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Overexpression of long non-coding RNA TUG1 alleviates TNF-α-induced inflammatory injury in interstitial cells of Cajal

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Abstract. – OBJECTIVE: Irritable bowel syndrome (IBS) is a common functional disorder in the gastrointestinal tract. Inflammatory response has been found to participate in the pathogenesis of IBS. This study aimed to explore the effects of long non-coding RNA taurine upregulated gene 1 (TUG1) on tumor necrosis factor alpha (TNF-α)-induced interstitial cells of Cajal (ICC) inflammatory injury, which was relevant to the pathogenesis of IBS.

PATIENTS AND METHODS: The expression levels of TUG1 and microRNA-127 (miR-127) were analyzed by qRT-PCR. Viability, apoptosis and the expression of apoptosis-associated factors were analyzed by CCK-8 assay, flow cytometry and Western blot, respectively. The mRNA and protein levels of pro-inflammatory cytokines were detected by qRT-PCR and Western blot, respectively. Finally, activations of nuclear factor kappa-B (NF-κB) and Notch pathways were evaluated by Western blot.

RESULTS: TNF-α treatment inhibited ICC viability, induced ICC apoptosis and promoted an inflammatory response in ICC. TUG1 was down-regulated in TNF-α-treated ICC. TUG1 overexpression protected ICC from TNF-α-induced apoptosis and pro-inflammatory cytokines expression. TUG1 suppression showed opposite effects. MiR-127 was negatively regulated by TUG1 and implicated in the action of TUG1 in ICC. MiR-127 up-regulation largely reversed the effects of TUG1 on TNF-α-treated ICC. Mechanistically, TUG1 inhibited TNF-α-induced activation of NF-κB and Notch pathways in ICC by down-regulating miR-127.

CONCLUSIONS: TUG1 attenuated TNF-α-caused apoptosis and inflammatory response in ICC by down-regulating miR-127 and then inactivating NF-κB and Notch pathways.

Key Words:

Irritable bowel syndrome, Interstitial cells of Cajal, Long non-coding RNA TUG1, MicroRNA-127, NF-κB pathway, Notch pathway.

Introduction

Irritable bowel syndrome (IBS), a kind of functional gastrointestinal disorders, is characterized by intermittent and recurrent abdominal pain with changes in stool frequency and form[1]. However, the pathogenesis of IBS remains unclear[2]. Many factors contribute to the occurrence of IBS, including gut dysmotility, imbalance of the intestinal micro-flora, abnormalities of the brain-gut axis, and poor diet habits[3,4]. Increasing numbers of reports provide evidence that there are links between interstitial cells of Cajal (ICC) and IBS[5,6]. ICC exists in the whole digestive system, which can act as mechanoreceptors to mediate signals from enteric neurons to smooth muscle cells[5,7]. Numerous studies in recent years demonstrated that the number and integrity change of ICC could induce the occurrence of IBS[8]. More importantly, the inflammatory response of ICC has been found in IBS animal model[9]. Therefore, it is worthy believing that a more clear understanding of ICC inflammatory response will be helpful for defining the pathogenesis of IBS. Long non-coding RNAs (lncRNAs) participate in the regulation of multiple important cellular processes, such as cell proliferation, cell differentiation and cellular responses to stress and immune agents[10,11]. Numerous research in recent years demonstrated that aberrant expression of lncRNAs was associated with the occurrence of many human diseases[12]. LncRNA taurine upregulated gene 1 (TUG1) was found to be implicated in the pathogenesis of many diseases, such as atherosclerosis[13], osteosarcoma and other cancer types[14,15], diabetes mellitus[16], and kidney diseases[17]. Besides, the previous study proved that TUG1 exerted anti-inflammatory effects and could protect mice livers from cold storage injury[18]. However, the effects of

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TUG1 on ICC subjected to inflammatory injury remain unclear. The biological functions of microRNAs (miRNAs, around 22 nt small non-coding RNAs) have been extensively studied[19]. Over two thousand miRNAs have been discovered in human cells and it is believed that they are broadly associated with the regulation of multiple cell functions and various diseases[20]. MiRNAs can be specifically regulated by some lncRNAs and further mediate the functions of these lncRNAs[21]. In this work, to clarify the regulatory mechanism of TUG1 on ICC, we also analyzed the regulatory effect of TUG1 on miRNA-127 (miR-127). ICC were treated by tumor necrosis factor alpha (TNF-α) to stimulate the *in vitro* inflammatory injury model occurred in IBS, and then the effects of TUG1 on cell viability, apoptosis, concentration of interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and monocyte chemotactic protein 1 (MCP-1) were evaluated. Moreover, the roles of miR-127 in inflammatory regulatory functions of TUG1 were investigated. This study will provide new evidence for further understanding the anti-inflammatory effects of TUG1 and provide potential targets to alleviating inflammation in ICC.

Materials and Methods

ICC Isolation

The female C57BL/6 mice (weighed from 13 g to 15 g) were obtained from the medical animal laboratory of The Affiliated Hospital of Qingdao University (Qingdao, China). All experiments on mice were approved by the Ethics Committee of The Affiliated Hospital of Qingdao University (Qingdao, China). The small intestine was removed (from 1 cm below the pyloric ring to the cecum) and opened along the mesenteric border. After washing luminal contents and removing mucosa, the obtained small tissue strips of intestine muscle were equilibrated using nominally Ca^{2+} free solution (KCl 5.36 mM, NaCl 12.5 mM, NaOH 0.34 mM, $NaHCO_3$ 0.44 mM, glucose 10 mM, sucrose 2.9 mM, and HEPES 11 mM PH 7.4) for 30 min. Cells were then dispersed in the enzyme solution (containing collagenase, bovine serum albumin, trypsin inhibitor, and ATP magnesium salt purchased from Sigma-Aldrich (St. Louis, MO, USA). Subsequently, ICC was isolated and identified as previously described[22].

TNF-α Treatment

ICC was treated by different concentration of TNF-α (Sigma-Aldrich, St. Louis, MO, USA, 10, 20, 30, and 40 ng/ml) to stimulate the inflammatory injury.

Cell Counting Kit-8 (CCK-8) Assay

CCK-8 assay (Dojindo Laboratories, Kumamoto, Japan) was used to evaluate the relative viability of ICC. Briefly, ICC was seeded into 96-well plate (Thermo Fisher Scientific, Waltham, MA, USA) with 5.000 cells per well. After TNF-α treatment and/or relevant transfection, 10 μl CCK-8 kit solution was added into the culture medium of each well and the plate was incubated for 2 h at 37°C in humidity incubator (Sanyo, Jencons, UK). After that, the absorbance of each well at 450 nm was measured using a Vmax Microplate Spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

Apoptosis Analysis

After relevant treatment and/or transfection, ICC was collected by centrifugation at 300 g and 4°C for 5 min. Then, ICC was washed with precold Phosphate-Buffered Saline (PBS; Beyotime Biotechnology, Shanghai, China) and centrifuged (300 g, 4°C, 5 min) two times. Collected cells (1-5 × 10^5) were re-suspended in 100 μl 1× binding buffer. Afterward, 5 μl Annexin V-fluoroscein isothiocyanate (FITC) and 10 μl Propidium Iodide (PI) staining solution were added in the binding buffer. After incubation at 25°C for 15 min in a dark place, samples were added with 1×400 binding buffer and placed on the ice after blending. Flow cytometry analysis was performed in 1 h by using a FACS can (Beckman Coulter, Fullerton, CA, USA).

Cell Transfection

The small interfering RNA (siRNA) against TUG1 (si-TUG1), TUG1-expressing plasmid (pc-TUG1), and miR-127 mimic were all purchased from Ribobio Corporation (Guangzhou, China). Lipofectamine 3000 reagent (Life Technologies, Gaithersburg, MD, USA) was used for cell transfection following the manufacturer's instruction. qRT-PCR was performed to verify the transfection efficacy.

qRT-PCR Analysis

All RNAs was extracted from ICC by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For qRT-PCR analyses of TUG1, IL-1β, IL-6, and MCP-1, 1 μg of RNA was reversely transcribed to cDNA and PCR analyses were performed us-

ing a Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan) and SYBR Premix ExTaq II kit (TaKaRa, Otsu, Shiga, Japan), respectively, and their expressions were normalized to GAPDH expression. For miR-127 detection, TaqMan MicroRNA Reverse Transcription Kit and TaqMan Universal Master Mix II (Applied Biosystems, Foster City, CA, USA) were used in turns for synthesizing cDNA and perform PCR. Its expression was normalized to U6 expression. The information of primer sequences was as follows: TUG1 forward primer: 5'-TAGCAGTTCCCCAATCCTTG-3', TUG1 reverse primer: 5'-CACAAATTCCCATCATTCCC-3'; miR-127 forward primer: 5'-GTTTGGGGAGAGCGTAAACG-3', miR-127 reverse primer: 5'-GTAAA CGAACACCGCACCG-3'; IL-1β forward primer: 5'-CCCCTCAGCAACACTCC-3', IL-1β reverse primer: 5'-GGTCAGAAGGGCAGAGA-3'; IL-6 forward primer: 5'-CGTGGAAATGAGAAAAGAGTTGTGC-3', IL-6 reverse primer: 5'-ATGCTT AGGCATAACGCACTAGGT-3'; MCP-1 forward primer: 5'-TCAGCCAGATGC AGTTAACGC-3', MCP-1 reverse primer: 5'-TGATCCTCTTGTAGCTCTCCA GC-3'; GAPDH forward primer: 5'-AC CAGGAAATGAGCTTGACA-3', GAPDH reverse primer: 5'-GACCACAGTCCATGCCATC-3'; U6 forward primer: 5'-TGGG GTTATACATTGTGAGAGGA-3', U6 reverse primer: 5'-GTGTGCTACGGAG TTCAGAGGTT-3'. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method[23].

Western Blot

After relevant treatment and/or transfection, ICC was harvested and lysed by Triton X-100 lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 4°C. Western blot system was established using a Bio-Rad Bis-Tris Gel system (Bio-Rad Laboratories, Hercules, CA, USA). After quantified using BCA Protein Assay kit (Beyotime Biotechnology, Shanghai, China), the proteins in equal concentration were electrophoresed in 12 % polyacrylamide gels and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking by 5% non-fat milk (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 1 h, PVDF membranes were incubated with primary antibodies (4°C, overnight) and secondary antibody marked by horseradish peroxidase (room temperature, 1 h). The primary antibodies against Bax (ab216494), Bcl-2 (ab692), pro-caspase-3 (ab208161), cleaved-caspase-3 (ab208161), IL-1β (ab200478), IL-6 (ab7737), MCP-1 (ab25124), p65 (ab16502), p-p65 (ab86299), inhibitor of nuclear factor kappa-B (IκBα, ab32518), p-IκBα (ab133462), Notch 1 (ab52627), Notch 2 (ab137665), and β-actin (ab8226), as well as the secondary antibody including goat anti-rabbit IgG (ab205718) and goat anti-mouse IgG (ab6789) were all provided from Abcam Biotechnology (Cambridge, MA, USA). β-actin was used as an internal control. The representative image from one of the three independent experiments was shown. Image-J software (National Institutes of Health, Bethesda, MD, USA) was used to quantify the band intensity.

Statistical Analysis

All experiments were repeated three times and all data were presented as the mean + standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA). Statistical comparisons were made using a one-way analysis of variance (ANOVA) with Sidak post-hoc test. *p*-value of < 0.05 was considered significantly different.

Results

TNF-α Inhibited Growth and Induced Inflammatory Response in ICC

The viability of ICC after different concentrations of TNF-α treatment was assessed using CCK-8 assay. We found that viability of ICC was significantly inhibited by 20, 30 or 40 ng/ml of TNF-α treatment ($p < 0.05$ or $p < 0.01$, Figure 1A). Apoptosis of ICC was significantly enhanced after 30 ng/ml TNF-α incubation ($p < 0.01$, Figure 1B). Simultaneously, the protein expression levels of Bax and cleaved-caspase-3 in ICC were up-regulated as well as the protein expression level of Bcl-2 was down-regulated in ICC after 30 ng/ml TNF-α incubation (Figure 1C). In addition, the mRNA and protein expression levels of IL-1β, IL-6, and MCP-1 were all increased in TNF-α-treated ICC ($p < 0.001$ in mRNA level, Figure 1D and 1E). These results suggested that TNF-α treatment inhibited ICC viability, induced ICC apoptosis and promoted inflammatory response in ICC.

TUG1 Participated in the Regulation of Inflammatory Injury in ICC

As shown in Figure 2A, TNF-α treatment down-regulated the expression of TUG1 in ICC ($p < 0.01$). To explore the effects of TUG1 on

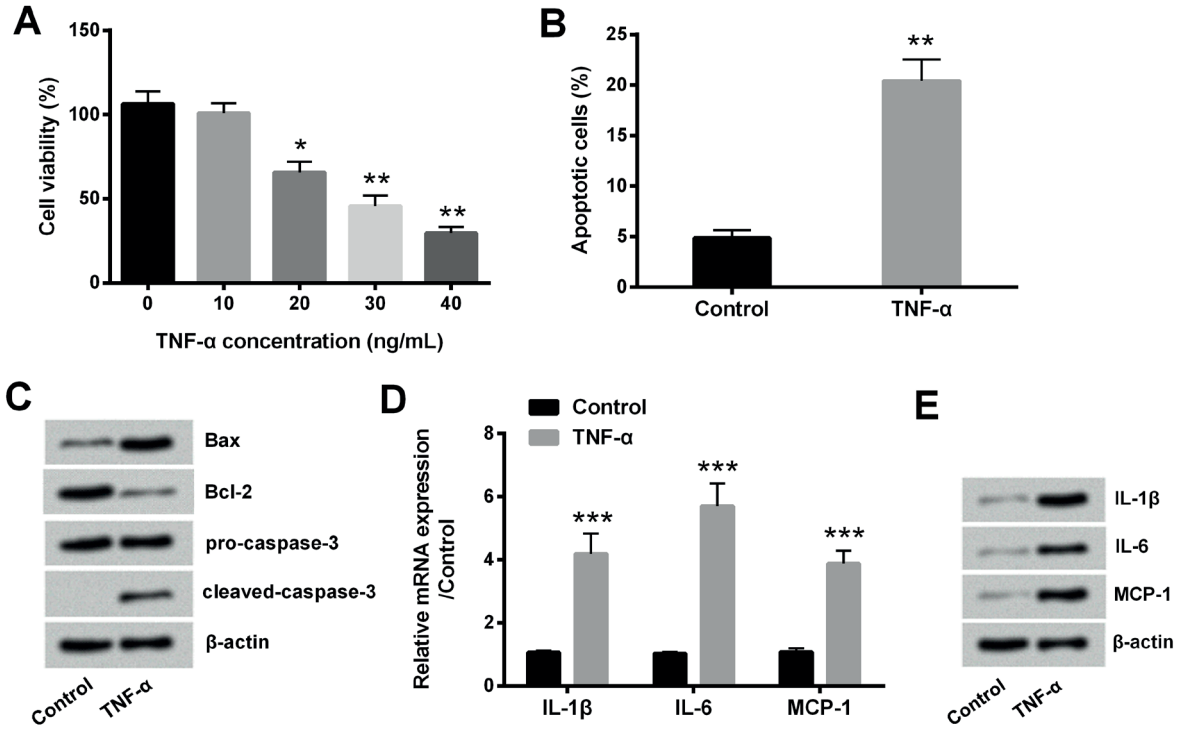

Figure 1. TNF-α induced ICC injury. After TNF-α treatment, ***A,*** ICC viability, ***B,*** ICC apoptosis, ***C,*** the expression levels of Bax, Bcl-2, Pro-caspase 3 and Cleaved-caspase 3 in ICC and ***D-E,*** the mRNA and protein levels of IL-1β, IL-6, and MCP-1 were detected, respectively. TNF-α: Tumor necrosis factor alpha; ICC: Interstitial cells of Cajal; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; MCP-1: Monocyte chemotactic protein 1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TNF-α-induced ICC apoptosis and pro-inflammatory cytokines expression, ICC was transfected with pc-TUG1 or si-TUG1, respectively. The results displayed that the expression level of TUG1 was significantly increased after pc-TUG1 transfection and decreased after si-TUG1 transfection ($p < 0.01$, Figure 2B and 2C). Figure 2D showed that TNF-α-induced ICC apoptosis was markedly inhibited by the up-regulation of TUG1 and exacerbated by the down-regulation of TUG1 ($p < 0.05$). Compared to the single TNF-α group, the protein expression levels of Bax and cleaved-caspase-3 were decreased and the protein expression level of Bcl-2 was increased in the TNF-α + pc-TUG1 group (Figure 2E). On the contrary, compared to the single TNF-α group, the protein expression levels of Bax and cleaved-caspase-3 were increased and the protein expression level of Bcl-2 was decreased in TNF-α + si-TUG1 group. Moreover, compared to the single TNF-α group, the mRNA and protein expression levels of IL-1β, IL-6 and MCP-1 in ICC were decreased in the TNF-α + pc-TUG1 group and increased in the TNF-α + si-TUG1 group ($p < 0.05$, $p < 0.01$ or $p < 0.001$ in mRNA level, Figure 2F and 2G). These findings indicated that TUG1 participated in the regulation of inflammatory injury in ICC.

Overexpression of TUG1 Protected ICC From TNF-α-Induced Inflammatory Injury by Down-Regulating miR-127

The expression level of miR-127 in ICC was significantly reduced after TUG1 overexpression and enhanced after TUG1 suppression ($p < 0.01$, Figure 3A and 3B). To analyze the roles of miR-127 in anti-inflammatory effects of TUG1 in TNF-α-treated ICC, miR-127 mimic was transfected into ICC ($p < 0.01$, Figure 3C). Compared to the TNF-α + pc-TUG1 group, the apoptosis of ICC was increased in the TNF-α + pc-TUG1 + miR-127 mimic group ($p < 0.05$, Figure 3D). The protein expression levels of Bax and cleaved-caspase-3 were enhanced, as well as the protein expression level of Bcl-2 was reduced in the TNF-α + pc-TUG1+miR-127 mimic group, compared to the TNF-α + pc-TUG1 group (Figure 3E). Moreover, the mRNA and protein expres-

sion levels of IL-1β, IL-6, and MCP-1 were all increased in the TNF-α + pc-TUG1 + miR-127 mimic group, compared to the single TNF-α + pc-TUG1 group ($p < 0.01$ or $p < 0.001$ in mRNA level, Figure 3F and 3G). These results suggested that miR-127 was involved in the effect of TUG1 on TNF-α-treated ICC and overexpression of TUG1 protected ICC from TNF-α-induced inflammatory injury at least partially by down-regulating miR-127.

Overexpression of TUG1 Inactivated nuclear factor kappa-B (NF-ÐB) and Notch Pathways in ICC by Down-Regulating miR-127

Finally, the activation of NF-κB and Notch pathways in ICC after TNF-α treatment and/or pc-TUG1 or miR-127 mimic transfection was evaluated. Figure 4A showed that the expression levels of p-IκBα and p-p65 in ICC were both increased after single TNF-α treatment ($p < 0.001$), but decreased by TNF-α treatment + pc-TUG1 transfection ($p < 0.001$). However, miR-127 mimic transfection abrogated the effects of TUG1 overexpression on p-IκBα and p-p65 expression levels decrease in TNF-α-treated ICC ($p < 0.01$ or $p < 0.001$). Similarly, the expression levels of Notch 1 and Notch 2 were both increased by TNF-α ($p < 0.001$), but decreased by TUG1 overexpression ($p < 0.001$). However, the effects of TUG1 on Notch 1 and Notch 2 expression levels decreases were impaired by miR-127 mimic transfection ($p < 0.001$, Figure 4B). These findings suggested that the overexpression of TUG1 suppressed TNF-α-induced activation of NF-κB and Notch pathways in ICC possibly by down-regulating miR-127.

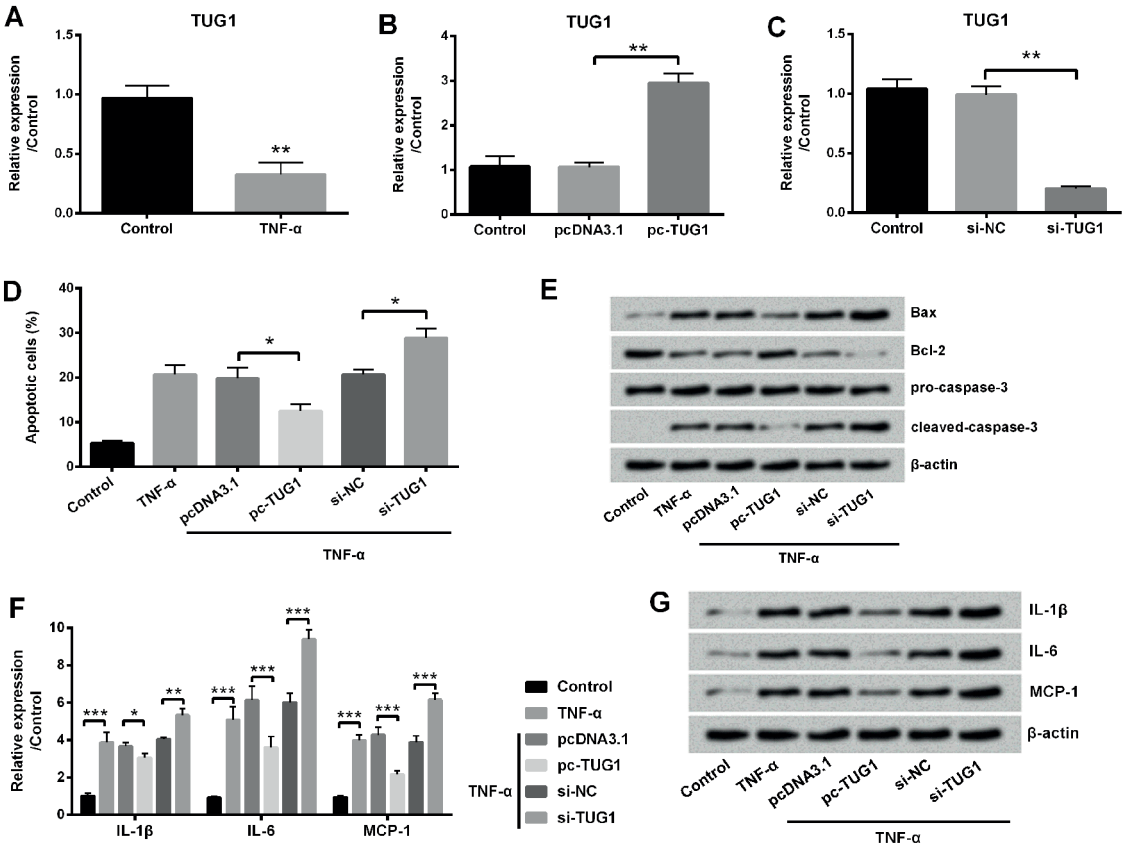

Figure 2. TUG1 participated in the regulation of TNF-α-induced inflammation injury in ICC. ***A,*** After TNF-α treatment, the expression of TUG1 in ICC was measured. ***B-C,*** After pc-TUG1 or si-TUG1 transfection, the expression of TUG1 in ICC was measured, respectively. After TNF-α treatment and/or pc-TUG1 (or si-TUG1) transfection, ***D,*** the apoptosis of ICC, ***E,*** the expression levles of Bax, Bcl-2, Pro-caspase 3 and Cleaved-caspase 3 in ICC and ***F-G,*** the mRNA and protein levels of IL-1β, IL-6, and MCP-1 were assessed, respectively. TUG1: Long non-cording RNA taurine upregulated gene 1; TNF-α: Tumor necrosis factor alpha; ICC: Interstitial cells of Cajal; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; MCP-1: Monocyte chemotactic protein 1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

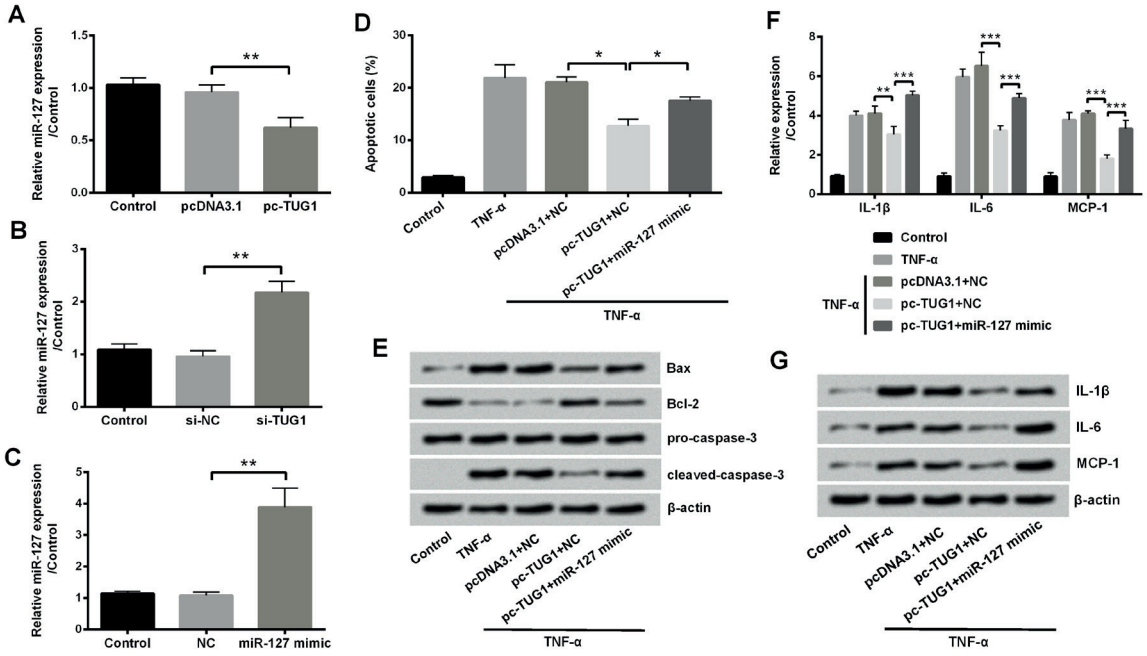

Figure 3. miR-127 was implicated in the effects of TUG1 on ICC. ***A-B,*** the expression of miR-127 in ICC was detected after pc-TUG1 or si-TUG1 transfection. ***C,*** The expression of miR-127 in ICC was measured after transfection with miR-127 mimic. After TNF-α treatment and/or pc-TUG1 or miR-127 mimic transfection, ***D,*** ICC apoptosis, ***E,*** the expression levels of Bax, Bcl-2, Pro-caspase 3 and Cleaved-caspase 3 in ICC, and ***F-G,*** the mRNA and protein levels of IL-1β, IL-6, and MCP-1 were assessed, respectively. TUG1: Long non-cording RNA taurine upregulated gene 1; miR-127: MicroRNA-127; TNF-α: Tumor necrosis factor alpha; ICC: Interstitial cells of Cajal; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; MCP-1: Monocyte chemotactic protein 1. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Discussion

IBS, a prevalent functional disorder occurred in gastrointestinal tract, has been discovered to be linked to infection and immune activation[24]. It has been reported that some pro-inflammatory cytokines are stimulated in patients with IBS[25]. In addition, the immune activation and mucosal inflammation, for example induced by inflammatory bowel disease, can alter intestinal motility in our body[6]. In inflamed intestine of patients, the immune system may target nerves, intestinal smooth muscle cells and the pacemaker system (ICC) and change gut functions[26]. Previous studies[5,9] indicated that the loss of ICC was closely related with the pathogenesis of IBS. Due to the reasons above, ICC was used for our study and treated by TNF-α to simulate the inflammatory condition resulting in IBS. TNF-α was frequently-used for induction of inflammatory models[25,27]. Our data showed that TNF-α treatment decreased ICC viability, increased ICC apoptosis, enhanced pro-apoptotic factor (Bax and cleaved-caspase-3) expression levels, reduced anti-apoptotic factor (Bcl-2) expression level and increased pro-inflammatory cytokines (IL-1β, IL-6, and MCP-1) expression levels. TUG1 was downregulated in ICC in response to TNF-α treatment. Whether TUG1 mediated cell apoptosis and inflammatory response remain unclear. Thus, apoptosis and degree of inflammation in ICC were evaluated when TUG1 expression was altered by transfection assay. The results indicated that the overexpression of TUG1 effectively declined apoptotic cell rate, reduced Bax and cleaved caspase-3 expressions and stimulated Bcl-2 expression in TNF-α-induced ICC, suggesting the apoptosis-inhibitory activity of TUG1. As for TUG1-silenced cells, apoptosis and expression levels of pro-inflammatory cytokines were enhanced, opposite to changes in the TUG1-overexpressed group. Most studies about TUG1 focused on its function in tumors. High TUG1 level enhances tumor growth and metastasis in lung adenocarcinoma but TUG1 silence impaired cell function by suppressing viability and promoting apoptosis[28]. This work also

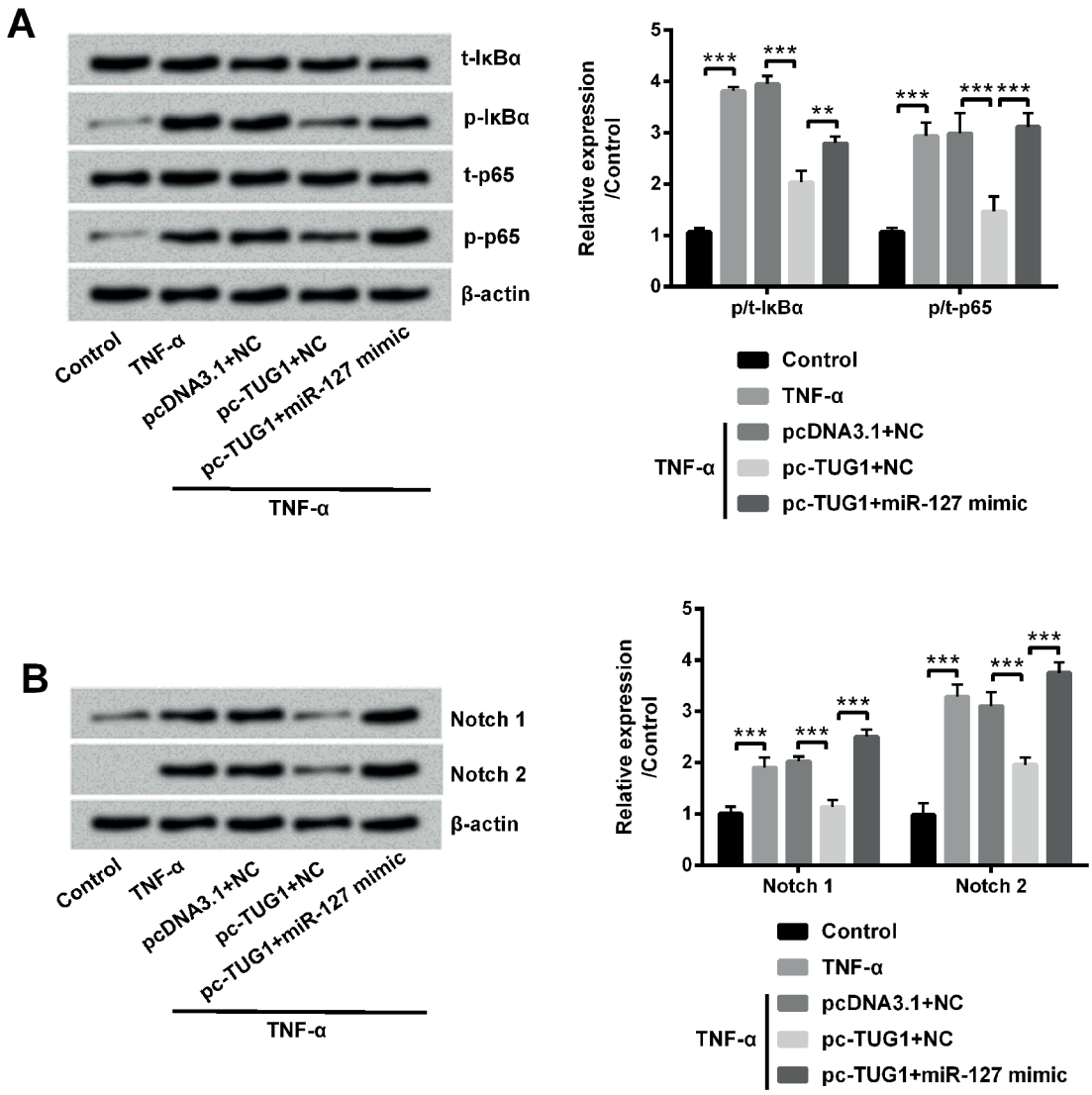

Figure 4. The overexpression of TUG1 suppressed NF-κB and Notch pathways by down-regulating miR-127 in TNF-α-treated ICC. ***A-B,*** After TNF-α treatment and/or pc-TUG1 or miR-127 mimic transfection, the expression levels of t-IκBα, p-IκBα, t-p65, p-p65, Notch 1, Notch 2 in ICC were evaluated, respectively. TUG1: Long non-cording RNA taurine upregulated gene 1; miR-127: MicroRNA-127; TNF-α: Tumor necrosis factor alpha; NF-κB: Nuclear factor kappa B; IκBα: Inhibitor of NF-κB. $^{**}p < 0.01$, $^{***}p < 0.001$.

proved that Bax was the downstream target of TUG1[28], which could explain the apoptosis-inhibitory effect of TUG1. Moreover, TUG1 was considered to be a promising target for preventing the cold-induced liver injury in liver transplantation[18], which was consistent with our study. Finally, we sought to reveal the potential underlying mechanism of protective effects of TUG1, and we found that miR-127 was negatively regulated by TUG1, which prompted us to explore the roles of miR-127 in protective activity of TUG1. MiR-127 has been found to play important roles in embryogenesis and oncogenesis, as well as inflammation[29]. The dysregulation of miR-127 was observed in tissues of inflammatory bowel disease patients[30]. According to our data, miR-127 overexpression abrogated the protective effects of TUG1 overexpression on TNF-α-treated ICC, which suggested that miR-127 acted as a pro-inflammatory mediator in this process. These results were consistent with the previous study, which reported that the up-regulation of miR-127 led to increased production of TNF-α, IL-1β, and IL-6 in macrophages and exaggerated pulmonary inflammation and

injury[31]. Interestingly, previous research demonstrated that miR-127 had the inhibitory effect on inflammation in chondrocytes[32], which appears to be paradoxical to our results. We inferred that the function of miR-127 might differ in different diseases. NF-κB is a key transcription factor in the process of inflammation and pain[33]. The NF-κB pathway can be activated in IBS and take part in the inflammation and visceral hypersensitivity[34,35]. Heat shock protein 70 (hsp70) was found to play protective effect by inhibiting NF-κB in mice suffered from IBS[36]. The findings of Ying et al[31] showed that miR-127 could activate the NF-κB signaling pathway in lung inflammation by targeting Bcl-6. The promoting effect of miR-127 on the NF-κB pathway was also shown in our work. We found that TUG1 inhibited TNF-α-induced inflammatory injury in ICC might by down-regulating miR-127 and then inactivating the NF-κB pathway. Additionally, studies showed that the Notch signaling pathway was also involved in the pathogenesis of IBS[37]. The effect of TUG1 on glioma was regulated by Notch pathway[15]. Our findings indicated the cross-talk between TUG1 and Notch pathway. TUG1 alleviated TNF-α-induced inflammatory injury also by inhibiting miR-127 and then inactivating Notch pathway.

Conclusions

We showed that TUG1 attenuated TNF-α-induced apoptosis and inflammatory response in ICC by decreasing miR-127 expression. Mechanistically, the inactivation of NF-κB and Notch signaling pathways regulated by TUG1-miR-127 axis might contribute to the protective effects of TUG1. This study may provide possible targets to alleviate inflammation in ICC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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