RESEARCH ARTICLE

TL1A mediates fibroblast-like synoviocytes migration and Indian Hedgehog signaling pathway via TNFR2 in patients with rheumatoid arthritis

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To cite this article: Al-Azab M, Wei J, Ouyang X, Elkhider A, Walana W, Sun X, Tang Y, Wang B, Li X. TL1A mediates fibroblast-like synoviocytes migration and Indian Hedgehog signaling pathway via TNFR2 in patients with rheumatoid arthritis. *Eur. Cytokine Netw.* 2018; 29(1): 27-35 doi:10.1684/ecn.2018.0405

ABSTRACT. Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joints inflammation. One of the aggressive characteristics of RA fibroblast-like synoviocytes (FLS) is the tendency for migration in the local environment, which plays a central role in the RA pathogenesis. Tumor Necrosis Factor (TNF)-like cytokine 1A (TL1A) is a member of TNF superfamily, which has a role in autoimmunity and influences the RA-FLS behavior through TNF receptor 2 (TNFR2). We investigated the effect of TNF-like cytokine 1A (TL1A) on RA-FLS migration using patients' samples. Specifically, we examined the hedgehog signaling pathway which is a key regulator in chondrocyte growth and differentiation. We found that TL1A increased significantly the hedgehog homologue Indian hedgehog (IHH) and its receptor Patched 1, 2 (PTCH 1, 2) in RA-FLS. In addition, TL1A-stimulated RA-FLS promoted significantly IHH protein expression. However, both mRNA and protein levels decreased substantially after blocking TL1A with TNFR2 antagonist. The migratory property of RA-FLS was enhanced after stimulation of RA-FLS with TL1A, but was compromised following TL1A blockage. In conclusion, our study has revealed that TL1A modulated RA-FLS migration and Indian hedgehog signaling pathway using TNFR2.

Keywords: rheumatoid arthritis, TNF-like cytokine 1A, fibroblast-like synoviocytes, Indian hedgehog, patched1, 2 receptors, TNF-receptor2

Rheumatoid arthritis (RA) is a common autoimmune chronic inflammatory disease which affects joints. The majority of research outputs from molecular studies and clinical reports indicate the involvement of autoreactivity of immune system and dysregulation of tissue matrix, which lead to chronic inflammation and joints destruction [1, 2]. Fibroblast-like synoviocytes (FLS), a major component of the hyperplastic synovial pannus, play a central role in the pathogenesis of RA. The RA-FLS shares many pathologic features with tumor cells, including excessive proliferation, resistance to apoptosis, enhanced migration, invasive properties, and immune system regulation [3–5]. The enhanced migration characteristic enables RA-FLS to migrate and attach to the cartilage and bone, and invade the local environment. Understanding the mechanisms regulating the migration of FLS in RA could provide strategies for novel treatment [6, 7].

Hedgehog (HH) signaling pathway is one of the key regulators of animal development including chondrocyte growth and differentiation. There are three hedgehog homologues: Desert (DHH), Indian (IHH), and Sonic (SHH). Patched1, 2 (PTCH1, 2) and Smoothened (SMO) are transmembrane receptors which respond to the three members of Hh signaling. The SHH signaling pathway is activated in the joint synovium of RA [8]. When IHH is present, its cholesterol moiety binds to the sterol-sensing domain in PTCH, which then inhibits the activity of SMO to activate hedgehog signaling pathway. SHH and SMO have been identified as regulators in FLS migration via Rho GTPase signaling [9]. Previous studies have reported IHH as a player in osteoarthritis [10]. An elevated level of IHH protein is observed in the synovial fluid of the patient with osteoarthritis and in the serum of patients with ankylosing spondylitis (AS). The increased expression of IHH in these pathological conditions suggests its involvement in rheumatic diseases. However, the exact role of IHH in FLS remains unclear [11, 12].

Tumor necrosis factor (TNF)-like ligand 1A (TL1A) is a longer variant TL1 type II transmembrane protein, also referred to as vascular endothelial growth inhibitor (VEGI)-251, and is a member of the tumor necrosis factor superfamily (TNFSF) ligands, which was characterized by Migone *et al*. in 2002 [13]. Generally, TNF has an important contribution in tissues destruction and remodeling by human synovial cells [14]. TNF-α converting enzyme

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doi: 10.1684/ecn.2018.0405

(TACE) catalyzes the TL1A-ectodomain shedding as a soluble protein [15]. Lymphocytes, antigen-presenting cells, human umbilical vein endothelial cells, and FLS are all TL1A expressing cells. TL1A is upregulated by stimulation with proinflammatory cytokines such as TNF-α, IL-1, and phorbol myristate acetate (PMA), a potent activator of protein kinase C [13, 16]. Similarly, Toll-like receptor (TLR) ligands, enteric bacteria, and Fcγ receptor (Fcγ R) crosslinking also upregulate TL1A [17–19]. Death receptor 3 (DR3) is a cell surface receptor of the tumor necrosis factor receptor superfamily (TNFRSF) with a typical death domain, the decoy receptor (DcR3). DR3 is expressed in immune cells, for example, NK cells, Th1, Th2, Th17, and T regulatory cells [20]. TL1A-DR3 signaling has been established as a NF-κB activator and apoptosis inducer in vitro. In addition, TL1A-DR3 signaling is required for effective T-cell immune response in the target organs of T-cell-mediated autoimmune and inflammatory diseases. Although DR3 and DcR3 are the conventional receptors for TL1A, our previous findings concluded that TL1A may have an influence on RA FLS through binding to TNFR2 [21], a little characterized receptor compared to detailed characterization for TNFR1 [22]. There is growing evidence that TL1A has a pivotal role in autoimmunity mediation. This is supported by evidence that there is increased expression of TL1A and its two receptors (DR3 and DcR3) in the serum and inflamed tissues in autoimmune diseases such as inflammatory bowel disease (IBD), RA, and AS [23, 24].

In the present study, our purpose was to study the effect of TL1A in RA-FLS migration and IHH signaling pathway. Because of their key role in HH signaling pathway, we decided to investigate the effect of TL1A in PCHD1, 2 hedgehog receptors. Furthermore, we investigated the TNFR2 as a novel receptor for TL1A in RA-FLS.

MATERIALS AND METHODS

Patients

Twenty-five patients with RA were enrolled in this study. Synovial tissues were collected from the Orthopedics Department of the Second Hospital of Dalian Medical University, Dalian, China. Patients were diagnosed according to the RA 1987 revised classification criteria of the American College of Rheumatology [25]. All patients were undergoing total joint replacement surgery. The approval to conduct this study was granted by the ethical committee of human and animal research of the Dalian Medical University.

RA-FLS isolation

Enzymatic digestion of synovial tissues was performed to get FLS. The collected synovial tissues were finely minced into pieces and treated with collagenase type I from *Clostridium histolyticum* (Biotopped, China) at a concentration of 3 mg/mL in Dulbecco's modified Eagle's medium (DMEM) (Biological Industries, USA) in a petri dish. Ensuring condition, the dish was incubated at 37 °C on a shaker for 2 h. The digested tissues were then filtered through nylon mesh of pore diameter 70 μM to get desired FLS. The filtrate was centrifuged for 5 min at 300 g. Finally, the sediment was resuspended in adequate volume of DMEM supplemented with 20% fetal bovine serum (FBS) (Biological Industries, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin, and cultured in humidified environment incubator at 37 °C and 5% CO_2. Three to five passages of RA-FLS were used for all experiments.

Cell culture and stimulation

At approximately 80-100% confluency, RA-FLS were trypsinized, harvested, resuspended, divided for cells propagation in DMEM with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated at 37°C, and 5% CO_2 in humidified environment. Three to five passages of RA-FLS showed typical morphological characters under phase contrast microscope. To study the effect of TL1A on RA-FLS migration, and IHH and PCHD1, 2 genes expression, RA-FLS were pretreated with 1.5 μg/mL TNFR2 antibodies (R&D, USA) before stimulating cells with TL1A (100 ng/mL) in serum-free DMEM for 24 h. For total RNA extraction and migration assays, cells were incubated for 24 h, while the incubation time was extended to 48 h for Western blotting.

Gene expression measurement

Total mRNA isolation

Two to four million RA-FLS were used for a single test. The cells were scraped in 1 mL RNAiso (Takara Bio, Japan) and the cell lysate collected into a 1.5 mL Eppendorf tube. 250 μL chloroform was added to the lysate and then shook vigorously. The mixture was centrifuged at 10,000 rpm for 5 min at 4 °C. The aqueous phase was transferred into a new tube. This was followed by the addition of 550 μL isopropanol and mixed gently. The mixture was then centrifuged at 14,000 rpm for 30 min 4 °C. The supernatant was poured off and the barely visible pellet gently mixed with 1mL 75% ethanol in DEPC-treated H_2O. The tube was recentrifuged at 9,500 rpm for 5 min at 4 °C. The air-dry pellet was dissolved in 10 μL RNase-freeH_2O, and the RNA concentration measured with NanoDrop 2000 full-spectrum (Thermo Scientific™, USA).

Reverse transcription PCR

Less than 1μg of mRNA was used to synthesize cDNA using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio, Japan). RNA template, oligo dT primer, dNTP mixture, and RNase-free H_2O were mixed together and incubated for 5 min at 65 °C, and then cooled immediately on ice. 5X primeScript buffer, RNase inhibitor, RTase, and RNase-free H_2O were added to the previous mixture to get 20 μL according to the manufacturer's instructions. The mixture was mixed and incubated for 60 min at 42 °C and then at 95 °C for 5 min and cooled on ice. PCR was performed to amplify GAPDH housekeeping gene as an internal control, IHH, PTCH1, and PTCH2. 2XPower Taq PCR MasterMix (Bio Teke Corporation, China), cDNA, RNase-free H_2O, and primers (Takara Bio, Japan) were mixed thoroughly according to the manufacturer's instructions. The microtubes were loaded in the thermocycler (Bio-Rad, USA), and each cycle consisted of 30 s for denaturation at 95 °C, 30 s of annealing at 56.5 °C, and 30 s for extension at 72 °C, for a total of 35 cycles. The primers used were as follows: GAPDH (272 bp): forward (TGACCACAGTCCATGCCATCAC), reverse

(CGCCTGCTTCACCACCTTCTT), IHH (247 bp): forward (GAACTCGCTGGCTATCTCGG), reverse (CTCGGACTTGACGGAGCAAT), PCHD1 (298 bp): forward (TGTCGCACAGAACTCCACTC), reverse (ACCAAGAGCGAGAAATGGCA), and PCHD2 (431 bp): forward (TTACCGCAACTGGCTACAGG), reverse (CGATGGCCTCCACAAAGTCT). Electrophoresis was performed using 2% agarose gels for all genes. PCR bands were analyzed by Image Lab detection system (Bio-Rad, USA).

Protein expression measurement

Protein extraction and measurement

RA-FLS with 100% confluency in 6 wells plate were harvested by centrifugation at 3000 RPM for 3 min at 4 °C after scraped using ice-cold phosphate buffer saline (PBS) and cold plastic scraper. Lysis buffer containing an appropriate quantity of proteinase inhibitor, phosphate inhibitor, and phenylmethanesulfonyl fluoride (PMSF) according to the kit protocol (KeyGen Biotech, China) was added to the harvested cells. The resulting mixture was vigorously shook by sonication for 40 min and centrifuged at 12,000 RPM for 15 min at 4 °C. The supernatant was used as the total protein extract. The concentration of the total protein was estimated with bicinchoninic acid (BCA) (KeyGen Biotech, China), in accordance with the manufacturer's instruction. Standard absorbance curve was established using standard protein and concentrations of the samples extrapolated from the curve. Absorbance was detected with a Multiaskan Ascent plate reader (Thermo, USA) at a wavelength of 540 nm. Protein concentration of 20 μg was used for Western blotting.

Western blotting

12% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to separate 20 μg of protein lysates and then transferred to a polyvinylidene fluoride (PVDF) membrane. Nonspecific antigens were blocked by shaking the membranes in blocking buffer (5% skimmed milk in Tris-buffered saline tween 20, TBST) at room temperature for 1h and then probed with primary antibodies overnight at 4 °C. The primary antibodies were rabbit anti-IHH (1:1000, Abcam, USA) and β-actin (1:1000, Abcam, USA). After washing with TBST, the membranes were incubated with secondary antibody conjugated with horseradish peroxidase at room temperature for 1 h. The membranes were washed again to remove the unbinding secondary antibodies. Enhanced chemiluminescent (ECL) detection system (Bio-Rad, USA) was used to measure immobilized proteins. The bands density quantification was performed by Image Lab software (Bio-Rad, USA).

Transwell cell migration assay

RA-FLS were detached from the tissue culture plate using 0.25% Trypsin-EDTA solution, and pellet obtained after centrifugation. The cells were resuspended in 5% FBS in DMEM medium. Transwell cell culture chamber apparatus with 8 μm pore membrane (Costar, USA) was used in this experiment. The cells suspension of 100 μL (1×10^5 cells/mL) was loaded into the upper chamber of the transwell inserts. Chemoattractant of 350 μL (20% FBS in DMEM medium) was added to the bottom of the lower chamber in a 24-well plate. After 24h incubation at 37 °C and 5% CO_2, the nonmigrated cells were carefully removed from the inner part of the insert by a cotton-tipped applicator. Transwell inserts were placed in the 70% ethanol for 10 min to allow cells fixation and in 0.2% crystal violet at room temperature for 20 min. FLS Migration was quantified by counting cells in five random fields at 100 magnifications in each membrane.

Cell culture wound closure assay

RA-FLS were plated in a 12-well plate to get 100% confluency for 24 h. Using a 200 μL pipette tip, a vertical wound was generated down through the cells monolayer, and the culture medium was replaced by enough culture media to remove cells debris. Initial pictures of the wound were taken (0 h) using an inverted microscope (Olympus 1X71, Japan). Culture plate was placed in an incubator at 37 °C and 5% CO_2. Snapshots were taken every 6 h for 24 h. Evaluation of RA-FLS migration was done by measuring the wounds widths.

Statistical analysis

Data were statistically analyzed by paired-sample t-test using GraphPad Prism Version 6.07 (San Diego, USA). All experiments were performed in triplicates, and data were presented as mean ± SD. The differences between groups were considered statistically significant where P value is <0.05.

RESULTS

IHH and PTCH1 and 2 are overexpressed in TL1A-stimulated RA-FLS

Because of the role of hedgehog signaling pathway in development and its special role in joints and cartilage, we wondered whether this pathway might have an effect on RA-FLS migration. We stimulated RA-FLS isolated from 5 patients with TL1A at a concentration of 100 ng/mL and for a duration of 24 h. PCR was performed to assess the effect of TL1A on IHH mRNA expression. We observed relatively that TL1A treatment significantly induced IHH overexpression (*figure 1A*). Subsequently, we extended TL1A-stimulation time to 48 h for IHH protein expression assay. In accordance with PCR test results, our Western Blot results showed significantly increased IHH protein in TL1A-stimulated RA-FLS (*figure 1B*), indicating that TL1A promotes IHH protein expression. PTCH receptors have been implicated in a variety of diseases, and down-regulation of PTCH suppressed the activity of the signal transduction pathway of cells. We therefore assessed the mRNA expression levels of PTCH1 and PTCH2 with or without stimulation. TL1A-stimulated RA-FLS exhibited significant increased levels of PTCH1 and PTCH2 expression compared to nonstimulated cells (*figure 1C and D*). These results suggested that TL1A stimulated IHH expression and also seemed to influence the upregulation of PTCH1, 2 hedgehog receptors.

TL1A increases migration of RA-FLS

Migration is one of the vital characteristics of RA-FLS which aid them to infiltrate in bones and cartilages. In view

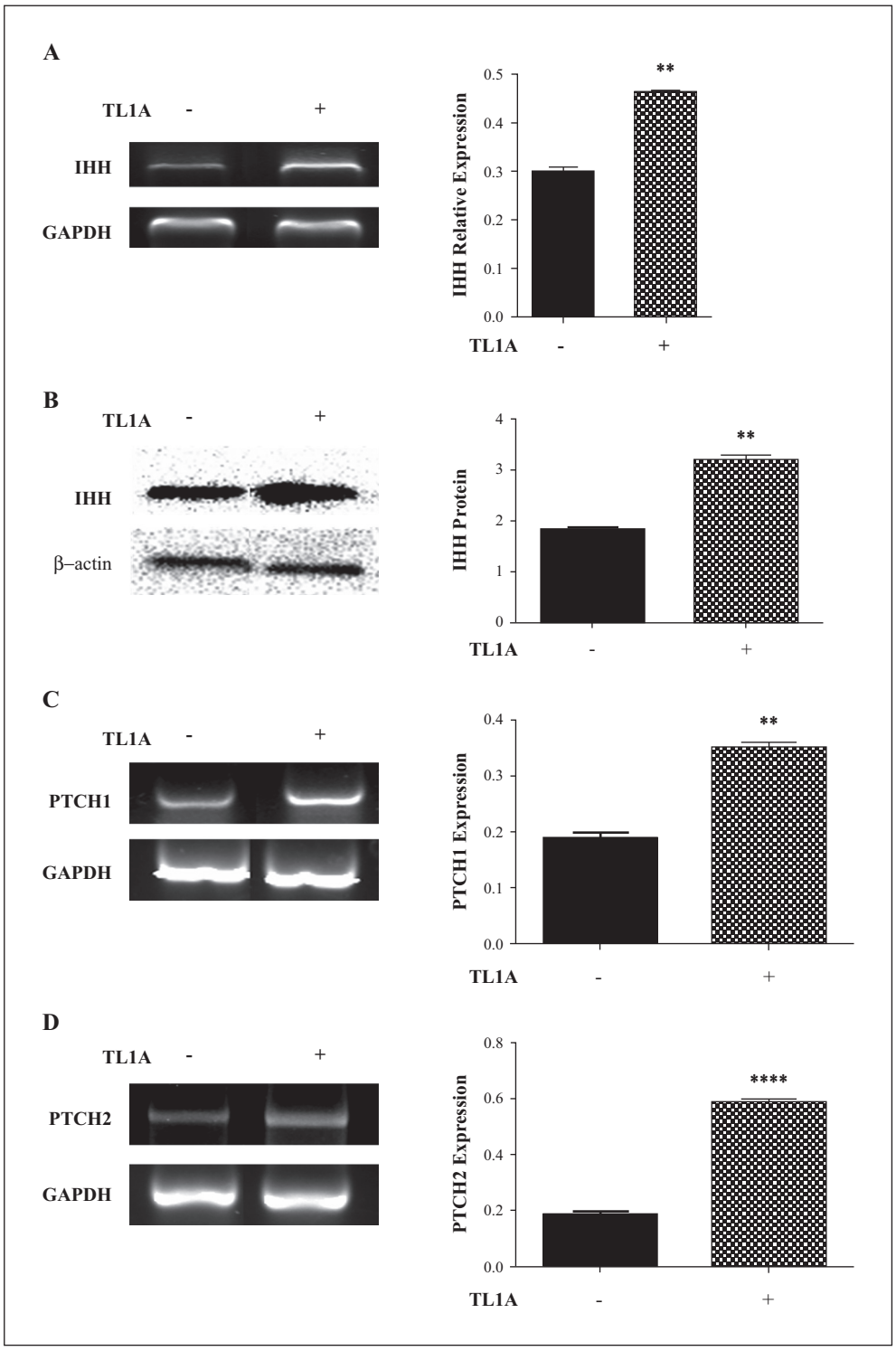

Figure 1

IHH and PTCH1, 2 expression increased by TL1A in RA-FLS. **(A)** RA-FLS ($n = 5$) were stimulated with and without TL1A (100 ng/mL) for 24 h. IHH gene expression was measured by RT-PCR. GAPDH was used as a housekeeping gene. Data were normally distributed around mean. Statistically, the significant difference was detected between stimulated and nonstimulated groups with $^{**}P = 0.0020$. **(B)** Isolated RA-FLS ($n = 5$) were incubated with and without 100 ng/mL TL1A for 48 h. IHH protein expression was measured by Western Blot. β-actin was used as an internal control. Normally distributed data were reported with the statistically significant difference compared with nonstimulated groups $^{**}P = 0.0014$. **(C)** RA-FLS ($n = 5$) were stimulated by TL1A (100 ng/mL) for 24 h, RA-FLS without TL1A were used as a control. PTCH1 gene expression was tested by RT-PCR, normalized to GAPDH. Data were distributed normally around mean. The statistically significant difference was detected between stimulated groups and nonstimulated with $^{**}P = 0.0039$. **(D)** Isolated RA-FLS ($n = 5$) were incubated with and without 100ng/mL TL1A for 24 h. *PTCH2* gene expression was measured by RT-PCR. GAPDH was used as an internal control. Normally distributed data were reported with the statistically significant difference compared with nonstimulated groups $^{****}P < 0.0001$.

of this, we assessed the effect of TL1A on RA-FLS migration, using the transwell migration test. In all FLS samples from five RA patients were used, we observed significantly enhanced migration in TL1A-stimulated RA-FLS compared to their non-stimulated counterparts (*figure 2A*). RA-FLS migration was also evaluated by cell culture wound healing assay. Similar to the outcome of the transwell migration assay, the scratch test showed relatively

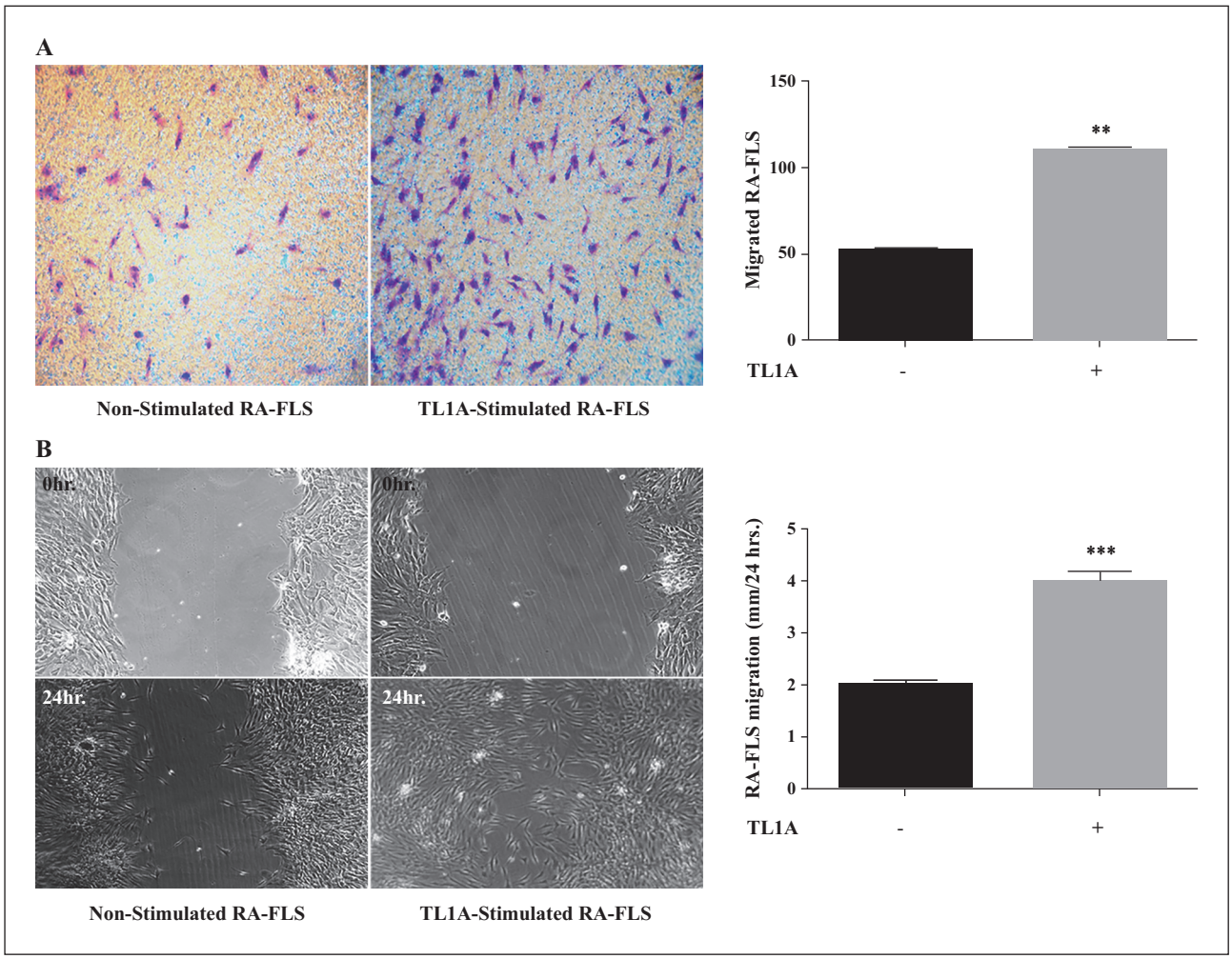

Figure 2

TL1A increased migration of RA-FLS. **(A)** RA-FLS ($n = 5$) were stimulated with and without TL1A (100 ng/mL) for 24 h. RA-FLS migration was evaluated by transwell migration test. Data were distributed normally around mean. The difference between stimulated and nonstimulated groups was statistically significant with $^{**}P = 0.0023$. **(B)** RA-FLS ($n = 5$) with 100% confluency were incubated with and without 100 ng/mL TL1A for 24 h. RA-FLS migration was tasted by wound assay. Normally distributed data were reported with the statistically significant difference compared with nonstimulated groups $^{***}P = 0.0008$.

faster healing in TL1A-stimulated RA-FLS monolayer compared with the nonstimulated (*figure 2B*). Our results suggested that TL1A enhanced FLS migration in patients with RA.

TL1A blocking mediated by TNFR2 antagonist restricts RA-FLS migration and downregulates IHH and PTCH1, 2 expressions

The previous studies have proven that TL1A can preferentially activate TNF receptor 2 (TNFR2) in RA instead of its conventional receptors (DR3 and DcR3) [21]. However, the expression of DR3 and DcR3 soluble receptors in RA-FLS is not clear. We therefore inhibited TL1A activity using TNFR2 antibodies to confirm the previous results. TL1A was added to stimulate RA-FLS pretreated with 1.5 μg/mL TNFR2 antibodies. The results of transwell migration test, scratch test, RT-PCR, and Western Blot were as follows, respectively: decreased RA-FLS migration in transwell and scratch assays (*figure 3A, B*), decreased IHH and PTCH1, 2 genes expressions (*figure 3A, C*), and IHH protein synthesis (*figure 4B*). Five patients were involved in these experiments. Our results revealed that TL1A might interact with TNFR2 that increased the production of IHH which activated PTCH1, 2 hedgehog receptors to mediate the migration of RA-FLS.

DISCUSSION

Recent reports have underscored the significance of hedgehog signaling pathways in the pathogenesis of RA [26, 27]. This pathway is crucial in bone and cartilage development, and its dysregulation causes severe bone diseases. Among the key proteins in this pathway is the IHH protein. Zhou *et al*. have reported the upregulation of IHH expression in human osteoarthritis (OA) cartilage, and this elevation correlates with OA progression and changes in chondrocyte morphology [28]. Consistent with this finding, we observed overexpression of both IHH mRNA and protein in FLS from patients with RA, following stimulation with TL1A. Even though IHH elevation has a positive effect on bone repair, particularly fracture [29], its excessive expression predisposes to chondrocytes hypertrophy, and MMP-13 upregulation in osteoarthritic cartilage [30]. We found from the present study that mRNA levels of PTCH1, 2 receptors are increased in TL1A-stimulated

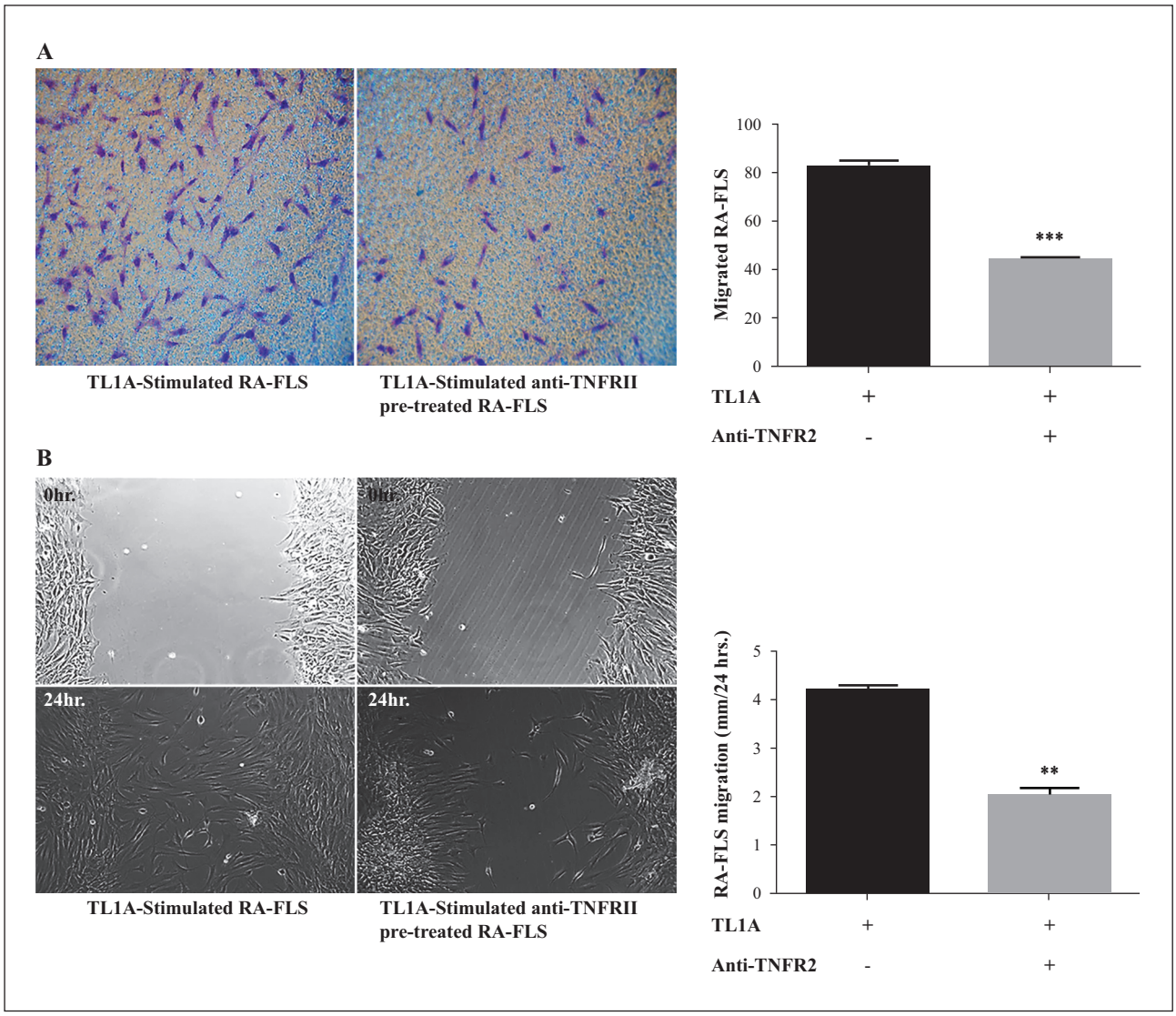

Figure 3

TL1A blocking by anti-TNFR2 decreased migration of RA-FLS. **(A)** RA-FLS ($n = 5$) pretreated with and without 1.5 μg/mL TNFR2 antibodies and then stimulated by TL1A (100 ng/mL) for 24 h. Transwell migration test was performed to measure RA-FLS migration. Data were distributed normally around mean. The difference between anti-TNFR2 pretreated and non-pretreated groups was statistically significant with ***$P = 0.0005$. **(B)** Isolated 100% confluency RA-FLS pretreated with and without 1.5 μg/mL TNFR2 antibodies ($n = 5$) were incubated with 100ng/mL TL1A for 24 h. RA-FLS migration was tasted by wound healing assay. Normally distributed data were reported with the statistically significant difference compared with non-pretreated groups **$P = 0.0045$.

RA-FLS. Among the major factors in the hedgehog pathway are the receptors to IHH, PTCH1, 2. Blocking of these receptors will mean silencing the downstream effect of the HH pathway, which include IHH, SHH, and DHH proteins. Previous studies have indicated that blocking, downregulation, or mutation in PTCH1, 2 has been associated with type 1 diabetic accompanied by cardiac dysfunction and naevoid basal cell carcinoma (Gorlin) syndrome conditions [31, 32]. On the other hand, elevated mRNA expression levels of three IHH associated genes, GLI1, PTCH1, and Hh-interacting protein (HHIP), have been shown to have positive correlation with lumbar facet joint OA pathological scores [33]. In addition, increased expression PTCH, SMO, and glioma-associated oncogene-1 (Gli1) have been found to promote the progression of RA and regulated the expression of the downstream MMPs, vascular endothelial growth factor (VEGF), and angiopoietin-2 (Ang-2) [34]. Most of the studies reported the involvement of PTCH1 in other diseases. However, the role of PTCH1, 2 in RA has been relatively unclear. In this study, we found that both PTCH1 and PTCH2 receptors are activated in TL1A-stimulated RA-FLS and that both receptors could be involved in RA, making them possible for therapeutic target.

A major future of RA is the ability of RA-FLS to migrate and invade local compartment of joints. RA-FLS migration and invasion play effective role in osteoclastogenesis and destruction of joints bones and cartilages. A previous study has revealed that RA-FLS migration is enhanced through overexpression of L-type amino acid transporter 1 (LAT1), mediated by IL-17, via the mTOR/4E-BP1 pathway [35]. In addition, peroxisome proliferator-activated receptor-γ (PPAR-γ) has been reported to have a contributory role on the proliferation and migration of FLS in RA by activation of Wnt/β-catenin signaling [36]. Furthermore, upregulation of long noncoding RNA ZFAS1 has been showed to promote RA-FLS migration and invasion through suppression of miR-27a [37]. The novelty of our study is that TL1A might enhance RA-FLS migration via upregulation of IHH ligand and PTCH1, 2 receptors. Our study showed

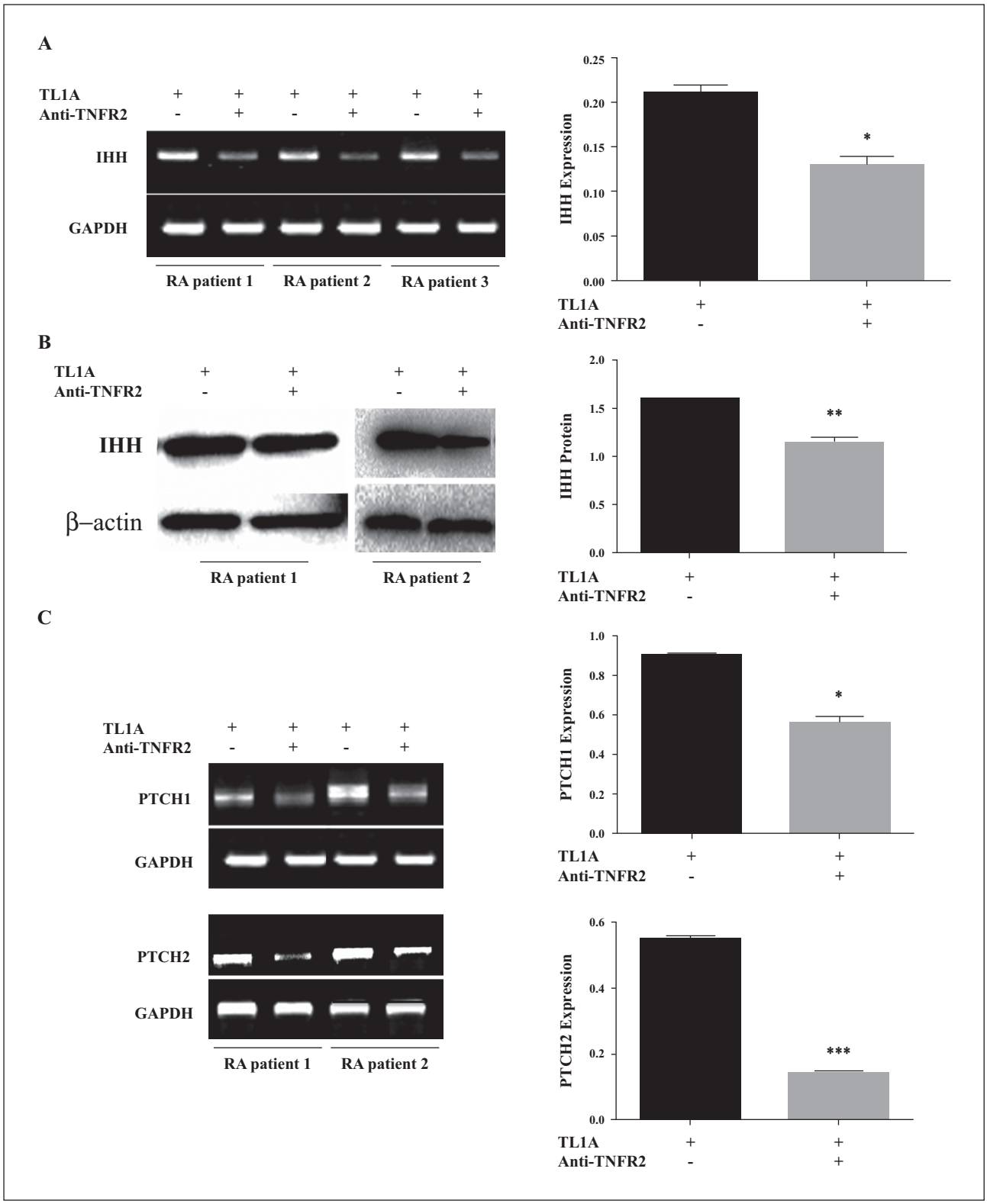

Figure 4

TL1A blocking by anti-TNFR2 decreased IHH and PTCH1, 2 expressions. **(A)** RA-FLS pretreated with and without 1.5 μg/mL TNFR2 antibodies ($n = 5$) and then incubated with TL1A (100 ng/mL) for 24 h. IHH gene expression was tested by RT-PCR, normalized to GAPDH. Data were distributed normally around mean. The statistically significant difference was detected between anti-TNFR2 pretreated and non-pretreated groups with $^{*}P = 0.0316$. **(B)** Isolated RA-FLS pretreated with and without 1.5 μg/mL TNFR2 antibodies ($n = 5$) and then incubated with TL1A (100 ng/mL) for 48 h. IHH protein was tested by Western Blot. β-actin was used as an internal control. Normally distributed data were reported with the statistically significant difference compared with anti-TNFR2 non-pretreated$^{**}P = 0.0057$. **(C)** Anti-TNFR2 (1.5 μg/mL) pretreated and non-pretreated RA-FLS ($n = 5$) were incubated with TL1A (100 ng/mL) for 24 h. RT-PCR was performed to measure genes expressions of PTCH1, 2. GAPDH was used as an internal control. Normally distributed data were reported with the statistically significant difference compared with non-pretreated groups$^{*}P = 0.0198$, $^{****}P = 0.0004$, respectively.

that TL1A may stimulate RA-FLS migration by activation of hedgehog signaling pathway through its members, IHH ligand, and PTCH1, 2 receptors.

TL1A action on immunologic cells is mediated by the DR3 receptor. However, DR3 expression remains undetected in FLS. In addition, there is homology for DR3

to TNFR1 domains, and also homology for extracellular part of TL1A to TNF domain. TNFR1 is activated through both soluble and membrane TNF-α, whereas TNFR2 is mainly activated by mTNF-α [38]. Although a recent study revealed that TNFR2 may have a therapeutic implication in arthritic disease [39], our previous results revealed that TL1A-stimulated FLS elevated Th17 percentage. Then, we used TNFR2 antagonist with FLS of RA and observed decreased IL-6 level in cell culture supernatant and IL-6 mRNA expression [21]. Clinically, antagonizing TNF is a widely used treatment option in RA management. In the present study, we observed significant decrease in the migration of RA-FLS with TNFR2 antagonist, and this was associated with low expressions of IHH and PTCH1, 2 genes, as well as IHH protein. The involvement of a variety of proinflammatory components in the pathogenesis of RA was reported, for example, TNF-α, IL-1β, and IL-6, IFN-γ, IL-15, and CXCL6 [40, 41]. The cell signaling pathways also have implication on the basis of RA. Novel inhibitors have targeted the intracellular signaling molecules which crucially involved in RA pathogenesis, for example, p38-mitogen-activated protein kinase (MAPK), and Janus kinase-signal transducer and activator of transcription (JAK-STAT) [42, 43]. According to the previous studies, the cytokines can cooperate together with some intracellular signaling pathways to promote FLS functions, for example, IL-17A through NF-κB/HIF-1α pathways were considered to have a role in FLS migration by increasing MMP-2 and MMP-9 expression [44]. In the present study, we showed how TL1A as proinflammatory factor can mediate RA-FLS migration and IHH signaling pathway through TNFR2.

CONCLUSION

In summary, we have explored a new effect of TL1A/TNFR2 interaction in RA-FLS migration and IHH signaling pathway using PTCH1, 2 transmembrane receptors. These findings may introduce a potential therapeutic strategy for targeting IHH/PTCH1, 2 axises and control RA-FLS pathological synovial invasion and suppress their undesired action. In addition, we confirmed the importance of clinical usage for anti-TNF drugs with patients with RA. However, the exact mechanisms of hedgehog signaling pathway and its effect in RA-FLS migration still need further study and explanation.

Disclosure. *Financial support: The research was funded by National Natural Science Foundation of China (81671606); Special Grant for Translational Medicine, Dalian Medical University (2015010); College Scientific Research Project of Education Department of Liaoning Province (LQ2017004); Flying aid program foundation of College of Basic Medical Science, Dalian Medical University. Conflict of interest: none.*

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