , because one of its best-known effect is the neutralization of the growth factor's biological activity [17]. This discovery was supported by the dramatic effect of decorin in preventing the deposition of ECM in injured glomeruli in an experimental glomerulonephritis model [18]. Previously we described that in human liver the deposition of decorin increased in parallel with that of TGF- $\beta$ 1 and with inflammatory activity in chronic hepatitis [19]. In patients with chronic hepatitis C, the IFN- $\alpha$ 2b treatment resulted in a decrease of decorin expression in all acinar areas and there was a significant correlation between decorin expression and the necroinflammatory activity score of chronic hepatitis C before IFN- $\alpha$ 2b treatment [20]. However, detailed analysis of fibrotic and cirrhotic human specimens did not provide any clue about the regulatory effect of decorin on TGF- $\beta$ 1 action in the liver. We could not decide if the increased deposition of decorin occurs simply as part of the matrix deposition or it indicates the attempt of decorin to up/or down regulate TGF- $\beta$ 1 as both effect has already been described [21, 22]. Activation of HSCs in primary culture was inhibited by anti-TGF- $\beta$ 1 treatments, as well as the by soluble TGF- $\beta$  type II receptor [23]. We hypothesized, that decorin might also antagonize the ECM production of these cells.

Therefore a set of pilot experiments were performed and in 2005 we first presented that decorin as natural inhibitory agent of fibrogenesis was able to antagonize the effect of TGF- $\beta$ 1 on rat HSCs. Later this observation was confirmed by others on an immortalized human HSC cell line [24]. To prove the protective effect of decorin against liver cirrhosis we induced cirrhosis in decorin $^{-/-}$  mice. The result of this experiment provided further support for the TGF- $\beta$ 1 inhibitory action of the proteoglycan [25, 26] However, there remained the question if all populations of nonparenchymal liver cells with fibrogenic potential response in a uniform manner to the exposure of TGF- $\beta$ 1 and decorin? To get an explanation for this question we measured the expression and production of a few important ECM proteins after stimulation and co-stimulation with TGF- $\beta$ 1 and decorin not only in primary cell cultures of HSCs but in portal MFs, as well. We also studied the intracellular signaling pathways using Western blot assays in both cell types.

## Materials and Methods

Unless other manufacturer is specified, chemicals were obtained from Merck (Darmstadt, Germany) and Sigma (St.Louis, MO, USA).

### Immunochemicals

Antibodies used are summarized on Table 1.

### Animals

Female Wistar rats were provided by Charles River (Sulzfeld, Germany) and received human care in compliance with the institutional guidelines and the National Institutes of Health guidelines.

### Isolation and Cultivation of Rat HSCs and Liver MFs

HSCs were isolated as described previously according to the method of de Leuw et al. [27]. Purity of freshly isolated HSCs was found to be  $85 \% \pm 3 \%$  [13]. Cells were plated onto 6-well Falcon plates (Beckton Dickinson, Heidelberg, Germany) with  $8 \times 10^5$  cell density, and Lab Tek tissue culture slides (Nunc, Naperville, IL, USA) with a density of 30,000 cells/cm<sup>2</sup>. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 15 % fetal calf serum (FCS), 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin and 1 % L-Glutamine. Culture medium was changed at day 2 after plating and every other day afterwards. Cells were incubated in culture at 37 °C in a 5 % CO<sub>2</sub> atmosphere and 100 % humidity. Rat liver myofibroblasts were obtained as described by Knittel et al. [28].

### Treatment Protocol

Freshly isolated HSCs and myofibroblasts from the 2–6 passages were counted and plated on six well dishes at  $8 \times 10^5$  cell number. At day 7 the cells were washed 3 times with Gey's balanced salt solution and incubated for 24 h in serum-reduced (0.3 % FCS) culture medium alone or in the presence of TGF- $\beta$ 1 and/or decorin (Sigma, St Louis, MO, USA). Previous studies suggested that TGF- $\beta$ 1 treatment resulted in maximal inhibition of apoptosis at 1 ng/mL concentration, and our preliminary dose-response data using different concentrations of decorin (1  $\mu$ g/mL, 5  $\mu$ g/mL, 10  $\mu$ g/mL, 20  $\mu$ g/mL) showed optimal response at 10  $\mu$ g/mL which was comparable with the concentrations used to neutralize TGF- $\beta$  activity on mink lung cells. Therefore, 1 ng/mL and 10  $\mu$ g/mL concentrations of TGF- $\beta$  and decorin were used, respectively [12]. Four parallel wells were used for each regimen. At the end we saved the culture media and collected the cells for RNA and protein analysis. For immunocytochemistry the

**Table 1** List of primary and secondary antibodies together with their sources and the dilutions applied

Primary antibodies	Species	Manufacturer*	Cat. No	Dilution for ICC	Dilution for Western blot/ELISA
Anti-rat decorin	Goat polyclonal	Kind gift of J.R. Woessner			1:500
Anti-collagen, type I	Rabbit polyclonal	Calbiochem	234,167		1:500
Anti-collagen, type III	Rabbit polyclonal	Calbiochem	234,189		1:500
Anti-mouse collagen, type IV	Rabbit polyclonal	Chemicon	AB756P		1:500
Phospho-Smad2	Rabbit polyclonal	Cell Signaling	3101	-	1:500
p44/42 MAP Kinase	Rabbit polyclonal	Cell Signaling	9102	-	1:1000
$\alpha$ -Smooth muscle actin	Rabbit monoclonal	Epitomics	1184-1	1:100	1:4000
Phospho-p44/42 MAP Kinase (Thr202/204)	Rabbit monoclonal	Cell Signaling	4370	-	1:1000
Anti-p38	Rabbit monoclonal	Cell Signaling	9212		1:1000
Anti-P-p38	Rabbit monoclonal	Cell Signaling	4511		1:1000
Anti-Thy-1	mouse monoclonal	Pharmigen	550,402	1:50	
Secondary antibodies	Species	Manufacturer*	Catalog number	Dilution for ICC	Dilution for Western blot/ELISA
Anti-rabbit immunoglobulins/HRP	Goat polyclonal	DakoCytomation	P 0448	-	1:2000
Anti-goat immunoglobulins/HRP	Rabbit polyclonal	DakoCytomation	P 0449	-	1:2000
Alexa Fluor 555 antimouse IgG	Donkey	Invitrogen	A-21,422	1:200	-
Alexa Fluor 488 antirabbit IgG	Donkey	Invitrogen	A-21,206	1:200	-

cells were grown on Lab-Tek (Nunc GmbH & Co.KG Wiesbaden, Germany) plates.

### RNA Isolation and cDNA Synthesis

Twenty-four hours after stimulation and co-stimulation, RNA was isolated using the RNeasy Kit (Qiagen, Hilden, Germany) and the quantity was determined using the Gene Quant (Pharmacia) device. First-strand cDNA was synthesized after DNase digestion with Deoxyribonuclease I - Amplification Grade (Sigma-Aldrich, St. Louis, MO, USA) from 1  $\mu$ g RNA using the SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen, Karlsruhe, Germany) applying Oligo(dT) priming under the conditions recommended by the manufacturer.

### Quantitative Analysis of Gene Expressions

Real-time PCR was performed by ABI Prism 7000 Sequence Detection System (Applied Biosystems, Welterstadt, Germany), using ABI Taqman Gene Expression Assays for rat procollagen I (assay ID: Rn01463848\_m1), procollagen III (assay ID: Rn01437681\_m1), procollagen IV (assay ID: Rn01482927\_m1), decorin (Rn01503161\_m1) according to the manufacturer's protocol. To demonstrate the effect of TGF- $\beta$ 1 treatment, the expression of the TGF- $\beta$ -inducible early growth response gene (TIEG) was also measured (assay ID: Rn00579697\_m1). Rat  $\beta$ -actin (Rn00667869\_m1) or 18S rRNA (4319413E)) was used as endogenous control All

samples were run in duplicates in 20  $\mu$ l with 50 ng of cDNA. Results were obtained as threshold cycle ( $C_T$ ) values. Expression levels were calculated by using the  $2^{-\Delta\Delta C_T}$  method.

### Western Blot Analysis

Cells were lysed in a buffer containing 20 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 10 mM NEM and 0.5 % Triton X-100. The media were concentrated by filtering through Centriplus tubes (Millipore Co., Bedford, MA, USA). Protein concentrations were measured as described before by Bradford [29]. Fifteen microgram of total proteins were mixed with loading buffer containing  $\beta$ -mercaptoethanol and were incubated at 95  $^{\circ}$ C for 5 min. Denatured samples were loaded onto a 10 % polyacrylamide gel and were run for 30 min at 200 V on a Mini Protean vertical electrophoresis equipment (Bio-Rad, Hercules, CA). Proteins were transferred onto a nitrocellulose membrane (Millipore) by blotting for 1.5 h at 100 V. Ponceau staining was applied to determine blotting efficiency. Membranes were blocked with 3 w/v% non-fat dry milk (Bio-Rad) in TBS for 1 h followed by incubation with the primary antibodies at 4  $^{\circ}$ C for 16 h. Membranes were washed 5 times with TBS containing 0.5 v/v% Tween-20, then were incubated with appropriate HRP-conjugated secondary antibodies for 1 h. Immunodetection was performed by peroxidase reaction using diaminobenzidine (DAB) or ECL (Pierce, Rockford, IL, USA). A detailed list of the antibodies used in this study is presented in Table 1.

## Collagen ELISA

Culture media were diluted in carbonate-bicarbonate buffer (50 mM, pH 9.0) to a final protein concentration of 25 µg/ml, and 100 µl-s were loaded into the wells of EIA/RIA plate (96 well, Corning Incorporated, Costar). Four parallels were prepared from each sample. For the effective coating, the plate was incubated overnight at 4 °C. The wells were washed 3 times in PBS-0.05% Tween-20 (PBST). Samples were blocked with 3 % BSA (PBS) for 30 min at 37 °C followed by a washing step. Primary antibodies were diluted in 1:500 by 1 % BSA (PBS), and incubated for 1,5 h at 37 °C. For negative controls 1% BSA was applied. The following antibodies were used: anti-collagen, type I, anti-collagen, type III (both from Calbiochem, San Diego CA USA) anti-collagen type IV (Chemicon, Millipore, Billerica MA USA). After applying the primary antibody, wells were washed 3 times with PBST, and incubated with goat anti-rabbit-HRP secondary antibody (polyclonal goat anti-rabbit immunoglobulins/HRP, Dako Cytomation, Glostrup, Denmark) in 1:2000 dilution for 30 min at room temperature. After washing the wells, tetramethyl benzidine (TMB) substrate (Sigma) was added to the wells to detect the HRP activity. The reaction was stopped by applying 1 N H<sub>2</sub>SO<sub>4</sub> solution. The absorbance was measured at 450 nm with a 620 nm reference filter by EIA Reader (Labsystems Multiskan MS). Data were analyzed in Excel, for statistical analysis 2-sided Student's t-test was chosen.

## Results

### Characterization of Isolated HSCs and MFs

In order to confirm that the isolated cells represent different phenotypes,  $\alpha$ -SMA and Thy-1 double immunostaining was carried out. Therefore all control and treated cells were stained with the two antibodies parallel. Figure 1 shows, that both HSCs and MFs were positive for  $\alpha$ -SMA, but only cells phenotyped as MFs were positive for Thy-1.

### Alterations in mRNA Expression

When HSCs were exposed to TGF- $\beta$ 1 only, we found that the growth factor significantly raised the mRNA levels of COL1 and COL3 (Fig. 2a). Remarkably, although decorin exerted statistically non-significant stimulatory tendency on COL1 and also on COL3 mRNA levels, the up-regulatory effect of TGF- $\beta$ 1 on the mRNA expression of the same genes was abolished by simultaneous treatment with decorin. COL4 mRNA expression of HSCs was low and decorin did not increase its steady state level, but provided protection against TGF- $\beta$ 1 effect. In contrast to HSCs, TGF- $\beta$ 1 increased only

the expressions of COL4 of MFs (Fig. 2b). Decorin administered either alone or in combination with TGF- $\beta$ 1 significantly decreased the mRNA steady state level of COL1 and COL3. Its effect was not significant in case of COL4.

To confirm the efficacy of TGF- $\beta$ 1 on the two cell types we measured the TIEG mRNA expression after stimulation with the growth factor alone and in combination with decorin. As a surprise in contrast to HSCs, TGF- $\beta$ 1 did not affect TIEG expression of MFs neither alone nor in combination with decorin (Fig. 2a, b). Decorin was capable to antagonize the TGF- $\beta$ 1 effect on HSCs (Fig. 2a).

### Protein Production

The amounts of type I and type III collagens as well as decorin were determined from cell lysates (CLs) and cell culture media (CCMs) on Western blots (Fig. 3a, b). The Western blots on Fig. 3a are representatives of three independent experiments. The most remarkable effect of TGF- $\beta$ 1 on HSCs was the increase of type I collagen in the culture media, but an increase in type III collagen production was also detectable. Decorin in combination with TGF- $\beta$ 1 resulted in a decrease of both type I and type III collagens in the media when compared to the effect of TGF- $\beta$ 1 alone (Fig. 3a). In MFs, no response on the protein level was detected after treatment or co-treatment with TGF- $\beta$ 1 or decorin (Fig. 3b). These results were in a good agreement with the findings on mRNA-level.

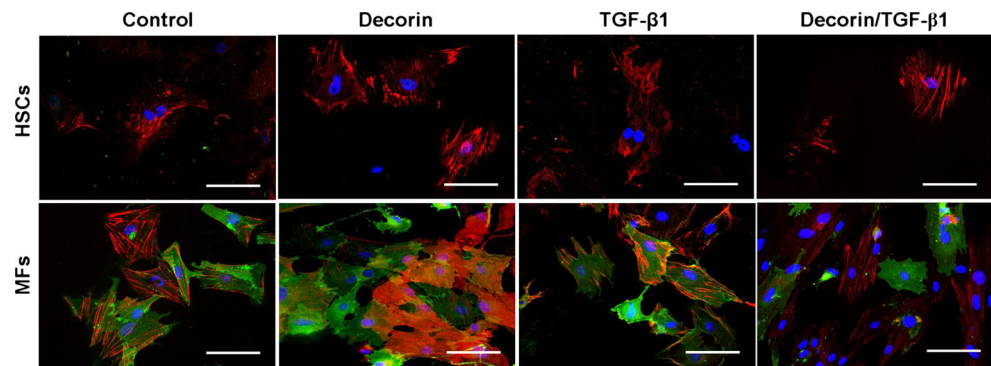
Collagens in media were quantified by ELISA, as well (Fig. 3c, d). These measurements supported the results of Western blots (Fig. 3a, b). They revealed the changes of type IV collagen, as well which was elevated after TGF- $\beta$ 1 treatment in both cell types (Fig. 3c, d).

### TGF- $\beta$ 1 Signal Transduction

To explain the different response of HSCs and MFs to TGF- $\beta$ 1 stimulus, we determined the levels of proteins that can be involved in TGF- $\beta$ 1 signaling (Fig. 4). In HSCs, decorin upregulated the p38, p-p38 MAPK proteins as well as the amount of p-Smad2. The major effect of TGF- $\beta$ 1 was the enhancement of p-Smad2, accompanied by moderate decrease of p-Erk1/2. Co-treatment with decorin and TGF- $\beta$ 1 caused a slight decrease in the amount of p38 and p-p38, compared to the treatment with TGF- $\beta$ 1 alone, but no significant change on the phosphorylation of Smad2 could be detected. In contrast, in MFs the Smad-dependent signaling pathway seemed to be downregulated after TGF- $\beta$ 1 and to a lesser extent after decorin treatment and co-treatment, compared to untreated controls. In the meantime, these cells responded to TGF- $\beta$ 1 with the increased phosphorylation of both p38 and Erk1/2. Co-treatment with decorin partially reversed the phosphorylation of p38 but increased that of the Smad2 (Fig. 4).



**Fig. 1** Double immunocytochemistry of isolated HSCs and MFs. Cells were incubated with monoclonal rabbit anti- $\alpha$ -SMA (red) and monoclonal mouse anti-Thy-1 (green). HSCs do not react with the Thy-1 antibody, whereas a great number of MFs shows double positivity. Scale bars: 100  $\mu$ m

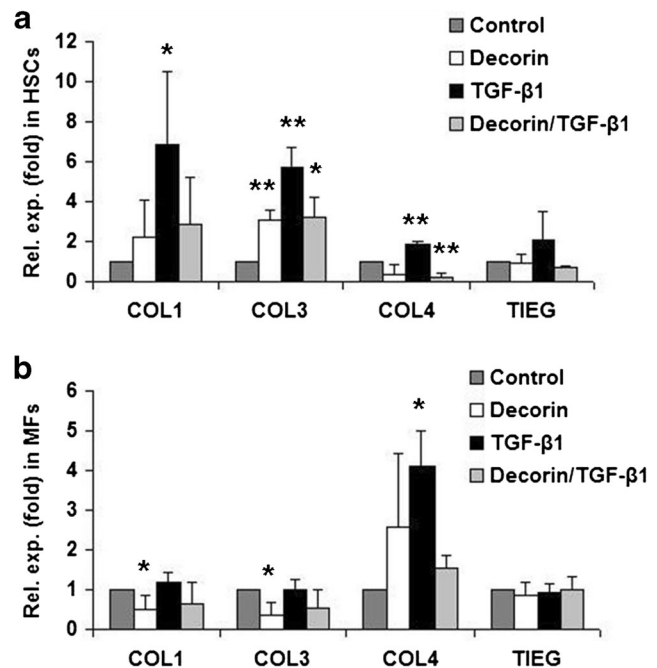


## Discussion

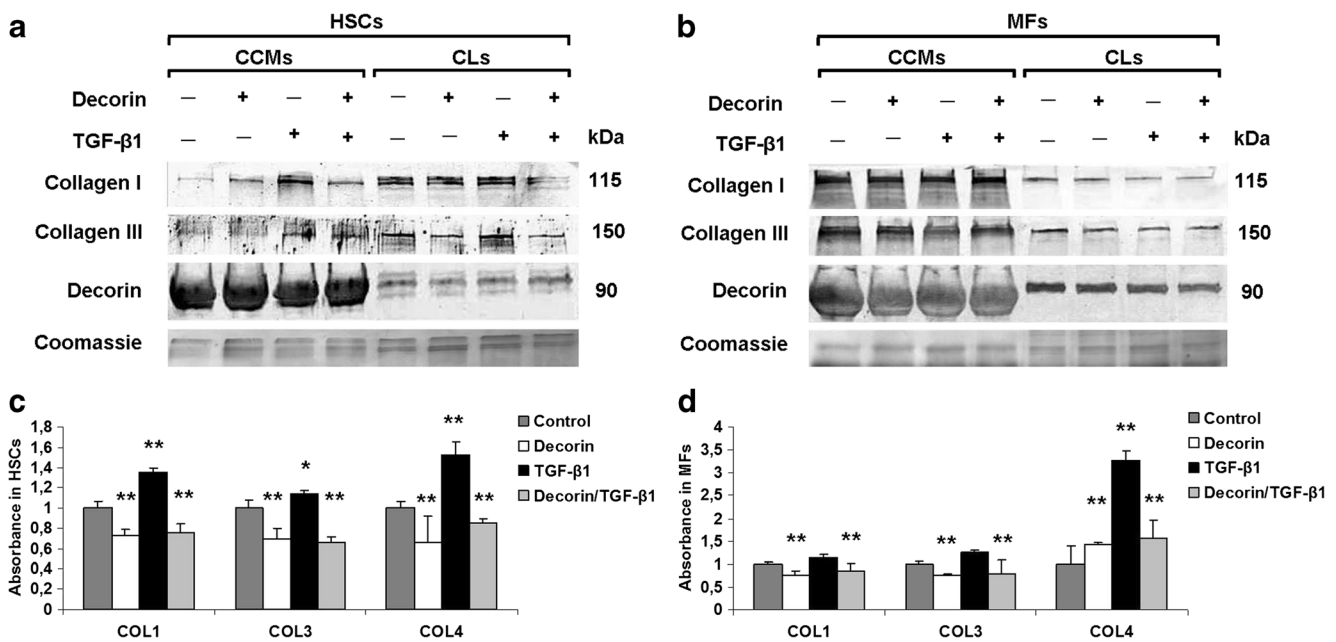
We demonstrated that decorin may not only act as a natural inhibitory agent of fibrogenesis in experimental glomerulonephritis in the kidney, but also plays an active role in the regulation of fibrogenesis in the liver. Previous transgenic models overexpressing TGF- $\beta$ 1 provided evidence for the etiological role of this growth factor in hepatic fibrosis and apoptotic death of hepatocytes [30, 31]. In these experiments, a marked perisinusoidal deposition of ECM was seen by immunohistochemistry due to the activation of HSCs as shown by  $\alpha$ -SMA expression. We proved the protective role of decorin against the development of liver fibrosis in decorin<sup>-/-</sup> mice which confirmed our unpublished pilot studies, showing that decorin was able to prevent the TGF- $\beta$ 1-induced increase of ECM mRNA expression and protein production of HSCs [25, 26]. Another study has also confirmed that the recombinant human core protein of decorin can modulate the proliferation of LX-2 cells upon stimulation of TGF- $\beta$ 1 [24]. However the latter study by Shi et al. is not likely to be considered as comprehensive due to the lack of comparisons between HSCs and MFs. In harmony with the results of others in our hands the  $\alpha$ -SMA-Thy-1 double immunostaining clearly demonstrated the existence of two distinct nonparenchymal cell populations with fibrogenic potential [32, 33]. This fact initiated our question if the pro-fibrogenic effect of TGF- $\beta$ 1 and its inhibition by decorin takes place in a uniform manner in myofibroblast of different origin, or in spite of gaining similar morphology, these cells are set for different response? As to the nature of the nonparenchymal cells with Thy-1 positivity there is a disagreement among authors, nonetheless this does not involve  $\alpha$ -SMA-Thy-1 positive myofibroblast population [32–34].

We provided evidence that the response to TGF- $\beta$ 1 differed from each other in HSCs and MFs. In contrast to the HSCs in which TGF- $\beta$ 1 upregulated mRNA levels and increased synthesis of collagen type I, III and IV, MFs responded to the exposure of growth factor by the sole enhancement of type IV collagen production. Our in vitro stimulation studies on HSC are in accordance with previous data where COL1 mRNA showed the strongest response to a TGF- $\beta$ 1 on-off regimen [35]. Western blot and ELISA proved

that changes on protein level went parallel with the response of mRNA. Signaling in case of HSC utilized Smad pathway whereas alternative MAPK pathways were activated in myofibroblasts. This finding points out the distinct role of these cell types in pathological tissue remodeling. Thus, it is conceivable, that MFs are rather involved in basement membrane synthesis in vivo, whereas activated HSCs may prevail



**Fig. 2** Real-time PCR analysis of COL1, COL3, COL4 and TIEG mRNA expression in HSCs (a) and MFs (b) after treatment and co-treatment with decorin and TGF- $\beta$ 1. Expression values were compared to untreated controls, and normalized to ACTB. The administration of 1 ng/ml growth factor resulted in a significant increase in all types of collagen mRNA level which effect was destroyed with the simultaneous administration of 10  $\mu$ g/ml decorin (\*  $p < 0.001$ ; \*\*  $p < 0.05$ ) in HSCs. Decorin treatment alone induced a moderate, non-significant elevation of the COL1 and a significant increase of COL3 mRNA in HSCs. HSCs respond to TGF- $\beta$ 1 (1 ng/mL) treatment with TIEG upregulation, however, in the co-treatment the two substances interfere and stimulation is abrogated. (a). TGF- $\beta$ 1 did not exert effect on mRNA steady-state levels of COL1 and COL3, but increased the expression of COL4. Decorin alone or in combination caused significant downregulation of the messages of COL1 and COL3 (\*  $p < 0.001$ ) in MFs. MFs are virtually unresponsive to the treatments (b)



**Fig. 3** Representative Western blot showing the effect of TGF- $\beta$ 1 and decorin on collagen type I and III, as well as on decorin production of HSCs (a) and MFs (b). Extracts of cell culture media (CCMs) and cell lysates (CLs) were immunoblotted against antibodies for type I and III collagens and decorin. Consistent changes in protein production were observed in HSCs only. TGF- $\beta$ 1 (1 ng/mL) increased the amount of type I and III collagens in the culture medium of HSCs. Decorin (10  $\mu$ g/mL) in the co-treatment, suppressed the stimulatory effect of TGF- $\beta$ 1. Collagen

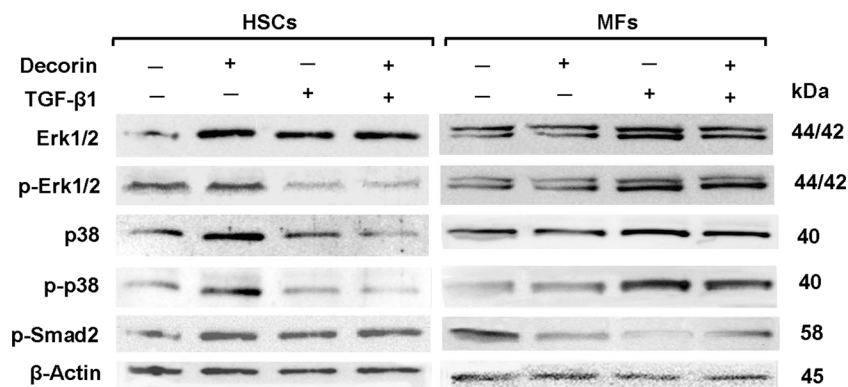
ELISA made from the culture media of the HSCs (c) and MFs (d). TGF- $\beta$ 1 significantly enhanced the amount of collagens and decorin alone or in combination with the growth factor decreased the amount of collagens in HSCs (c). In MFs TGF- $\beta$ 1 affected only the level of type IV collagen, whereas decorin lowered the level of all types of collagens and reverted the increase of type IV collagen induced by TGF- $\beta$ 1 (d). \*  $p < 0.001$ ; \*\*  $p < 0.05$

in the massive deposition of interstitial matrix. The cell type-specific dominance of one or the other signaling pathway should also be taken into account when interpreting the different reaction of HSCs and MFs to TGF- $\beta$ 1.

Although the TGF- $\beta$ 1 inhibitory potential of decorin was unequivocal both on HSC and myofibroblast cells we failed to discover the exact mode of its action, so far. Decorin did not inhibit the TGF- $\beta$ 1 induced upregulation of pSMAD2 of

HSC or the increased phosphorylation of ERK1/2 of myofibroblasts. Only the decrease of p38 phosphorylation was detected in both cell types.

The response of HSCs to decorin showing activation both the Smad-dependent and -independent signaling pathways was unexpected. Thus, it seems decorin, when administered alone, stimulates signaling pathways overlapping with those of TGF- $\beta$ 1. It is conceivable, that beside the ability of the



**Fig 4** Western blots showing the effect of TGF- $\beta$ 1 and decorin on signaling in HSCs and MFs. After TGF- $\beta$ 1 exposure the level of p-Smad2, in HSC, but decreased in MFs. In contrast, the protein expression and phosphorylation of Erk1/2 and p38) increased in MFs, and seemed to be unchanged or inhibited in HSCs. Surprisingly, decorin exerted a stimulatory effect on both the Smad-dependent and -independent pathways in HSCs. In

fact in HSCs decorin does not seem to counteract TGF- $\beta$ 1 via MAPK or Smad pathways as phosphoprotein levels after combined treatment are identical with those after TGF- $\beta$ 1 exposure. In case of MFs modest decrease in the expression of p38 and p-p38 levels and increase of p-Smad2 level can be seen after combined treatment when compared to those in the TGF- $\beta$ 1 treated group

proteoglycan to bind and inactivate TGF- $\beta$ 1 in the extracellular space, its interference could be exerted somehow by the activation tyrosine kinase receptors. It is noteworthy to mention that decorin is proved as ligand for EGF, IGF and Met receptors [36–38], it inhibits the binding of PDGF to its receptor [39] however no data is available on its binding capacity to TGF- $\beta$ 1 receptors.

Here we have demonstrated that decorin as natural inhibitory agent of fibrogenesis was able to suppress ECM production of HSCs, as well as myofibroblasts activated by TGF- $\beta$ 1. Thus, decorin might potentially be considered as a candidate molecule for future antifibrotic therapies. However, the complexity of this issue is well demonstrated by the finding that activated mouse stellate cells have a profound T-cell inhibitory activity in vitro; furthermore, activated stellate cells were able to prolong the survival of insulin-expressing islet grafts, probably by enhancing apoptosis in the infiltrating immunocytes [40, 41]. On the other way around, decorin proved to be the ligand of innate immunity receptor TLR2 and TLR4 capable to initiate pro-inflammatory signaling [42]. Hence, when antifibrotic strategies directed against HSCs activation are planned, other factors such as the immune-regulatory activity of HSCs and decorin should also be taken into account in order to avoid enhancement of immune attack on the liver.

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