

Deficiency of two-pore segment channel 2 contributes to systemic lupus erythematosus via regulation of apoptosis and cell cycle

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Abstract

Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease, and the mechanism of SLE is yet to be fully elucidated. The aim of this study was to explore the role of two-pore segment channel 2 (*TPCN2*) in SLE pathogenesis.

Methods: Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the expression of *TPCN2* in SLE. We performed a loss-of-function assay by lentiviral construct in Jurkat and THP-1 cell. Knockdown of *TPCN2* were confirmed at the RNA level by qRT-PCR and protein level by Western blotting. Cell Count Kit-8 and flow cytometry were used to analyze the cell proliferation, apoptosis, and cell cycle of *TPCN2*-deficient cells. In addition, gene expression profile of *TPCN2*-deficient cells was analyzed by RNA sequencing (RNA-seq).

Results: *TPCN2* knockdown with short hairpin RNA (shRNA)-mediated lentiviruses inhibited cell proliferation, and induced apoptosis and cell-cycle arrest of G2/M phase in both Jurkat and THP-1 cells. We analyzed the transcriptome of knockdown-*TPCN2*-Jurkat cells, and screened the differential genes, which were enriched for the G2/M checkpoint, complement, and interleukin-6-Janus kinase-signal transducer and activator of transcription pathways, as well as changes in levels of forkhead box O, phosphatidylinositol 3-kinase/protein kinase B/mechanistic target of rapamycin, and T cell receptor pathways; moreover, *TPCN2* significantly influenced cellular processes and biological regulation.

Conclusion: *TPCN2* might be a potential protective factor against SLE.

Keywords: *TPCN2*; Systemic lupus erythematosus; RNA sequencing analysis; Apoptosis; Cell cycle

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by the production of various autoantibodies and immune complexes that interact with multiple organs such as kidneys, joints, and blood.^[1] SLE affects people of all races, genders, and ages.^[2] In particular, SLE mostly affects adult women, occurring approximately three to six times more often in women than in men.^[3] Due to the completion of the Human Genome Project and the HapMap Project, it is now possible to identify genes that indicate susceptibility to complex diseases, including SLE. Our extensive genetic research on SLE using genome-wide association studies (GWAS) and exome-wide association studies have identified many susceptibility genes for SLE.^[4-6] Though these previous studies indicated that genetic factors contribute to SLE development,^[7] the exact causes remain unclear.

Apoptosis, also called programmed cell death, is a key mechanism regulating the balance between cell growth and death in many tissues.^[8,9] Dysregulation of apoptosis has an important role in the induction of autoimmunity, as apoptotic bodies produced during apoptosis are a major source of autoantigens.^[10] Dysfunctional clearance of apoptotic cells can lead to the accumulation of apoptotic bodies, generation of autoantibodies, and exacerbation of inflammatory responses.^[8] The development and exacerbation of symptoms in SLE may be due to the impairment of apoptosis, while inefficient clearance of apoptotic cells may underlie the autoimmune reactions observed in SLE.^[10] It has been reported that peripheral blood mononuclear cells (PBMCs) undergo apoptosis in SLE patients, and that there is a positive correlation between the rate of apoptosis and the severity of SLE.^[11,12] Clearly, apoptosis is an important factor in SLE pathogenesis.

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Two-pore channels (TPCs), important members of the voltage-gated ion channel superfamily, localize to acidic Ca^{2+} stores within the endolysosomal system,^[13] and are widely expressed in various tissue types.^[14-18] Two-pore segment channel 2 (TPCN2) is a member of a family of TPCs linked to Parkinson's disease, non-alcoholic fatty liver disease, Ebola virus disease, diabetes, and cancer.^[18-22] Although these diseases have distinct pathologies, TPCs are potential therapeutic targets in each of them.^[21,23,24] In addition, our previous study, a large-scale exome-wide study in the Han Chinese population to identify SLE susceptibility genes, showed that *TPCN2* was a susceptibility gene. The novel intergenic variant rs10750836 exhibited an expression quantitative trait locus effect on the *TPCN2* gene in immune cells. Clones containing this novel single-nucleotide polymorphism exhibited gene promoter activity for *TPCN2*, the expression level of which was significantly reduced in patients with SLE.^[25] However, the role of *TPCN2* in the pathogenesis of SLE remains unclear.

Here, we performed an *in vitro* study using Jurkat and THP-1 cell lines. Our results indicate that silencing *TPCN2* inhibited cell proliferation and induced apoptosis and cell-cycle arrest in both Jurkat and THP-1 cells. Moreover, RNA sequencing (RNA-seq) was used to understand the molecular mechanism of SLE in *TPCN2*-deficient cells, and we assessed whether *TPCN2* could be a potential protective factor against SLE. Our findings could help to understand the mechanism of action of *TPCN2* in SLE, and thereby, provide insight into novel therapeutics for SLE.

Methods

Ethical approval

The study protocol was approved by the Ethics Committee of the China-Japan Friendship Hospital (No. 2021-107-K68). Each participant provided written informed consent.

Clinical samples

Six SLE patients and seven age- and sex-matched healthy volunteers, who visited the Department of Dermatology at the China-Japan Friendship Hospital (Beijing, China), were recruited for this study. The information relating to SLE patients is provided in Supplementary Table 1, <http://links.lww.com/CM9/A854>. All patients met the European League Against Rheumatism/American College of Rheumatology 2019 classification criteria for SLE.^[26] Lupus disease activity was assessed using the SLE Disease Activity Index.^[27] All samples were collected in sterile tubes (BD Biosciences, Franklin Lakes, NJ, USA) containing anticoagulants. PBMCs were obtained from 5 mL of peripheral blood and enriched by density centrifugation using a Ficoll-Paque (GE Healthcare, Uppsala, Sweden).

Cell lines and cell culture

The human Jurkat T cell line and human monocyte cell line THP-1 were cultured in RPMI-1640 medium (Gibco, Invitrogen, Grand Island, NY, USA) containing 10% fetal

bovine serum (FBS) (Gibco) and 1% penicillin-streptomycin (Gibco) at 37°C with 5% CO_2 . HEK 293T cells were grown in complete Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS. Jurkat, THP-1, and HEK 293T cells were obtained from China Infrastructure of Cell Line Resource (Beijing, China).

Lentiviral vector infection

HEK 293T cells were transfected with target gene plasmids (GV112) (normal control [NC], shTPCN2#1 [sh#1], and shTPCN2#2 [sh#2]) (Genechem Co. Ltd., Shanghai, China) along with packaging vectors psPAX2 and pMD2G, using TurboFect reagent (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. Two lentivirus targets of *TPCN2* are presented in Supplementary Table 2, <http://links.lww.com/CM9/A854>. The lentiviral supernatants were collected after 72 h and used for the infection of the Jurkat and THP-1 cells. We selected the stable *TPCN2*-deficient cells by 3 $\mu\text{g}/\text{mL}$ puromycin (Yeasen, Shanghai, China) after infection.

Cell proliferation assay

Cell proliferation was assessed using the Cell Count Kit-8 kit (CCK-8, Dojindo, Kumamoto, Japan) assay, as per the manufacturer's instructions. Briefly, the infected cells (4000 cells/well) were seeded into 96-well plates and cultured for 24 to 72 h. The optical density was then measured at a wavelength of 450 nm. Each experiment was independently performed at least three times with each group having five replicates.

Cell apoptosis assay

The infected cells were stained with annexin V-fluorescein isothiocyanate (FITC-Annexin V) and propidium iodide (PI) as per the manufacturer's instructions (BD Biosciences). The cells were washed with cold phosphate-buffered saline (PBS) and resuspended in a binding buffer. Then, 100 μL of this solution was transferred to a tube and incubated with 5 μL of FITC-Annexin V and 5 μL of PI. After incubation for 15 min at 37°C in the dark, 400 μL of binding buffer was added to the solution and flow cytometric analysis within 1 h. FlowJo software (Tree Star Inc, Ashland, USA) was used to analyze the data.

Cell-cycle assay

For the cell-cycle assays, the infected cells were washed with cold PBS and then fixed in ice-cold 70% ethanol at -20°C overnight. The fixed cells were stained with 500 μL of PI (BD Biosciences) for 15 min at room temperature in the dark. Finally, the cell cycle was analyzed using flow cytometry (CytoFLEX, Beckman, CA, USA).

Western blot analysis

The cells were lysed with a Radio-Immunoprecipitation Assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) containing protease and phosphatase inhibitors (NCM Biotech, Suzhou, China). The protein concentration of the cells was determined with a

bicinchoninic acid [BCA] kit (ComWin Biotech, Beijing, China), and 40 µg of protein was prepared for a sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride [PVDF] membrane (Millipore, Burlington, MA, USA). The membranes were blocked with 5% nonfat dry milk for 1 h, and then incubated with rabbit anti-TPCN2 monoclonal antibodies (1:200; Alomone Labs, Jerusalem, Israel) overnight at 4°C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:8000; Proteintech, Rosemont, IL, USA) was used as the loading control. The results were visualized using a gel image analysis system (Bio-Rad, Hercules, CA, USA) as per the manufacturer's instructions.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the cell samples using TRIzol reagent (Invitrogen, USA) as per the manufacturer's instructions. Reverse transcription was performed using the PrimeScript™ RT reagent kit (Takara, Tokyo, Japan). The cDNA was amplified using SYBR Green qPCR mix (Takara) and loaded onto a 7500 real-time PCR system (Applied Biosystem, Waltham, MA, USA). GAPDH was used as an internal control and relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ comparative threshold cycle method. All PCR primers used in this study are listed in Table 1.

RNA-seq analysis

Total RNA was isolated from the infected cells using TRIzol reagent (Invitrogen), and Majorbio Co. Ltd. (Shanghai, China) performed RNA-seq analysis, including cDNA library construction, purification, and sequencing. Gene expression levels were quantified using the RNA Sequencing by Expectation-Maximization (RSEM) software package. The significantly affected genes were determined by setting a fold change of ≥ 2 . Differentially expressed genes (DEGs) were analyzed using the Illumina Bioinformatics platform (San Diego, CA, USA).

Statistical analysis

The results are expressed as means \pm standard deviation. Student's *t* test and one-way analysis of variance were used to analyze the significant differences between two groups and for multi-sample analysis, respectively. Statistical analyses were performed using Prism 8.3.0 software (GraphPad, La Jolla, CA, USA). A *P* value < 0.05 was considered to indicate a statistically significant result.

Results

Knockdown of TPCN2 inhibits cell proliferation in SLE

In accordance with our previous study, we found that the expression level of TPCN2 was significantly decreased in SLE PBMCs [Figure 1A]. To investigate the functional role of TPCN2, we knocked down TPCN2 in Jurkat [Figure 1B] and THP-1 [Figure 1C] cells. Following infection, the mRNA and protein levels of TPCN2 were both significantly suppressed by the TPCN2-knockdown, as compared to the NC group. Further, the CCK-8 assay results indicate that, compared with the NC group, TPCN2 silencing significantly suppressed cell viability in both the Jurkat [Figure 1D] and THP-1 [Figure 1E] cell groups.

TPCN2-knockdown induces apoptosis and G2/M cell-cycle arrest in SLE

Given that TPCN2 could regulate cell proliferation, we investigated the effect of TPCN2 on both cell apoptosis and cell cycle. As shown in Figure 2A, the proportion of Annexin V-positive Jurkat cells indicating early- and late-stage apoptosis was three times as high in the sh#2 group than in the NC group. We observed similar results in the THP-1 [Figure 2B] cells: TPCN2-knockdown induced 13.86% and 20.26% apoptosis in the sh#1 and sh#2 groups, respectively. Additionally, flow cytometry demonstrated that suppressing TPCN2 expression modulated the cell cycle by inducing G2/M arrest in the Jurkat [Figure 2C] and THP-1 [Figure 2D] cell lines.

Table 1: The primers used in the quantitative reverse transcription polymerase chain reaction.

Genes	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
TPCN2	GGTGTCGTCTGTCATCTGGGTC	CTGGTAGGTGGCCTCTGGGG
CX3CR1	ATCGTGGTCTTTGGGACTGTGTTT	TGGGCTTCTTGCTGTTGGTGAG
AHNAK	CCCCTGTTCGGAGGAGGTCTTC	GGCAGGTTACATCACATCCAGAG
ARHGDI1B	TCCAGTTGAGGAGGCTCCCAAG	TGCTTGTTCATCGTCGGTGAAGAAG
ERO1A	AAGAAGGGACTGTGCTGTCAAACC	TTCATCCACTGCTCCAAGTCGTTC
GRB10	ACCATCCGTAACACCAGGACAAGG	CATATCGTTCACCAGGGCTTCCAG
NCOA3	GCAGCAACAGCAACAGCAACAG	GCGGAGGAGCTTGTGGCATTG
PMEPA1	GTGCCTGCTGAGCCACTACAAG	GGGATTCCGTTGCCTGACACTG
S100A8	TTGCTAGAGACCGAGTGTCTCTCAG	GCCACGCCATCTTTATCACCAG
GAPDH	GTGAAGGTCGGAGTCAACG	TGAGGTCAATGAAGGGGTC

AHNAK: AHNAK nucleoprotein; ARHGDI1B: Rho GDP dissociation inhibitor beta; CX3CR1: C-X3-C motif chemokine receptor 1; ERO1A: Endoplasmic reticulum oxidoreductase 1 alpha; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GRB10: Growth factor receptor bound protein 10; NCOA3: Nuclear receptor coactivator 3; PMEPA1: Prostate transmembrane protein, androgen induced 1; S100A8: S100 Calcium Binding Protein A8; TPCN2: Two-pore segment channel 2.

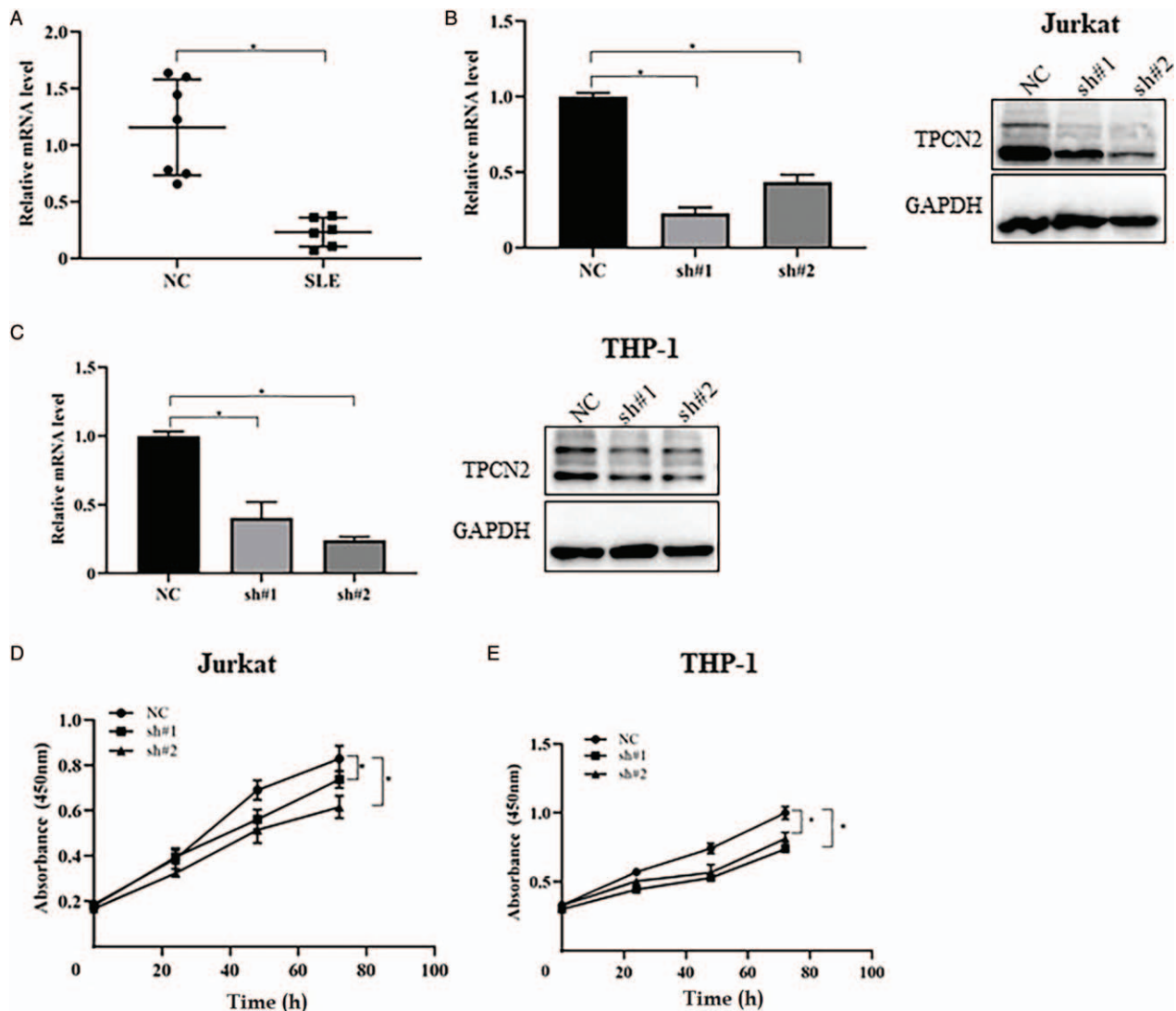


Figure 1: *TPCN2*-knockdown inhibits the proliferation in SLE. (A) Expression level of *TPCN2* in PBMCs of SLE cases ($n=6$) and healthy controls ($n=7$). $*P < 0.05$, Student's t test. Knockdown of *TPCN2* with two independent shRNA in Jurkat (B) and THP-1 (C) cells; the left panel represents the relative expression levels of *TPCN2* assessed by qRT-PCR; the right panel represents protein expression level of *TPCN2* that was detected by Western blotting. The data represent the mean ($n=3$) \pm SD. $*P < 0.05$, Student's t test. Cell viability of silencing *TPCN2* in Jurkat (D) and THP-1 (E) cells were determined by CCK-8 assay. The results represent the means ($n=5$) \pm SD. Significant differences were evaluated using Student's t test. $*P < 0.05$. CCK-8: Cell count kit-8; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NC: Normal control; PBMCs: Peripheral blood mononuclear cells; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; SD: Standard deviation; Sh#1: shTPCN2#1; Sh#2: shTPCN2#2; shRNA: Short hairpin RNA; SLE: Systemic lupus erythematosus; *TPCN2*: Two-pore segment channel 2.

Molecular profiling of *TPCN2*-deficient cells in SLE

To analyze the function of *TPCN2* in SLE, RNA-seq was used to analyze the expression profiles of *TPCN2*-deficient Jurkat cells. We obtained approximately 6.52 GB of clean data from each sample using the Illumina Novaseq 6000 platform (Illumina, San Diego, USA). Q30 percentages of clean data for all samples were higher than 94.34%. The RNA-seq results showed that 906 genes were upregulated and 312 were downregulated in the sh#1 group, whereas 362 genes were upregulated and 598 were downregulated in the sh#2 group (compared with the NC group) [Figure 3A and 3B].

We further analyzed DEGs using the Gene Ontology (GO) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. GO analysis was

performed to elucidate the genetic regulatory networks, including the following categories: biological process, cellular component, and molecular function. Silencing *TPCN2* significantly influenced cellular processes and biological regulation, as shown in Figure 3C and Supplementary Figure 1A, <http://links.lww.com/CM9/A854>. Furthermore, we analyzed DEGs using KEGG pathway analysis. The pathway functional enrichment results for upregulated or downregulated genes are shown in Figure 3D. We found that most DEGs were involved in multiple pathways, such as the forkhead box O (FoxO), thyroid hormone, and T cell receptor pathways, which is consistent with the effect of *TPCN2* on cell proliferation and cell-cycle processes.

Moreover, we conducted Gene Set Enrichment Analysis (GSEA) using the NC group and the sh#1/sh#2 group to

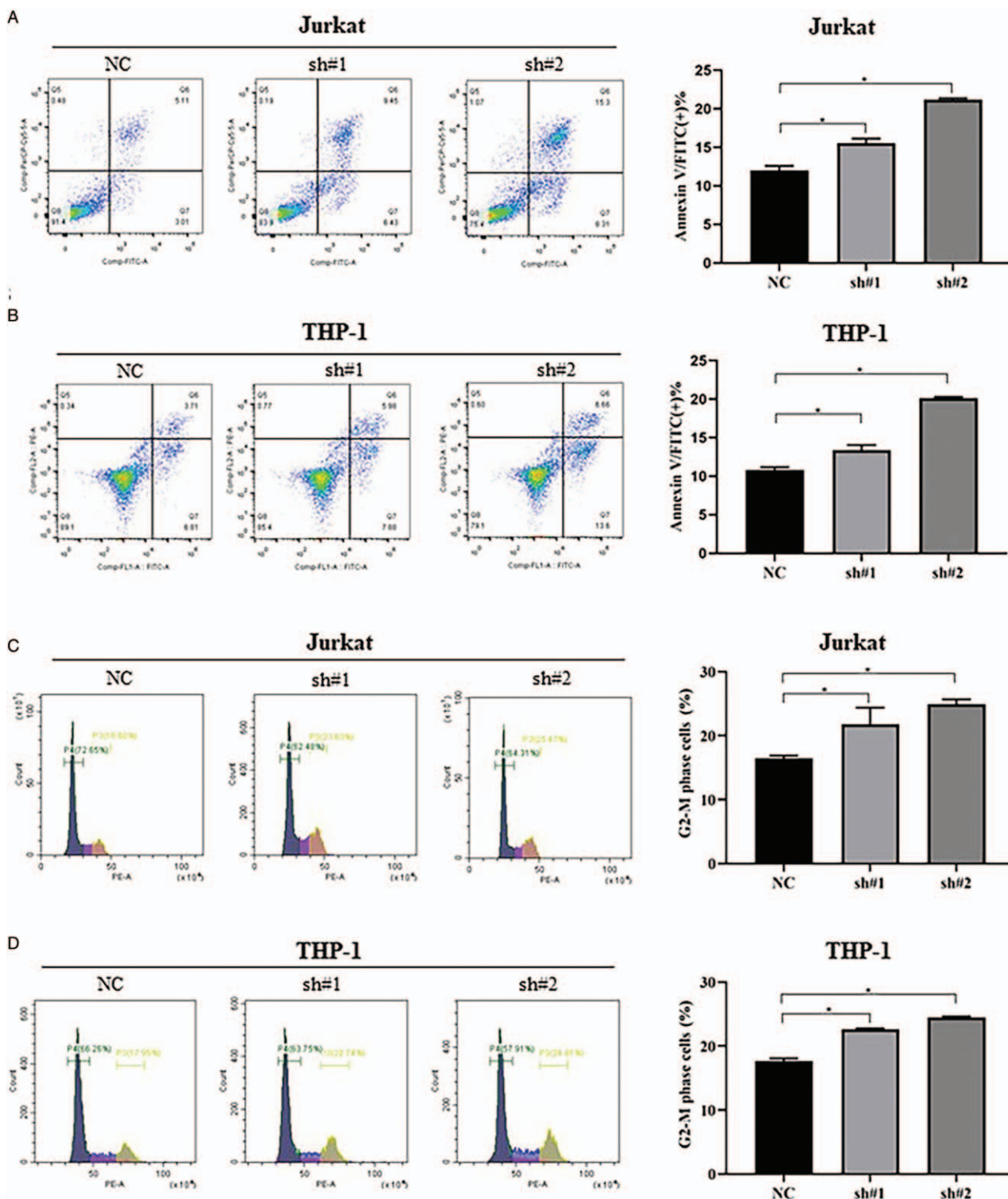


Figure 2: Knockdown of *TPCN2* induces apoptosis and cell-cycle arrest in SLE. Apoptosis was detected by flow cytometry with Annexin V and PI in jurkat-*TPCN2*-knockdown cells (A) and THP-1-*TPCN2*-knockdown cells (B). *TPCN2*-knockdown induced G2/M cell-cycle arrest. The percentage of G2-M phase cells of Jurkat (C) and THP-1 (D) was assessed by flow cytometry. **P* < 0.05. NC: Normal control; PI: Propidium iodide; Sh#1: shTPCN2#1; Sh#2: shTPCN2#2; SLE: Systemic lupus erythematosus; *TPCN2*: Two-pore segment channel 2.

identify the role of *TPCN2* in Jurkat cells. As shown in Figure 4A and consistent with previous results, silencing *TPCN2* affected the G2/M checkpoint. Thus, *TPCN2* seems to be involved in Jurkat cell-cycle regulation. In addition, *TPCN2* deficiency was associated with inflammatory response [Figure 4B], IFN- γ signaling [Supplementary

Figure 1B, <http://links.lww.com/CM9/A854>], and the complement system [Figure 4C and Supplementary Figure 1C, <http://links.lww.com/CM9/A854>]. Also, the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT)-mechanistic target of rapamycin (mTOR) [Figure 4D] and interleukin-6-Janus kinase-signal transducer and activator of transcription

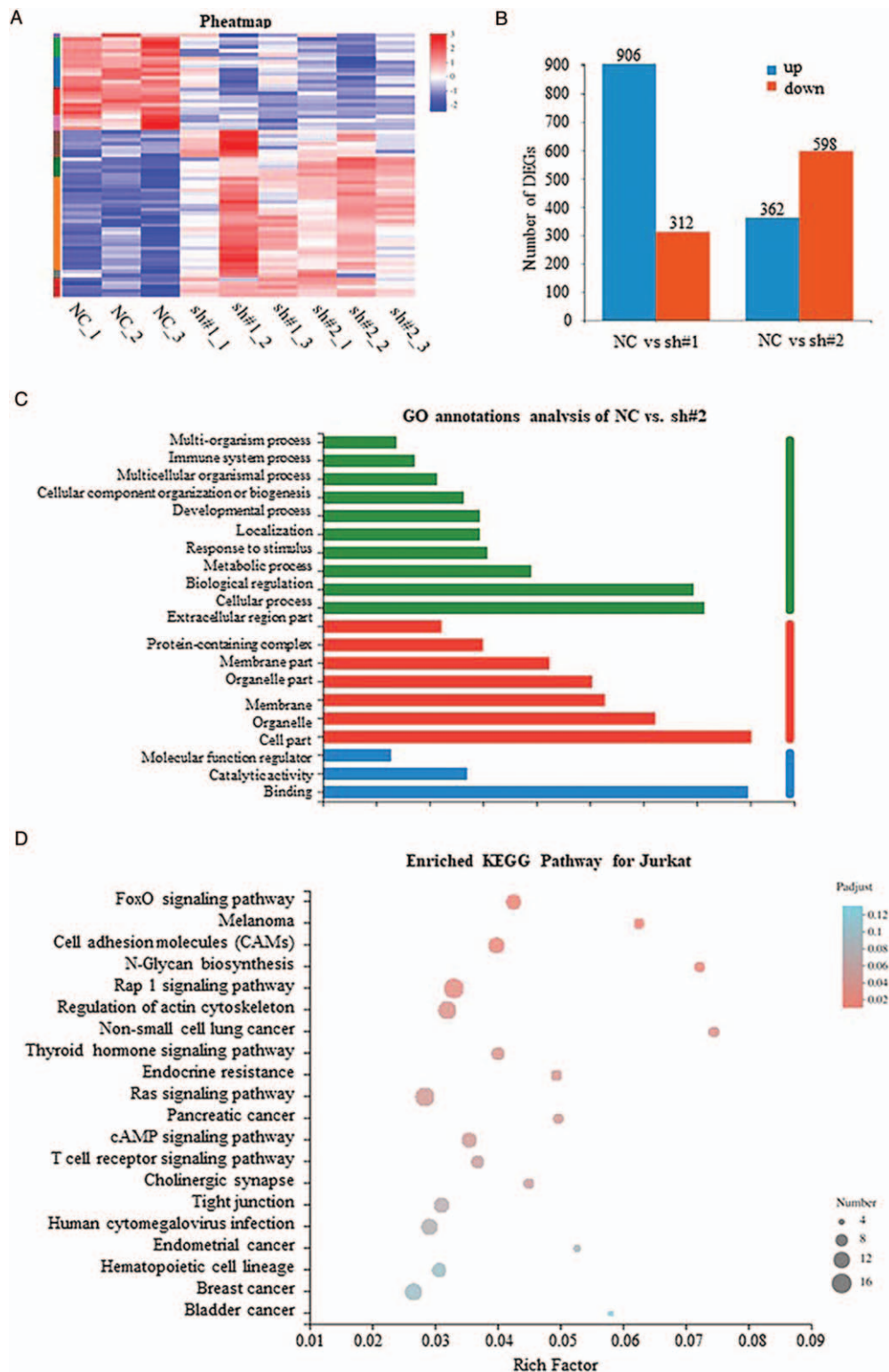


Figure 3: RNA-seq analyses of the effect of *TPCN2*-knockdown on the gene expression profile. (A) The heatmap shows differential expression genes in sh#1 and sh#2 (in comparison with NC group). (B) The differential statistics of DEGs in NC groups vs. sh#1/sh#2. Blue columnar represent up-regulated DEGs. Red column represents down-regulated DEGs. (C) GO classification analysis of DEGs. NC group vs. sh#2. Molecular function – blue; Cellular components – red; Biological process – green. (D) Top 20 enriched KEGG pathways after silencing *TPCN2* in Jurkat. The x-axis is the enrichment score, and the y-axis is the enriched pathways. DEGs: Differentially expressed genes; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; NC: Normal control; RNA-seq: RNA sequencing; Sh#1: shTPCN2#1; Sh#2: shTPCN2#2; *TPCN2*: Two-pore segment channel 2.

signaling pathways [Supplementary Figure 1D, <http://links.lww.com/CM9/A854>] were enriched in the sh#2 and sh#1 groups, respectively.

Next, using qRT-PCR, we validated the key DEGs in Jurkat cells [Figure 4E], including *NCOA3*, endoplasmic reticulum oxidoreductase 1 alpha [*ERO1A*], *AHNAK*

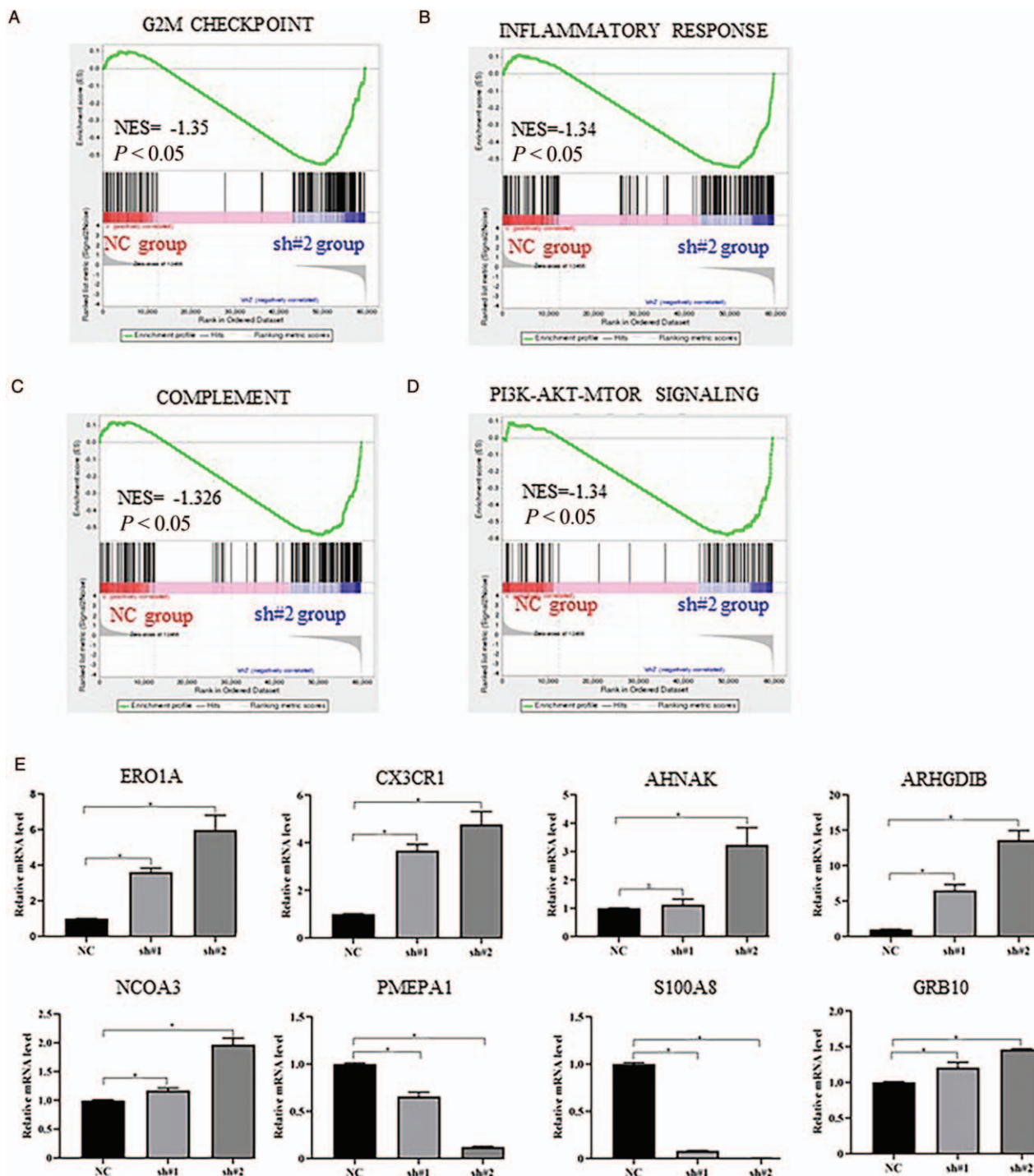


Figure 4: Representative enriched pathways in high-risk shTPCN2#2 through GSEA analysis. GSEA results showed that the G2/M checkpoint (A), inflammatory response (B), complement (C), and PI3K-AKT-mTOR (D) pathways were enriched in the sh#2 expression group. Top panels indicate the enrichment scores for each gene. Bottom panels show the ranking metrics of each gene. Y-axis: ranking metric values; X-axis: ranks for all genes. (E) The mRNA expression of some DEGs in Jurkat cells were detected by qRT-PCR. The RNA was extracted from cells knocked down of *TPCN2* with two independent shRNA. The results were shown as the mean \pm SD from three independent experiments. * $P < 0.05$. AKT: Protein kinase B; DEGs: Differentially expressed genes; GSEA: Gene set enrichment analysis; mTOR: Mechanistic target of rapamycin; NC: Normal control; NES: Normalized enrichment score; PI3K: Phosphatidylinositol 3-kinase; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; SD: standard deviation; Sh#1: shTPCN2#1; Sh#2: shTPCN2#2; shRNA: Short hairpin RNA; *TPCN2*: Two-pore segment channel 2.

nucleoprotein [*AHNAK*], Rho GDP dissociation inhibitor beta [*ARHGDI B*], growth factor receptor bound protein 10 [*GRB10*], *PMEPA1*, S100 Calcium Binding Protein A8 [*S100A8*], and C-X3-C motif chemokine receptor 1

[*CX3CR1*]. Consistent with the RNA-seq results, we found that *NCOA3*, *ERO1A*, *AHNAK*, *GRB10*, *PMEPA1*, and *S100A8* were significantly upregulated and that the expression of *ARHGDI B* and *CX3CR1* was

significantly downregulated. In addition, we observed similar results in THP-1 cells [Supplementary Figure 1E, <http://links.lww.com/CM9/A854>].

Discussion

GWAS have identified many genes for SLE susceptibility. However, the molecular mechanisms related to these genes are not yet well understood. In a previous study, we found that *TPCN2* is a susceptibility gene for SLE.^[25] In the present study, we verified that the expression level of *TPCN2* was significantly decreased in SLE PBMCs with a new cohort. Further, we found that *TPCN2* knockdown significantly inhibited cell growth and induced G2/M arrest in Jurkat and THP-1 cells. Furthermore, RNA-seq analysis showed that biological regulation and cellular processes were significantly affected by *TPCN2* silencing. Further, GSEA showed that the G2/M checkpoint pathway was enriched in the sh#2 group. These findings are consistent with those of a previous study which showed that TPCs play a role in mediating cytokinesis, which is also dependent on cell-cycle regulation.^[28]

Increasing evidence has demonstrated the important role of apoptosis in SLE pathogenesis.^[29] It has been reported that leukocyte apoptosis is significantly higher in SLE patients.^[30] In this study, we observed that knockdown of *TPCN2* induced apoptosis in both Jurkat and THP-1 cells. Similarly, RNA-seq analysis showed that the FoxO, thyroid hormone, and T cell receptor pathways were affected by the dysregulation of the DEGs. Increasing evidence indicates that FoxO family members (including FoxO 1, 3, 4, and 6) play critical roles in immunoregulation.^[31,32] FoxO affects cell proliferation, autophagy, response to reactive oxygen species, the cell cycle, and apoptosis through phosphorylation, acetylation, and ubiquitylation; these are processes which rely on the proteins Akt, STAT, Smad, and sirtuin-1 [SIRT1].^[33,34] It has been reported that FoxO3a is a potential target for SLE therapy.^[35] Foxo are downstream targets of PI3K, which control cell cycle and apoptosis in numerous cell types.^[36] Interestingly, GSEA show that *TPCN2* is a regulatory factor in PI3K/AKT/mTOR pathway. Consequently, we speculate that *TPCN2* may play an important role in FoxO signaling pathway. However, the precise mechanisms involved in mediating cellular functions remain unknown. Further investigation is needed to elucidate the molecular mechanisms underlying the role of *TPCN2* in regulating apoptosis.

Furthermore, we assessed the expression of key genes at the mRNA level in *TPCN2*-deficient Jurkat cells, which indicated that *NCOA3*, *S100A8*, *AHNAK*, *GRB10*, *PMEPA1*, and *ERO1A* expression was significantly upregulated and that *ARHGDI1* and *CX3CR1* expression was significantly downregulated; similar results were observed in THP-1 cells. These genes play crucial roles in cell growth and apoptosis.

NCOA3 plays an important role in several biological processes, such as cell proliferation, apoptosis, and migration.^[37,38] *S100A8*, a member of the S100 family of proteins that can be found in neutrophils, monocytes/macrophages, dendritic cells, and osteoclasts, induces

apoptosis via Toll-like receptor 4 signaling.^[39] *AHNAK* is a tumor suppressor that mediates the negative regulation of cell growth via modulation of the TGF β /Smad signaling pathway.^[40] *GRB10* has been implicated in apoptotic signaling, and is highly concentrated in the mitochondria, an organelle involved in the regulation of apoptosis.^[41] Since these genes are crucial mediators of the cell cycle and of apoptosis, our study highlights the importance of *TPCN2* in these processes.

In this study, we investigated the biological effects and regulatory mechanisms of *TPCN2* *in vitro*. Our findings revealed that *TPCN2* knockdown induces apoptosis and G2/M cell-cycle arrest in both Jurkat and THP-1 cells, indicating that *TPCN2* might be a potential protective factor against SLE. These results elucidate the mechanism of action of *TPCN2* in SLE and could potentially enable novel therapeutic approaches against SLE, upon further research.

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Conflicts of interest

None.

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