Pro-Fibrotic Phenotype of Human Skin Fibroblasts Induced by Periostin via Modulating TGF- β Signaling

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ABSTRACT

Background Periostin is a matricellular protein, one of a class of extracellular matrix (ECM)-related molecules defined by their ability to modulate cell–matrix interaction. We previously reported an elevated level of circulating periostin in patients with systemic sclerosis (SSc) and its association with severity of skin sclerosis.

Objective We examined the role of periostin in transforming growth factor- β (TGF- β) signaling mediating fibrosis.

Methods We first determined periostin levels in skin and lung fibroblasts obtained from SSc patients. ECM proteins and pro-fibrotic factors protein levels were further evaluated in periostin-expressing human skin fibroblasts in the presence or absence of TGF- β . Following TGF- β stimulation, the effects of periostin on Smad proteins were also evaluated by immunoblotting and immunofluorescence staining. Finally, Smad 7 protein levels were assessed in fibroblasts under periostin RNA interference.

Results Periostin was more strongly expressed in skin and lung primary SSc fibroblasts than in healthy subjects. Recombinant periostin (rP)-treated fibroblasts and periostin-expressing fibroblasts produced significantly more ECM proteins in the presence of TGF- β than control fibroblasts stimulated with TGF- β alone. Overexpression of periostin enhanced the induction of Egr-1 and α -SMA in the presence of TGF- β . In addition, R-Smad activity induced by TGF- β was not significantly affected by periostin, but the level of Smad 7 was reduced in periostin-expressing fibroblasts under TGF- β stimulation. On the other hand, the level of Smad 7 was increased in fibroblasts under periostin RNA interference in the presence of TGF- β .

Conclusion These results suggest that periostin may contribute to fibrosis by enhancing TGF- β signaling via Smad 7 inhibition, which leads to further ECM deposition and periostin generation. Periostin may be a therapeutic target molecule in fibrosis.

INTRODUCTION

- Systemic sclerosis (SSc) results in significant morbidity and mortality due to organ fibrosis characterized by the increased deposition of extracellular matrix (ECM).
- We previously reported an elevated level of circulating periostin in SSc patients and its association with the severity of skin sclerosis.
- Periostin is a matricellular proteins, one of a class of ECM-related molecules defined by their ability to modulate cell–matrix interaction.
- Recent studies have revealed that periostin serves as a critical regulator of wound healing, epithelial mesenchymal transition, and fibrosis.
- Periostin plays an important role as a co-factor of TGF- β in promoting ECM production and differentiation to myofibroblasts.

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- TGF- β transduces its signal from the membrane to the nucleus through Smad proteins.
- R-Smads are directly phosphorylated to form a complex with Co-Smad and translocate into the cell nuclei, where they regulate gene transcription in co-operation with other co-factors.
- Smad 7 down-regulates TGF- β signaling.

PURPOSE

To examine the role of periostin in transforming growth factor- β (TGF- β) signaling mediating fibrosis.

METHODS & RESULTS

Periostin levels in skin and lung fibroblasts obtained from SSc patients

A. Immunohistochemical analysis of periostin in human skin obtained from patients with systemic sclerosis –diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc)– and a healthy control. Haematoxylin-eosin (HE) staining of skin from the same subjects is also shown. Scale bars=100 μ m.

B. Periostin mRNA and protein levels in normal and SSc fibroblasts –normal skin fibroblasts (NS), scleroderma fibroblasts (SSc), lesional fibroblasts (L), non lesional fibroblasts (N) and normal lung fibroblasts (NL)–. **p < 0.01, T-test with Welch's correction, n=3.

Periostin was more strongly expressed in skin and lung primary SSc fibroblasts than in healthy subjects.

ECM production by recombinant periostin in human fibroblasts in the presence or absence of TGF- β

Type I collagen α 1 protein levels in supernatants of cultured healthy fibroblasts were confirmed by immunoblotting at 24h after stimulation with recombinant periostin (rP) and/or TGF- β . *p < 0.05; **p < 0.01, one-way analysis of variance (post-hoc Tukey), n=4.

Although stimulation with rP alone did not increase type I collagen protein levels, co-stimulation with rP and TGF- β enhanced type I collagen production.

ECM production in periostin-expressing fibroblasts in the presence or absence of TGF- β

To enhance the function of periostin, a retrovirus expressing human periostin was transfected into human fibroblasts to prepare periostin-expressing fibroblasts.

A. Human fibroblasts were infected with periostin-expressing retrovirus (RV-hPn) or a control retrovirus (RV-mock).

B. Type I collagen α 1 and fibronectin protein levels in supernatants of cultured RV-mock and RV-hPn cells were confirmed by immunoblotting at 6, 24 and 48h after stimulation with TGF- β . The levels of each protein at 48h were quantified using densitometry and expressed as the ratio of each protein to GAPDH. *p < 0.05; **p < 0.01; ***p < 0.001, one-way analysis of variance (post-hoc Tukey), n=4.

Overexpression of periostin enhanced the induction of ECM proteins in the presence of TGF- β .

Pro-fibrotic factors in periostin-expressing fibroblasts in the presence or absence of TGF- β

A. Egr-1 and α -SMA protein levels in lysates of cultured RV-mock and RV-hPn cells were confirmed by immunoblotting at 6, 24 and 48h after stimulation with TGF- β . The levels of each protein at 48h were quantified using densitometry and expressed as the ratio of each protein to GAPDH. *p < 0.05; **p < 0.01, one-way analysis of variance (post-hoc Dunnett), n=4.

B. Matrix metalloproteinase-1 (MMP-1) protein levels in supernatants of cultured RV-mock and RV-hPn cells were confirmed in the same way. *p < 0.05; **p < 0.01; ***p < 0.001, one-way analysis of variance (post-hoc Tukey), n=4.

C. MMP-1 activity was detected by casein zymography for each sample in the same way. Overexpression of periostin enhanced the induction of Egr-1 and α -SMA in the presence of TGF- β and decreased the expression of MMP-1.

R-Smad activity in periostin-expressing fibroblasts in the presence or absence of TGF- β

A Smad Reporter Assay was performed on the RV-mock or RV-hPn cells using a Cignal SMAD Reporter Assay kit® 24h prior to TGF- β stimulation and R-Smad activity was examined at 18h after TGF- β stimulation. *p < 0.05; ***p < 0.001; ns, not significant, one-way analysis of variance (post-hoc Dunnett), n=4.

R-Smad activity induced by TGF- β was not significantly affected by periostin.

Smad 7 induced by TGF- β in periostin-expressing fibroblasts

A. Smad 7 protein levels in lysates of cultured PV-hPn and RV-mock cells were confirmed by immunoblotting at 0, 10, 30, 60 and 90 minutes after stimulation with TGF- β . *p < 0.05; ns, not significant, one-way analysis of variance (post hoc Dunnett), n=3.

B. At 30 minutes after TGF- β stimulation, RV-hPn and RV-mock cells were stained for Smad 7 and periostin. DAPI was used to identify the nuclei. Scale bars=100 μ m.

Smad 7 levels decreased in periostin-expressing fibroblasts under TGF- β stimulation.

Smad 7 induced by TGF- β in periostin-silenced fibroblasts

A. Smad 7 and periostin protein levels in the lysates of cultured periostin-silenced fibroblasts and control fibroblasts were confirmed by immunoblotting at 0, 10, 30, 60 and 90 minutes after stimulation with TGF- β . *p < 0.05, one-way analysis of variance (post-hoc Dunnett), n=5.

B. At 30minutes after stimulation of TGF- β , periostin-silenced fibroblasts and control fibroblasts were stained for Smad 7 and periostin. DAPI was used to identify the nuclei. Scale bars=100 μ m.

Smad 7 levels increased in fibroblasts under periostin RNA interference in the presence of TGF- β .

SUMMARY

- Periostin was more strongly expressed in skin primary SSc fibroblasts than in healthy subjects.
- Overexpression of periostin enhanced the induction of ECM production and pro-fibrotic factors in the presence of TGF- β .
- Levels of Smad 7, a down-regulator of TGF- β signaling, were reduced in periostin-expressing fibroblasts and increased in fibroblasts with periostin RNA interference.

CONCLUSION

These results suggest that periostin may contribute to fibrosis by enhancing TGF- β signaling via Smad 7 inhibition, which leads to further ECM deposition and periostin generation. Periostin may be a therapeutic target molecule in fibrosis.