

## Original Article

# Pituitary tumor transforming gene PTTG2 induces psoriasis by regulating vimentin and E-cadherin expression

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Received July 6, 2015; Accepted August 21, 2015; Epub September 1, 2015; Published September 15, 2015

**Abstract:** Psoriasis is a common and intractable skin disease affecting the physical and mental health of patients. This study focused on the roles of pituitary tumor transforming gene 2 (PTTG2) in psoriasis. Using real-time quantitative PCR and western blot, the expression patterns of PTTG2 were compared in psoriatic epidermis cells and normal cells, from both mRNA levels and protein levels. Knockdown of PTTG2 by siRNA was conducted in HaCaT cells to investigate the changes in cell viability and migration in vitro. Expression changes of vimentin and E-cadherin were also detected in the transfected cells. Results showed PTTG2 was significantly overexpressed in the psoriatic epidermis cells ( $P < 0.05$ ). The cell viability and migration were inhibited by the knockdown of PTTG2. Besides, knockdown of PTTG2 resulted in down-regulation of vimentin and up-regulation of E-cadherin, with significant differences compared to the siRNA control group ( $P < 0.05$ ). This study indicated the involvement of PTTG2 in mediating epidermis cell viability and migration and in pathogenesis of psoriasis. PTTG2 might be a potential therapeutic target for psoriasis through inducing epithelial-to-mesenchymal transition (EMT) via regulating the expression of vimentin and E-cadherin.

**Keywords:** Psoriasis, PTTG2 (pituitary tumor transforming gene 2), vimentin, E-cadherin, EMT (epithelial-to-mesenchymal transition)

## Introduction

Psoriasis is a common inflammatory skin disease affecting 2-4% of the general population in western countries [1]. Now it is considered that psoriasis is not purely a skin disease and can lead to comorbidities, such as cardiovascular diseases, psoriatic arthritis and associated depressive illness [2], which means psoriatic patients may suffer both physically and mentally. Psoriasis can be caused by genetic factors triggered by environmental elements [3]. Existed studies have proved some genes related to psoriasis. Genome-wide scans have found the psoriasis susceptibility 1 locus that is associated with psoriasis within the major histocompatibility complex class I region on chromosome 6 [4]. T helper cells Th1 and Th17 exist in psoriatic skin and interleukin-17 plays regulatory roles [5]. Some factors, like cystatin, can cause the genetically determined skin barrier dysfunction contributing to psoriasis development [6, 7]. Though related targeted immune

therapies are being studied, the detailed mechanisms have not been fully understood.

Pituitary tumor transforming genes (PTTGs) are proto-oncogenes that are expressed abnormally in human cancers [8, 9]. They are attracting increasing attentions these years. One of the PTTG isoforms, PTTG1, has been proved to associate with psoriasis. Its mRNA level in psoriatic epidermis cells is about five folds more than that in normal cells [10]. Besides, PTTG1 protein level is increased in the epidermis of psoriasis, and it may participate in the pathophysiology of psoriasis via modulating the production of some angiogenic or proinflammatory cytokines [11]. Unlike PTTG1, PTTG2 does not bind to separase [12, 13], and its mRNA expresses at lower levels in human tissues [14] and can be detected in both normal and tumorous pituitary [15]. But PTTG2 mRNA has been detected to be up-regulated in the malignant progression of glioma by microarray analysis [16]. Generally, PTTG2 is considered to be a

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regulator of the cell circadian clock [17], functioning in cell mitosis, differentiation and apoptosis. Still, little is known about its functions in epidermis hyperplasia diseases, such as psoriasis.

The aim of this study was to determine the potential roles of PTTG2 in psoriasis. The expression pattern of PTTG2 was detected and compared in psoriatic epidermis cells and in normal cells. Then cell viability, migration ability and expression changes of related factors, namely vimentin and E-cadherin, were analyzed after PTTG2 was down-regulated by its specific siRNA in the HaCaT cell line, which was used as a psoriasis model in vitro. These data provided evidence that PTTG2 played important roles in the pathogenesis of psoriasis, implicating its potential for being a therapeutic target of psoriasis.

### Materials and methods

#### *Culture of epidermal keratinocytes*

Epidermis samples were peeled off from the dermis of normal persons and psoriatic patients at the hospital. The samples were incubated in 0.25% (w/v) trypsin (Sigma-Aldrich, Shanghai, China) for 10 min, neutralized by 10% (v/v) fetal bovine serum (FBS) (Sigma-Aldrich), and then centrifuged at 1,000 rpm. The keratinocytes were cultured at 37°C in a 5% (v/v) CO<sub>2</sub> humidified atmosphere in keratinocyteserum-free medium with keratinocyte growth factor until they reached confluence of 65%.

#### *RNA extraction and qRT-PCR*

The extraction and purification of RNA were performed with Trizol reagent (Invitrogen, Auckland, CA, USA). First-strand cDNA was synthesized from 2 µg total RNA using SuperScript II Reverse Transcriptase (Invitrogen). Real-time quantitative PCR (qRT-PCR) was conducted in 20 µL reacting systems with 20 ng template and SYBR GREEN PCR Master Mix (Applied Biosystems, Foster City, CA, USA). *GAPDH* was used as the internal reference (forward 5'-GAGGTGAAGGTCGGAGTCAAC-3' and reverse 5'-CAGAGTAAAAGCAGCCCTGGT-3'). A pair of specific primers was designed to amplify PTTG2 (forward 5'-TAGTGCTTTGGAGTTTGTGTGT-3' and reverse 5'-AACAAAGGACAGCTGAGACA-3').

#### *Western blot*

Cells (untreated and transfected for 48 h) were collected and lysed in lyses buffer and the

extracts were centrifuged at 12,000 rpm for 5 min. Cell protein concentration was determined by Bradford method [18]. A total of 80 µg protein for each sample was boiled in loading buffer for 5 min to denature. Then the protein samples were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The blot was blocked by 5% skim milk in TBST (TBS containing 0.1% Tween 20) for 2 h at room temperature and then incubated with anti-PTTG2, anti-actin, anti-vimentin or anti-E-cadherin antibodies (Abgent, San Diego, USA) overnight at 4°C. After washed for three times in TBST, the blot was incubated with the horseradish peroxidase-conjugated second antibody in TBST for one h. Protein levels were normalized to the β-actin protein level. The relative protein expression quantity was analyzed by a densitometer.

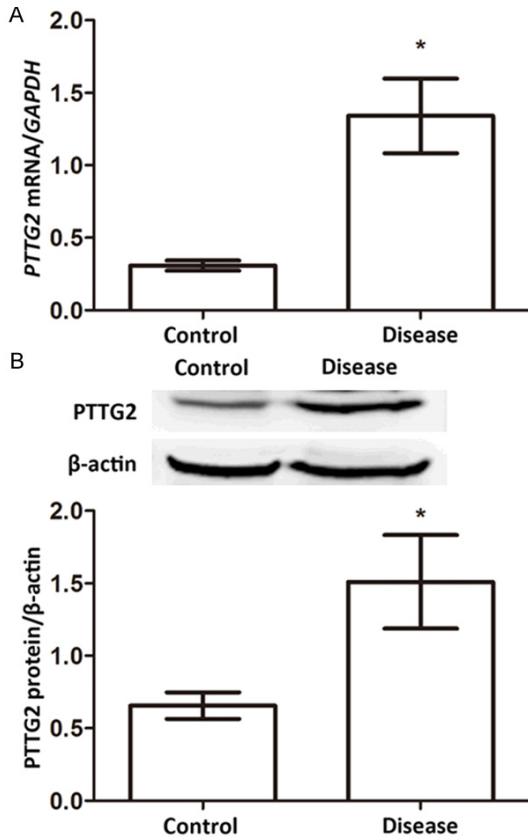
#### *Culture of HaCaT cells and transfection*

The HaCaT cell line was used as a model of psoriasis and the cells transformed keratinocytes from histologically normal human skin spontaneously. The cells were cultured as monolayer in Dulbecco's Modified Eagle Medium (DMEM) high glucose with 2 mM L-glutamine, 10% FBS and 1% antibiotic, at 37°C with 5% CO<sub>2</sub>. Cells were passaged two times by tripsinization per week. The PTTG2-specific siRNA (PTTG2-siRNA), scrambled siRNA, Lentivector and piLenti-siRNA-GFP were obtained from Applied Biological Materials Inc. (Richmond, BC, Canada). *E. coli* DH5α was used to amplify the Lentivector. Lentivirus packaging and HaCaT infection were conducted based on the manuals. Infected cells with stable PTTG2 knock-down were selected by 2 µg/mL puromycin (Invitrogen) and then maintained in DMEM with 0.5 µg/mL puromycin and 10% FBS.

#### *Cell viability analysis*

After transfected by PTTG2-siRNA for 72 h, the viability of HaCaT cells was measured using Vybrant MTT Cell Proliferation Assay Kit (Invitrogen) according to the manuals. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well and the cells were incubated for 4 h at 37°C. Then media was removed and the cells were lysed by dimethyl sulfoxide (Sigma-Aldrich). Absorbance was measured at 570 nm by microplate reader SpectraMax M2e (Bucher Biotec, Basel, Switzerland). All data was measured in triplicate.

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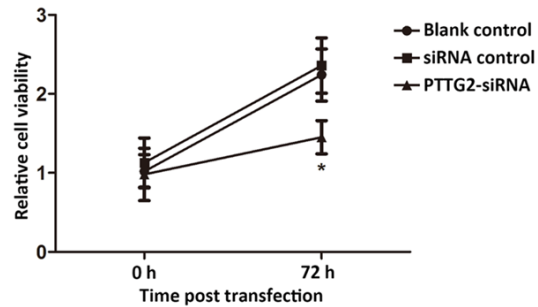
**Figure 1.** Expression levels of PTTG2 in psoriatic epidermis cells. A: qRT-PCR results showed the mRNA expression level of PTTG2 in psoriatic epidermis cells compared to normal cells, with GAPDH mRNA as the internal control; B: Western blot showed the protein expression level of PTTG2 in psoriatic epidermis cells compared to normal cells, with  $\beta$ -actin protein as the internal control. The signal strengths of western blot were indicated in the histogram below. \*indicated the difference was significant ( $P < 0.05$ ).

### Cell migration analysis

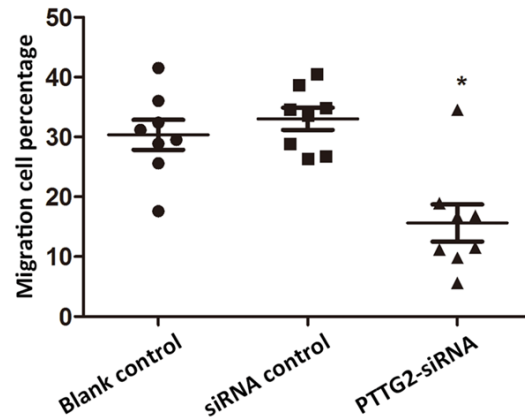
The HaCaT cells were cultured in six-well plates to more than 90% confluency and transfected. Scratches were made on the plates. Then the plates were carefully washed for two times with serum-free medium, and fresh culture medium was added to the plates. After 48 h, photographs were randomly taken from ten visual fields. The mean percentages of migrated cells were analyzed.

### Statistical analysis

All data were first tested for the normal distribution using one-sample K-S test. Enumeration data were analyzed by chi-square or rank-sum test, and measurement data were tested by Student's *t*-test (for two groups) or ANOVA (analysis of variance, for more than three groups).



**Figure 2.** Relative cell viabilities of PTTG2-knock-down cells. The cell viability of blank control, siRNA control and PTTG2-siRNA groups was detected at 0 h post transfection and 72 h post transfection. \*indicated the significant difference between viability of the PTTG2-siRNA group and siRNA control ( $P < 0.05$ ).



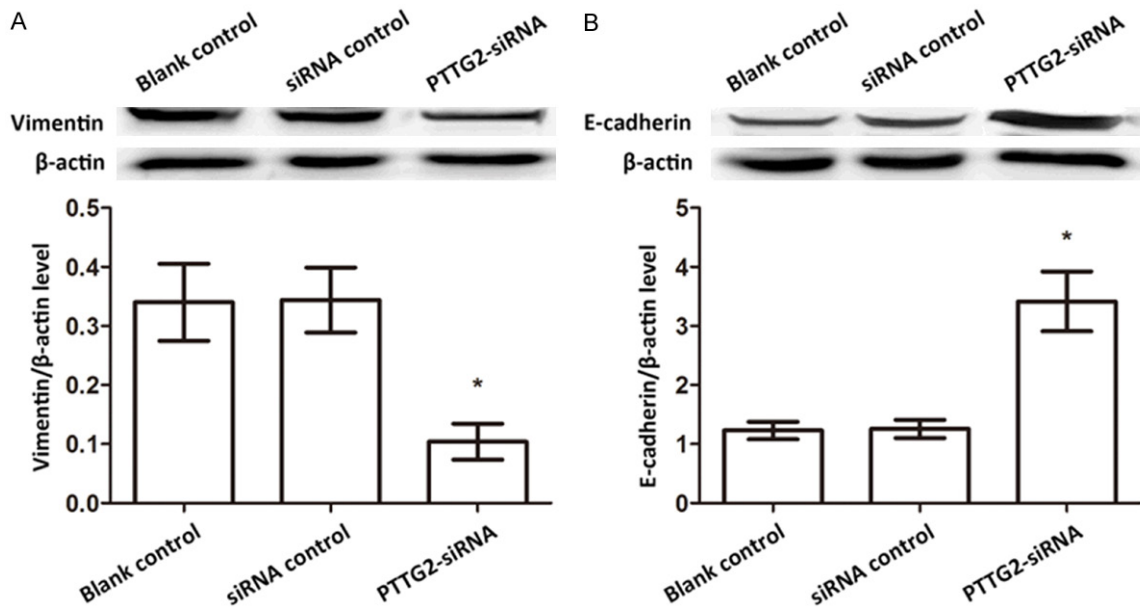
**Figure 3.** Migration cell percentages in transfected cells. The percentage of migrated cells in blank control, siRNA control and PTTG2-siRNA groups was detected at 48 h after transfection. \*indicated the significant difference between migrated cell percentage of the PTTG2-siRNA group and siRNA control ( $P < 0.05$ ).

Between-group-comparison was performed by post-hoc Tukey's test. The difference was defined statistically significant if  $P < 0.05$ .

## Results

### PTTG2 overexpressed in psoriatic epidermis cells

The expression of PTTG2 was detected from both the mRNA level and the protein level in psoriatic epidermis cells and normal cells. qRT-PCR results showed the expression of PTTG2 mRNA was significantly higher ( $P < 0.05$ ) in psoriatic epidermis cells, almost three times of that in normal cells (**Figure 1A**). Western blot showed similar results as to the protein levels of PTTG2 (**Figure 1B**), which was also signifi-



**Figure 4.** Regulation of PTTG2 on the protein levels of vimentin and E-cadherin detected by western blot. A: The relative expression levels of vimentin protein in blank control, siRNA control and PTTG2-siRNA groups; B: The relative expression levels of E-cadherin protein in blank control, siRNA control and PTTG2-siRNA groups.  $\beta$ -actin was used as the internal reference. The signal strengths of western blot were indicated in the histogram below. \*indicated the significant difference of protein expression levels between the PTTG2-siRNA group and siRNA control ( $P < 0.05$ ).

cantly higher in psoriatic epidermis cells ( $P < 0.05$ ). These results obviously indicated that PTTG2 was overexpressed at both the mRNA level and the protein level in psoriatic epidermis cells, which could be a sign of psoriasis.

#### Knockdown of PTTG2 inhibited HaCaT cell viability

At 72 h after transfection, the viability of HaCaT cells was detected with MTT method. Compared to the blank control group, the cell viability of siRNA control group did not change significantly (**Figure 2**). But the viability of cells transfected with PTTG2-siRNA was decreased after 72 h, showing significant difference compared to the siRNA control ( $P < 0.05$ ). These results indicated knockdown of PTTG2 could inhibit the viability of HaCaT cells, implying the involvement of PTTG2 in regulating the proliferation ability and viability of epidermis cells.

#### Knockdown of PTTG2 inhibited cell migration

The percentage of migrated cells was detected at 48 h post transfection. Cell migration of blank control and siRNA control showed no difference (**Figure 3**). But transfected cells exhibited lower migration percentage compared to siRNA control, with significant difference ( $P < 0.05$ ). These results indicated knockdown of

PTTG2 could inhibit HaCaT cell migration. So PTTG2 might participate in the regulation of epidermis cell migration.

#### PTTG2 regulated the expression of E-cadherin and vimentin

Two possible downstream molecules of psoriasis, vimentin and E-cadherin (epithelial-cadherin), were selected to analyze the related mechanism between PTTG2 and psoriasis. Western blot results showed that the protein level of vimentin was significantly down-regulated in transfected cells compared to the siRNA control ( $P < 0.05$ ) (**Figure 4A**). Unlike vimentin, the expression of E-cadherin protein was increased after the knockdown of PTTG2, exhibiting significant difference compared to siRNA control ( $P < 0.05$ ) (**Figure 4B**). Since knockdown of PTTG2 could result in lower vimentin levels and higher E-cadherin levels, interfering with-PTTG2 might affect the pathogenesis of psoriasis by regulating related factors, like E-cadherin and vimentin.

#### Discussion

In this study, PTTG2 is proved to function in psoriasis regulation, possessing significant higher expression levels in psoriatic cells than in normal cells. Knockdown of PTTG2 can inhibit

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it the viability and migration of HaCaT cell, and result in down-regulation of vimentin and up-regulation of E-cadherin. PTTG2 is likely to induce psoriasis and play key roles in the regulatory system of psoriasis. This study provides references for future research on PTTG molecules and psoriasis.

Cell cycle regulatory factors can be involved in the regulation of psoriasis. Nuclear factor- $\kappa$ B mediates pathogenesis of psoriasis by regulating survivin [19]. Expression levels of cyclin D1 and proliferating cell nuclear antigen are suppressed following treatment of psoriasis [20, 21]. PTTG1 is also a crucial cell cycle regulator which promotes cancer cell proliferation [22] and participates in psoriasis pathogenesis [10]. PTTG2 is an intronless homolog of PTTG1 [15] and may possess homologous functions as PTTG1. Its function in regulating psoriasis has not been reported yet. In this study, PTTG2 was detected overexpressing in the epidermis cells of psoriatic patients, which implied the association of PTTG2 with psoriasis pathogenesis. Besides, interfering with PTTG2 would affect the viability and migration ability of HaCaT cells, indicating PTTG2 could promote the viability and migration of epidermis cells, and down-regulation of PTTGs might suppress psoriasis pathogenesis. These data further verified that, as an important cell cycle regulator, PTTG2 played vital roles in controlling the proliferation of psoriasis cells. Therefore, we speculated that PTTG2 silencing might suppress psoriasis progression through inhibiting cell proliferation and migration ability.

E-cadherin, encoded by CDH1 gene, is an important glycoprotein of the classical cadherin members. Generally, E-cadherin mediates cell adhesion in normal adult epithelial tissues, and loss of it can be a marker of epithelial-to-mesenchymal transition (EMT) [23], whose uncontrolled status is considered to lead to tumor invasion and metastatic spread [24]. Existing studies have found the down-regulation of E-cadherin in the granular layer, upper spinous layer and basal layer of psoriasis lesional tissues [25, 26]. Vimentin is one of the intermediate filaments (IFs) members, which usually serves as a major mesenchymal marker expressed mainly in EMT. It has been proved to execute positive regulation of EMT via up-regulating several EMT-linked genes [27, 28]. Besides, the expression level of autoantibodies targeting mutated citrullinated vimentin is high-

er in patients with psoriatic arthritis than in patients without joint symptoms [29]. Since studies have found both E-cadherin and vimentin can be involved in EMT, and increased vimentin expression and decreased E-cadherin expression are related to the cell invasion pattern of cervical squamous cell cancer [30]. The two factors are also proved to be negatively correlated in EMT of diseases, like hepatocellular carcinoma [31] and oral squamous cell carcinomas [32], with higher vimentin expression and lower E-cadherin expression than normal tissues. So the negative correlation of vimentin and E-cadherin expression levels found in this study implied the down-regulation of PTTG2 might connect with EMT. Based on the former studies, decreased expression of vimentin and increased expression of E-cadherin could be an indicator that EMT was at normal levels in HaCaT cells with the knockdown of PTTG2. Together with the observations in psoriatic epidermis cells where PTTG2 was overexpressed, it could be deduced that PTTG2 could induce abnormal EMT by regulating vimentin and E-cadherin, thus causing psoriasis.

Interestingly, among the few studies on PTTG2, some results have found distinct cellular roles between PTTG2 and PTTG1. For example, microarray-based gene expression analysis results show knockdown of PTTG2 leads to the down-regulation of E-cadherin and the up-regulation of vimentin levels, differing from the function of PTTG1 [12], which can induce EMT via regulating related factors [33, 34]. However, in this present study, PTTG2 silencing could down-regulate vimentin but up-regulate E-cadherin level, which might be a sign of EMT inhibition. Usually, EMT is indicated to be accompanied with enhanced cell migration by many proofs [35, 36], and HaCaT cell migration was inhibited by PTTG2 knockdown in this study. So PTTG2 seemed to resemble PTTG1 as to the regulation of vimentin and E-cadherin, even EMT. Still, the structural and functional relationship among PTTG molecules needs to be investigated in order to get a profound knowledge about the regulatory mechanisms of diseases.

In conclusion, this study demonstrates the relationship of PTTG2 and psoriasis. PTTG2 can mediate cell viability and migration, and induce EMT through regulating the expression of E-cadherin and vimentin, thus causing psoriasis. These results provide evidence for the usage of cell cycle regulatory factor, especially PTTG2,

as promising targets for psoriasis treatment. Further studies are still necessary to reveal the intrinsic mechanisms between pathogenesis of psoriasis and crucial regulatory factors.

### Disclosure of conflict of interest

None.

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