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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-[illegible]Z) were analyzed by qRT-PCR. Viability, a[illegible]sis and the expression of apoptosis-asso[illegible]ed factors were analyzed by CCK-8 assay, flo[illegible]tometry and Western blot, respectively. The [illegible]NA and protein levels of pro-inflammatory cy[illegible]kines were detected by qRT-P[illegible] Weste[illegible]blot, respectively. Finally, a[illegible]tio[illegible]f nuclear factor kappa-B (NF-κB) [illegible] Notch [illegible]thways were evaluated by Weste[illegible]ot.

RESULTS: TNF-α tr[illegible]th[illegible]hi[illegible]bility, induced ICC [illegible]tosi[illegible]romoted an inflammatory resp[illegible]e in ICC. [illegible]was downregulated in TN[illegible]ated ICC. [illegible]overexpression pro[illegible]ec[illegible] from TNF-[illegible]induced apoptosis an[illegible]pro-in[illegible]atory cytokines expression. [illegible]G1 suppr[illegible] showed opposite eff[illegible] MiR-127 was [illegible]ively regulated by TU[illegible]and in[illegible]licated in th[illegible]action of TUG1 in I[illegible]MiR-12[illegible]p-regulation largely reversed the[illegible]s o[illegible]G1 on [illegible]F-α-treated ICC. Mechanisti[illegible]G1 inh[illegible]d TNF-α-induced acti[illegible]n o[illegible]B a[illegible]otch pathways in ICC by [illegible]regu[illegible]-127.

C[illegible]CLUSI[illegible] TUG1 attenuated TNF-α-ca[illegible]d apoptosis and inflammatory response in I[illegible]egulating miR-127 and then inacti[illegible]ng NF-κB and Notch pathways.

Ke[illegible]rds:
Irritable bowel syndrome, Interstitial cells of Cajal, Long non-coding RNA TUG1, MicroRNA-127, NF-κB pathway, Notch pathway.

Introductio[illegible]

Irritable bowel sy[illegible]e (IBS), a kind of function[illegible]intestinal d[illegible]rs, is characterized b[illegible]ntermittent and recu[illegible]ent abdominal pain [illegible] changes in st[illegible]ol frequency and form[1]. How[illegible] the pathog[illegible]sis of IBS remains unclear[2]. M[illegible] factors co[illegible]bute to the occurrence of IBS, incl[illegible]gut [illegible]otility, imbalance of the intestinal fl[illegible]a, abnormalities of the brain-gut [illegible]xis, and poor diet habits[3,4]. Increasing numbers [illegible]rts provide evidence that there are links [illegible] 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 (... 13 g to 15 g) were obtained from the ...ical an... laboratory of The Affiliated Hosp... Qing... ty (Qingdao, China). A... xpe... mice ...e approved by the Ethi... ommitt... he Affiliated Hospital of Qing... niversity (Q... o, China). The small intes... moved (from ...m below the pyloric rin... the ce... and opened along the mesenteri... der. After w... luminal contents and re... ng mucosa, the ...ed small tissue strip... intesti... uscle were equilibrated using no... Ca... e solution (KCl 5.36 mM, NaCl 12.5 ... 0.34 ... $NaHCO_3$ 0.44 mM, glu... 10 ... ucros... mM, and HEPES 11 mM ...) for 3... ils were then dispersed in the enz... e soluti... containing collagenase, bovine se... min, trypsin inhibitor, and ATP magne... ...ased from Sigma-Aldrich (St. Lou... MO, USA). Subsequently, ICC was isolated and ...ified 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 Laboratori... tumamo... to, Japan) was used to evaluate th... tive viability of ICC. Briefly, ICC was seede... 96-well plate (Thermo Fisher Scient... Wal... MA, USA) with 5.000 cells p... ell. After ... treatment and/or relevan... nsfecti... 10 µl ... 8 kit solution was ad... nto th... ture medium of each well and th... p... cubate... 2 h at 37°C in hum... incu... (Sany... ncons, UK). After th... e absorba... f... n well at 450 nm wa... d using a ... Microplate Spectrop... mete... olecular Devices, Sunnyvale, CA, USA).

Apoptosis Analysis

...fter relevant ...eatment and/or transfection, ... was collecte... y centrifugation at 300 g and 4... r 5 min. T... , ICC was washed with precol... phat... ffered Saline (PBS; Beyotime Biotech... , Shanghai, China) and centrifuged (300 g, 4°C, 5 min) two times. Collected cells (1-5 ... were re-suspended in 100 µl 1× binding ... 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 GAAATGAGCTTGACA-3', GAPDH primer: 5'-GACCACAGTCCATGCCATC- U6 forward primer: 5'-TGGG GTTATACAT GAGAGGA-3', U6 reverse primer: 5'-GTGTC TACGGAG TTCAGAGGTT-3'. lative expression was calculated using ethod[23].

Western Blot

After relevant trea ansfec , ICC was harvested ysed by n X-100 lysis buffer (Thermo Scientific, am, MA, USA) supplem protease in tor cocktail (Sigma-A ich, St is, MO, USA) for 30 min at 4°C estern blot was established using -Rad Bis-Tris G ystem (Bio-Rad Labo ies, H ales, CA, USA). After quantifi ing Protein Assay kit (Beyotime Biotec Shang China), the proteins in al co ation e electrophoresed in 12 % p rylam and transferred onto polyvinyl e diflu e (PVDF) membranes (Millipo ica, MA, USA). After blocking by 5% fat igma-Aldrich, St. Louis, MO, USA) room temperature for 1 h, PVDF membranes incubated with primary antibodies (4°C, o ght) 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), N 1 (ab52627), Notch 2 (ab137665), a (ab8226), as well as the secondary body in cluding goat anti-rabbit IgG (ab2) and goat anti-mouse IgG (ab6789) were all p ed from Abcam Biotechnology (Cam idge, USA). β-actin was used as an int l control. resentative image from of the ree inde dent experiments wa own. J ge-J software (National Institute o Bethes MD, USA) was used t antify and in ity.

Statistical is

All exp ments repeated three times and all data were presen the mean + standard devi D). Statistica lysis was conducted u GraphPad Prism 6.0 software (GraphPad S ware Inc., La lla, CA, USA). Statistical comp ons were m using a one-way analysis of v e (ANOV with Sidak post-hoc test. *p*-value nsidered 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

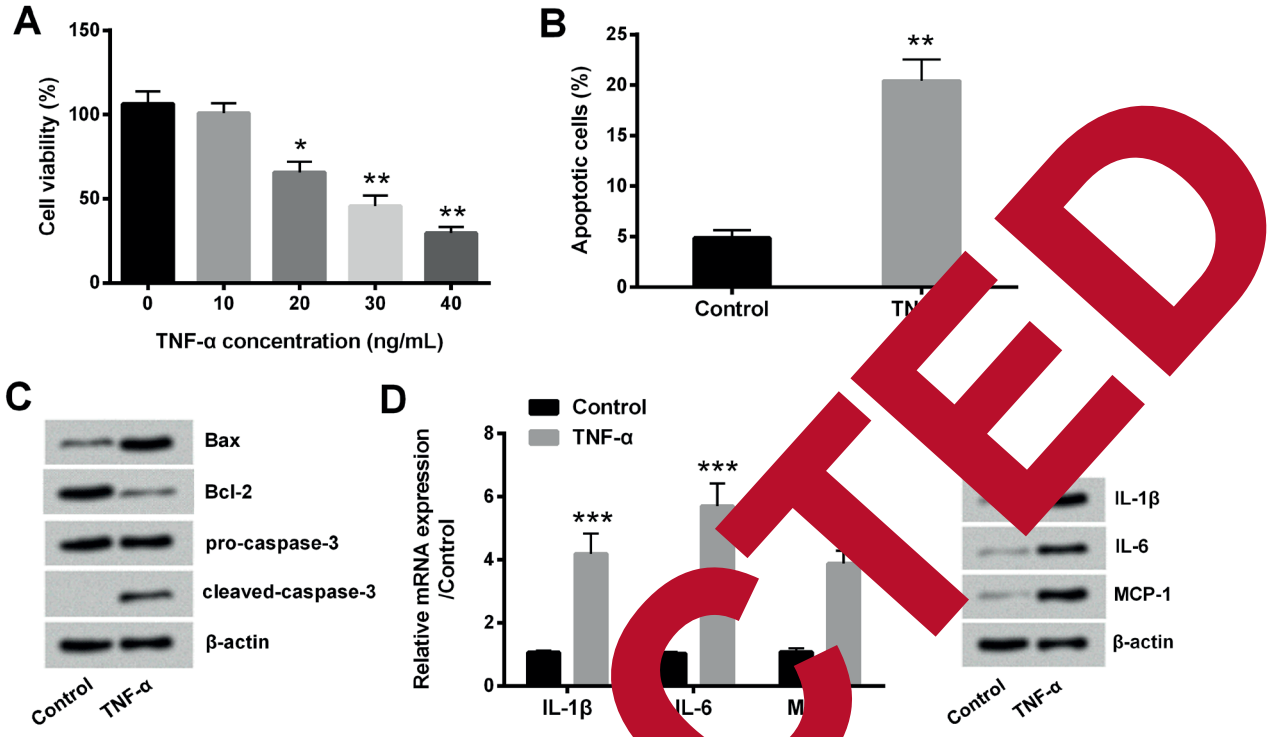

Figure 1. TNF-α induced ICC injury. After TNF-α treatment, ***A,*** ICC [illegible] apoptosis, ***C,*** the expression levels of Bax, Bcl-2, Pro-caspase 3 and Cleaved-caspase 3 in [illegible] ***D-E,*** the mRNA and protein levels of IL-1β, IL-6, and MCP-1 were detected, respectively. TNF-α: Tumor necrosis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

TNF-α-induced ICC apopto[illegible] inflammatory cytokines expres[illegible], ICC [illegible] transfected with pc-TUG1 o[illegible] UG1. The results displayed [illegible] of TUG1 was sig[illegible] ed after pc-TUG1 transfecti[illegible] decreased [illegible] si-TUG1 transfection ([illegible] ure 2B and [illegible]). Figure 2D showed th[illegible] TNF-[illegible] uced ICC apoptosis was mar[illegible] inhibited [illegible] he up-regulation of TUG[illegible] nd exacerbated b[illegible] e down-regulation [illegible] UG1 ([illegible] 0.05). Compared to the single [illegible]-α g[illegible], the protein expression levels of Ba[illegible] eaved-[illegible]ase-3 were decreased [illegible] the [illegible] in ex[illegible]ion level of Bcl-2 was [illegible]sed i[illegible]-α + pc-TUG1 group (Figure [illegible]). On t[illegible]ontrary, compared to the single [illegible] group, the protein expression levels [illegible] cleaved-caspase-3 were increased [illegible]d the protein expression level of Bcl-2 was de[illegible]sed in TNF-α + si-TUG1 group. Moreover, c[illegible]red 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-ĐB) 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- transfection abrogated the effects of TUG1 overexpression on p-IκBα and p-p65 e sion levels decrease in TNF-α-treated ICC (p or $p <$ 0.001). Similarly, the expressi levels tch 1 and Notch 2 were both in ed by TN 0.001), but decreased by G1 ove xpressi < 0.001). However, th ects o G1 on Notch 1 and Notch 2 exp decrea were impaired by mi / mi ransfe ($p <$ 0.001, Figure These fin sted that the overexp f TUG1 su ed TNF-α-induced a ation F-κB and Notch pathways in ICC possibly by d egulating miR-127.

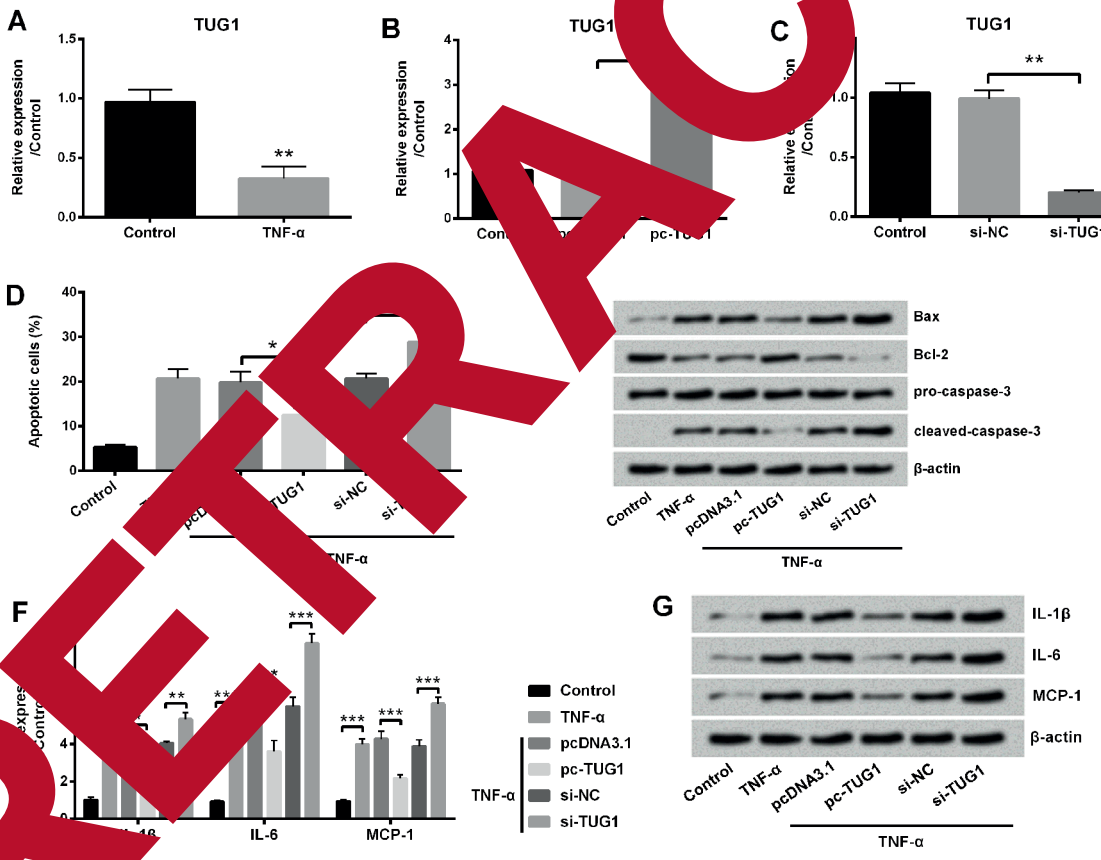

Figure 2. TUG1 participated in the regulation of TNF-α-induced inflammation injury in ICC. ***A,*** After TNF-α treatment, the ssion of TUG1 in ICC was measured. ***B-C,*** After pc-TUG1 or si-TUG1 transfection, the expression of TUG1 in ICC was d, respectively. After TNF-α treatment and/or pc-TUG1 (or si-TUG1) transfection, ***D,*** the apoptosis of ICC, ***E,*** the expres n 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. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

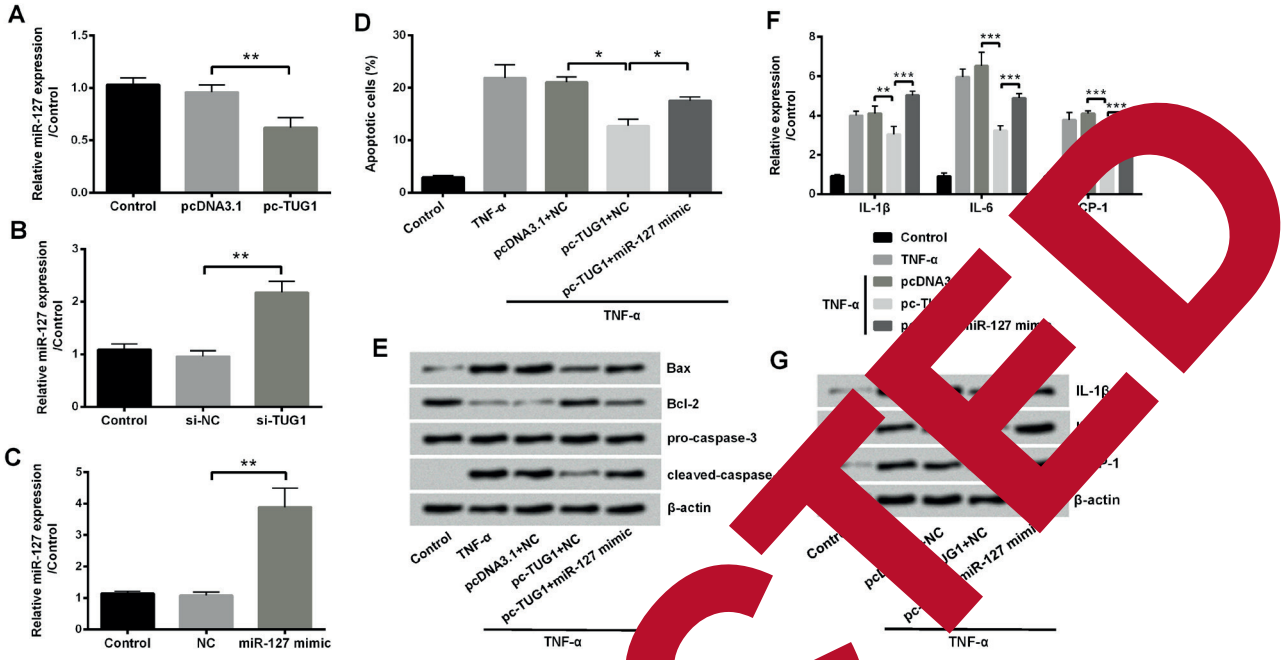

Figure 3. miR-127 was implicated in the effects of TUG1 on ICC. ... the expressio... miR-127 in ICC was detected after pc-TUG1 or si-TUG1 transfection. ***C,*** The expression of miR-127 in I... measu... er transfection with miR-127 mimic. After TNF-α treatment and/or pc-TUG1 or miR-127 mimic transfection... tosis, ***E,*** the expression levels of Bax, Bcl-2, Pro-caspase 3 and Cleaved-caspase 3 in ICC... the mRNA and p... ein levels of IL-1β, IL-6, and MCP-1 were assessed, respectively. TUG1: Long non-cording R... lated gene 1; miR-127: MicroRNA-127; TNF-α: Tumor necrosis factor alpha; ICC: Interstitial cells of Cajal; I... Inter... IL-6: Interleukin 6; MCP-1: Monocyte chemotactic protein 1. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Discuss...

IBS, a prevalent fu...tion... r occ... d in gastrointestinal ..., has b... iscovered to be linked to inf... and immu... tivation[24]. It has been re... some pro-i... mmatory cytokines are ...imulat... patients with IBS[25]. In additi... e immune ...tion and mucosal inflam...on, for example i... d by inflammatory ...el dis..., can alter intestinal motility in ...ody[6]... inflamed intestine of patients, the i... stem n... arget nerves, intestinal ...oth ... cel... d the pacemaker system (... and c... t functions[26]. Previous studies ...dicated ... the loss of ICC was closely re... ...th the pathogenesis of IBS. Due to the ..., ICC was used for our study and ...eated by TNF-α to simulate the inflammatory ...dition resulting in IBS. TNF-α was frequently ... 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

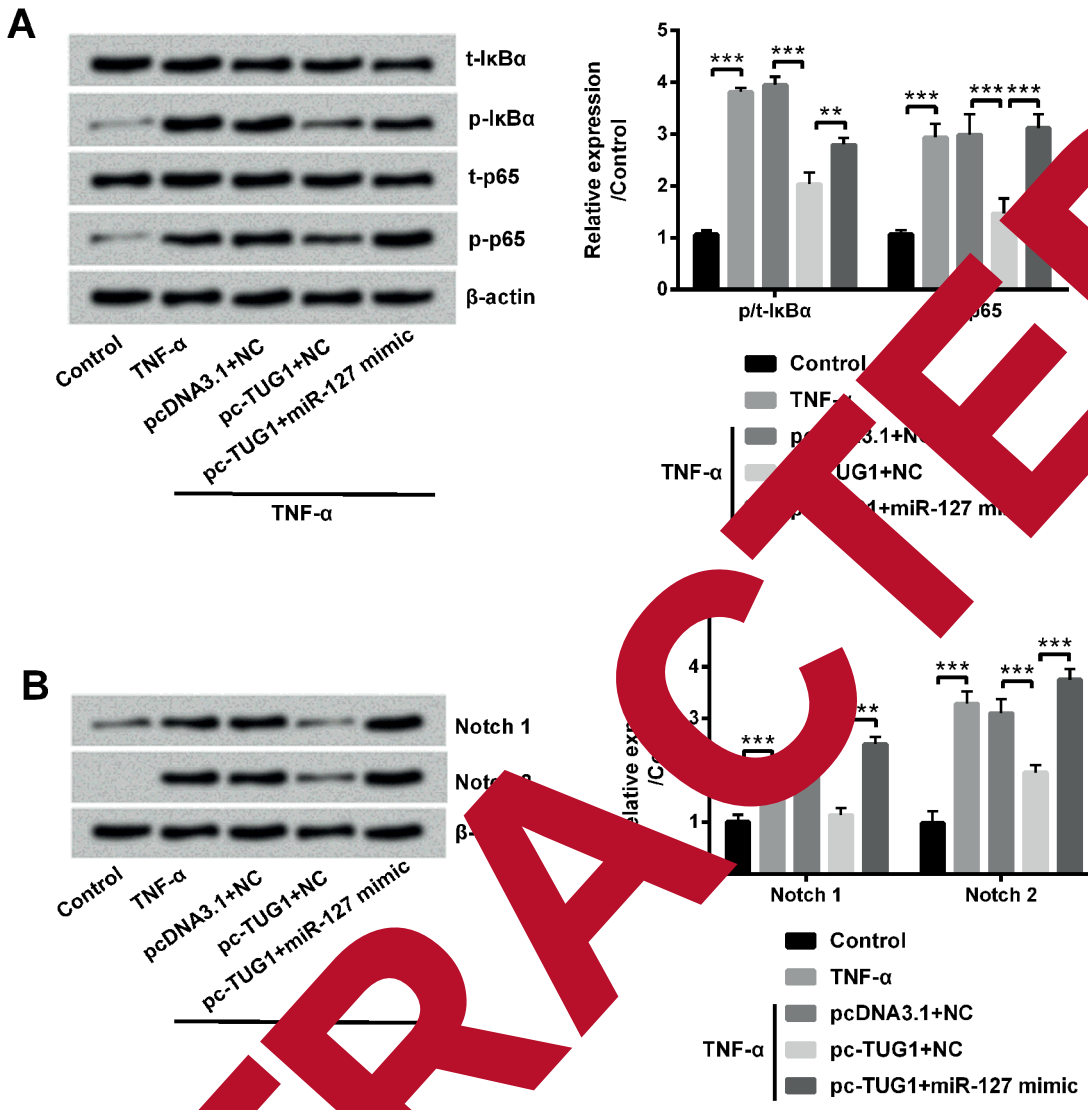

Figure 4. The overe ion of TUG1 ed NF-κB and Notch pathways by down-regulating miR-127 in TNF-α-treated ICC. ***A-B,*** After T ent and/or pc 1 or miR-127 mimic transfection, the expression levels of t-IκBα, p-IκBα, t-p65, p-p65, Nc , No ICC were evaluated, respectively. TUG1: Long non-cording RNA taurine upregulated gene 1; miR-127: M roRNA-127, : Tumor necrosis factor alpha; NF-κB: Nuclear factor kappa B; IκBα: Inhibitor of NF-κB. $^{**}p < 0.01$ < 0.001.

prove x was downstream target of G1[28], coul plain the apoptosis-inhib- ffect Moreover, TUG1 was con- side to be a p ising target for preventing the co d liver injury in liver transplantation[18], ch sistent with our study. Finally, we ught to reveal the potential underlying mecha- of protective effects of TUG1, and we found th R-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-ind d inflammatory injury also by inhibiting n and then inactivating Notch pathway.

Conclusion

We showed that TUG1 nuated F-α-induced apoptosis and im atory in ICC by decreasing m 12 on. Mechanistically, the inact on of 3 and Notch signaling pathw gulated by -miR-127 axis might co the protecti ffects of TUG1. This st y may ide possible targets to alleviate i mmation in

Co of I est

The Au re that t ave no conflict of interest.

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