TMEM88 inhibits TGF-β1-induced-extracellular matrix (ECM) accumulation and epithelial-mesenchymal transition (EMT) program in human pleural mesothelial cells through modulating TGF-β1/Smad pathway

Zhongmin Sun
First Affiliated hospital of Xi'an jiaotong University
Ning Qian
Firrst Affiliated hospital of Xi'anjiaotong University
Hong Li
First affiliated hospital of Xi'an jiaotong University
Tinghua Hu
First affiliated hospital of Xi'an university
Ling Tang
First affiliated hospital of Xi'an jiaotong University
Qing Wen
First affiliated hospital of Xi'an jiaotonguniversity
Liangrong Shen liangrong_shen@126.com (liangrong_shen@126.com)
The first affiliated hospital of Xi'an jiaotong Unkiversity

Research

Keywords: TMEM88, pleural fibrosis, TGF-β1/Smad pathway, extracellular matrix (ECM), epithelial-mesenchymal transition (EMT)

Posted Date: April 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22919/v1

Abstract

Pleural fibrosis is an irreversible pathological process occurred in the development of several lung diseases. TMEM88 is a member of transmembrane (TMEM) family and has been found to be involved in the regulation of fibrogenesis. However, the role of TMEM88 in pleural fibrosis remains unknown. In this study, we aimed to explore the role of TMEM88 in pleural fibrosis *in vitro* using TGF-β1-induced human pleural mesothelial cell line MeT-5A cells. Our results showed that the expression levels of TMEM88 were downregulated in pleural fibrosis tissues and TGF-β1-treated Met-5A cells. Overexpression of TMEM88 inhibited the proliferation of Met-5A cells under TGF-β1 stimulation. In addition, TMEM88 overexpression prevented TGF-β1-induced extracellular matrix (ECM) accumulation and epithelial-mesenchymal transition (EMT) in Met-5A cells with decreased expression levels of Col I and fibronectin, increased levels of cytokeratin-8 and E-cadherin, as well as decreased levels of vimentin and α-SMA. Furthermore, overexpression of TMEM88 inhibited the expression of TGF-β receptor I (TβRI) and TβRII and suppressed the phosphorylation of Smad2 and Smad3 in Met-5A cells. In conclusion, these results indicated that TMEM88 exhibited an anti-fibrotic activity in pleural fibrosis via inhibiting the activation of TGF-β1/Smad signaling pathway.

1. Introduction

Pleural fibrosis is a pathological process occurred in the development of several lung diseases. Pleural fibrosis is defined as an excessive deposition of extracellular matrix (ECM) components, which finally leads to the destruction of normal pleural tissue architecture and compromised function [1]. It is generally considered that the pathogenesis of pleural fibrosis is associated with inflammatory cells, profibrotic mediators and coagulation, and fibrinolytic pathways are integral to pleural remodeling and fibrosis [2, 3]. The pleura is lined by a monolayer of mesothelial cells that rest on a thin basement membrane [4]. Increasing evidence suggests that pleural mesothelial cells (PMCs) play a significant role in the pathogenesis of this condition through initiating inflammatory responses and producing ECM components [5, 6]. Targeting PMCs may be adequate therapy and can be developed to prevent pleural fibrosis from occurring.

Transforming growth factor (TGF)-β1 is an isoform of TGF-β superfamily that has been well documented to be a master regulator of fibrosis [7]. TGF-β1 was found to promote ECM proteins production by regulation of transcriptional activation of the relevant genes [8]. Thus, activation of TGF-β1 is important to fibrosis process during wound repair in many organs. In addition, many studies have revealed that TGF-β1 signaling is a major mechanism for the pleural fibrosis [9, 10, 11].

The transmembrane (TMEM) family consists of many proteins that spans the entire width of the lipid bilayer [12]. Many TMEM proteins function as channels to permit the transport of specific substances and play important roles in various physiological functions including fibrogenesis [13]. TMEM88, belonging to the TMEM family, was found to be involved in the regulation of fibrogenesis. TMEM88 attenuates liver fibrosis via regulating Wnt/β-catenin and Bcl-2/Bax/Caspase3 signaling pathways [14].

Zhao *et al*. [15] reported that TMEM88 inhibits hyper-proliferation and migration of fibroblasts and suppresses excess ECM deposition in TGF-β1-stimulated keloid-derived fibroblasts. TMEM88 inhibits fibrosis in renal proximal tubular epithelial cells (HK2 cells) by suppressing the TGF-β1/Smad signaling pathway [16]. However, the role of TMEM88 in pleural fibrosis remains unknown.

In this study, we explored the role of TMEM88 in pleural fibrosis using TGF-β1-induced human pleural mesothelial cell line MeT-5A cells. The results showed that TMEM88 exhibited anti-fibrotic activity in pleural fibrosis via regulating TGF-β1/Smad signaling pathway.

2. Materials And Methods

2.1. Specimen collection

Totally of 30 patients with tuberculous pleuritis diagnosed at The First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) were enrolled in the current study. The pleural tissues were collected for the HE staining to identify the fibrotic tissues (n = 15) and non-fibrotic tissues (n = 15). The clinical tissues were used for the detection of TMEM88 expression. All patients have signed the informed consent prior to the study. The study was approved by the Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University.

2.2. Cell culture and treatment

MeT-5A cells (ATCC, Manassas, VA, USA) were incubated in RPMI medium and maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The medium was supplemented with 10% FBS, 100 units/ml penicillin G, 100 mg/ml streptomycin, and 2 mM L-glutamine. Cells were cultured in the absence or presence of TGF-β1 (5 ng/ml; Sigma-Aldrich, St. Louis, MO, USA) for different times.

2.3. Cell transfection

The full-length cDNA for TMEM88 was obtained by RT-PCR. The cDNA was inserted into pcDNA3.0 vector for building the TMEM88-overexpressing plasmid pcDNA3.0-TMEM88. Cell transfection was performed using Lipofectamine 2000 Reagent (Life Technologies, Grand Island, NY, USA) when MeT-5A cells grew to 80%-90% confluence.

2.4. Cell proliferation assay

Cell proliferation was measured using the Cell Counting Kit (CCK-8) (Dojindo, Kumamoto, Japan). Met-5A cells (5×10^4 cells/ml) were plated in 96-well plates (100 μl medium per well). After indicated treatments, 10 μl CCK-8 solution was added into the culture medium and incubated for 4 h. The absorbance (A_{450}) was measured using a microplate reader (Molecular Devices, Sunnyvale, California, USA).

2.6. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA of pleural tissues and MeT-5A cells were extracted by the TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). The isolated RNA (1 μg) was subjected to reverse transcription for the cDNA synthesis using PrimeScrip reverse transcription reagent kit (TaKaRa, Otsu, Shiga, Japan). The PCR reaction was performed using a SYBR Prellix Ex Taq™ Real-Time PCR Kit (Takara) on an Applied Biosystems 7500 Sequence Detection System (Applied Biosystem, Foster, CA, USA). Specific primer sequences (sense/antisense) were designed as follows: TMEM88 forward 5'-GCTGCCTTCAATCTTCTCCTG-3', reverse 5'-ATAAAGGCTCGGCTGTAG G-3'; β-actin forward, 5'-ATCACCATTGGCAATGAGCG-3' and reverse 5'-TTGA AGGTAGTTTCGTGGAT-3'.

2.7. Western blot analysis

MeT-5A cells were harvested and lysed in RIPA lysis buffer (Invitrogen). Protein lysates were separated through 10% SDS-PAGE and electroblotted onto a PVDF membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were then blocked with 5% skimmed milk in TBST buffer (10 mM Tris-base, 100 mM NaCl, and 0.01% Tween 20) for 1 h at room temperature, followed by incubation with various specific primary antibodies against TMEM88 (Abcam, Cambridge, MA, USA), E-cadherin (Abcam), cytokeratin-8 (Abcam), α-SMA (Santa Cruz Biotechnology, Santa Cruz, CA, USA), vimentin (Santa Cruz), collagen I (Col I; Santa Cruz), fibronectin (Santa Cruz), TGF-β receptor I (TβRI; Abcam), TGF-β receptor II (TβRII; Abcam), Smad2 (Cell Signaling Technology, CST, Boston, MA, USA), p-Smad2 (CST), Smad3 (CST), p-Smad3 (CST) and β-actin (Santa Cruz) for 12 h. The membranes were then washed with TBST buffer for three times and incubated with the HRP-linked secondary antibody (Santa Cruz) for 1 h at 37 °C. The antigen-antibody complexes on the membranes were visualized using an enhanced chemiluminescence system (Bio-Rad).

2.8. Statistical analysis

Results were analyzed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) and shown as the mean ± SEM. Differences between two groups were analyzed using unpaired t-test. Differences among more than two groups were analyzed using one-way analysis of variance (ANOVA). A *p* value less than 0.05 was considered as statistically significant.

3. Results

3.1. TMEM88 expression is down-regulated in pleural fibrosis tissues

The TMEM88 expression levels in pleural fibrosis tissues and non-fibrotic tissues were determined using qRT-PCR and western blot. The results showed that the mRNA level of TMEM88 in pleural fibrosis tissues was significantly lower than that in non-fibrotic tissues (Fig. 1A). Similarly, the protein expression levels of TMEM88 were also down-regulated in pleural fibrosis tissues (Fig. 1B).

3.2. TMEM88 expression is down-regulated in TGF-β1-treated Met-5A cells

To investigate the role of TMEM88 in pleural fibrosis *in vitro*, Met-5A cells were treated with TGF-β1 (10 ng/ml) for 24 h. The mRNA and protein levels of TMEM88 were measured using qRT-PCR and western blot analysis. As shown in Fig. 2A, the mRNA level of TMEM88 was significantly decreased after treatment with TGF-β1. Meanwhile, the protein level of TMEM88 was also downregulated in TGF-β1-treated Met-5A cells (Fig. 2B).

3.3 Overexpression of TMEM88 inhibits the proliferation of Met-5A cells under TGF-β1

Given that TMEM88 was down-regulated in pleural fibrosis tissues and TGF-β1-treated Met-5A cells, we transfected pcDNA3.0-TMEM88 into Met-5A cells. As indicated in Fig. 3A, the protein expression level of TMEM88 was greatly increased by pcDNA3.0-TMEM88 in TGF-β1-stimulated Met-5A cells. Functional assay was conducted to further validate the role of TMEM88. We found that overexpression of TMEM88 inhibited the cell proliferation of Met-5A cells under TGF-β1 stimulation (Fig. 3B).

3.4 Overexpression of TMEM88 prevents TGF-β1-induced ECM accumulation in Met-5A cells

To investigate the role of TMEM88 in ECM accumulation, the expression levels of ECM proteins including Col I and fibronectin were determined using western blot. Overexpression of TMEM88 caused significant decreases in the expression levels of Col I and fibronectin in TGF-β1-induced Met-5A cells (Fig. 4).

3.5. Overexpression of TMEM88 prevents TGF-β1-induced epithelial-mesenchymal transition (EMT) in Met-5A cells

EMT is considered as another essential mechanism that governs the progression of fibrosis process [17, 18]. Next, we explored the effect of TMEM88 on EMT through detecting the protein levels of epithelial phenotypic markers (cytokeratin-8 and E-cadherin) and mesenchymal phenotypic markers (vimentin and α-SMA). The expression levels of cytokeratin-8 and E-cadherin were decreased, while the expression levels of vimentin and α-SMA were increased by TGF-β1 treatment. However, these effects were reversed by pcDNA3.0-TMEM88 (Fig. 5).

3.6. Overexpression of TMEM88 inhibits the expression of TβRI and TβRII in Met-5A cells

TGF-β1 initiates a series of signal transduction upon binding with TGF-β receptors, such as TβRI and TβRII [19]. Next, we assessed the effect of TMEM88 on the expression of TβRI and TβRII. As indicated in

Fig. 6, as compared with the control group, the expression levels of TβRI and TβRII were markedly increased in TGF-β1-stimulated Met-5A cells. However, TMEM88 significantly reduced the protein expression levels of TβRI and TβRII in TGF-β1-stimulated Met-5A cells.

3.7. Overexpression of TMEM88 inhibits the activation of TGF-β1/Smad pathway in Met-5A cells

TGF-β1/Smad pathway has been found to be related to the fibrosis process [20]. Thus, we examined the effect of TMEM88 on TGF-β1/Smad pathway activation in Met-5A cells. The results showed that overexpression of TMEM88 greatly inhibited TGF-β1-upregulated the expression levels of p-Smad2 and p-Smad3. The results indicated that TMEM88 overexpression suppressed the activation of TGF-β1/Smad pathway in Met-5A cells (Fig. 7).

4. Discussion

TMEM88 is a disheveled-binding protein belonging to TMEM family. TMEM88 has been demonstrated to be implicated in the organ fibrosis, such as liver fibrosis and kidney fibrosis. TMEM88 expression is decreased in the human liver fibrotic tissues. *In vitro* assays prove that TMEM88 inhibits activation and proliferation of hepatic stellate cells (HSCs) by blocking Wnt/β-catenin pathway, and promotes the activated HSCs apoptosis by initiating Bcl-2/Bax/Caspase3 pathway [14]. TMEM88 was also found to be downregulated in renal fibrotic tissues and TGF-β1-treated HK2 cells. TMEM88 overexpression inhibits TGF-β1-induced cell proliferation and migration, and production of ECM proteins including α-SMA, Col I, and Col III in HK2 cells [16]. In the current study, we investigated the role of TMEM88 in pleural fibrosis. Our results showed that the expression level of TMEM88 was downregulated in pleural fibrosis tissues and TGF-β1-treated Met-5A cells. The findings implied that TMEM88 might be involved in the progression of pleural fibrosis, which were consistent with the previous studies.

Fibrosis is the consequence of dysfunctional response of wound healing mechanisms after tissue injury [21]. It is a basic connective tissue lesion accompanied by extensive expression and accumulation of ECM components [22]. ECM deposition results in the disruption of the proper three-dimensional structure of tissue and homeostatic imbalance [23]. During the fibrotic event, various regulators including growth factors, proteolytic enzymes, angiogenic factors, and fibrogenic cytokines have been observed to stimulate the deposition of ECM [24]. Among these, TGF-β is a major pro-fibrotic mediator [25]. EMT, an essential mechanism in embryonic development and tissue repair, governs the progression of many diseases, including organ fibrosis and cancer [26, 27, 28]. Transcription program switching in EMT is induced by various signaling pathways, including epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-β, and platelet-derived growth factor (PDGF) [29]. In addition, ECM proteins induce various signaling mechanisms in the fibrotic event, and certain ECM proteins, such as Col I, provide a suitable microenvironment for EMT, thereby promoting the organ fibrosis [29, 30]. Further gain-of-function assays demonstrated that overexpression of TMEM88 inhibited the proliferation of Met-5A cells under TGF-β1 stimulation. Besides, overexpression of TMEM88 prevented TGF-β1-induced ECM accumulation and EMT

in Met-5A cells with decreased expression levels of ECM proteins (Col I and fibronectin), increased levels of epithelial phenotypic markers (cytokeratin-8 and E-cadherin), as well as decreased levels of mesenchymal phenotypic markers (vimentin and α-SMA). These data indicated that TMEM88 suppressed the progression of pleural fibrosis.

According to the published articles, TGF-β signaling begins when the activated TGF-β ligand binds to the TβRII subunits on the cell surface, which leads to the recruitment of TβRI subunits [31]. The TβRI subunits become phosphorylated and activated by TβRII, leading to the activation of Smad-dependent TGF-β1 signaling (canonical pathway) [19, 32]. The Smad2 and Smad3 are recruited and subsequently phosphorylated by TβRI, thereby assembles with co-Smad4 and translocates to the nucleus [32]. Finally, the activation of TGF-β1-Smad results in the regulation of gene expression through interaction with co-activators and cell-specific transcription factors [20]. In the current study, our results demonstrated that overexpression of TMEM88 inhibited the expression of TβRI and TβRII, as well as suppressed the phosphorylation of Smad2 and Smad3 in Met-5A cells. The findings suggested that TMEM88 prevented the activation of TGF-β1/Smad pathway in Met-5A cells, which might contribute to its anti-fibrotic activity.

5. Conclusion

In conclusion, our results indicated that TMEM88 inhibited TGF-β1-induced cell proliferation, ECM accumulation and EMT process in Met-5A cells. Furthermore, TMEM88 inhibited the expression of TβRI/II and activation of TGF-β1/Smad pathway in Met-5A cells. Therefore, TMEM88 might be a novel and promising therapeutic approach for the treatment of pleural fibrosis.

Declarations

Conflict of interest statement

No conflict of interests.

Funding

This study was supported by the National Natural Science Foundation of China (81170073).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Authors' contributions

The research was conceived and designed by Liangrong Shen, Zhongmin Sun. The experiments was carried out by Qian Ning, Hong Li and Tinghua Hu. The manuscript was wrote by Ling Tang, Qing Wen and Zhongmin Sun.

References

1. Jantz MA, Antony VB. Pleural fibrosis. Clin Chest Med. 2006;27:181–91.
2. Mahjoubi I, Khalil A, Carette MF. [Inflammation and pleural fibrosis]. Rev Pneumol Clin. 2006;62:97–110.
3. Mutsaers SE, Prele CM, Brody AR, Idell S. Pathogenesis of pleural fibrosis. Respirology. 2004;9:428–40.
4. Mutsaers SE. The mesothelial cell. Int J Biochem Cell Biol. 2004;36:9–16.
5. Batra H, Antony VB. Pleural mesothelial cells in pleural and lung diseases. J Thorac Dis. 2015;7:964–80.
6. Kawanishi K. Diverse properties of the mesothelial cells in health and disease. Pleura Peritoneum. 2016;1:79–89.
7. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-beta: the master regulator of fibrosis. Nat Rev Nephrol. 2016;12:325–38.
8. Kim KK, Sheppard D, Chapman HA. TGF-beta1 Signaling and Tissue Fibrosis. Cold Spring Harb Perspect Biol. 2018;10.
9. Decologne N, Kolb M, Margetts PJ, Menetrier F, Artur Y, Garrido C, et al. TGF-beta1 induces progressive pleural scarring and subpleural fibrosis. J Immunol. 2007;179:6043–51.
10. Jiang N, Zhang Z, Shao X, Jing R, Wang C, Fang W, et al. Blockade of thrombospondin-1 ameliorates high glucose-induced peritoneal fibrosis through downregulation of TGF-beta1/Smad3 signaling pathway. J Cell Physiol. 2020;235:364–79.
11. Wang M, Xiong L, Jiang LJ, Lu YZ, Liu F, Song LJ, et al. miR-4739 mediates pleural fibrosis by targeting bone morphogenetic protein 7. EBioMedicine 2019;41: 670–682.
12. Marx S, Dal Maso T, Chen JW, Bury M, Wouters J, Michiels C, et al. Transmembrane (TMEM) protein family members: Poorly characterized even if essential for the metastatic process. Semin Cancer Biol. 2020;60:96–106.
13. Schmit K, Michiels C. TMEM Proteins in Cancer: A Review. Front Pharmacol. 2018;9:1345.
14. Cai SP, Cheng XY, Chen PJ, Pan XY, Xu T, Huang C, et al. Transmembrane protein 88 attenuates liver fibrosis by promoting apoptosis and reversion of activated hepatic stellate cells. Mol Immunol. 2016;80:58–67.
15. Zhao H, Lu F, Cui S, Zhang X, Wang W, Si E, et al. TMEM88 inhibits extracellular matrix expression in keloid fibroblasts. Biomed Pharmacother. 2017;95:1436–40.

16. Li H, Wang Y, Chen B, Shi J. TMEM88 inhibits fibrosis in renal proximal tubular epithelial cells by suppressing the transforming growth factor-β1/Smad signaling pathway. RSC Adv. 2019;9:6928–34.
17. Strippoli R, Moreno-Vicente R, Battistelli C, Cicchini C, Noce V, Amicone L, et al. Molecular Mechanisms Underlying Peritoneal EMT and Fibrosis. Stem Cells Int. 2016;2016:3543678.
18. Kriz W, Kaissling B, Le Hir M. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? J Clin Invest. 2011;121:468–74.
19. Hata A, Chen YG. TGF-beta Signaling from Receptors to Smads. Cold Spring Harb Perspect Biol 2016;8.
20. Hu HH, Chen DQ, Wang YN, Feng YL, Cao G, Vaziri ND, et al. New insights into TGF-beta/Smad signaling in tissue fibrosis. Chem Biol Interact. 2018;292:76–83.
21. Jones RE, Foster DS, Hu MS, Longaker MT. Wound healing and fibrosis: current stem cell therapies. Transfusion. 2019;59:884–92.
22. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol. 2008;214:199–210.
23. Walraven M, Hinz B. Therapeutic approaches to control tissue repair and fibrosis: Extracellular matrix as a game changer. Matrix Biol. 2018;71–72:205–24.
24. Sutariya B, Jhonsa D, Saraf MN. TGF-beta: the connecting link between nephropathy and fibrosis. Immunopharmacol Immunotoxicol. 2016;38:39–49.
25. Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-beta) isoforms in wound healing and fibrosis. Wound Repair Regen. 2016;24:215–22.
26. Nieto MA, Huang RY, Jackson RA, Thiery JP. Emt: 2016 Cell. 2016;166:21–45.
27. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol. 2003;15:740–6.
28. Lim J, Thiery JP. Epithelial-mesenchymal transitions: insights from development. Development. 2012;139:3471–86.
29. Gonzalez DM, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition. Sci Signal. 2014;7:re8.
30. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8.
31. Vander Ark A, Cao J, Li X. TGF-beta receptors: In and beyond TGF-beta signaling. Cell Signal. 2018;52:112–20.
32. Lodyga M, Hinz B. TGF-beta1 - A truly transforming growth factor in fibrosis and immunity. Semin Cell Dev. Biol 2019.

Figures

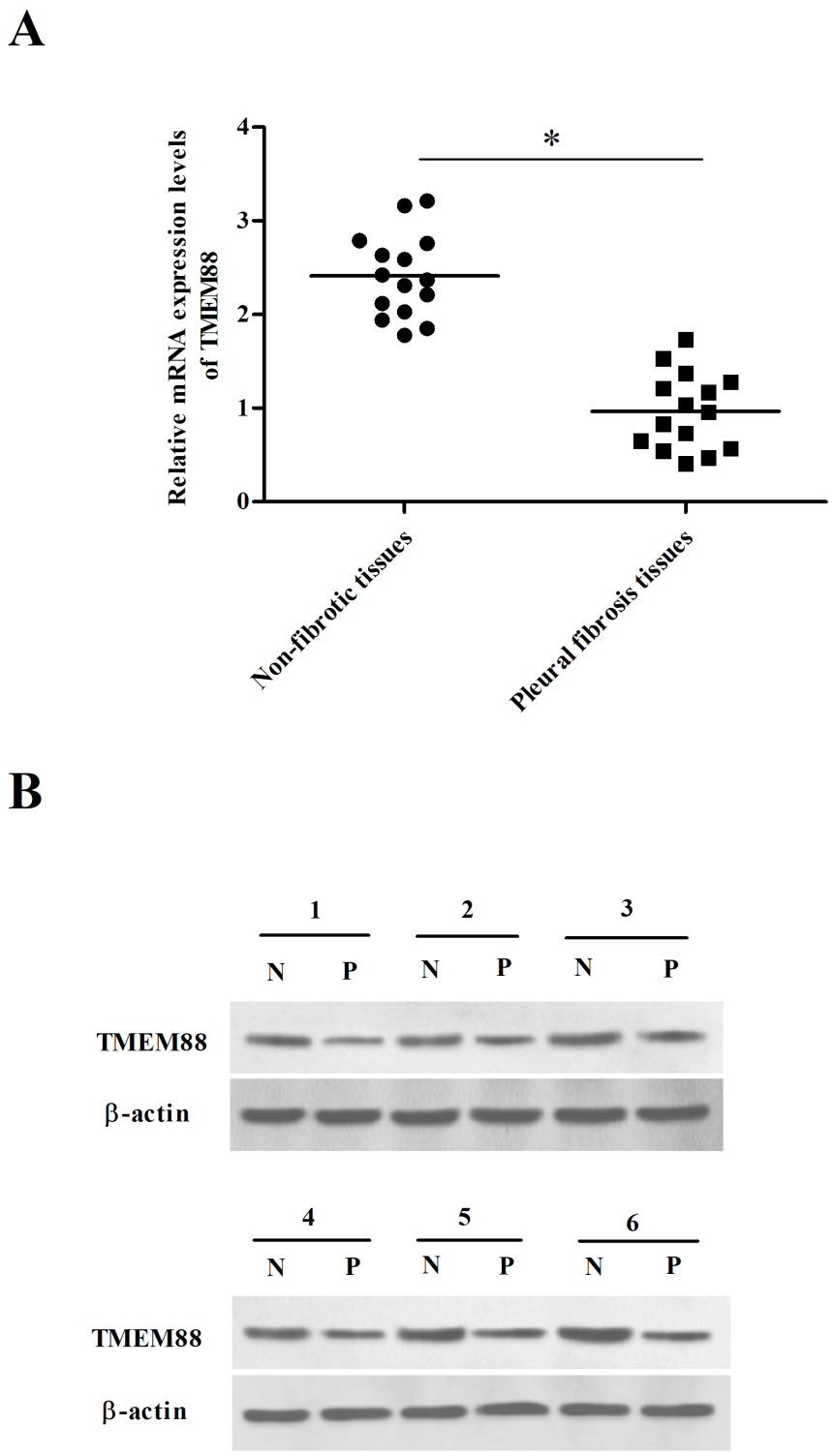

Figure 1

Quantitative PCR analysis of TMEM88 expression in pleural fibrosis tissues. Pleural fibrosis tissues and non-fibrotic tissues were collected for the detection of TMEM88 expression levels. (A) Quantification of TMEM88 mRNA levels was performed using qRT-PCR. (B) The protein expression levels of TMEM88 were measured using western blot. N represents “Non-fibrotic tissues”. N represents “Pleural fibrosis tissues”. *$p < 0.05$.

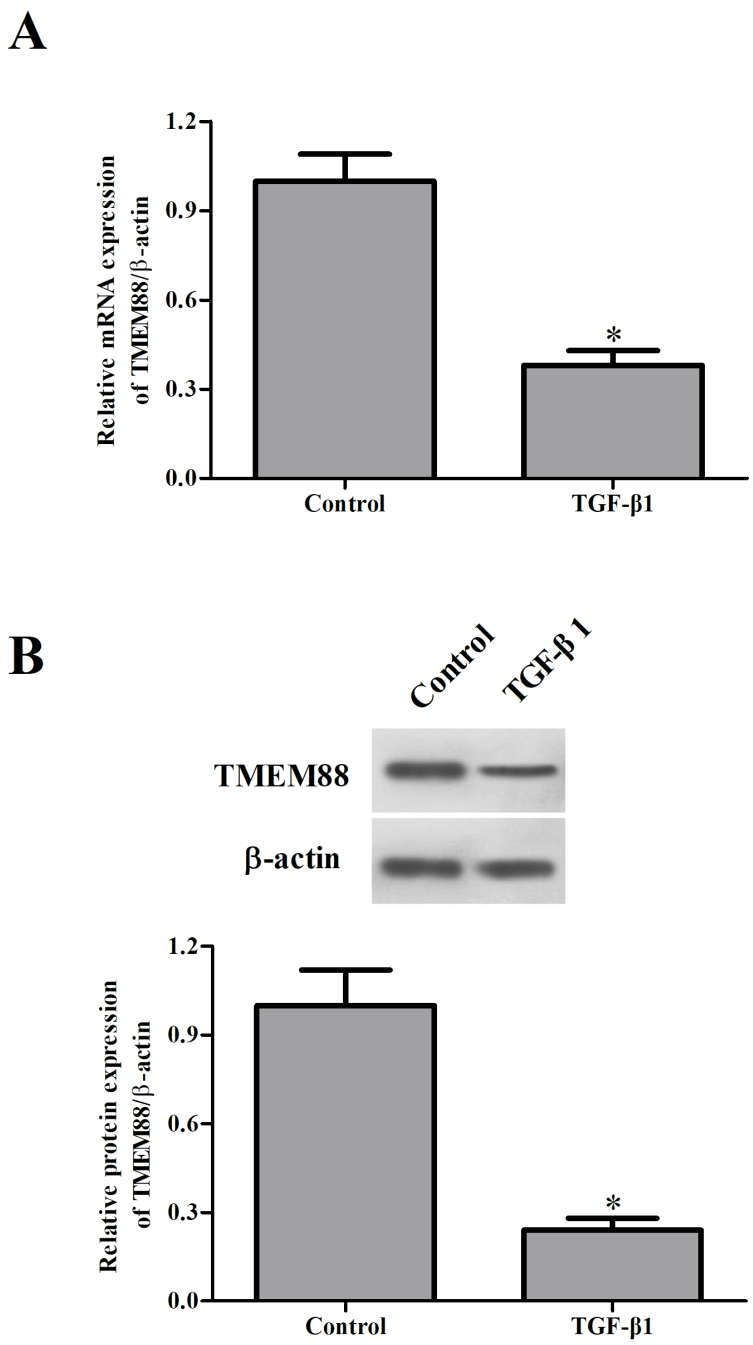

Figure 2

TMEM88 expression is down-regulated in TGF-β1-treated Met-5A cells. Met-5A cells were treated with TGF-β1 (5 ng/ml) for 24 h. (A and B) The mRNA and protein levels of TMEM88 were measured using qRT-PCR and western blot analysis. *p < 0.05 control group.

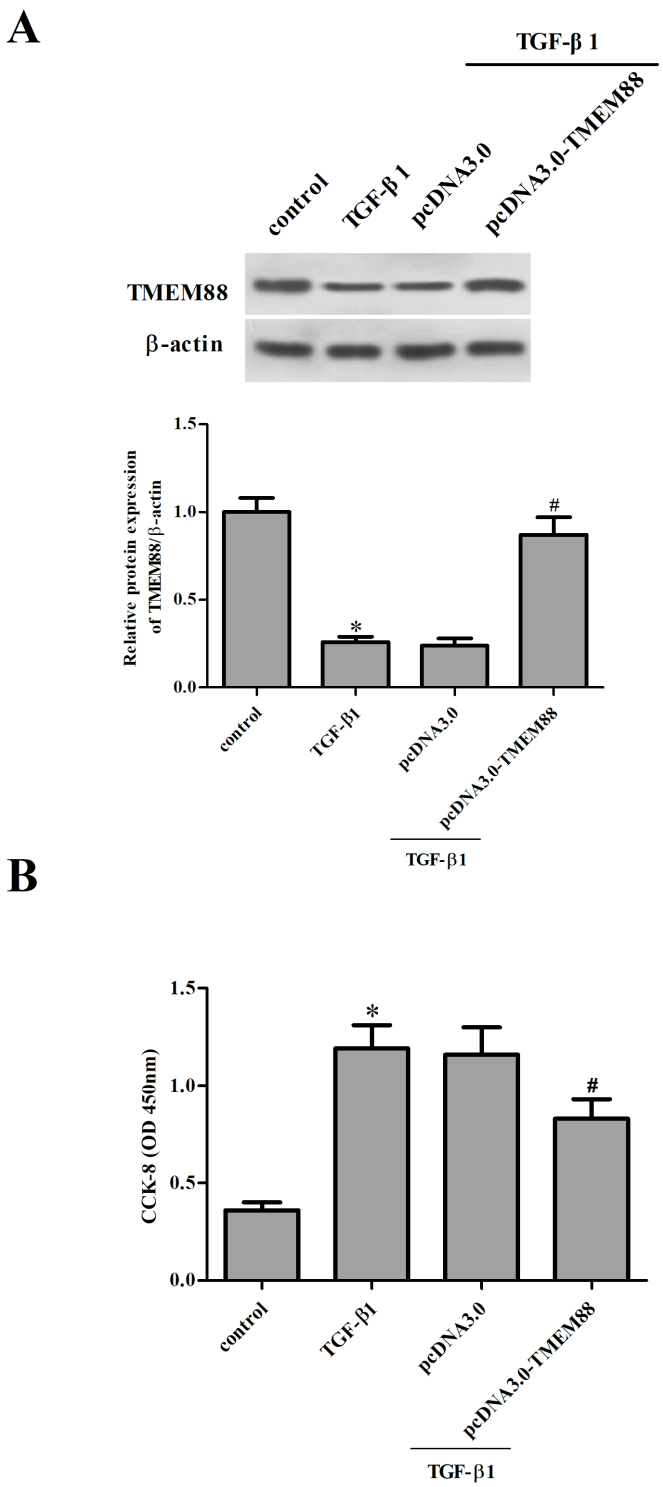

Figure 3

Schematic illustration showing the inhibitory effect of TMEM88 on Met-5A cells proliferation under TGF-β1. Met-5A cells were transfected with pcDNA3.0-TMEM88 or pcDNA3.0, and then treated with TGF-β1 (5 ng/ml) for 24 h. (A) The protein level was performed using western blot. (B) CCK-8 assay was conducted to evaluate cell proliferation of Met-5A cells. *p < 0.05 control group; #p < 0.05 pcDNA3.0 + TGF-β1 group.

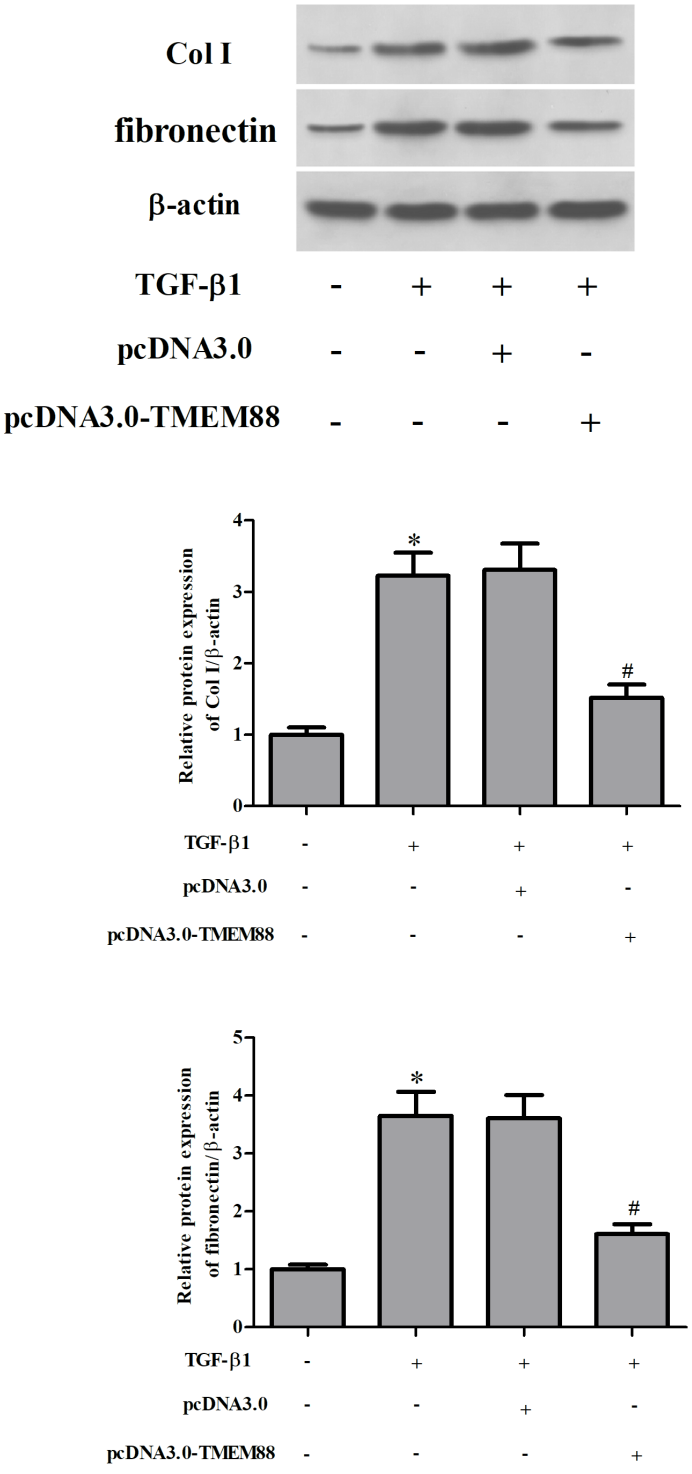

Figure 4

Schematic illustration showing the inhibitory effects of TMEM88 on ECM accumulation in TGF-β1-induced Met-5A cells. Met-5A cells were transfected with pcDNA3.0-TMEM88 or pcDNA3.0, and then treated with TGF-β1 (5 ng/ml) for 24 h. Analysis of ECM proteins including Col I and fibronectin in Met-5A cells. *p < 0.05 control group; #p < 0.05 pcDNA3.0 + TGF-β1 group.

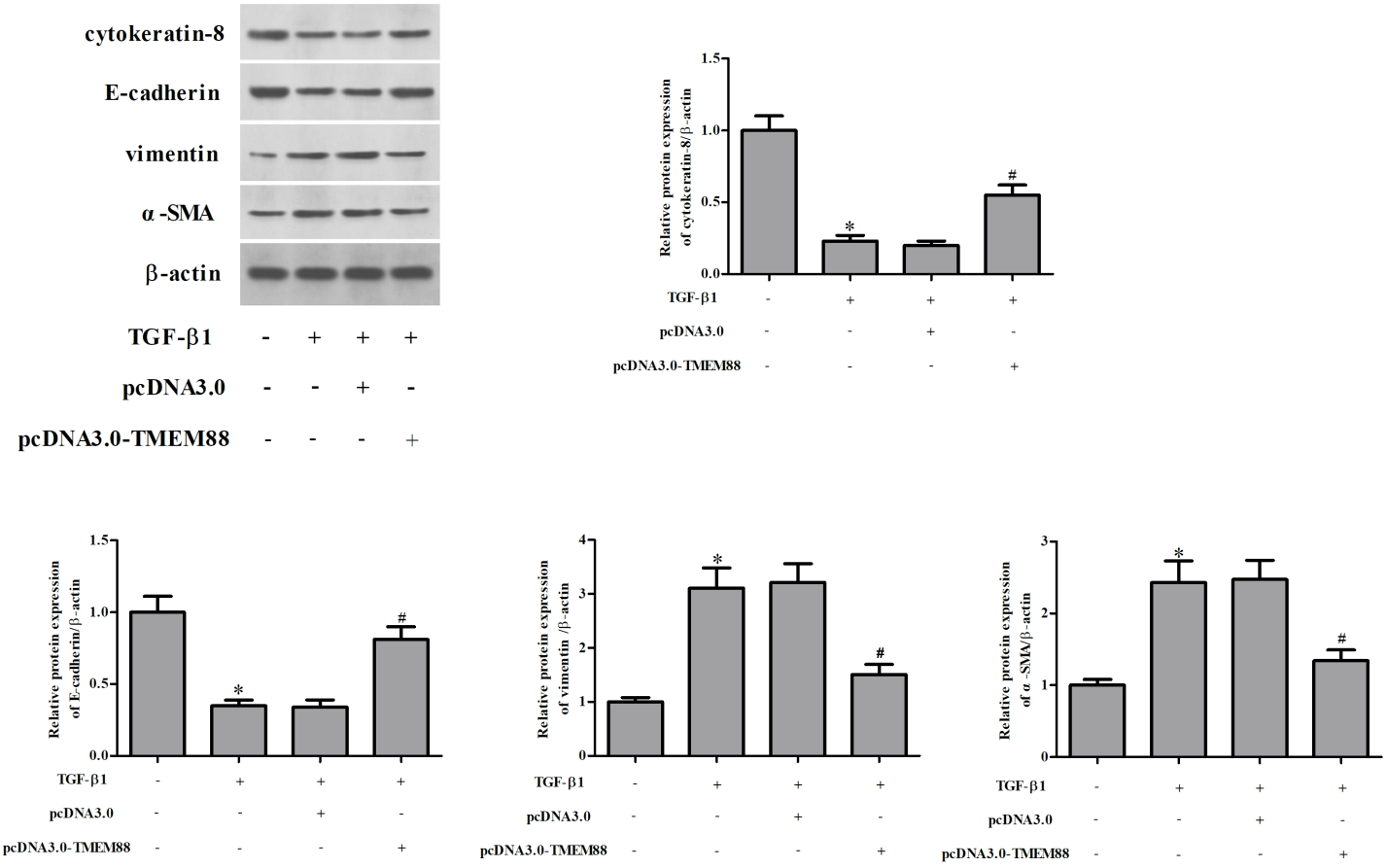

Figure 5

Schematic illustration showing the inhibitory effects of TMEM88 on EMT in TGF-β1-induced Met-5A cells. Met-5A cells were transfected with pcDNA3.0-TMEM88 or pcDNA3.0, and then treated with TGF-β1 (5 ng/ml) for 24 h. Analysis of epithelial phenotypic markers (cytokeratin-8 and E-cadherin) and mesenchymal phenotypic markers (vimentin and α-SMA) in Met-5A cells. *p < 0.05 control group; #p < 0.05 pcDNA3.0 + TGF-β1 group.

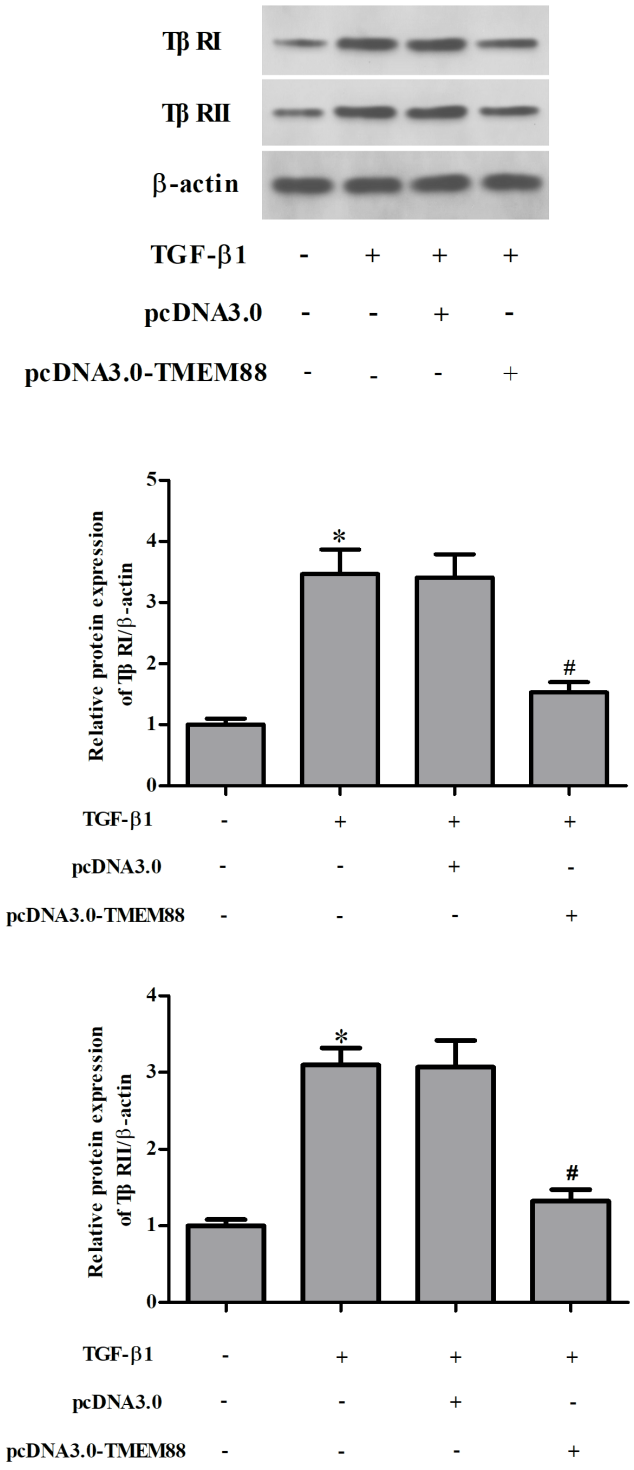

Figure 6

Western blot analysis of TβRI and TβRII expression in Met-5A cells. Met-5A cells were transfected with pcDNA3.0-TMEM88 or pcDNA3.0, and then treated with TGF-β1 (5 ng/ml) for 24 h. Quantification of TβRI and TβRII expression levels was performed and showed that TMEM88 inhibits the expression levels of TβRI and TβRII in Met-5A cells. *p < 0.05 control group; #p < 0.05 pcDNA3.0 + TGF-β1 group.

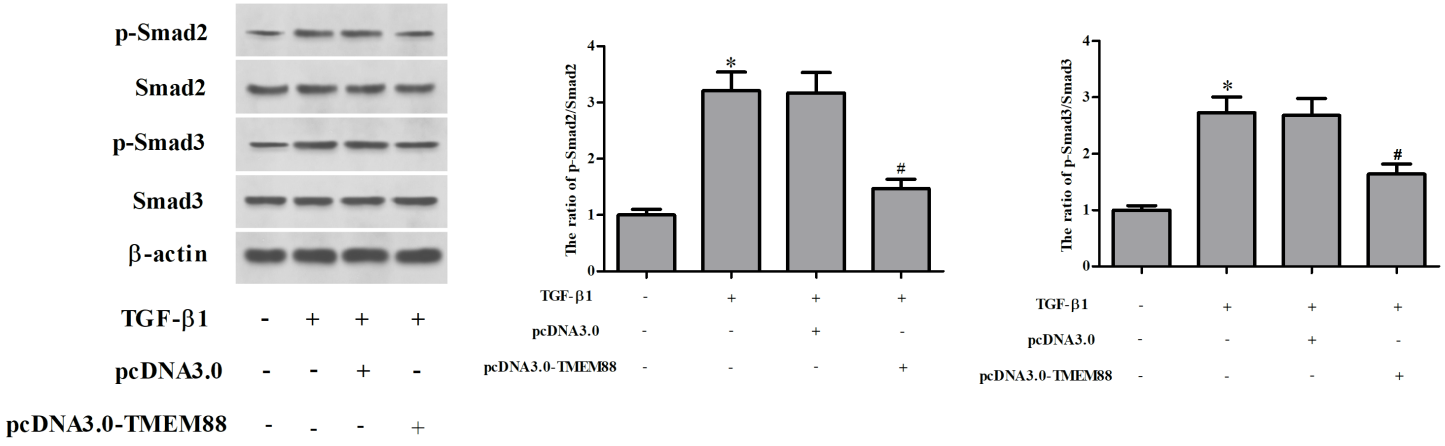

Figure 7

Western blot analysis of Smad2, p-Smad2, Smad3 and p-Smad3 in Met-5A cells. Met-5A cells were transfected with pcDNA3.0-TMEM88 or pcDNA3.0, and then treated with TGF-β1 (5 ng/ml) for 1 h. The expression levels of p-Smad2 and p-Smad3 were significantly inhibited by overexpression of TMEM88 in Met-5A cells. *p < 0.05 control group; #p < 0.05 pcDNA3.0 + TGF-β1 group.