Endoglin Expression Regulates Basal and TGF- β 1-induced Extracellular Matrix Synthesis in Cultured L₆E₉ Myoblasts

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Key Words

Endoglin • Extracellular matrix • Connective tissue growth factor • Myoblasts • Transforming growth factor beta

Abstract

Background/aims: TGF- β 1 plays a major role in extracellular matrix (ECM) accumulation in tissue fibrosis. Connective tissue growth factor appears to play a critical role in this effect. Endoglin is a component of the transforming growth factor b (TGF- β) receptor complex. Endoglin is upregulated by TGF- β 1, but its functional role in ECM regulation is unknown. Using rat myoblasts as a model system, we have assessed the role of endoglin on regulating CTGF expression and ECM synthesis and accumulation in the presence or absence of TGF- β 1. **Methods:** L6E9 myoblast cell line was transfected with human endoglin, and collagen, fibronectin and CTGF production was assessed by Western blot and by proline incorporation to collagen proteins. **Results:** Northern blot analysis revealed that parental rat myoblasts L₆E₉ do not express endogenous endoglin. Upon endoglin transfection, endoglin-expressing cells displayed a decreased CTGF expression and

decreased collagen and fibronectin accumulation respect to mock transfectants. Northern blot analysis also revealed a decreased α_2 (I) procollagen mRNA expression in endoglin transfectants. TGF- β 1 treatment induced an increase in CTGF expression and collagen synthesis and accumulation in L6E9 myoblasts. This effect was significantly lower in endoglin-transfected than in mock-transfected cells. **Conclusion:** These results demonstrate that endoglin expression negatively regulates basal and TGF- β 1-induced CTGF and collagen expression and synthesis.

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Introduction

In several pathological conditions, the excessive accumulation of extracellular matrix (ECM) in tissues leads to dysfunction. This matrix accumulation, a key part of the process of sclerosis, may cause thickening of basement membranes, disruption of normal cell-cell interactions, and loss of tissue compliance or elasticity in conditions such as atherosclerosis of the blood vessels

[1], pulmonary fibrosis [2], liver cirrhosis [3], and glomerulosclerosis [4]. Moreover, ECM is an important determinant of cellular behaviour, regulating cell adhesion, migration, proliferation, and differentiation. Among the growth factors, none of them has the diversity of effects on ECM ascribed to transforming growth factor- β (TGF- β) family. TGF- β plays a pivotal role in the extracellular matrix accumulation observed in chronic progressive tissue fibrosis [5, 6]. TGF- β has been found to increase mRNA levels for most of the matrix proteins in which this has been examined and to result in increased secretion of the protein. Indeed, there are unequivocal data implicating TGF- β in the pathogenesis of several chronic inflammatory diseases including glomerulonephritis [6, 7], and atherosclerosis [8, 9]. Each of these diseases is characterized by increased expression of TGF- β protein and excessive accumulation of ECM.

TGF- β not only directly induces ECM protein synthesis, but also induces expression of other profibrotic mediators. One such protein is connective tissue growth factor (CTGF) which induces collagen synthesis in mesenchymal cells [10, 11] and fibronectin synthesis in mesangial cells [12] and seems to mediate TGF- β -induced fibronectin and collagen synthesis in renal fibroblasts [13]. TGF- β 1 induces CTGF expression in mesangial cells [14], and it is overexpressed in numerous fibrotic disorders, being considered a molecular marker of the fibrotic response [15, 16].

TGF- β exerts their function through binding to a large family of specific receptors, including receptors type I (R-I), type II (R-II), betaglycan and endoglin [17, 18]. Among these, the serine-threonine kinase receptors R-I and R-II are necessary for all tested biological responses to TGF- β and transmit the signal to down-stream substrates through their kinase activity. By contrast, endoglin and betaglycan have been postulated as regulators of TGF- β access to the signalling receptors [19]. Endoglin, also known as CD105, is a 180-KD homodimeric membrane glycoprotein expressed by human endothelial cells [20], macrophages [21], vascular smooth muscle cells [22] and mesangial cells [23]. Endoglin binds with high affinity TGF- β 1, TGF- β 3, activin, BMP-2 and BMP-7 and associates with the TGF- β signalling receptors, as evidenced by co-immunoprecipitation experiments [24-27]. In addition, we have demonstrated that endoglin is overexpressed in several experimental models of renal fibrosis [28, 29] and that it is up-regulated by TGF- β 1 in mesangial cells [23]. Several studies have suggested that endoglin is able to inhibit some cellular responses to TGF- β 1 [30, 31].

In this study, we investigated the possible regulation by endoglin of CTGF and ECM synthesis in basal conditions and in the presence of TGF- β 1. For this purpose, we have used the rat myoblast cell line L₆E₉, which, here we demonstrated, does not express endoglin neither in basal conditions nor after incubation with TGF- β 1. Then, the transfection of this cell line with human endoglin allows us to compare cells with and without endoglin expression. In addition, previous studies have demonstrated that TGF- β 1 induces collagen and fibronectin synthesis in this cell line [32, 33]. Elucidating the cellular effects of endoglin on extracellular matrix synthesis is critical to understand the mechanisms underlying the role of endoglin in the development of fibrotic processes.

Materials and Methods

Stable transfectants

The rat myoblast cell line L₆E₉ was cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) containing 10% FBS (Biowhittaker) and 100 U/ml of penicillin/streptomycin at 37°C in a 5% CO₂ atmosphere. Rat myoblasts transfectants expressing human L-endoglin were generated by co-transfecting pcEXV-EndoL vector and psV2neo at a 10:1 ratio. Ten micrograms of plasmid DNA were mixed with 20 μ g Lipofectin (Life Technologies, Inc) in serum-free medium according to the protocol provided by the manufacturer. Positive clones were selected in the presence of 400 μ g/ml of the antibiotic G418 and identified by flow cytometry. Parallel transfections with psV2neo alone yielded endoglin-negative mock transfectants. Pooled clones were used in biochemical and functional characterizations. No significant differences were observed between parental and mock transfectants in biochemical and functional studies as we previously described [30]. Cells were plated at an identical density in 6 x 4 well plates (2 x 10⁴ cells/well), for ECM and TGF- β 1 measurements, and in 100-mm culture dishes (4 x 10⁵ cells/dish) for Northern and Western blot analysis. Twenty-four hours later, cells were serum starved for 24 hours, and after adding fresh serum-free medium, cells were treated with various concentrations of active, human recombinant TGF- β 1 (R & D Systems; dilutions made from a 1 μ g/ml stock solution in 4mM HCl containing 1 mg/ml BSA) or control vehicle during 24 hours.

Retroviral infection

For retrovirus production, full length L-Endoglin was subcloned into the EcoRI site of the pLZR-IRES-GFP vector. The DNA construct was transformed and propagated in DH5 α *Escherichia coli* and isolated with plasmid isolation columns from Promega (Madison, WI, USA). For transient generation of retroviruses, 293T cells were plated in 60 mm-diameter dishes (1.0 x 10⁶ cells in 3 ml of DMEM with 10% FBS) and allowed to attach overnight as previously described [34]. In brief, five

minutes prior to transfection, 25 mM chloroquine was added to each plate. The transfection solution contained DNA (2.5 µg of pMD-G, 5µg of pNGVL-MLV-gag-pol, 3 µg of retroviral vector [pLZR-Sense-L-endo-IRES-GFP, or pLZR-Antisense-L-endo-IRES-GFP for the control transfection]), 61µl of 2 M CaCl₂, and double-distilled H₂O to 500 µl. After mixing, 0.5 ml of 2X HBS (pH 7.0) was added and the solution was bubbled for 15 s. The HBS-DNA complex was then dropped onto cells. Eight hours later, the medium was replaced with fresh complete culture medium that, 24 to 32 h post-transfection, was again replaced with 3 ml of fresh virus-collecting medium. Twenty-four hours after the medium change, the supernatant from transfected cells was collected and filtered with a 0.45 µm filter (Millipore). Twenty-four hours before infection, L₆E₉ rat myoblast cells were plated at 120,000 cells in 60 mm-diameter dishes and infected with viral supernatants containing Polybrene 6 µg/ml. Twenty four hours later, infected cells were incubated in DMEM with 10% FBS.

Western blot analysis

Protein extraction for Western blot analysis was done according to previously described methods [23, 33]. Equal amount of proteins or culture medium were loaded on an 8 or 12% SDS-polyacrylamide gel and transferred to PVDF membrane (Pall-Gelman). Membranes were blocked with blocking buffer and incubated with the mouse anti-human endoglin mAb P3D1 [35], rabbit anti-rat Collagen I antibody (Chemicon) or goat anti-human CTGF antibody (Santa Cruz). Membranes were washed, incubated with the secondary antibody conjugated with horseradish peroxidase (goat anti-mouse or goat anti-rabbit IgG; Bio-Rad Laboratories) for 30 minutes and washed extensively. Blots were developed by chemiluminescence using the ECL Western blotting system (Amersham-Pharmacia-Biotech).

ECM production

The collagen content in the medium was quantified by measuring the incorporation of [³H]-Proline into collagen proteins, as previously described [23, 31]. L-[³H]-proline (2 µCi/well) was added at the same time as TGF-β1 addition. Fibronectin accumulated in the culture medium was quantified by enzyme-linked immunoabsorbent assay (ELISA), using a procedure previously described [36]. Concentrations were determined from a standard curve, generated in each experiment using fibronectin (Gibco BRL). Protein content of cell extracts was determined by the Lowry assay (Bio-Rad).

RNA isolation and Northern blot analysis

Total RNA was isolated from myoblasts with the guanidinium thiocyanate-phenol-chloroform method, size-fractionated by electrophoresis (20 µg/lane) through denaturing 1% agarose-1.1% formaldehyde gels, transferred to Hybond membranes (Amersham), and UV cross-linked before hybridization. Hybridization was performed at 60°C for 12-16 h, and membranes were then washed at final stringency conditions of 60°C, 2 X SSC, 1% SDS, two times for 30 min each, and at 20°C, 0.2 X SSC, 0.1% SDS for 1 hour, and exposed on XAR Kodak film, using a intensifying screen. The probe used for rat

endoglin was a 360-bp SacI/SacII fragment of rat endoglin cDNA in pGEM-T kindly given by Dr. Calvin PH Vary (The Center for Molecular Medicine, Maine Medical Center Research Institute, South Portland, Maine, USA). The probe used for human endoglin was derived from a 2.3-kb Eco RI fragment corresponding to the full length coding sequence of human endoglin contained within a 6.62-kb pCMV5 plasmid vector. Probe for rat α₂(I) collagen (0.6-kb fragment) was obtained by digestion of constructs inserted in plasmid PUC18, and were kindly provided by Dr. Diego Rodríguez-Puyol (University of Alcalá de Henares, Madrid, Spain). The probe of mouse TGF-β1, that hybridizes with rat TGF-β1 mRNA, is a fragment of 451 bp obtained as a PCR product kindly gifted by Dr Jean Pierre Girolami and Jean Loup Bascand (U388 INSERM, Toulouse, France). 18S ribosomal subunit probe (1.5-kb) was used as internal loading control. The autoradiograms were analyzed by optical scanning densitometry and values expressed as percentage of control with normalization of the results according to the corresponding transcripts levels for the ribosomal 18S gen.

Transforming growth factor-β1 assay

Total TGF-β1 protein was measured in the culture supernatants from myoblasts stimulated for 1h with TGF-β1. After washes, culture medium was replaced by serum-free medium without TGF-β1. Twenty-four hours later, supernatants were analysed by a sandwich ELISA kit according to the manufacturer's instructions (Quantikine™, R&D Systems). We measured the level of TGF-β1 at time zero of stimulation, which is after the washes, to ensure that is equal to baseline. Samples were first activated with 1N HCl followed by neutralization with 1.2N NaOH/0.5M HEPES.

Statistical analysis

Statistical differences between experimental groups were determined by analysis of variance using NCSS 2000 software for PC. Values of *p* < 0.05 were considered significant.

Results

Rat myoblasts L₆E₉ as a model system to study the role of endoglin on ECM synthesis

Endoglin has been demonstrated to be expressed on several tissues and some cell lines in culture, but there are not published data about the endoglin levels in the rat myoblast cell line L₆E₉. Northern blot analysis revealed that the rat myoblasts L₆E₉ do not express endogenous endoglin (Fig. 1A). As a positive control, Northern blot analysis using the same probe identified endoglin transcripts in rat tissues (Fig. 1A). Figure 1A shows human-endoglin mRNA expression in endoglin-transfected myoblasts. Furthermore, Western blot analysis revealed a band corresponding to the dimeric form of endoglin, specifically recognized by anti-endoglin antibody P3D1.

Fig. 1. Endoglin expression in rat myoblasts L_6E_9 and stable endoglin transfectants. (A) Endoglin mRNA expression in mock and endoglin-transfected myoblast. Cells were harvested, RNA extracted, and rat and human mRNA endoglin expression measured by Northern blot analysis. The 18S ribosomal RNA is shown to indicate RNA loading. RNA from rat tissue (kidney and lungs) was used as a positive control for rat-endoglin mRNA. (B) Extracts from mock and endoglin-transfected myoblast were electrophoresed and transferred to a PVDF membrane. The presence of human endoglin was revealed with the anti-endoglin mAb P3D1 using a chemiluminescence assay.

Fig. 2. Effects of endoglin expression on collagen and fibronectin accumulation in L_6E_9 myoblast. Extracellular matrix proteins were measured in mock and endoglin transfected myoblast serum deprived for 48 hours. (A) Total collagen accumulation in the culture medium was measured by metabolic radiolabeling with [3 H]-proline during 24 hours. (B) Fibronectin formation was evaluated by ELISA according to its medium concentration. Each value represents the mean $\pm$ SEM of six samples. * $P < 0.05$ versus mock.

No specific reactivity could be detected in mock-transfected cells (Fig. 1 B).

Endoglin expression in L_6E_9 myoblasts interferes with ECM synthesis and accumulation

Given the lack of endogenous endoglin expression in the myoblast cell line L_6E_9 , it was of interest to study the effect of endoglin expression on ECM synthesis and accumulation. Endoglin-transfected myoblasts showed a significantly lower collagen and fibronectin accumulation respect to mock-transfectants after 48 hours of incubation in serum-free medium (Fig. 2).

We also analyzed the effects of TGF- β 1 on collagen and fibronectin accumulation in mock and endoglin transfected myoblasts. Incubation with exogenous TGF- β 1 induced a dose-dependent increase on collagen accumulation in mock-transfected myoblasts, with a marked increase observed at 10 ng/ml (about 2.5-fold respect control), whereas the overall response was much lower in endoglin-transfected cells (about 1.5-fold respect

control) (Fig. 3A). Collagen I and collagen III amount were analyzed by Western blot in the cell layer as well as in the culture media. Figure 3B shows a significantly lower basal collagen I expression in endoglin-transfected compared with mock-transfected myoblast. In the culture media TGF- β 1-induced collagen I increase was significant in mock-transfected but not in endoglin-transfected myoblasts. In the cell layer, the relative increase induced by TGF- β 1 is 3.5 times in mock-transfected cells and only 1.8 times in endoglin-transfected myoblasts. Similar numbers can be obtained with collagen I protein production. In addition, total amount of collagen I and total collagen production in endoglin-transfected myoblasts after incubation with TGF- β 1 was about a half of that observed in mock-transfected cells. No significant differences were found between mock and endoglin transfectants in basal and TGF- β 1 induced collagen III expression (data not shown).

We also analysed the collagen I mRNA expression in L_6E_9 myoblast. In absence of TGF- β 1, α_2 (I) collagen

Fig. 3. Effect of TGF- β 1 on collagen synthesis and accumulation. Quiescent mock and endoglin-transfected myoblast were incubated with or without increasing concentrations of TGF- β 1 (0.1-10 ng/ml) for 24 hours. (A) Total collagen accumulation in culture media was measured by metabolic radiolabeling with [3 H]-proline. * P <0.05 versus control; + P <0.05 versus mock+TGF- β 1 (10 ng/ml). (B) Western blot for type I collagen in the culture media and cell layer. A representative blot is shown on top panel. Results of densitometric analysis are shown at bottom. Results are expressed as percentage over mock control. Each value represents the mean $\pm$ SEM of 3 experiments. * P <0.05 versus mock control.

mRNA expression was lower in endoglin than in mock-transfectants (Fig. 4). α_2 (I) Collagen mRNA expression increased after treatment with the TGF- β 1 (10 ng/ml) in both endoglin and mock-transfected myoblasts (Fig. 4). In agreement with the Western blot and collagen accumulation experiments, α_2 (I) collagen mRNA increase was significantly higher in mock than in endoglin-transfected myoblasts: the relative increase induced by

TGF- β 1 is 3.5 times in mock-transfected cells and only 1.8 times in endoglin-transfected myoblasts. (Fig. 4).

Fibronectin accumulation in response to TGF- β 1 was found to be rather small (data not shown). Nevertheless, the fibronectin accumulation in the presence or absence of TGF- β 1 was 50% lower in endoglin than in mock-transfected cells.

Fig. 4. Effect of TGF- β 1 on collagen I mRNA expression in myoblasts transfectants. Mock and endoglin transfectants were incubated with or without TGF- β 1 (10 ng/ml) for 24 hours and the levels of specific transcripts of $\alpha_2(I)$ collagen mRNA detected by Northern blot analysis. RNA blots were stained with ethidium bromide to visualize the integrity of the 18S ribosomal subunit. The levels of collagen mRNA in mock and endoglin transfected myoblasts were analyzed by densitometric analysis using the imageQuant software. Results are expressed as percentage over mock control. Each value represents the mean $\pm$ SEM of 3 experiments. *P<0.05 versus control. +P<0.05 versus mock+ TGF- β 1.

Fig. 5. Effect of transient endoglin expression on fibronectin and collagen I expression in L_6E_9 rat myoblast. Cells were infected with pLZR-Sense-L-endo-IRES-GFP (sense-Eng+) or pLZR-Antisense-L-endo-IRES-GFP (α -sense-Eng+) and analyzed for endoglin, collagen and fibronectin expression by Western blotting. A representative blot is shown on top panel. Results of densitometric analysis are shown at bottom. Results are expressed as percentage over α -sense Eng+. Each value represents the mean $\pm$ SEM of 3 experiments. *P<0.05 versus α -sense-Eng+ control.

To assess that the differences in ECM expression between mock and endoglin stable transfectants were not due to clone selection of the stable transfected cell lines, we determined ECM in myoblasts transiently infected with endoglin using retrovirus methodology. For this purpose, L_6E_9 rat myoblasts were infected with pLZR-Sense-L-endo-IRES-GFP (sense-Eng+) and pLZR-

Antisense-L-endo-IRES-GFP (α -sense-Eng+) as described above. Analysis of endoglin expression by Western blotting with the anti-human endoglin antibody P3D1 reveals that cells infected with pLZR-Sense-L-endo-IRES-GFP show notable amounts of endoglin, whereas those infected with the pLZR-Antisense-L-endo-IRES-GFP, as expected, did not show endoglin

Fig. 6. Effect of TGF- β 1 on CTGF expression in myoblasts transfectants. Quiescent mock and endoglin transfectants were incubated with or without TGF- β 1 (10 ng/ml) for 24 hours and the levels of CTGF were assessed by Western blot analysis. A representative blot of CTGF expression in basal conditions and after TGF- β 1 stimulation is shown on top. Densitometric analysis is shown at the bottom. The results shown are the mean $\pm$ SEM of 3 blots performed in the same conditions. * p <0.05 vs Mock basal; + p <0.05 vs Endoglin basal; # p <0.05 vs Mock+ TGF- β 1.

expression (Fig. 5). Collagen I and fibronectin protein expression, assessed by western blot, were lower in endoglin expressed myoblast (Fig. 5).

Endoglin modifies CTGF expression in L_6E_9 myoblasts

As CTGF seems to be a key mediator of the pro-fibrotic effects of TGF- β 1, we assessed whether endoglin expression could modify CTGF expression. We observed that in basal conditions endoglin-transfected myoblasts had a lower CTGF expression than the mock-transfected ones. Incubation with TGF- β 1 induced a similar percent increase in CTGF expression in both types of myoblasts (Fig.6). However, the amount of CTGF detected in endoglin-transfected myoblasts after incubation with TGF- β 1 was less than 60 % of that observed in mock-transfected cells.

Fig. 7. Effect of exogenous TGF- β 1 on TGF- β 1 mRNA expression and total TGF- β 1 production in myoblasts transfectants. (A) Mock and endoglin transfectants were incubated with or without increasing concentrations of TGF- β 1 (0.1-10 ng/ml) for 24 hours and the levels of specific transcripts of TGF- β 1 mRNA detected by Northern blot analysis. Results are expressed as percentage over mock control. Each value represents the mean $\pm$ SEM of 3 experiments. (B) Endogenous TGF- β 1 was measured by ELISA in supernatants from mock and endoglin transfected myoblast in basal conditions and after incubation with TGF- β 1 (500 pM) as described in materials and methods. Each value represents the mean $\pm$ SEM of four samples. * p <0.05 versus control. + p <0.05 versus Mock+ TGF- β 1.

Endoglin expression interferes with TGF- β 1 synthesis and release in L₆E₉ myoblasts

As TGF- β 1 induces its own expression, our next aim was to test the effect of endoglin expression on endogenous TGF- β 1 synthesis and release. Incubations with TGF- β 1 induce a dose-dependent increase in TGF- β 1 mRNA. TGF- β 1 mRNA expression after incubation with TGF- β 1 was about 70% in endoglin-transfected compared with mock-transfected cells. (Fig. 7A). We also measured total TGF- β 1 in the culture media of mock and endoglin myoblast in basal conditions and upon TGF- β 1 treatment. In basal conditions we did not find significant differences in TGF- β 1 concentration in culture media in mock and endoglin transfected myoblast. The stimulation with TGF- β 1 for 24 h increased the amount of TGF- β 1 released by myoblasts. TGF- β 1-induced increase in TGF- β 1 release was of about 4.5 times over basal in mock-transfected cells and only 2.5 times in endoglin-transfected myoblasts (Fig. 7B).

Discussion

The purpose of the present study has been to assess the role of endoglin on basal and TGF- β 1-induced ECM synthesis and CTGF production. For this purpose we have used the rat myoblast cell line L₆E₉, and their stable transfectants expressing human endoglin. Previous studies have shown that TGF- β 1 induces collagen and fibronectin synthesis in this cell line [32, 33]. In the present study we demonstrate that the parental line of these cells and mock-transfected L₆E₉ myoblasts do not express endoglin either in basal conditions or after treatment with TGF- β 1. This fact allow us to compare the response of the same cell line with (endoglin-transfected) or without (mock-transfected) endoglin. Our results demonstrate that the presence of endoglin inhibits both basal and TGF- β 1-induced collagen accumulation, and α_2 (I) collagen mRNA expression.

To confirm that the differences in ECM expression between mock and endoglin stable transfectants were not caused by clone selection of the transfected cell lines, we induced transient endoglin expression in myoblasts by retroviral infection. Analysis of endoglin expression by Western blotting revealed that cells transfected with endoglin shows notable amounts of endoglin, whereas those infected with the antisense plasmid did not show endoglin expression. Collagen and fibronectin expression, assessed by western blot, were lower in endoglin-

transfected cells than in cells transfected with the antisense plasmid.

These findings are in agreement with some previous studies demonstrating that the presence of endoglin can inhibit some cellular responses to TGF- β 1. For instance, it has been already shown that endoglin transfection in L₆E₉ myoblasts decreased the synthesis of the plasminogen activator inhibitor 1 (PAI-1), as endoglin transfectants show decreased PAI-1 protein levels and PAI-1 gene promoter activity [30]. In the monocyte cell line U-937, endoglin transfection also decreased the TGF- β 1-induced fibronectin synthesis [31]. In addition, in the mouse fibroblast cell line L929 transfected with endoglin, collagen and fibronectin production as well as Collagen I mRNA expression were significantly lower than mock-transfected cells either in absence or presence of TGF- β 1 [37, 38]. Taken together, these results suggest that endoglin expression decreases the basal synthesis and accumulation of extracellular matrix components such as collagen I and fibronectin, and impairs the TGF- β 1-induced stimulation of collagen synthesis and accumulation.

CTGF expression seems to be a mediator of TGF- β 1-induced fibrosis. Thus, TGF- β 1 induces CTGF synthesis in several cell types such as mesangial cells or normal fibroblasts [14, 16]. In addition, CTGF expression is increased in several fibrotic disorders, and CTGF levels correlate with the progression and severity of the fibrosis [15, 16, 39]. We have assessed whether CTGF expression is modified by the presence of endoglin. Our results demonstrate that the presence of endoglin decreases CTGF expression both in basal conditions and after incubation with TGF- β 1.

The effect of endoglin expression on collagen and CTGF synthesis in basal conditions could be probably explained because endoglin antagonizes the effect of TGF- β 1 present in the culture medium, as we have observed that myoblasts synthesize and release TGF- β 1 that can be recovered in the conditioned medium. Then, we evaluated whether endoglin was able to regulate the induction by TGF- β 1 of its own synthesis. The present study and previous ones [31] demonstrate that TGF- β 1 is able to induce its own synthesis. Although in endoglin-transfected cells, TGF- β 1-induced TGF- β 1 release is slightly reduced, we have not enough evidence to suggest that endoglin overexpression could also play a role modulating not only TGF- β 1 effects but also TGF- β 1 production.

The inhibition of the TGF- β 1 responses in endoglin-transfected cells is compatible with the requirement of

the R-II for the TGF- β 1 binding to endoglin [27, 30]. The receptor II-induced ligand binding to endoglin might cause a deflection of the ligand to the signalling core, which could lead to a sequestering of the ligand, associated with a loss of cellular responses to TGF- β 1 [30]. Alternatively, endoglin could be actively involved in the modulation of the downstream signalling of TGF- β 1, although the exact mechanism of endoglin action remains to be determined.

In conclusion, our results demonstrate that endoglin expression impairs the effects of TGF- β 1 on CTGF expression and ECM synthesis in rat myoblasts. Thus, it is tempting to speculate that the increased expression of endoglin in several cell types in the presence of TGF- β 1 contributes to regulate the effects of TGF- β 1 on increased ECM production.

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