



TRIM37 inhibits PDGF-BB-induced proliferation and migration of airway smooth muscle cells

Ying Dai^{*,1}, Ying Li¹, Ruiduo Cheng, Jie Gao, Yanyang Li, Chunyan Lou

Department of Pediatrics, Henan University Huaihe Hospital, Kaifeng, 475000, Henan Province, PR China

ARTICLE INFO

Keywords:

Asthma
TRIM37
Airway smooth muscle cells (ASMCs)
Proliferation
Migration

ABSTRACT

Tripartite motif 37 (TRIM37) belongs to the TRIM family of proteins and has been reported to be involved in the progression of asthma. However, the effects of TRIM37 on airway smooth muscle cells (ASMCs) proliferation and migration are still unknown. This study aimed to investigate the effects of TRIM37 on cell proliferation and migration in platelet-derived growth factor BB (PDGF-BB)-stimulated ASMCs, and the potential molecular mechanisms was also explored. Our data demonstrated that the expression of TRIM37 was significantly decreased in ASMCs stimulated with PDGF-BB. In addition, overexpression of TRIM37 efficiently suppressed PDGF-BB-induced ASMCs proliferation and migration. Furthermore, overexpression of TRIM37 obviously inhibited the protein expression levels of β -catenin, c-Myc and cyclinD1 in PDGF-BB-stimulated ASMCs. The Wnt/ β -catenin pathway activator LiCl significantly reversed the inhibitory effects of TRIM37 on cell proliferation and migration in PDGF-BB-stimulated ASMCs. Taken together, these results demonstrate that TRIM37 inhibits the proliferation and invasion of ASMCs cultured with PDGF-BB through suppressing the Wnt/ β -catenin signaling pathway.

1. Introduction

Childhood asthma is one of the most common chronic diseases in childhood, and the prevalence has been increasing in the past years [1]. It is characterized by airway inflammation, reversible airway obstruction, hyperresponsiveness and remodeling [2]. The proliferation and migration of airway smooth muscle cells (ASMCs) play important roles in the pathogenesis of asthma [3]. Increased ASMCs proliferation and migration are responsible for the change of airway smooth muscle thickness and contribute to the progression of airway remodeling [4]. In addition, several studies showed that the level of platelet-derived growth factor BB (PDGF-BB) was significantly up-regulated in asthmatic tissues [5,6]. PDGF-BB could induce ASMCs proliferation and migration, resulting in the progression of asthma [7]. Previous studies showed that PDGF-BB treatment can promote the proliferation and migration of ASMCs through activating various signaling pathways, such as the PI3K/Akt, ERK and JAK/STAT pathways [8–10]. Therefore, blocking of PDGF-BB-induced ASMCs proliferation and migration represents a potential therapeutic strategy for the treatment of asthma.

The tripartite motif (TRIM) protein family is composed of more than 70 members and is characterized by three zinc-binding domains, a

RING, a B-box type 1, and a B-box type 2, followed by a coiled-coil region. TRIM proteins play diverse physiological and pathological roles in regulating cell cycle, migration, autophagy and innate immunity [11–13]. Tripartite motif 37 (TRIM37) belongs to the TRIM family of proteins and located in the 17q23 chromosomal region. Emerging studies have indicated that TRIM37 plays a critical role in the development and progression of cancer [14–16]. In addition, TRIM37 was reported to be involved in the progression of asthma. Shin *et al.* reported that the expression of TRIM37 was significantly down-regulated in the peripheral blood mononuclear cells of patients with asthma [17]. However, the effects of TRIM37 on ASMCs proliferation and migration are still unknown. This study aimed to investigate the effects of TRIM37 on cell proliferation and migration in PDGF-BB-stimulated ASMCs, and the potential molecular mechanisms was also explored.

2. Materials and methods

2.1. Cell culture

Human ASMC line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's

* Corresponding author at: Department of Pediatrics, Henan University Huaihe Hospital, 115 Ximen Street, Kaifeng 475000, Henan Province, PR China.

E-mail address: daiyingkf@126.com (Y. Dai).

¹ Authors contributed equally to this work.

modified Eagle's medium (DMEM; Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin and 100 µg/ml of streptomycin (Sigma). The cells were incubated at a humidified atmosphere at 37 °C in a 5%-CO₂ incubator. ASMCs in passages 4 to 9 were used.

2.2. Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from ASMCs using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNA was synthesized using M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA). qPCR was performed on the ABI prism 7000 sequence detection system (Applied Biosystems, Eugene, OR, USA) using the SYBR Green PCR Master Mix (Applied Biosystems). The specific primers were as follows: TRIM37, forward: 5'-AACAGAGCGTGGAGAGCATT-3' and reverse 5'-CTTCTGCCCAACGACAATTT-3'; and β-actin, forward: 5'-AGAAAATCTGGCACCACACC-3', reverse: 5'-TAGCACAGCTGGATAGCAA-3'. The PCR procedure was as follows: 94 °C for 3 min; 94 °C for 20 s, 55 °C for 30 s; 72 °C for 25 s; 2 s for plate reading for 35 cycles; and a melting curve from 65 to 95 °C. Gene expression was normalized to β-actin, and the relative mRNA levels were calculated by the method of 2^{-ΔΔCt} [18].

2.3. Western blot

Cells were homogenized and lysed using RIPA lysis buffer (Beyotime Biotech. CO., China). Protein extracts (30 µg of total protein/sample) were separated by 12% SDS-PAGE and transferred to the polyvinylidene difluoride (PVDF) membranes (Sigma, St. Louis, MO, USA). The membranes were blocked with 5% defatted milk in TBST buffer for 1 h at room temperature before hybridization with anti-TRIM37, anti-β-catenin, anti-c-Myc, anti-cyclinD1 and anti-GAPDH antibodies (Santa Cruz, CA, USA) overnight at 4 °C. After washing five times in TBST, the membranes were incubated with horseradish peroxidase conjugated secondary antibodies for 1 h at room temperature. Finally, the protein bands were visualized using enhanced chemiluminescence reagents (Bio-Rad, Hercules, CA).

2.4. Construction of the pcDNA3.1-TRIM37 vector and cell transfection

Full-length human TRIM37 was generated from human peripheral blood mononuclear cells (PBMC) cDNA by PCR and cloned into pcDNA3.1 vector. For *in vitro* transfection, ASMCs (1 × 10⁵ cells/well) were transfected with pcDNA3.1-TRIM37 or empty vector using Lipofectamine™2000 (Invitrogen), respectively, according to the manufacturer's protocols.

2.5. Cell proliferation assay

Cell proliferation was evaluated using the MTT assay. Briefly, ASMCs at a density of 1 × 10⁵ cells/well were plated into a 96-well plate. The cells were treated with or without PDGF-BB in the presence or absence of TRIM37 for 24 h. Then, 20 µl of MTT solution (0.2 mg/ml, Sigma) was added to each well, and the cells were further incubated in 5% CO₂ at 37 °C for 4 h. Subsequently, the medium was removed and 150 µl of dimethyl sulfoxide (DMSO; Sigma) was added to each well to solubilize the formazan crystals. The absorbance was assessed at 490 nm using a microplate reader (Invitrogen).

2.6. Assessment of cell proliferation via BrdU assay

ASMCs (1 × 10⁵ cells/well) were transfected with pcDNA3.1-TRIM37 or empty vector in the presence or absence of PDGF-BB (10 nM) for 24 h. Cell proliferation was assessed by a BrdU-based Cell Proliferation ELISA according to the manufacturer's protocol (Roche Applied Science, Mannheim, Germany). The relative proliferation ratio

was absorbance of the treated ASMCs to the absorbance of control ASMCs. BrdU incorporation of the control group was set to 100%.

2.7. Cell migration assay

Cell migration was evaluated using the Transwell migration assay. In brief, ASMCs (1 × 10⁵ cells/well) transfected with TRIM37 were resuspended in 200 µl of serum-free DMEM and added into the upper compartment. 500 µl of DMEM medium containing 10% FBS with or without PDGF-BB was added into the lower chamber. After 24 h, cells on the upper membrane surface were removed with cotton-tipped swabs, and those that had migrated to the lower surface of the filter were fixed in methanol and stained with 0.05% crystal violet for 15 min. The average number of migrated cells from six random optical fields was evaluated under a Leica microscope (Leica Microsystems GmbH, Wetzlar, Germany).

2.8. Data analysis

All data are expressed as mean ± SD. Statistical analysis involved using the Student's *t* test for comparison of 2 groups. Multiple comparisons were assessed by one-way ANOVA followed by Dunnett's tests. Values of *P* < 0.05 were regarded as statistically significant.

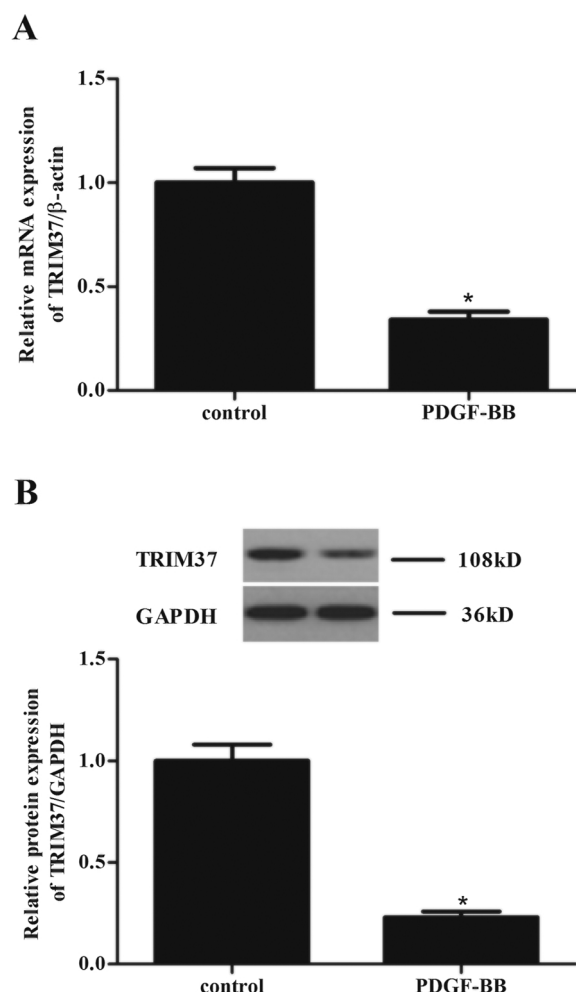


Fig. 1. TRIM37 is down-regulated in PDGF-BB-stimulated ASMCs. Human ASMCs were treated with PDGF-BB (10 nM) for 24 h. (A) The mRNA expression level of TRIM37 was measured using qRT-PCR analysis. (B) The protein expression level of TRIM37 was measured using western blot analysis. GAPDH served as a loading control. Data were expressed as means ± SD. **P* < 0.05 compared with the control group.

Download English Version:

<https://daneshyari.com/en/article/8525371>

Download Persian Version:

<https://daneshyari.com/article/8525371>

[Daneshyari.com](https://daneshyari.com)