

Role of TGM2 in T-cell lymphoblastic lymphoma via regulation of IL-6/JAK/STAT3 signalling

YUYAN WANG*, NI ZHENG*, TINGTING SUN, HUI ZHAO, YING CHEN and CONGCONG LIU

Department of Laboratory, Shengli Oilfield Central Hospital, Dongying, Shandong 257034, P.R. China

Received August 19, 2021; Accepted November 16, 2021

DOI: 10.3892/mmr.2022.12592

Abstract. Transglutaminase 2 (TGM2) is a Ca^{2+} -dependent enzyme that is closely associated with cancer progression; however, the function of TGM2 in T-cell lymphoma remains unclear. In the present study, TGM2 was identified as an upregulated gene by bioinformatics analysis of the microarray datasets GSE132550 and GSE143382 from the Gene Expression Omnibus database. The effects and mechanisms of TGM2 on T-cell lymphoma cells were evaluated using the Cell Counting Kit-8, colony formation assay, 5-ethynyl-2'-deoxyuridine (EdU) assay, flow cytometry, reverse transcription-quantitative polymerase chain reaction, western blotting and gene set enrichment analysis (GSEA). TGM2 expression was shown to be elevated in formalin-fixed paraffin-embedded skin biopsies from patients with T-cell lymphoma relative to skin tissue from healthy cases. TGM2 expression was also increased in T-cell lymphoma cell lines compared with that in CD4^+ T cells. Transfection with TGM2 small interfering RNAs (siRNAs) decreased the number of EdU-positive cells, and the viability and colony formation of T-cell lymphoma cells. Furthermore, TGM2 siRNAs enhanced the apoptosis of T-cell lymphoma cells potentially via cleavage of caspase-3 and poly ADP-ribose polymerase. GSEA identified the IL-6/JAK/STAT3 pathway as a potential downstream signalling pathway of TGM2. Notably, the effects of TGM2 siRNAs on T-cell lymphoma cells were attenuated by IL-6 and accelerated by IL-6/JAK/STAT3 inhibitor AG490. These findings indicated that TGM2 siRNAs inhibited the proliferation of T-cell lymphoma cells by regulating the IL-6/JAK/STAT3 signalling pathway; therefore, TGM2 may function as a potential therapeutic target for T-cell lymphoma.

Introduction

T-cell lymphoblastic lymphoma (T-LBL) is an aggressive cancer of precursor T cells, which accounts for ~90% of lymphoblastic lymphoma cases worldwide (1). The majority of T-LBL cases occur in children and young adults, particularly in male patients (1). T-LBL is predicted to originate from malignant thymocytes at a specific stage during intrathymic T-cell differentiation (1). The development of T-LBL can lead to metastases of lymphoblasts to the spleen, bone marrow and central nervous system, which can be life-threatening (2). Although the 5-year event-free survival rate has been greatly improved by contemporary chemotherapy (~85%), salvage remains poor with <25% event-free and overall survival rates for relapsed disease in Australia (3). Therefore, exploration of the mechanisms underlying T-LBL malignancy, and the identification of novel and effective therapeutic targets is crucial.

Transglutaminase 2 (TGM2) is a ubiquitously expressed member of the transglutaminase family of Ca^{2+} -dependent enzymes. TGM2 is responsible for catalysing the formation of covalent bonds between peptide-bound glutamine and a variety of primary amines, including the γ amino group of peptide-bound lysine, or mono- and polyamines, thus producing cross-linked or aminated proteins, respectively (4-6). Previous studies have suggested a close association between TGM2 and cancer progression, including apoptosis, angiogenesis, cell stemness and drug resistance (7-9). The effects of TGM2 on various types of cancer have previously been reported, including osteosarcoma (8), colorectal cancer (CRC) (7), glioma (9) and endometrial cancer (10). As for lymphoma, TGM2 has been shown to facilitate the survival of malignant B cells and promote lymphoma progression (11). Additionally, TGM2 may modulate autophagy in mantle cell lymphoma cells by activating NF- κ B, which can confer cell survival (11).

IL-6 is a cytokine that mediates the homeostatic process, and has a great impact on various immune and non-immune cell types (12). IL-6 has been reported to modulate the survival, proliferation, maturation and cytokine secretion of B and T cells (12) and, has an important role in activating oncogenic signalling pathways in cancer (13,14). Furthermore, IL-6 can activate multiple intracellular signalling pathways, such as GTPase RAS and mitogen-activated protein kinase signalling, which modulate cell proliferation and differentiation, as well as the well-known JAK family and STAT family of transcription factors (15). Mounting evidence has suggested that IL-6

Correspondence to: Dr Hui Zhao, Department of Laboratory, Shengli Oilfield Central Hospital, 31 Jinan Road, Dongying, Shandong 257034, P.R. China
E-mail: huizhao3316@126.com

*Contributed equally

Key words: T-cell lymphoma, proliferation, apoptosis, transglutaminase 2, IL-6/JAK/STAT3 signalling

may mediate resistance against lymphoma treatment. Notably, levels of IL-6 and phosphorylated (p)-STAT3 were revealed to be increased in duvelisib [a phosphatidylinositol-3-kinase (PI3K) PI3K inhibitor]-resistant T-cell lymphoma cells, and the upregulated expression of IL-6/STAT3 possibly mediated the resistance in lymphoma (16). ERK-dependent IL-6 positive feedback regulatory signalling has also been shown to cause resistance of lymphoma to the PI3K inhibitor BKM120, whereas blockade of ERK activity could reduce IL-6 secretion and improve antitumor outcomes (17).

The present study aimed to explore the novel mechanisms underlying T-LBL progression. Bioinformatics analysis of microarray data from the GSE132550 and GSE143382 datasets revealed a significantly higher expression of TGM2 in T-LBL samples compared with that in normal samples. The effects of TGM2 on T-LBL cell proliferation and apoptosis were determined. Furthermore, whether TGM2 exerted its effects on T-LBL cells through regulating IL-6/JAK2/STAT3 signalling was revealed. The findings of present study may present a novel therapeutic target for T-LBL treatment.

Materials and methods

Bioinformatics analysis. The GSE132550 (18) and GSE143382 datasets (19) were selected for targeted chip research from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo). The GSE132550 dataset comprised 80 formalin-fixed paraffin-embedded (FFPE) tumour cases, including 68 peripheral T-cell lymphoma, not otherwise specified cases and 12 Lennert lymphoma cases. Peripheral T-cell lymphoma cases and 10 CD4⁺ T-cell samples from healthy donors were subjected to gene expression profiling in the GSE132550 dataset. The mRNA profiling was carried out on all tumour samples and 10 normal CD4⁺ T-cell samples from healthy donors and analysed using GeneSpring version GX 12 (Agilent Technologies, Inc.). The GSE143382 dataset comprised mRNA information from early-stage mycosis fungoides biopsies (43 cases), dermatitis biopsies (29 cases) and 12 healthy skin tissue controls, which were analysed by an 800 gene NanoString panel (NanoString Technologies, Inc.). Differentially expressed genes were screened using the 'limma' package in R (Linear Models for Microarray Data, <http://www.bioconductor.org/packages/release/bioc/html/limma.html>). The mechanism underlying TGM2-mediated T-cell lymphoma was analysed based on microarray data using the gene set enrichment analysis (GSEA) software (version 2.0.14, <http://www.broadinstitute.org/gsea/index.jsp>). Gene Ontology (GO) term function annotation and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the ClusterProfiler package in R language (20) to annotate differentially expressed genes under the 'biological process', 'cellular component' and 'molecular function' categories, and the enrichment pathways were analysed. Biological processes and pathways with significant differences were selected, and their enrichment scores and P-values were ranked. Visualisation of these pathways with $P < 0.05$ was completed using the R software package.

Tissue sample collection. This study was approved by the ethics committee of Shengli Oilfield Central Hospital (Dongying, China; approval no. DYSLYT20191201). Written informed consent was obtained from all the enrolled

individuals. The skin biopsies were collected from 36 patients with stage III~IV cutaneous T-cell lymphoma who underwent tumor resection or biopsy procedure at the Shengli Oilfield Central Hospital between January 2020 and January 2021. In total, 21 patients were male and 15 were female with a median age of 36 years (ranged from 19 to 64 years). The skin biopsies were fixed by using 10% formalin at room temperature ($22 \pm 2^\circ\text{C}$) for 24 h, embedded in paraffin, and sectioned at 4- μm thickness. No samples were collected following the treatment of first-line therapy, such as hyper-CVAD. T-cell lymphoma was diagnosed by two physicians depending on the skin biopsy. A total of 36 normal skin tissues collected from sex- and age-matched healthy individuals were used as controls. Peripheral blood was collected from four healthy human adults (two male and two female subjects) with a medium age of 45 years for the following CD4⁺ T cell isolation.

CD4⁺ cell collection. For isolation of peripheral blood mononuclear cells (PBMCs), peripheral blood diluted in phosphate buffer saline (PBS; 1:1) was added into a 15 ml centrifuge tube together with 5 ml Ficoll-Paque media (Cytiva). After centrifugation at 400 x g for 20 min, the supernatant was collected and washed in PBS twice. A sample of 1×10^{10} /l PBMCs was incubated with anti-CD4 primary antibody (cat. no. 612761; BD Biosciences) at 25°C for 30 min and loaded on a FACSCalibur (BD Biosciences) to obtain CD4⁺ T cells. The obtained CD4⁺ T cells were used in the following reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blotting.

Cell culture. T-cell lymphoma cell lines, including Jurkat, MJ, HH and SUP-T1, were purchased from American Type Culture Collection (ATCC). MOTN-1 cells were purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. ATN-1 cells were purchased from RIKEN BioResource Center. Jurkat, MOTN-1, ATN-1, HH and SUP-T1 cells were maintained in RPMI-1640 medium (HyClone; Cytiva) containing 10% foetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (MilliporeSigma). MJ cells were maintained in Iscove's modified Dulbecco's medium (ATCC) supplemented with 20% FBS and 1% penicillin/streptomycin. All cells were cultured in a humidified incubator containing 5% CO₂ at 37°C .

siRNA transfection. siRNAs targeting TGM2 (si-TGM2-1 and si-TGM2-2) and the scrambled control were synthesised by Shanghai GenePharma Co., Ltd. Jurkat and SUP-T1 cells in 6-well plates at a density of 1×10^5 cells/well were transfected with 30 nM siRNAs using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37°C . Following transfection, the cells were cultured for 48 h at 37°C and used in subsequent experimentation. The siRNA sequences were as follows: si-TGM2-1, 5'-GAGCTGGTCTTAGAG AGGTGT-3'; si-TGM2-2, 5'-GCAACCTTCTCATCGAGT ACT-3'. A scrambled sequence (5'-GATGGCATGGCGGAG TGATTT-3') was used as the negative control. Following transfection, Jurkat and SUP-T1 cells were treated with 60 ng/ml IL-6 (MedChemExpress) for 6 h or 100 μM AG490 (MedChemExpress) for 24 h at 37°C .

Cell Counting Kit-8 (CCK-8). CCK-8 (Dojindo Molecular Technologies, Inc.) was used to evaluate cell viability. Briefly, 3×10^3 Jurkat and SUP-T1 cells were inoculated in a 96-well plate. After incubation for the 24, 48 and 72 h at 37°C, 10 μ l CCK-8 reagent was added to each well and incubated for a further 2 h at 37°C. The absorbance values at 450 nm were measured using a microplate detector (PerkinElmer, Inc.).

5-ethynyl-2'-deoxyuridine (EdU) assay. An EdU-labelling kit (Thermo Fisher Scientific, Inc.) was used to determine cells in the S phase. Briefly, 3×10^3 Jurkat and SUP-T1 cells were seeded in 96-well plates overnight, followed by incubation with 50 μ M EdU reagent for 1 h at 37°C. Nuclei were stained with 0.1 μ g/ml DAPI (MilliporeSigma) for 20 min at room temperature (22±2°C) and the samples were observed under a fluorescence microscope (Leica Microsystems GmbH).

Colony formation. Jurkat or SUP-T1 cells (1×10^3) were seeded in 6-well plates as single cell suspensions and were cultured for 2 weeks. Subsequently, visible colonies with >50 cells were stained with 0.1% crystal violet (Beyotime Institute of Biotechnology) for 20 min at room temperature (22±2°C), and were observed and captured under a light microscope (Leica Microsystems GmbH).

Cell apoptosis. Apoptosis was detected using an Annexin V-FITC/PI kit (Beyotime Institute of Biotechnology). A sample of 1×10^5 Jurkat or SUP-T1 cells were collected after the indicated treatment, suspended in binding buffer, and incubated with FITC-conjugated Annexin V and PI reagent in the dark for 10 min at room temperature (22±2°C). The samples were then analysed using flow cytometry (BD LSRFortessa™ X-20; BD Biosciences) and the cytometry data were analysed using FlowJo software version 7.6.1 (FlowJo LLC).

RT-qPCR. RNA was extracted from FFPE biopsy samples, CD4⁺ T cells and T-cell lymphoma cells lines using RecoverAll™ Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Inc.) and TRIzol® Reagent (Invitrogen; Thermo Fisher Scientific, Inc.), respectively. Subsequently, cDNA was obtained using a High-Capacity cDNA RT kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's protocol. mRNA expression was evaluated using SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Inc.) and normalised to GAPDH using the $2^{-\Delta\Delta C_q}$ method (21). The following thermocycling conditions were used: Enzyme activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 1 min and extension at 72°C for 20 sec. The primers used were as follows: TGM2, forward 5'-GAGGAGCTGGTCTTA GAGAGG-3' and reverse 5'-CGGTCACGACACTGAAGG TG-3'; and GAPDH, forward 5'-GTTGCAACCGGAAG GAAAT-3' and reverse 5'-GCCCAATACGACCAAATC AGA-3'.

Western blotting. Proteins were extracted from Jurkat or SUP-T1 cells using ice-cold radioimmunoprecipitation assay buffer (Thermo Fisher Scientific, Inc.) containing protease and phosphatase inhibitors (Thermo Fisher Scientific, Inc.). Protein concentration of the extracts was determined by using

a Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime Institute of Biotechnology). Equal amounts (20 μ g) of protein were separated via 10~12% SDS-PAGE and blotted onto PVDF membranes, which were blocked with 5% non-fat milk at room temperature (22±2°C) for 1 h. Subsequently, the membranes were incubated with specific primary antibodies against TGM2 (1:1,000; cat. no. ab2386; Abcam), cleaved caspase-3 (1:500; cat. no. ab2302; Abcam), cleaved poly ADP-ribose polymerase (PARP) (1:1,000; cat. no. ab32561; Abcam), JAK2 (1:5,000; cat. no. ab108596; Abcam), p-JAK2 (1:2,000; cat. no. ab195055; Abcam), STAT3 (1:2,000; cat. no. ab68153; Abcam), p-STAT3 (1:2,000; cat. no. ab32143; Abcam) and GAPDH (1:2,000; cat. no. ab9485; Abcam) overnight at 4°C. The next day, the blots were probed with secondary goat anti-mouse (1:2,000; cat. no. ab6789; Abcam) or goat anti-rabbit antibodies (1:2,000; cat. no. ab205718; Abcam) at room temperature (22±2°C) for 1 h. The blots were visualised using an ECL system (Thermo Fisher Scientific, Inc.) and analysed via Image Lab software (version 5.0, Bio-Rad Laboratories, Inc.).

Statistical analysis. Data are presented as the mean ± SD from three independent experiments. Statistical analysis was performed using SPSS 22.0 (IBM Corp.). An unpaired t-test was used for two-group comparisons. One-way ANOVA followed by Dunnett's multiple comparisons test was used for multiple-group comparisons. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

TGM2 is highly expressed in T-cell lymphoma samples and cells. To explore the potential oncogenes in the development of T-cell lymphoma, an overlap analysis was performed based on the upregulated genes from the GSE132550 and GSE143382 datasets. Eight overlapping genes were identified, namely MMP12, CCL19, MMP9, CD180, CCL18, FPR3, TNC and TGM2. TGM2 was selected for further analysis, because its role in lymphoma is not well understood (Fig. 1A). Bioinformatics analysis based on the GEO database from the GSE132550 dataset revealed that TGM2 was significantly upregulated in T-cell lymphoma samples compared with that in the normal group ($P < 0.05$; Fig. 1B). Furthermore, the present study validated that the expression of TGM2 was elevated in the FFPE skin biopsies from patients with T-cell lymphoma ($n=36$) relative to the skin tissue from healthy patients ($n=36$) ($P < 0.05$; Fig. 1C). Further analysis revealed that the mRNA and protein expression levels of TGM2 were increased in T-cell lymphoma cell lines, including Jurkat, MOTN-1, ATN-1, MJ, HH and SUP-T1 cells, compared with CD4⁺ T cells collected from four healthy controls ($P < 0.05$; Fig. 1D and E). Collectively, these results suggested that TGM2 may be highly expressed in T-cell lymphoma samples and cells.

TGM2 siRNAs suppress the viability and proliferation of T-cell lymphoma cells. The function of TGM2 in T-cell lymphoma cell proliferation was studied. For this purpose, T-cell lymphoma Jurkat and SUP-T1 cells, which had higher expression of TGM2, were transfected with TGM2 siRNAs. As shown in Fig. 2A, TGM2 siRNAs markedly reduced the protein expression levels of TGM2 in both Jurkat and SUP-T1 cells, indicating that TGM2

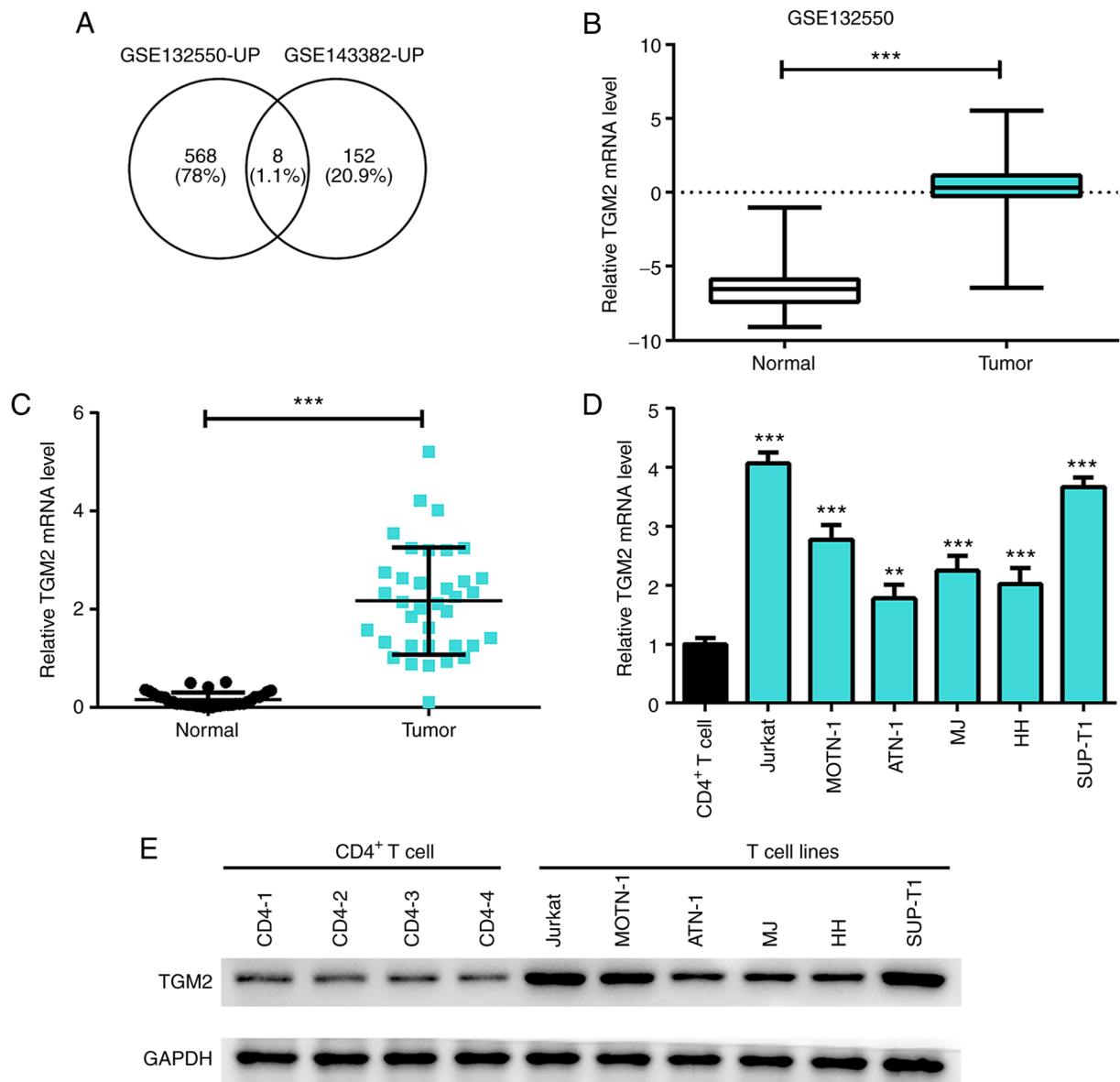


Figure 1. TGM2 is highly expressed in T-cell lymphoma samples and cell lines. (A) Overlap analysis was conducted by comparing the upregulated genes in microarray data from the GSE132550 and GSE143382 datasets; eight genes were identified, including MMP12, CCL19, MMP9, CD180, CCL18, FPR3, TNC and TGM2. (B) Expression of TGM2 was analysed in T-cell lymphoma samples and normal samples from the Gene Expression Omnibus database. (C) Formalin-fixed paraffin-embedded skin biopsies from patients with T-cell lymphoma (n=36) were collected and skin tissues from healthy individuals were used as controls (n=36). The expression of TGM2 in the samples was detected by RT-qPCR. (D) mRNA expression levels of TGM2 in CD4⁺ T cells and T-cell lymphoma cell lines (Jurkat, MOTN-1, ATN-1, MJ, HH and SUP-T1) were measured by RT-qPCR. (E) Protein expression levels of TGM2 in CD4⁺ T cells and T-cell lymphoma cell lines (Jurkat, MOTN-1, ATN-1, MJ, HH and SUP-T1) was detected by western blotting. Data are presented as the mean \pm SD. **P<0.01, ***P<0.01 as indicated or vs. CD4⁺ T cells. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; TGM2, transglutaminase 2.

was silenced successfully. In addition, TGM2 siRNAs decreased the viability of Jurkat and SUP-T1 cells (P<0.05; Fig. 2B and C). Furthermore, the number of EdU-positive Jurkat and SUP-T1 cells was reduced by TGM2 siRNAs (P<0.05; Fig. 2D and E). Consistently, TGM2 siRNAs suppressed the colony-forming ability of Jurkat and SUP-T1 cells (P<0.05; Fig. 2F-H). Taken together, these results suggested that TGM2 siRNAs were effective at suppressing the viability and proliferation of T-cell lymphoma cells *in vitro*.

TGM2 siRNAs stimulate the apoptosis of T-cell lymphoma cells. The present study analysed the effect of TGM2 on the apoptosis of T-cell lymphoma cells. TGM2 siRNAs

enhanced the apoptosis of Jurkat and SUP-T1 cells (P<0.05; Fig. 3A and B). Furthermore, the expression levels of cleaved caspase-3 and cleaved PARP were increased by TGM2 siRNAs in Jurkat and SUP-T1 cells (Fig. 3C and D).

IL-6/JAK/STAT3 signalling is a downstream pathway of TGM2. The mechanism underlying TGM2-mediated T-cell lymphoma was studied. To this end, bioinformatics analysis based on microarray data using the GSEA software was performed. GO and KEGG pathway enrichment analyses were conducted using the ClusterProfiler package, and the activation and suppression pathways of TGM2 were analysed. The IL-6/JAK/STAT3 pathway was predicted to be

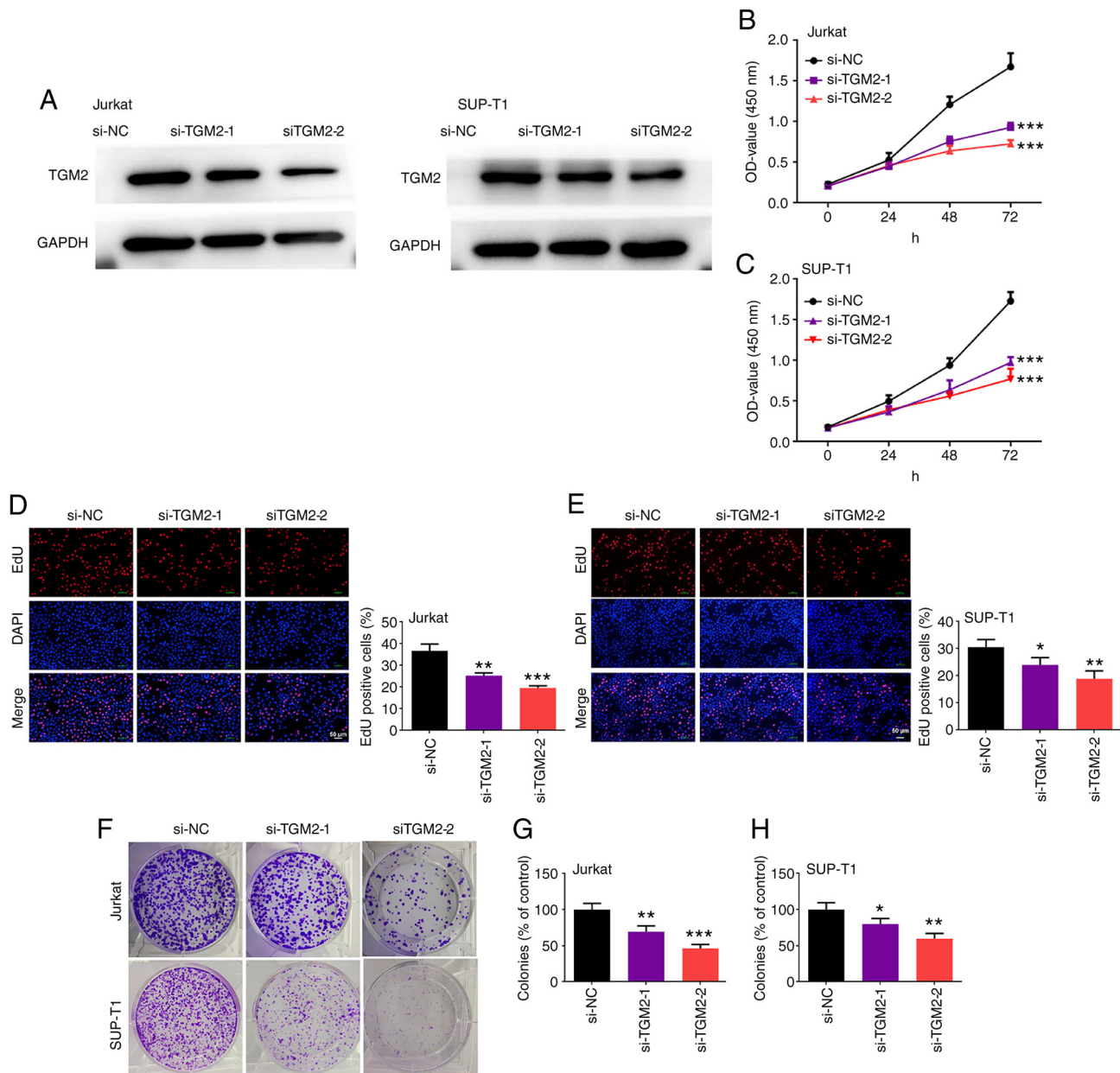


Figure 2. TGM2 siRNAs suppress the viability and proliferation of T-cell lymphoma cells. Jurkat and SUP-T1 cells were transfected with TGM2 siRNAs. (A) Protein expression levels of TGM2 were analysed by western blotting. (B and C) Cell viability was measured by Cell Counting Kit-8 assay. (D and E) Cell proliferation was detected by the EdU assay. (F-H) Cell survival was determined via a colony formation assay. Data are presented as the mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the si-NC group. EdU, 5-ethynyl-2'-deoxyuridine; NC, negative control; si/siRNA, small interfering RNA; TGM2, transglutaminase 2.

activated by TGM2 and was selected for subsequent analysis (Fig. 4A). Subsequently, it was confirmed that TGM2 siRNAs suppressed the phosphorylation of JAK2 and STAT3 in Jurkat and SUP-T1 cells ($P < 0.05$; Fig. 4B and C). Collectively, these data suggested that IL-6/JAK/STAT3 signalling may be a downstream pathway of TGM2.

TGM2 siRNAs suppress T-cell lymphoma by regulating the IL-6/JAK/STAT3 pathway. The present study validated whether TGM2 affected T-cell lymphoma by regulating the IL-6/JAK/STAT3 pathway. TGM2 siRNAs suppressed the number of EdU-positive cells and the colony-forming ability of Jurkat and SUP-T1 cells, whereas the IL-6/JAK/STAT3 activator IL-6 reversed these effects and the IL-6/JAK/STAT3 inhibitor AG490 enhanced these effects ($P < 0.05$; Fig. 5A and B).

Furthermore, apoptosis of Jurkat and SUP-T1 cells was induced by TGM2 siRNAs, whereas IL-6 reversed it; however, AG490 enhanced the effect of TGM2 siRNAs on apoptosis ($P < 0.05$; Fig. 5C). Taken together, these results suggested that TGM2 siRNAs may suppress T-cell lymphoma by regulating the IL-6/JAK/STAT3 pathway.

Discussion

T-LBL is a malignant tumour of precursor T cells and accounts for ~90% of lymphoblastic lymphoma cancer cases worldwide (1). In the present study, an overlap analysis based on the upregulated genes from the GSE132550 and GSE143382 datasets was performed. Eight overlapping genes were identified, namely MMP12, CCL19, MMP9, CD180, CCL18, FPR3, TNC

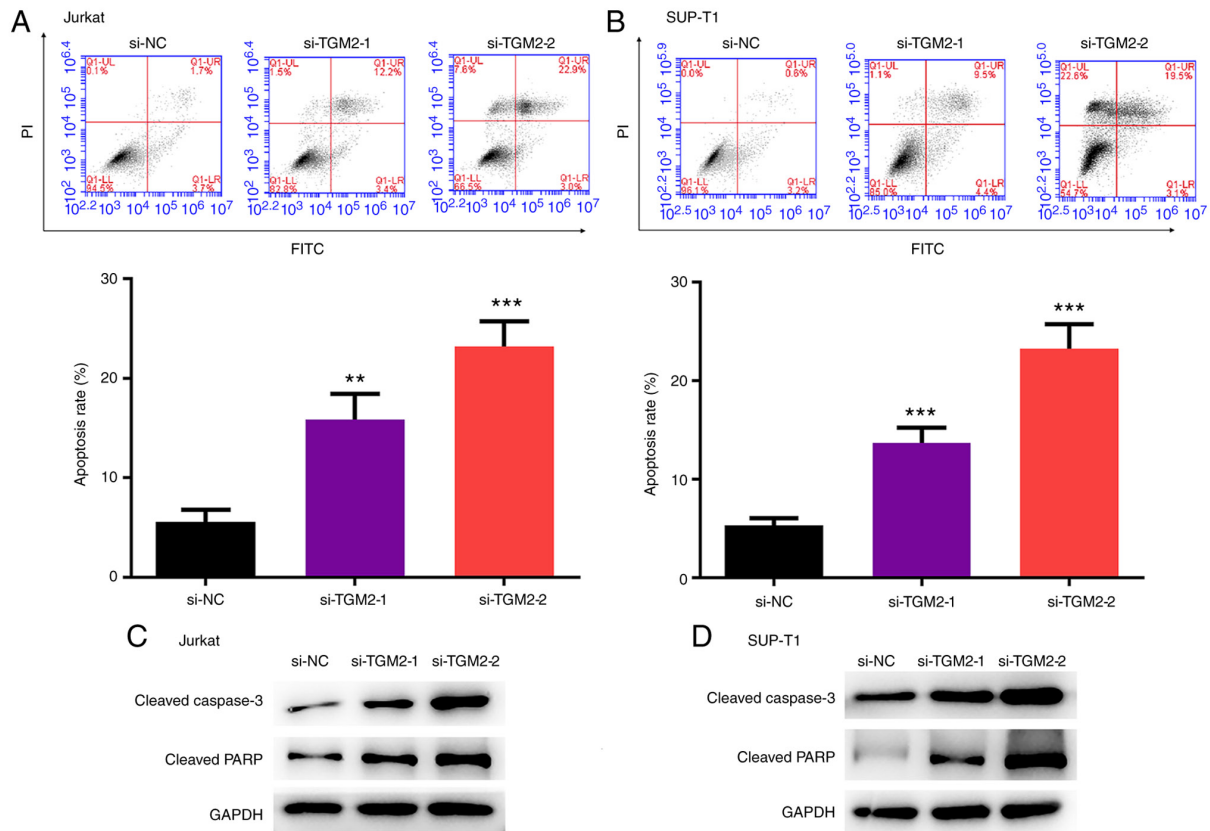


Figure 3. TGM2 siRNAs stimulate the apoptosis of T-cell lymphoma cells. Jurkat and SUP-T1 cells were transfected with TGM2 siRNAs. (A and B) Cell apoptosis (early + late apoptosis) was measured using an Annexin V/PI detection kit. (C and D) Expression levels of cleaved caspase-3 and cleaved PARP were detected by western blotting. Data are presented as the mean \pm SD. ** $P < 0.01$, *** $P < 0.001$ vs. the si-NC group. NC, negative control; PARP, poly ADP-ribose polymerase; si/siRNA, small interfering RNA; TGM2, transglutaminase 2.

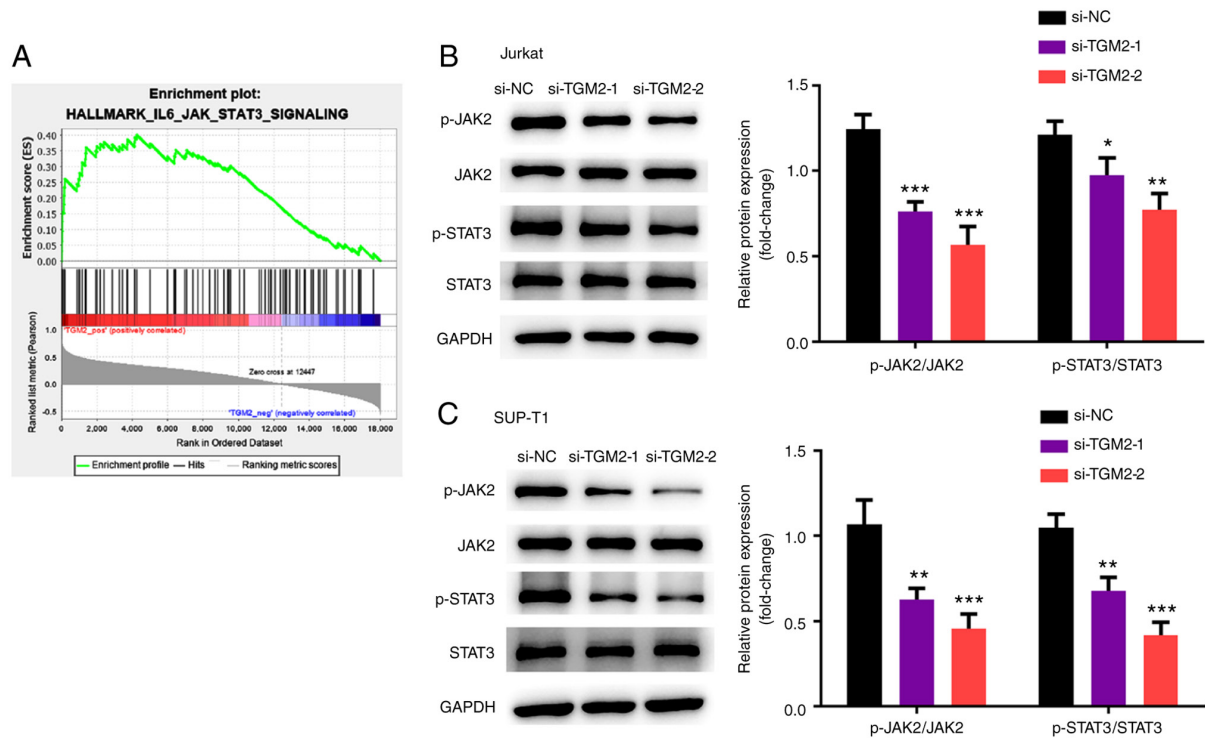


Figure 4. IL-6/JAK/STAT3 signalling is a downstream signalling pathway of TGM2. (A) Expression data from the microarray was analysed using gene set enrichment analysis software to obtain signalling pathways that can be activated or suppressed by TGM2. Among them, the IL-6/JAK/STAT3 signalling pathway was selected for subsequent experimental analysis. (B and C) Jurkat and SUP-T1 cells were transfected with TGM2 siRNAs. The expression levels of JAK2, p-JAK2, STAT3 and p-STAT3 were examined by western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the si-NC group. NC, negative control; p-, phosphorylated; si/siRNA, small interfering RNA; TGM2, transglutaminase 2.

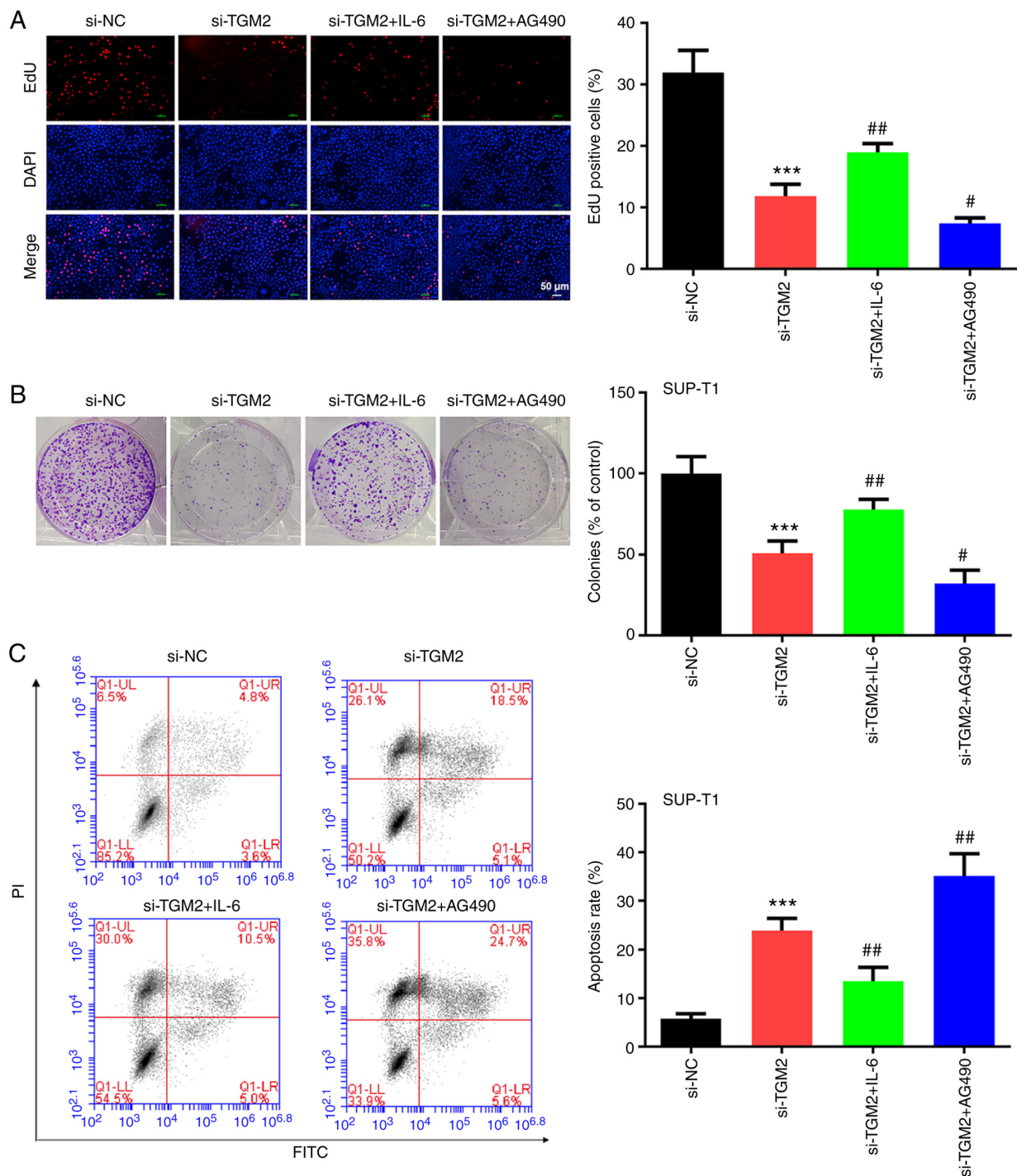


Figure 5. TGM2 siRNAs suppress T-cell lymphoma by regulating the IL-6/JAK/STAT3 signalling pathway. Jurkat and SUP-T1 cells were transfected with TGM2 siRNAs, and then treated with IL-6 (60 ng/ml) for 6 h or AG490 (100 μ M) for 24 h. (A) Cell proliferation was detected by EdU assay and (B) cell survival was assessed via a colony formation assay. (C) Cell apoptosis (early + late apoptosis) was measured using an Annexin V/PI detection kit. Data are presented as the mean \pm SD. *** P <0.01 vs. the si-NC group; # P <0.05, ** P <0.01 vs. the si-TGM2 group. EdU, 5-ethynyl-2'-deoxyuridine; NC, negative control; si/siRNA, small interfering RNA; TGM2, transglutaminase 2.

and TGM2. Among these genes, the roles of MMP12, CCL19, MMP9, CD180 and CCL18 have been well established in various types of cancer, including lymphoma (22-24). FPR3 is a member of the formyl peptide receptor family, which is mainly expressed in bone marrow cells and mature neutrophils (25). Its main function is relative to immune responses (25) and less is currently known regarding its role in cancer. TNC is a member of the extracellular matrix (ECM) protein family, the

main function of which is tissue regeneration via its participation in wound healing and inflammation (26). Since TNC is a glycoprotein of the ECM, its roles in several solid tumours have been demonstrated (27). TGM2 is a member of the transglutaminase family, and its effects on various types of cancer have been previously reported, including osteosarcoma (8), CRC (7), glioma (9) and endometrial cancer (10). However, less is currently known about its role in lymphoma; therefore,

TGM2 was selected for further studies. The present study identified the innovative role of TGM2 in T-cell lymphoma proliferation and apoptosis.

TGM2 has been shown to participate in the modulation of cancer development. It has been reported that TGM2 may serve as a biomarker for cisplatin resistance in non-small cell lung cancer (28). In a previous study, inhibition of TGM2 suppressed docetaxel resistance and epithelial-to-mesenchymal transition in breast cancer (29). Furthermore, kaempferol has been reported to promote reactive oxygen species-related apoptosis via the TGM2-regulated Akt/mTOR pathway in pancreatic cancer cells (30). TGM2 was also revealed to be correlated with the prognosis of CRC and may serve as a potential treatment target (31). In addition, TGM2 depletion can inhibit cisplatin chemoresistance in osteosarcoma cells (8), and TGM2 may modulate apoptosis and angiogenesis via Wnt/ β -catenin signalling in CRC (7). Furthermore, microRNA (miR)-532-3p has been shown to attenuate the progression of CRC by targeting the ETS1/TGM2-regulated Wnt/ β -catenin pathway (32). These previous studies suggested the key roles of TGM2 in human cancer. In the present study, TGM2 was revealed to be highly expressed in FFPE skin biopsies from patients with T-cell lymphoma (n=36) relative to the skin tissue from healthy individuals (n=36). The mRNA and protein expression levels of TGM2 were also higher in T-cell lymphoma cell lines compared with those in CD4⁺ T cells. Silencing of TGM2 via siRNA transfection decreased cell viability, EdU-positive numbers and colony formation of T-cell lymphoma cells. In addition, silencing of TGM2 was able to enhance the apoptosis of T-cell lymphoma cells potentially by regulating the cleavage of caspase-3 and PARP. These data indicated a novel function of TGM2 in regulating proliferation and apoptosis in T-cell lymphoma, suggesting that TGM2 may be considered a new therapeutic target of T-cell lymphoma. The clinical significance of TGM2 needs to be confirmed by further studies.

The function of the IL-6/JAK/STAT3 pathway in lymphoma has been widely studied. It has been reported that JAK/STAT3 signalling is activated in mantle cell lymphoma (33). Furthermore, benzoxathiole was shown to inhibit the proliferation and survival of lymphoma cells by inactivation of the JAK3/STAT3 pathway (34). In a previous study, miR-155 modulated the apoptosis and proliferation of lymphoma cells by regulating JAK/STAT3 signalling (35). Generation sequencing has also revealed a novel STAT3-JAK2 fusion in anaplastic large cell lymphomas (36). Furthermore, inhibition of JAK3/STAT3 signalling may serve as a treatment option for NK/T-cell lymphoma (37). Kinase fusion and convergent mutations may result in activation of STAT3 in the anaplastic large cell lymphoma (38). The results of the present study revealed that the IL-6/JAK/STAT3 pathway may be a potential downstream signalling pathway of TGM2. Silencing of TGM2 suppressed the phosphorylation of JAK2 and STAT3 in T-cell lymphoma cells. Further study confirmed that the silencing of TGM2 reduced the number of EdU-positive cells and the colony-forming ability of T-cell lymphoma cells, whereas the IL-6/JAK/STAT3 activator IL-6 blocked this effect and the IL-6/JAK/STAT3 inhibitor AG490 enhanced it. Consistently, the apoptosis of T-cell lymphoma cells was induced by TGM2 silencing, whereas IL-6 reversed it and

AG490 enhanced the effect of TGM2 silencing on the cells. These findings indicated that TGM2 may regulate T-cell lymphoma proliferation and apoptosis, possibly through the activation of IL-6/JAK/STAT3 signalling. A previous study demonstrated that activation of IL-6/IL-6 receptor/STAT3 can promote TGM2 expression to induce epithelial-mesenchymal transition in hepatocellular carcinoma (39). Although the results cannot be compared, these findings indicate an association between TGM2 and IL-6/JAK/STAT3 signalling in human cancer. IL-6/JAK/STAT3 signalling may be one of the downstream pathways of TGM2-mediated cancer progression and other mechanisms should be explored in future studies.

In conclusion, silencing of TGM2 inhibited the growth of T-cell lymphoma by regulating IL-6/JAK/STAT3 signalling. These findings indicated that TGM2 may function as a potential therapeutic target for T-cell lymphoma.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YW and NZ designed the study. TS, YC and CL performed the experiments. YW and HZ analysed the data. NZ prepared the manuscript. YW and NZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol of this research has been approved by the Ethics Committee of Shengli Oilfield Central Hospital (approval no. DYSLYT20191201; Dongying, China). All patients have signed written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Cortelazzo S, Ferreri A, Hoelzer D and Ponzoni M: Lymphoblastic lymphoma. *Crit Rev Oncol Hematol* 113: 304-317, 2017.
2. Burkhardt B and Hermiston ML: Lymphoblastic lymphoma in children and adolescents: Review of current challenges and future opportunities. *Br J Haematol* 185: 1158-1170, 2019.
3. Rietz EA and Teachey DT: T-cell acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* 2016: 580-588, 2016.

4. Leicht DT, Kausar T, Wang Z, Ferrer-Torres D, Wang TD, Thomas DG, Lin J, Chang AC, Lin L and Beer DG: TGM2: A cell surface marker in esophageal adenocarcinomas. *J Thorac Oncol* 9: 872-881, 2014.
5. Tovar-Vidales T, Clark AF and Wordinger RJ: Transforming growth factor-beta2 utilizes the canonical Smad-signaling pathway to regulate tissue transglutaminase expression in human trabecular meshwork cells. *Exp Eye Res* 93: 442-451, 2011.
6. Lai TS and Greenberg CS: TGM2 and implications for human disease: Role of alternative splicing. *Front Biosci (Landmark Ed)* 18: 504-519, 2013.
7. Yang P, Yu D, Zhou J, Zhuang S and Jiang T: TGM2 interference regulates the angiogenesis and apoptosis of colorectal cancer via Wnt/ β -catenin pathway. *Cell Cycle* 18: 1122-1134, 2019.
8. Li C, Cai J, Ge F and Wang G: TGM2 knockdown reverses cisplatin chemoresistance in osteosarcoma. *Int J Mol Med* 42: 1799-1808, 2018.
9. Fu J, Yang QY, Sai K, Chen FR, Pang JC, Ng HK, Kwan AL and Chen ZP: TGM2 inhibition attenuates ID1 expression in CD44-high glioma-initiating cells. *Neuro Oncol* 15: 1353-1365, 2013.
10. Torres A, Pac-Sosińska M, Wiktor K, Paszkowski T, Maciejewski R and Torres K: CD44, TGM2 and EpCAM as novel plasma markers in endometrial cancer diagnosis. *BMC Cancer* 19: 401, 2019.
11. Zhang H and McCarty N: Tampering with cancer chemoresistance by targeting the TGM2-IL6-autophagy regulatory network. *Autophagy* 13: 627-628, 2017.
12. Hunter CA and Jones SA: IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 16: 448-457, 2015.
13. West AJ, Tsui V, Styli SS, Nguyen HPT, Morokoff AP, Kaye AH and Luwor RB: The role of interleukin-6-STAT3 signalling in glioblastoma. *Oncol Lett* 16: 4095-4104, 2018.
14. Burger R: Impact of interleukin-6 in hematological malignancies. *Transfus Med Hemother* 40: 336-343, 2013.
15. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G and Schaper F: Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 374: 1-20, 2003.
16. Kim JH, Kim WS and Park C: Interleukin-6 mediates resistance to PI3K-pathway-targeted therapy in lymphoma. *BMC Cancer* 19: 936, 2019.
17. Liu J, Hong J, Ahn KS, Go J, Han H, Park J, Kim D, Park H, Koh Y, Shin DY and Yoon SS: ERK-dependent IL-6 positive feedback loop mediates resistance against a combined treatment using danusertib and BKM120 in Burkitt lymphoma cell lines. *Leuk Lymphoma* 60: 2532-2540, 2019.
18. Etebari M, Navari M, Agostinelli C, Visani A, Peron C, Iqbal J, Inghirami G and Piccaluga PP: Transcriptional analysis of lemming lymphoma reveals a unique profile and identifies novel therapeutic targets. *Front Genet* 10: 780, 2019.
19. Nielsen PR, Eriksen JO, Lindahl LM, Wehkamp U, Bzorek M, Andersen G, Woetmann A, Iversen L, Ødum N, Litman T and Gjerdrum LMR: Diagnostic two-gene classifier in early-stage mycosis fungoides: A retrospective multicenter study. *J Invest Dermatol* 141: 213-217.e5, 2021.
20. Yu G, Wang LG, Han Y and He QY: ClusterProfiler: An R package for comparing biological themes among gene clusters. *Omics* 16: 284-287, 2012.
21. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
22. Gobin E, Bagwell K, Wagner J, Mysona D, Sandirasegarane S, Smith N, Bai S, Sharma A, Schleifer R and She JX: A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential. *BMC Cancer* 19: 581, 2019.
23. Korbecki J, Kojder K, Barczak K and Simińska D: Hypoxia alters the expression of CC chemokines and CC chemokine receptors in a tumor-A literature review. *Int J Mol Sci* 21: 5647, 2020.
24. Mayeur-Rousse C, Guy J, Miguet L, Bouyer S, Geneviève F, Robillard N, Solly F, Maar A, Bené MC and Mauvieux L: GEIL (Groupe d'Etude Immunologique des Leucémies): CD180 expression in B-cell lymphomas: A multicenter GEIL study. *Cytometry B Clin Cytom* 90: 462-466, 2016.
25. Stempel H, Jung M, Pérez-Gómez A, Leinders-Zufall T, Zufall F and Bufer B: Strain-specific loss of formyl peptide receptor 3 in the murine vomeronasal and immune systems. *J Biol Chem* 291: 9762-9775, 2016.
26. Tucker RP and Chiquet-Ehrismann R: The regulation of tenascin expression by tissue microenvironments. *Biochim Biophys Acta* 1793: 888-892, 2009.
27. Lowy CM and Oskarsson T: Tenascin C in metastasis: A view from the invasive front. *Cell Adh Migr* 9: 112-124, 2015.
28. Park KS, Kim HK, Lee JH, Choi YB, Park SY, Yang SH, Kim SY and Hong KM: Transglutaminase 2 as a cisplatin resistance marker in non-small cell lung cancer. *J Cancer Res Clin Oncol* 136: 493-502, 2010.
29. He W, Sun Z and Liu Z: Silencing of TGM2 reverses epithelial to mesenchymal transition and modulates the chemosensitivity of breast cancer to docetaxel. *Exp Ther Med* 10: 1413-1418, 2015.
30. Wang F, Wang L, Qu C, Chen L, Geng Y, Cheng C, Yu S, Wang D, Yang L, Meng Z and Chen Z: Kaempferol induces ROS-dependent apoptosis in pancreatic cancer cells via TGM2-mediated Akt/mTOR signaling. *BMC Cancer* 21: 396, 2021.
31. Miyoshi N, Ishii H, Mimori K, Tanaka F, Hitora T, Tei M, Sekimoto M, Doki Y and Mori M: TGM2 is a novel marker for prognosis and therapeutic target in colorectal cancer. *Ann Surg Oncol* 17: 967-972, 2010.
32. Gu C, Cai J, Xu Z, Zhou S, Ye L, Yan Q, Zhang Y, Fang Y, Liu Y, Tu C, *et al*: MiR-532-3p suppresses colorectal cancer progression by disrupting the ETS1/TGM2 axis-mediated Wnt/ β -catenin signaling. *Cell Death Dis* 10: 739, 2019.
33. Yared MA, Khoury JD, Medeiros LJ, Rassidakis GZ and Lai R: Activation status of the JAK/STAT3 pathway in mantle cell lymphoma. *Arch Pathol Lab Med* 129: 990-996, 2005.
34. Kim BH, Min YS, Choi JS, Baeg GH, Kim YS, Shin JW, Kim TY and Ye SK: Benzoxathiol derivative BOT-4-one suppresses L540 lymphoma cell survival and proliferation via inhibition of JAK3/STAT3 signaling. *Exp Mol Med* 43: 313-321, 2011.
35. Li XD, Li XM, Gu JW and Sun XC: MiR-155 regulates lymphoma cell proliferation and apoptosis through targeting SOCS3/JAK-STAT3 signaling pathway. *Eur Rev Med Pharmacol Sci* 24: 7577, 2020.
36. Quesada AE, Zhang Y, Ptashkin R, Ho C, Horwitz S, Benayed R, Dogan A and Arcila ME: Next generation sequencing of breast implant-associated anaplastic large cell lymphomas reveals a novel STAT3-JAK2 fusion among other activating genetic alterations within the JAK-STAT pathway. *Breast J* 27: 314-321, 2021.
37. Liu J, Liang L, Li D, Nong L, Zheng Y, Huang S, Zhang B and Li T: JAK3/STAT3 oncogenic pathway and PRDM1 expression stratify clinicopathologic features of extranodal NK/T-cell lymphoma, nasal type. *Oncol Rep* 41: 3219-3232, 2019.
38. Crescenzo R, Abate F, Lasorsa E, Tabbo' F, Gaudiano M, Chiesia N, Di Giacomo F, Spaccarotella E, Barbarossa L, Ercole E, *et al*: Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell* 27: 516-532, 2015.
39. Jia C, Wang G, Wang T, Fu B, Zhang Y, Huang L, Deng Y, Chen G, Wu X, Chen J, *et al*: Cancer-associated Fibroblasts induce epithelial-mesenchymal transition via the Transglutaminase 2-dependent IL-6/IL6R/STAT3 axis in hepatocellular carcinoma. *Int J Biol Sci* 16: 2542-2558, 2020.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.