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Original Article

Calpain 1 regulates TGF-β1-induced epithelial-mesenchymal transition in human lung epithelial cells via PI3K/Akt signaling pathway

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Abstract: Cell proliferation, transformation, and epithelial-mesenchymal transition (EMT) are key processes involved in the development of idiopathic pulmonary fibrosis (IPF). This study investigated the regulatory factors and signaling pathways that mediate EMT in the human type II alveolar epithelial A549 cell line. A549 cells were cultured in RPMI-1640 medium and allocated to the following four groups: blank control group or treated with transforming growth factor-β1 (TGF-β1), TGF-β1 + PD 150606 (a calpain 1 inhibitor), or PD 150606. We examined E-cadherin (E-cad), α-smooth muscle actin (α-SMA), and calpain 1 mRNA transcript and protein expression levels in these four groups by performing RT-PCR and western blot analyses. The results indicated that TGF-β1 treatment significantly downregulated E-cad and upregulated α-SMA expression compared with that of the blank control group ($P<0.05$). TGF-β1 also enhanced calpain 1 expression compared with that of the blank control group ($P<0.05$). By contrast, treatment with the calpain 1 inhibitor PD 150606 increased E-cad expression and decreased α-SMA expression. Furthermore, PD 150606 treatment antagonized TGF-β1-mediated increase in Akt/phospho-Akt in A549 epithelial cells. However, TGF-β1-induced ETM was not correlated with the ERK and JNK signaling pathways. These combined results indicate that calpain 1 could regulate EMT in TGF-β1-treated A549 epithelial cells via the PI3K/Akt signaling pathway.

Keywords: Idiopathic pulmonary fibrosis, epithelial-mesenchymal transition (EMT), transforming growth factor-β1 (TGF-β1), calpain 1, PI3K/Akt signaling

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, incurable, and fatal interstitial lung disease with unknown etiology [1, 2]. The occurrence of IPF is increasing, and life expectancy is poorer than that of some cancers [3, 4]. Currently, there are no pharmaceuticals or therapies that prevent IPF progression, and life expectancy is 2.5-3.5 years after diagnosis [5]. IPF also carries a burden of morbidity and poor quality of life [6]. IPF pathology causes increased deposition of extracellular matrix (ECM) and triggers the formation of fibro-proliferative foci [7]. IPF causes some cell types to transdifferentiate into activated myofibroblasts, which secrete large amounts of ECM that accumulates in lung tissues and ultimately destroys alveolar structure [8]. Therefore, cell proliferation and transformation are the key processes underlying IPF [9]. Lung epithelial cells can transdifferentiate into myofibroblasts when they acquire the mesenchymal phenotype via epithelial-mesenchymal transition (EMT) [10, 11].

Many factors induce EMT, including transforming growth factor-β1 (TGF-β1), epithelial growth factor (EGF), insulin-like growth factor (IGF), and interleukin-17 (IL-17) [12]. TGF-β1 is an important factor that induces EMT under both pathological and physiological conditions by triggering EMT regulators such as hypoxia-inducible factor-1 (HIF-1), Snail, zinc finger E-box binding homeobox 1 (ZEB1), and Twist [13].

EMT is always accompanied by downregulation of epithelial cell markers, such as E-cadherin (E-cad), and upregulation of mesenchymal cell markers such as α-smooth muscle actin (α-

Table 1. Primer sequences used for gene amplification

Genes	Primer sequence	Length (bp)
α-SMA	Forward: TCATGGTCGGTATGGGTCAG	258
	Reverse: CGTTGTAGAAGGTGTGGTGC	
Calpain-1	Forward: TGTCGGAGGAGATCATCACG	237
	Reverse: TCTTGGAGGAATTGGGACCC	
E-cad	Forward: CGTAGCAGTGACGAATGTGG	175
	Reverse: CTGGGCAGTGTAGGATGTGA	
GAPDH	Forward: AGAAGGCTGGGGCTCATTTG	150
	Reverse: AGGGGCCATCCACAGTCTTC	

SMA) [14, 15]. Calpain 1 is a non-lysosomal cysteine protease that is selectively triggered in response to changes in calcium levels. Calpain 1 regulates cellular functions such as cell transdifferentiation, cytoskeletal remodeling, apoptosis, and cell cycle progression [16-18]. Therefore, we hypothesized that calpain 1 may participate in EMT in lung epithelial cells.

In this study, we treated A549 cells (human lung epithelial cells) with TGF-β1 and/or the calpain 1 inhibitor PD 150606 (a calpastatin analog), and analyzed changes in E-cad and α-SMA expression. We show that calpain 1 regulates EMT in TGF-β1-treated A549 epithelial cells via the PI3K/Akt signaling pathway.

Materials and methods

Cell culture

The human type II alveolar epithelial cell line, A549, was obtained from the Scientific Research Department of Wuhan University, Wuhan, China. A549 cells were cultured in RPMI-1640 medium supplemented with 10% (w/v) fetal bovine serum (FBS, HyClone, Logan, UT, USA), 100 mg/l streptomycin, and 100 U/ml penicillin in a humidified 5% CO_2 atmosphere at 37°C.

Cell treatment and trial grouping

Cell cultures were adjusted to a density of 10×10^6 cells/ml using RMPI-1640 containing 10% (w/v) FBS, and then seeded into 6-well plates (100 μl per well). The cells were allocated into four groups: blank control group (only RMPI-1640 medium was added), TGF-β1 treatment group (5 ng/ml TGF-β1), TGF-β1 + calpain 1 inhibitor (PD 150606) treatment group (5 ng/ml TGF-β1 and 20 μg/ml PD 150606), and calpain 1 inhibitor treatment group (20 μg/ml PD 150606). TGF-β1 was purchased from Peprotech (Rocky Hill, NJ, USA). The calpain 1 inhibitor PD 150606 was purchased from Abcam (catalog no. ab141464; UK).

Total RNA isolation, cDNA synthesis, and RT-PCR analysis

Total RNA of A549 cells was extracted using the TRIzol total RNA extraction kit (catalog no. DP405; Tiangen, Beijing, China) according to the manufacturer's instructions. The cDNA was synthesized using the miRcute miRNA cDNA first chain synthesis kit (catalog no. KR201; Tiangen) according to the manufacturer's instructions. The mRNA of calpain-1, E-cad, and α-SMA were obtained. Gene expression was evaluated by RT-PCR (amplification conditions were as follows: 97°C for 2 min; 35 cycles of 97°C for 5 s and 55°C for 30 s) using the PrimeScript One-step RT-PCR kit ver. 2 (catalog No. RR055A; Takara Bio., Dalian, China). The primer sequences used for RT-PCR are listed in **Table 1**. The amplified DNA was analyzed on 1.5% agarose gels, and the images were digitally captured with a CCD camera. The results were analyzed using a gel imaging analyzer, NIH Imager beta version 2.0.

Western blot analysis

A549 cells were harvested and lysed in RIPA lysis buffer. The lysate was centrifuged at 12,000× *g* for 5 min at 4°C. The protein concentration was determined using the BCA assay kit (Pierce, Rockford, IL, USA). Equal amounts of proteins were loaded and separated by SDS-PAGE according to standard protocols and the manufacturer's instructions (Tiangen). Subsequently, the proteins were transferred to a PVDF membrane (Millipore, Bedford, MA, USA) for 2 h at 4°C. The membranes were blocked with 5% non-fat milk in phosphate buffer saline containing Tween 20 (PBST) and washed three times with PBST (5 min per wash). Then, the membranes were incubated overnight at 4°C with one of the following antibodies in PBST: rabbit anti-E-cad monoclonal antibody (catalog no. ab40772; 1:2000, Abcam), rabbit anti-calpain 1 polyclonal antibody (catalog no. ab28257; 1:2000, Abcam), or mouse anti-α-SMA monoclonal antibody (catalog no. sc-716-

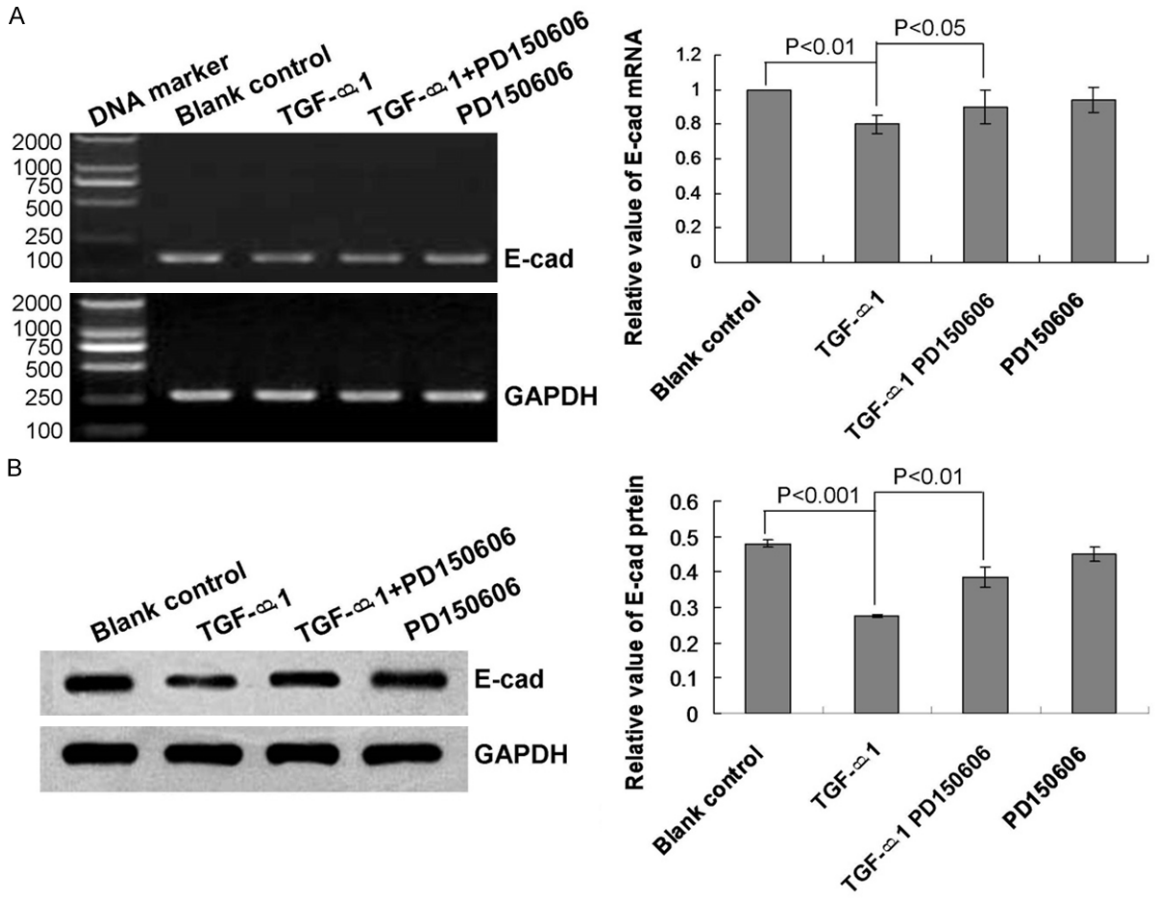

Figure 1. Analysis of E-cadherin mRNA transcript and protein expression levels in human lung epithelial cells. A. RT-PCR analysis of E-cad mRNA expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. B. Western blot analysis of E-cad protein expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. Statistical analyses and *P* values are given in the charts.

26; 1:4000, Santa Cruz, CA, USA). Subsequently, the membranes were washed three times with PBST (5 min per wash), and incubated for 2 h at room temperature with horseradish peroxidase-conjugated goat anti-mouse (catalog no. BA1051; 1:2000, Boster, Inc., Wuhan, China) or horseradish peroxidase-conjugated goat anti-rabbit (catalog no. BA1054; 1:2000, Boster, Inc., Wuhan, China). The membranes were examined for antibody binding using the western electrochemiluminescence (ECL) kit (catalog no. 32106; Pierce). Band intensities were detected and quantified using the Alpha-EaseFC image analyzer system (Alpha Innotech, Inc., CA, USA). Relative protein expression was normalized with respect to that of GAPDH (ratio of OD values of proteins and GAPDH).

Statistical analysis

All analyses were performed using SPSS 17.0 software (IBM). Multiple comparisons were analyzed with analysis of variance (ANOVA). Pairwise comparisons between means of two groups were analyzed with Student's *t*-test. Data are reported as the means ± SD (standard deviation). All experiments were performed independently and repeated at least three times. A *P* value less than 0.05 was considered statistically significant.

Results

TGF-β1 downregulates E-cad expression

Cells were treated with TGF-β1 to induce EMT, and mRNA transcript and protein expression levels of E-cad were examined by RT-PCR and western blotting, respectively. The results indicated that TGF-β1 treatment significantly reduced E-cad mRNA expression compared with that of the blank control group (**Figure 1A**, *P*<0.01). TGF-β1 treatment also reduced E-cad

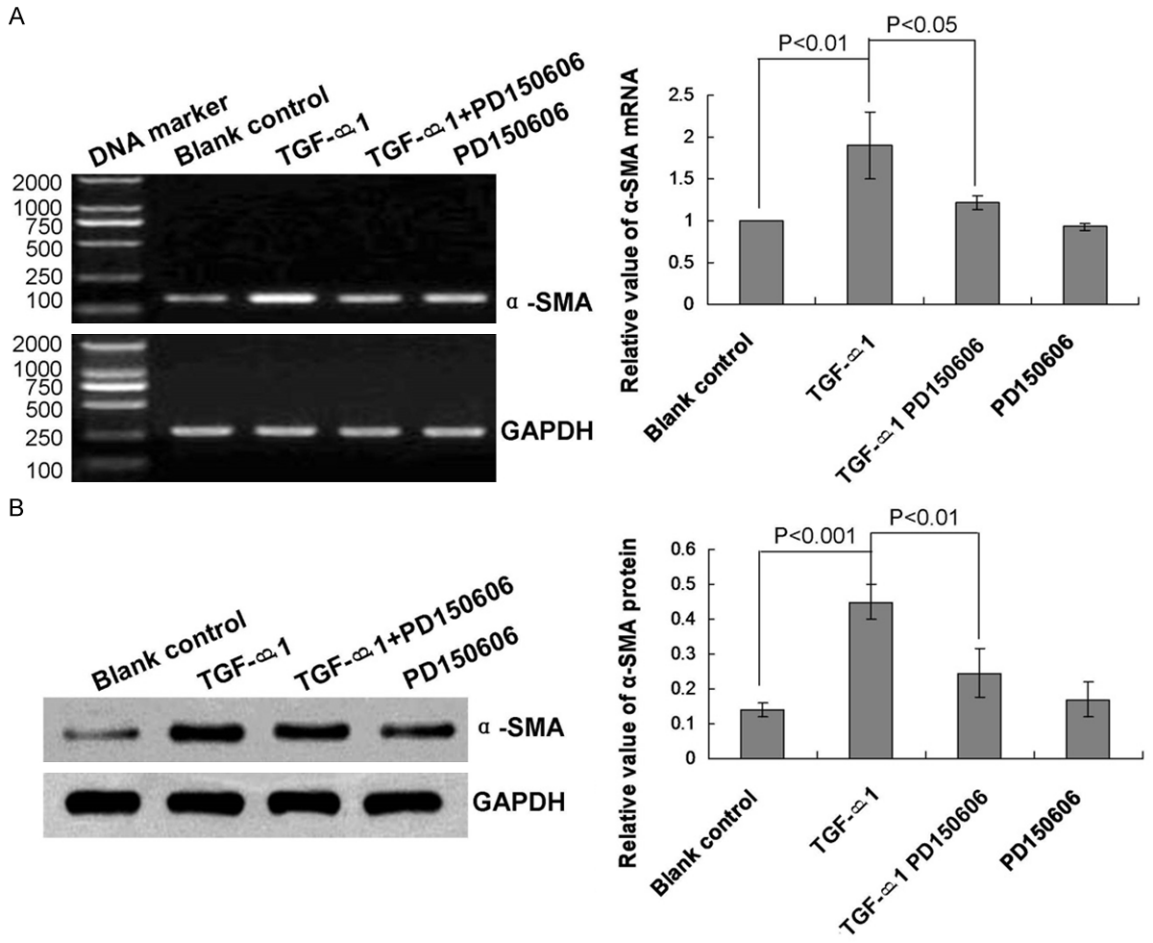

Figure 2. Analysis of α-smooth muscle actin mRNA transcript and protein expression levels in human lung epithelial cells. A. RT-PCR analysis of α-SMA mRNA expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. B. Western blot analysis of α-SMA protein expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. Statistical analyses and *P* values are given in the charts.

protein expression compared with that of the blank control group (**Figure 1B**, $P<0.001$).

TGF-β1 upregulates α-SMA expression

We also tested the effect of TGF-β1-induced EMT on α-SMA transcript and protein expression levels. The results indicated that TGF-β1 treatment significantly increased α-SMA mRNA (**Figure 2A**, $P<0.01$) and protein (**Figure 2B**, $P<0.001$) expression levels compared with that of the blank control group.

TGF-β1 enhances calpain 1 expression

We evaluated calpain 1 expression during TGF-β1-induced EMT to assess its role in lung epithelial cells. The results indicated that TGF-β1 treatment significantly enhanced calpain 1 mRNA (**Figure 3A**, $P<0.01$) and protein (**Figure 3B**, $P<0.001$) expression levels compared with that of the blank control group.

Calpain 1 inhibitor enhances E-cad expression and reduces α-SMA expression

We hypothesized that calpain 1 may be a specific factor associated with TGF-β1-induced downregulation of E-cad, upregulation of α-SMA, and EMT. Therefore, we treated lung epithelial cells with the calpain 1 inhibitor PD 150606 (a calpastatin analog). The results showed that PD 150606 treatment significantly attenuated the enhancement of calpain 1 mRNA and protein expression levels caused by TGF-β1 treatment (**Figure 3A** and **3B**, $P<0.05$). By contrast, treatment with PD 150606 also significantly attenuated the effects of TGF-β1

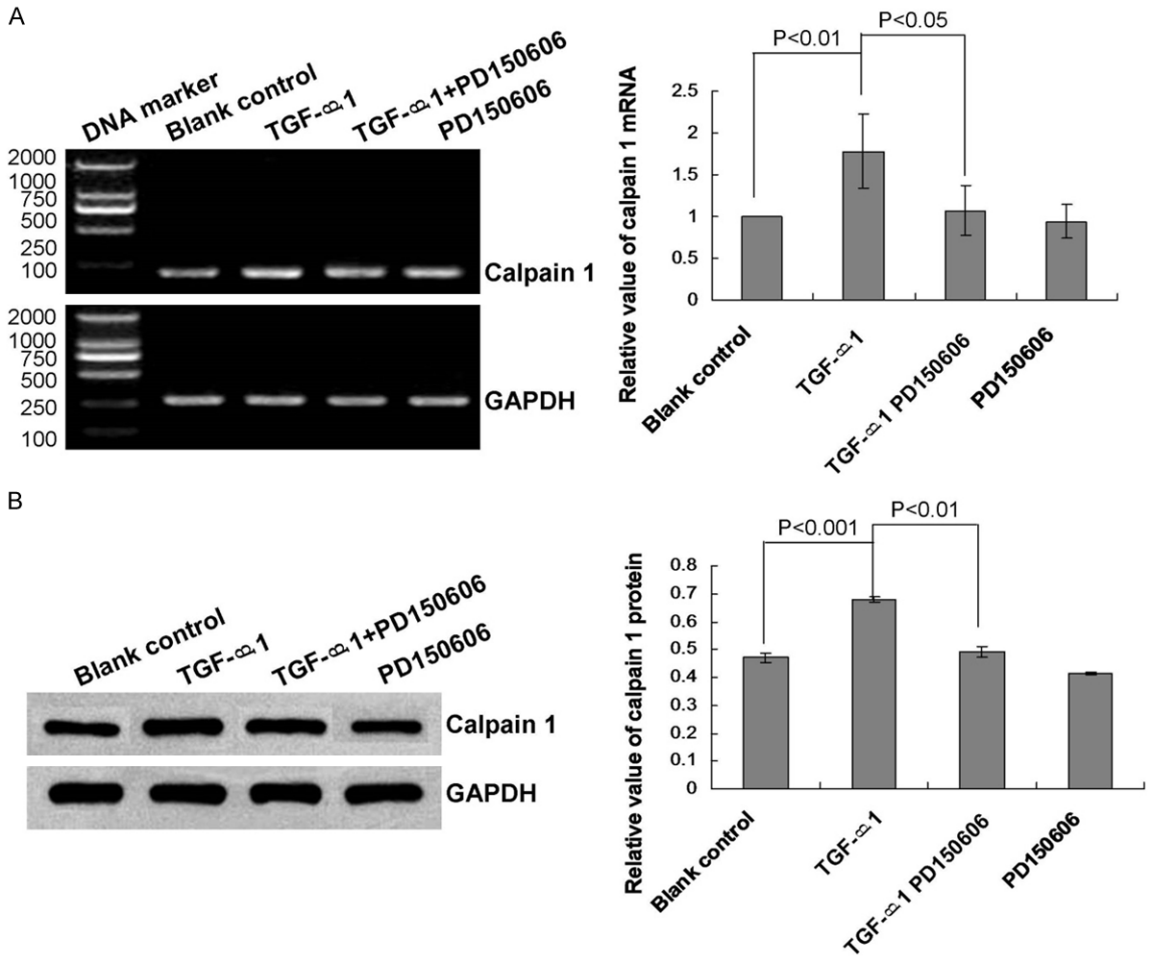

Figure 3. Analysis of calpain 1 mRNA transcript and protein expression levels in human lung epithelial cells. A. RT-PCR analysis of calpain 1 mRNA expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. B. Western blot analysis of calpain 1 protein expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. Statistical analyses and *P* values are given in the charts.

on E-cad (**Figure 1A** and **1B**, $P<0.05$) and α-SMA (**Figure 2A** and **2B**, $P<0.05$) expression levels.

Calpain 1 inhibitor antagonizes TGF-β1-induced increase in Akt/phospho-Akt

We investigated possible mechanisms and signaling pathways involved in human lung cell EMT and TGF-β1-induced changes in calpain 1 expression levels. The results indicated that PD 150606 treatment significantly attenuated TGF-β1-induced increase in Akt/phospho-Akt expression levels (**Figure 4A**, **4D**, and **4E**, $P<0.05$).

We also assessed whether other signaling pathways, such as the ERK and JNK pathways, might be involved in TGF-β1-induced EMT. The results showed that the TGF-β1-induced ETM was not correlated with ERK and JNK signaling (**Figure 4B-E**, $P>0.05$).

Discussion

Epithelial-mesenchymal transition in lung cells has an important role in the transdifferentiation of lung epithelial cells to myofibroblasts [19]. EMT in lung epithelial cells is associated with decreases in E-cad levels and increases in α-SMA levels [10]. Many studies [20-22] have confirmed the critical role of EMT in the development of idiopathic pulmonary fibrosis. EMT has an important role in maintaining cellular homeostasis in the tumor microenvironment, which includes oxidative stress, inflammation, and hypoxia [10]. The human A549 alveolar epithelial cell line is a model that can be used to simulate IPF via TGF-β1-induced cell proliferation and EMT. Previous studies confirmed that

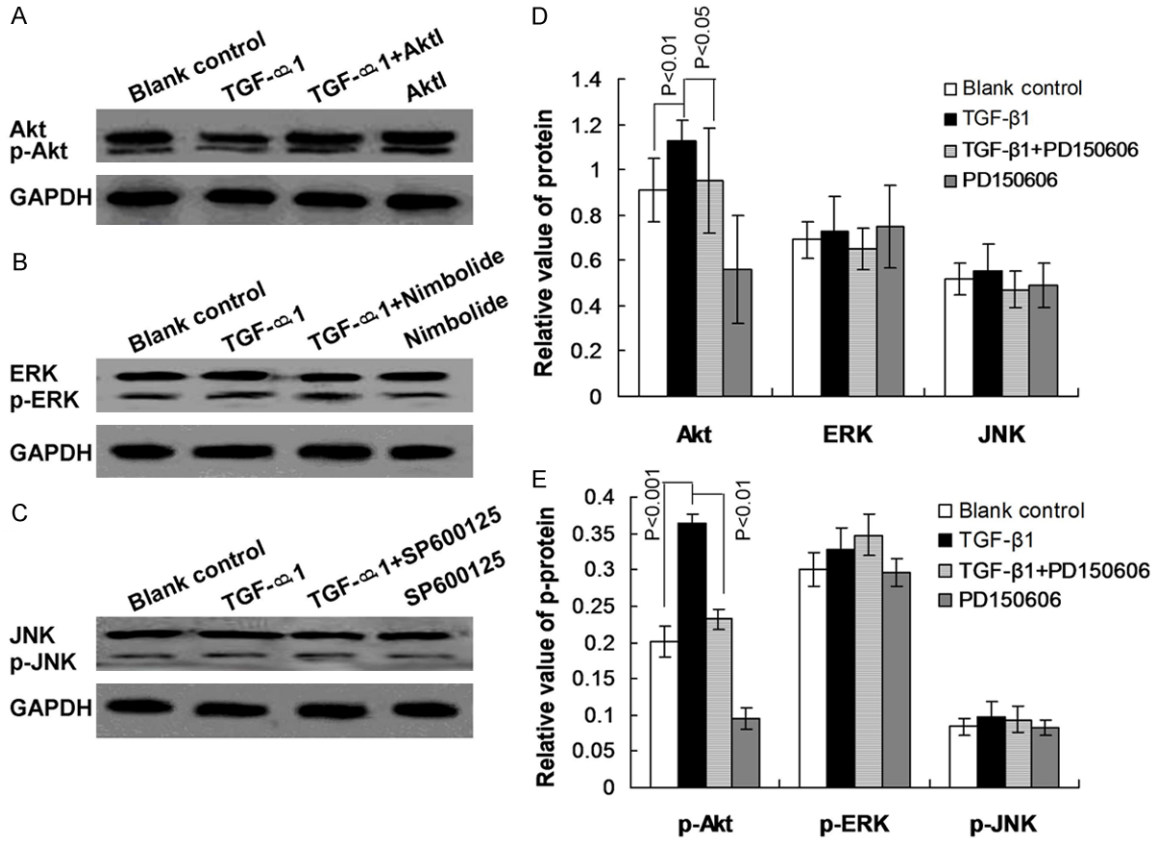

Figure 4. Analysis of Akt, ERK, and JNK signaling pathways during epithelial-mesenchymal transition in human lung epithelial cells. A. Western blot analysis of Akt/phospho-Akt expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. B. Western blot analysis of ERK/phospho-ERK expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. C. Western blot analysis of JNK/phospho-JNK expression in the blank control, or treated with TGF-β1, TGF-β1 + PD 150606, or PD 150606. D. Statistical analysis of Akt, ERK, and JNK expression levels. E. Statistical analysis of phospho-Akt, phospho-ERK, and phospho-JNK expression levels. *P* values are given in the charts.

TGF-β1 induces EMT [23, 24]. EMT has the characteristics of an invasive and migratory cellular phenotype, which is similar to that of tumor cells with increased metastatic potential. We treated A549 cells with TGF-β1 to induce EMT and observed corresponding changes in E-cad, α-SMA, and calpain 1-expression levels.

Calpain is a ubiquitous Ca^{2+}-regulated cysteine protease in many mammals and other organisms. Calpain also has a role in protein modification pathways that are involved in the limited proteolysis of target proteins [25]. Calpain activity is essential for cell mobility, cell cycle progression, cell migration, apoptosis, and necrosis [26, 27]. Recent studies report that calpain has an important role in EMT [28, 29]. Calpain is activated primarily by two different signaling pathways [30]: increased cytosolic free Ca^{2+} concentration and the ERK/MAPK signaling pathway activated by epithelial growth factor (EGF). Our results showed that treatment of A549 cells with TGF-β1 upregulated calpain expression, downregulated E-cad expression, and upregulated α-SMA expression. By contrast, treatment of A549 cells with the calpain inhibitor PD 150606 significantly increased E-cad expression and significantly decreased α-SMA expression. These results suggest that TGF-β1 triggers EMT by upregulating calpain 1 expression. However, the mechanism that mediates TGF-β1-induced increase in calpain 1 expression will be elucidated in future studies.

Other signaling pathways involved in EMT include the phosphoinositide 3-kinase (PI3K)/Akt pathway and the ERK and JKN MAPK pathways [31-34]. We investigated potential roles for the Akt, ERK, and JNK signaling pathways in TGF-β1-induced enhancement of calpain 1 expression during EMT in A549 cells. The results

showed that PD 150606-mediated inhibition of calpain 1 expression significantly downregulated Akt/phospho-Akt expression levels. However, treatment of A549 cells with PD 150606 did not significantly affect ERK/phospho-ERK or JNK/phospho-JNK expression levels, indicating that calpain activity is not involved in ERK and JNK signaling during EMT. This result does not support a previous study, which reported that TGF-β1 regulates EMT by activating ERK and JNK signaling.

In conclusion, this study has shown that calpain 1 could regulate EMT in TGF-β1-treated A549 cells via the PI3K/Akt signaling pathway. This result provides new insights into the cellular mechanisms that mediate EMT in human lung epithelial cells and may provide therapeutic targets for clinical studies on idiopathic pulmonary fibrosis.

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Disclosure of conflict of interest

None.

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References

[1] Duck A, Spencer LG, Bailey S, Leonard C, Ormes J, Caress AL. Perceptions, experiences and needs of patients with idiopathic pulmonary fibrosis. J Adv Nurs 2015; 71: 1055-1065.

[2] Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, Murphy E, Johnston SL, Schwartz DA, Wells AU, Cookson WO, Maher TM, Moffatt MF. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2014; 190: 906-913.

[3] Navaratnam V, Fleming KM, West J, Smith CJ, Jenkins RG, Fogarty A, Hubbard RB. The rising incidence of idiopathic pulmonary fibrosis in the UK. Thorax 2011; 66: 462-467.

[4] Ley B, Collard HR, King TE, King TE. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2010; 183: 431-440.

[5] King TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011; 378: 1949-1961.

[6] Schoenheit G, Becattelli I, Cohen A. Living with idiopathic pulmonary fibrosis: an in-depth qualitative survey of European patients. Chronic Respir Dis 2011; 8: 225-231.

[7] Li FZ, Cai PC, Song LJ, Zhou LL, Zhang Q, Rao SS, Xia Y, Xiang F, Xin JB, Greer PA, Shi HZ, Su Y, Ma WL, Ye H. Crosstalk between calpain activation and TGF-beta 1 augments collagen-1 synthesis in pulmonary fibrosis. Biochim Biophys Acta 2015; 1852: 1796-1804.

[8] Scotton CJ, Hambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest 2007; 132: 1311-1321.

[9] Phan SH. The myofibroblast in pulmonary fibrosis. Chest 2001; 122: 286-289.

[10] Yamada A, Aki T, Unuma K, Funakoshi T, Uemura K. Paraquat induces epithelial-mesenchymal transition like cellular response resulting in fibrogenesis and the prevention of apoptosis in human pulmonary epithelial cells. PLoS One 2015; 10: e0120192.

[11] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014; 15: 178-196.

[12] Willis BC, Borok Z. TGF-beta induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol 2007; 293: 525-534.

[13] Argast GM, Krueger JS, Thomson S, Sujka-Kwok I, Carey K, Silva S, O'Connor M, Mercado P, Mulford IJ, Young GD. Inducible expression of TGFbeta, snail and Zeb1 recapitulates EMT in vitro and in vivo in a NSCLC model. Clin Exp Metastasis 2011; 28: 593-614.

[14] Chapman HA. Epithelial-mesenchymal interactions in pulmonary fibrosis. Annu Rev Physiol 2011; 73: 413-435.

[15] Kage H, Borok Z. EMT and interstitial lung disease: a mysterious relationship. Curr Opin Pulm Med 2012; 18: 517-523.

[16] Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiol Rev 2003; 82: 731-801.

[17] Abe K, Takeichi M. NMDA-receptor activation induces calpain-mediated beta-catenin cleavages for triggering gene expression. Neuron 2007; 53: 387-397.

[18] Glading A, Lauffenburger DA, Wells A. Cotting to the chase: calpain proteases in cell motility. Trends Cell Biol 2002; 12: 46-54.

[19] Li S, Qin X, Li Y, Zhang X, Niu R, Zhang H, Cui A, An W, Wang X. MiR-133a suppresses the migration and invasion of esophageal cancer cells by targeting the EMT regulator SOX4. Am J Transl Res 2015; 7: 1390-1403.

[20] Chapman HA. Epithelial-mesenchymal interactions in pulmonary fibrosis. Annu Rev Physiol 2011; 73: 413-435.
[21] Kage H, Borok Z. EMT and interstitial lung disease: a mysterious relationship. Curr Opin Pulm Med 2012; 18: 517-523.
[22] Liu M, Zhou C, Zheng J. Cigarette smoking impairs the response of EGFR-TKIs therapy in lung adenocarcinoma patients by promoting EGFR signaling and epithelial-mesenchymal transition. Am J Transl Res 2015; 7: 2026-2035.
[23] Ohshio Y, Teramoto K, Hashimoto M, Kitamura S, Hanaoka J, Kontani K. Inhibition of transforming growth factor beta release from tumor cells reduces their motility associated with epithelial-mesenchymal transition. Oncol Rep 2013; 30: 1000-1006.
[24] Li L, Qi L, Liang Z, Song W, Liu Y, Wang Y, Sun B, Zhang B, Cao W. Transforming growth factor beta 1 induces EMT by the transactivation of epithelial growth factor signaling through HA/CD44 in lung and breast cancer cells. Int J Mol Med 2015; 36: 113-122.
[25] Sorimachi H, Hata S, Ono Y. Impact of genetic insights into calpain biology. J Biochem 2011; 150: 23-37.
[26] Cotten SW, Kornegay JN, Bogan DJ, Wadosky KM, Patterson C, Willis MS. Genetic myostatin decrease in the golden retriever muscular dystrophy model does not significantly affect the ubiquitin proteasome system despite enhancing the severity of disease. Am J Transl Res 2013; 6: 43-53.
[27] Van Ba H, Inho H. Significant role of calpain in proliferation/survival of bovine skeletal muscle satellite cells. In Vitro Cell Dev Biol Anim 2013; 49: 785-797.
[28] Wu SM, Lin WY, Shen CC, Pan HC, Keh-Bin W, Chen YC, Jan YJ, Lai DW, Tang SC, Tien HR, Chiu CS, Tsai TC, Lai YL, Sheu ML. Melatonin set out to ER stress signaling thwarts epithelial mesenchymal transition and peritoneal dissemination via calpain-mediated C/EBPbeta and NFkB cleavage. J Pineal Res 2016; 60: 142-154.
[29] Liu SH, Lee WJ, Lai DW, Wu SM, Liu CY, Tien HR, Chiu CS, Peng YC, Jan YJ, Chao TH, Pan HC, Sheu ML. Honokiol confers immunogenicity by dictating calreticulin exposure, activating ER stress and inhibiting epithelial-to-mesenchymal transition. Mol Oncol 2015; 9: 834-849.
[30] Glading A, Chang DA, Lauffenburger DA, Wells A. Epithelial growth factor receptor activation of calpain is required for fibroblast motility and occurs via an ERK/MAP kinase signaling pathway. J Biol Chem 2000; 275: 2390-2398.
[31] Steelman LS, Chappell WH, Abrams SL, Kempf RC, Long J, Laidler P, Mijatovic S, Maksimovic-Ivanic D, Stivala F. Role of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging (Albany NY) 2011; 3: 192-222.
[32] Yeh YH, Wang SW, Yeh YC, Hsiao HF, Li TK. Rhapontigenin inhibits TGF-beta mediated epithelial mesenchymal transition via the PI3K/Akt/mTOR pathway and is not associated with HIF-1 alpha degradation. Oncol Rep 2016; 35: 2887-2895.
[33] Watanabe-Takano H, Takano K, Hatano M, Tokuhisa T, Endo T. DA-Raf-mediated suppression of the Ras-ERK pathway is essential for TGF beta 1 induced epithelial-mesenchymal transition in alveolar epithelial type 2 cells. PLoS One 2015; 10: e0127888.
[34] Epstein Shochet G, Tartakover-Matalon S, Drucker L, Pasmanik-Chor M, Pomeranz M, Fishman A, Lishner M. Placenta-breast cancer cell interactions promote cancer cell epithelial mesenchymal transition via TGF-beta/JNK pathway. Clin Exp Metastasis 2014; 31: 961-975.
[35] Cao J, Zhang X, Wang Q, Wang X, Jin J, Zhu T, Zhang D, Wang W, Li X, Li Y, Shen B, Zhang J. Cyclic AMP suppresses TGF-beta mediated adaptive Tregs differentiation through inhibiting the activation of ERK and JNK. Cell Immunol 2014; 285: 42-48.
[36] Liu X, Hubchak SC, Browne JA, Schnaper HW. Epidermal growth factor inhibits transforming growth factor beta induced fibrogenic differentiation marker expression through ERK activation. Cell Signal 2014; 26: 2276-2283.