doi:10.4149/neo_210802N1083

Matrine exerted an anti-tumor effect on acute myeloid leukemia via the lncRNA LINC01116/miR-592-mediated JAK/STAT pathway inactivation

Ping-Ping ZHANG, Feng ZHANG, Kai ZHU, Jun-Feng ZHU, Yuan YUAN, Yan-Li YANG, Lin LIU, Meng WANG, Jia-Jia LI*

Department of Hematology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, China

**Correspondence: jiajiali0720@163.com*

Received August 2, 2021 / Accepted November 2, 2021

As a malignant hematological cancer, acute myeloid leukemia (AML) influences the health of many people. This study explored the anti-AML activity of matrine (a natural-derived alkaloid), as well as the internal molecular mechanism. *In vitro*, cell viability, apoptosis, and productions of inflammatory cytokines including IL-1β, IL-6, and TNF-α were tested by MTT, Annexin V-FITC/PI staining, and ELISA, respectively. The expression levels of LINC01116 and miR-592 were measured by qRT-PCR. Bcl-2 and PCNA expression, and JAK/STAT3 pathway activity were evaluated by western blotting. Besides, an AML mouse xenograft model was established to further analyze the anti-AML activity of matrine. We found that matrine suppressed cell proliferation and levels of inflammatory factors, induced cell apoptosis, reduced LINC01116 expression, and raised miR-592 expression in AML cells. LINC01116 directly bound to miR-592 and downregulated its expression. Both LINC01116 overexpression and miR-592 knockdown attenuated the effects of matrine on AML cells. Moreover, miR-592 overexpression reversed the influences of LINC01116 overexpression on matrine-treated AML cells. Matrine inactivated the JAK/STAT3 pathway in AML cells via modulating LINC01116/miR-592. Additionally, matrine inhibited tumor growth via modulating LINC01116/miR-592 *in vivo*. To sum up, matrine exhibited the anti-AML activity through regulating the LINC01116/miR-592 axis, thereby inactivating the JAK/STAT3 pathway.

Key words: acute myeloid leukemia, matrine, long non-coding RNA LINC01116, microRNA-592, JAK/STAT3 pathway

Acute myeloid leukemia (AML) is a common malignant hematological cancer caused by abnormal proliferation and differentiation of immature myeloid blasts in the bone marrow and peripheral blood [1]. In the United States, there are more than 20,000 people diagnosed with AML each year [2]. It is discovered that old people (age over 65) have a higher incidence of AML than people less than 65 [3]. Considering that the old people usually exert low tolerance to relevant treatment, the overall long-term survival rate of AML remains unsatisfactory [3]. Many factors are related to the pathogenesis of AML, such as chromosomal translocation, radionuclide, and chemical exposure, as well as genetic mutation [4–6]. Genetic mutations are verified in over 97% of AML patients [2]. More efforts are still needed to further explore the pathogenesis of AML and look for a new therapeutic strategy for this disease.

Matrine ($C_{15}H_{24}N_2O$, CAS number: 519-02-8) is a natural alkaloid separated from the dried roots, plants, and fruits of the legume plant *Sophora flavescens Ait* (Kushen) [7]. Earlier studies reported that matrine exerted excellent anti-cancer activity in multiple human cancers, including AML [8]. Zhang et al. [9] revealed that matrine showed an anti-cancer effect in AML via inducing mitochondrial-related cell apoptosis. Hao et al. [10] indicated that matrine caused AML cell apoptosis by suppressing the PI3K/AKT/mTOR pathway. Wu et al. [11] discovered that matrine could promote AML cell autophagy. However, more investigations are demanded to study the molecular mechanism regarding the anti-cancer activity of matrine in AML.

In recent years, lots of non-coding RNAs (ncRNAs), especially long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) are discovered to play indispensable regulatory roles in cell physiology and cellular functions [12]. More importantly, lncRNAs and miRNAs are found to participate in the occurrence and progression of multiple human cancers [13]. LncRNAs and miRNAs can regulate the expression of numerous genes in cancer cells at transcriptional and post-transcriptional levels [14]. Some lncRNAs are demonstrated to serve as functional and structural chromatin regulators in AML [15]. Studies have found that lncRNAs are related to the regulation of cell proliferation, apoptosis, and differentiation [16, 17], as well as therapeutic resistance in AML [18].

Matrine has been found to regulate lncRNA expression [19]. LINC01116 is an oncogenic lncRNA and contributes to the tumorigenesis and development of multiple human cancers, such as lung squamous cell carcinoma [20], gastric cancer [21], and glioma [22]. Nevertheless, whether LINC01116 takes part in the progression of AML is not clear. Especially, whether matrine can regulate LINC01116 expression in AML remains dim.

As a tumor-suppressive miRNA in human cancer cells, miR-592 was lowly expressed in the serum of AML patients and cells [23]. Overexpression of miR-592 inhibited the growth and induced apoptosis via targeting Rho-associated kinase 1 (ROCK1) in AML cells [23]. Earlier studies have reported that matrine could regulate miRNA expression, thus, playing anti-cancer activity [24]. Through bioinformatic analysis, we found that LINC01116 was associated with miR-592. In the current research, we further investigated the anti-cancer effect and mechanism of matrine in AML both *in vitro* and *in vivo*. About the molecular mechanism, we mainly focused on the LINC01116/miR-592 axis.

Patients and methods

Cell culture and matrine treatment. THP-1 and HL-60 cells were obtained from Procell Inc., (Wuhan, China). Bone marrow stromal HS-5 cells were purchased from the American Type Culture Collection (ATCC, VA, USA). THP-1 and HS-5 cells were cultured in RPMI-1640 (Sigma-Aldrich, MO, USA) containing 10% (v/v) fetal bovine serum (FBS, Hyclone, UT, USA), 1% (v/v) penicillin-streptomycin solution (Sigma-Aldrich). HL-60 cells were cultured in Iscove's Modified Dulbecco Medium (IMDM, Procell Inc.,) with 20% FBS and 1% penicillin-streptomycin solution. Cells were placed in a humidity incubator at 37 °C with 5% CO_2.

Matrine (purity 98.56%) was purchased from APExBIO (TX, USA) and dissolved into ultra-pure water with a storage concentration of 40 g/l. THP-1 and HL-60 cells were subjected to 0.5, 1, or 2 g/l (equal to 2, 4, or 8 nM) matrine for 72 h in this research.

Clinical samples. Fifteen AML patients and fifteen health individuals were recruited in our research. The AML samples and normal samples were collected. Informed consent was acquired from each subject. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical College [SYXK(Wan)-2017-001].

Table 1. Primers information for PCR.

Name		Sequence (5'-3')
LINC01116	F	GTTCAAGTGCGTCCGGGTTT
	R	CGGACTTCTTTTCCAGGCGG
GAPDH	F	AGGAGCGAGATCCCGCCAACA
	R	CGGCCGTCACGCCACATCTT
miR-592	F	TTGTGTCAATATGCGATGATGT
	R	GCGAGCACAGAATTAATAGCAC
U6	F	CTCGCTTCGGCAGCACA
	R	AACGCTTCACGAATTTGCGT

Cell transfection. The LINC01116 sequence was sub-cloned into pcDNA3.1 vector (Invitrogen, CA, USA) to form LINC01116 overexpressing vector (named LINC01116). Unloaded pcDNA3.1 vector was utilized as a negative control (NC). miR-592 mimics (named miR-592), anti-miR-592, and relative NC were supplied by Genechem Corporation (Shanghai, China). For cell transfection, Lipofectamine™ 2000 Reagent (Invitrogen) was used in line with the manufacturer's instructions. Transfection efficiencies were tested by quantitative reverse transcription PCR (qRT-PCR).

qRT-PCR. Total RNAs in cells and tissues were isolated using an RNAiso Plus kit (Takara Biomedical Technology, Beijing, China). cDNA was synthesized using a High-Capacity RNA-to-cDNA™ kit (Applied Biosystems, CA, USA). For LINC01116 expression, the TaqMan Non-coding RNA Assay (Applied Biosystems) was performed with the program as follows: 95 °C for 60 s, 40 cycles of 95 °C for 30 s, 60 °C for 40 s, and 72 °C for 30 s. To detect miR-592 expression, the Mir-X miRNA qRT-PCR TB Green kit (Clontech, CA, USA) was used with the program as follows: 94 °C for 3 min, 40 cycles of 90 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. Primer information is shown in Table 1. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. GAPDH or U6 served as an internal control for LINC01116 and miR-592, respectively.

Cell viability assay. Viabilities of THP-1 and HL-60 cells were examined by MTT assay. Briefly, 1×10^4 transfected or non-transfected cells were seeded in 96-well plates for 12 h and subjected to matrine treatment. Then, 20 μl MTT solution (2.5 mg/ml, Sigma-Aldrich) was added to the culture medium for 4 h at 37 °C. After removing the culture medium, 150 μl dimethyl sulfoxide (DMSO) was added to each well of the plate for 1 h. The absorbance at 570 nm was measured using a microplate reader (Bio-Tek Instruments, VT, USA). Results were displayed as the percentage of control.

Cell apoptosis assay. Apoptosis of THP-1 and HL-60 cells was evaluated by the Annexin V-FITC/PI Apoptosis Detection kit (Yeasen Biotechnology, Shanghai, China). Briefly, 1×10^5 transfected or non-transfected THP-1 and HL-60 cells were seeded in 6-well plates for 12 h and treated with matrine. Following trypsin solution incubation, cells in each group were collected, washed with phosphate buffer saline (PBS) solution, and mixed with 5 μl Annexin V-FITC and 10 μl PI solution for 20 min and 15 min, respectively, at 37 °C in the dark. Results were detected using a flow cytometer (Beckman Coulter, CA, USA) and the percentage of apoptotic cells was recorded.

Enzyme linked immunosorbent assay (ELISA). ELISA was performed to test the concentrations of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in THP-1 and HL-60 cells. In brief, after different treatments,

culture supernatants of cells were harvested. Human IL-1β (RAB0273), IL-6 (RAB0306), and TNF-α (RAB0459) ELISA kits, which were all from Sigma-Aldrich, were used in line with the manufacturer's instructions.

Dual-luciferase reporter assay. A dual-luciferase reporter assay was conducted to analyze the binding relationship between LINC01116 and miR-592. To establish the LINC01116-wild type (LINC01116-WT) report vector, the full-length sequence of LINC01116 was inserted into the psiCHECK-2 plasmid (Promega, WI, USA). To establish the LINC01116-mutated type (LINC01116-MUT) report vector, the full-length sequence of the LINC01116 containing the mutated miR-592 binding site was also sub-cloned into the psiCHECK-2 plasmid. Then, miR-592 and LINC01116-WT or LINC01116-MUT were co-transfected into THP-1 and HL-60 cells by using Lipofectamine™ 2000 Reagent. After 48 h, the relative luciferase activity was detected by the Dual-luciferase Reporter Assay System (Promega).

RNA immunoprecipitation (RIP) assay. The interaction between LINC01116 and miR-592 was confirmed using the EZ-Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, MA, USA). Briefly, following trypsin solution incubation, THP-1 and HL-60 cells were harvested, respectively. Then, cells were washed with PBS and mixed with PIPR Lysis Buffer (P0013B, Beyotime Biotechnology, Shanghai, China). The lysates of both cells were incubated with RIP buffer containing magnetic beads conjugated with anti-Argonaute-2 (Ago2) antibody (ab57113) or negative control immunoglobulin G (IgG, ab181236, Abcam Biotechnology, MA, USA), respectively. The enrichments of LINC01116 and miR-592 in extracted RNAs were analyzed by qRT-PCR.

Western blotting. Total proteins in THP-1 and HL-60 cells were extracted using xTractor™ Buffer (Clontech, CA, USA) supplemented with EDTA-Free Protease Inhibitor Cocktail (Clontech). A Bradford Protein Quantification kit (Yeasen Biotechnology) was used to test protein concentrations. Then, proteins with equal concentration were separated by SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). PVDF membranes were incubated with primary antibodies including anti-Proliferating cell nuclear antigen (PCNA) antibody (#13110, 1:1000), anti-Bcl-2 antibody (#15071, 1:1000), anti-p-JAK antibody (#66245, 1:1000), anti-JAK antibody (#50996, 1:1000), anti-p-STAT3 antibody (#9145, 1:2000), anti-STAT3 antibody (#9139, 1:1000), and anti-GAPDH antibody (#2118, 1:1000), which were all obtained from Cell Signaling Technology (MA, USA), for 12 h at 4 °C, followed by incubated with secondary antibodies [HRP-labeled Goat anti-rabbit (or anti-mouse) IgG (H+L), A0208, A0216, Beyotime Biotechnology] for 1 h at room temperature. Signals of proteins were visualized via enhanced chemiluminescence technique.

***In vivo* mouse xenograft model.** Fifteen BALB/c nude mice (male, 7–8-week-old) were purchased from Lebeiao Biotechnology Co., Ltd (Tongling, China). After being fed in our animal room for 1 week, mice were randomly divided into control, matrine, and matrine+LINC01116 groups, with 5 mice in each group. Following anesthesia with 40 mg/kg pentobarbital (Sigma-Aldrich), 0.1 ml of THP-1 cells (1×10^7 cells/ml) were subcutaneously injected into the flank of mice in the control and matrine groups, while 0.1 ml of THP-1 cells with LINC01116 overexpressing (1×10^7 cells/ml) were subcutaneously injected into the flank of mice in the matrine+LINC01116 group. Mice in matrine and matrine+LINC01116 groups were treated with matrine (60 mg/kg/day) via gastric lavage. The long and short diameters of tumors were measured every 4 days after day 7 to calculate tumor volume. After 27 days, mice were anesthetized via intraperitoneal injection of 40 mg/kg pentobarbital and then were sacrificed by cervical dislocation [25]. Tumor specimens were isolated and weighted. Two tumor specimens of each group were placed at −80 °C to detect the expression of LINC01116 and miR-592 using qRT-PCR, other three tumor specimens were fixed in 10% formalin solution to test PCNA expression and cell apoptosis by immunocytochemistry (IHC) and TUNEL staining, respectively.

All animal experimental procedures were performed in line with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical College [SYXK(Wan)-2017-001].

IHC assay. After gradient dehydration and paraffin embedding, the tissues were cut into 7 μm thick sections. Then, the sections were de-paraffinized with xylene solution for twice (each for 20 min), placed in 100% ethanol solution twice (each for 10 min), and hydrated in 95%, 90%, 80%, 70% ethanol, and distilled water (each for 5 min). After heat-mediated antigen retrieval using 10 mM citrate buffer (pH 6.0) for 15 min and incubating with 0.2% Triton X-100 solution for 15 min, the endogenous peroxidase activity was eliminated by 3% H_2O_2 solution for 10 min. Then, the sections were incubated with 5% normal goat serum for 15 min, anti-PCNA antibody (#13110, Cell Signaling Technology) overnight and horseradish peroxidase-conjugated secondary antibody for 1 h. Then, tissues were incubated with diaminobenzidine solution, stained with hematoxylin solution, and dehydrated with ethanol. Lastly, the sections were observed under a microscope (Nikon, Japan).

TUNEL assay. Click-iT™ TUNEL Colorimetric IHC Detection Kit (C10625, Invitrogen) was used to test cell apoptosis in line with the manufacturer's instruction *in vivo*. Briefly, after de-paraffinizing and hydrating as above mentioned, the sections were incubated with proteinase K for 15 min, PBS for 5 min, TdT reaction buffer for 10 min at 37 °C, 2× SCC solution for 15 min, PBS for 5 min, 3% H_2O_2 solution for 5 min, Click-iT™ TUNEL Colorimetric Reaction cocktail for 30 min in the dark, PBS for 5 min, 1× Streptavidin-Peroxidase Conjugate for 30 min, PBS for 5 min, DAB Reaction Mixture for 3 min, hematoxylin solution for 2 min, and ethanol solution for 15 s. Results were observed under a microscope (Nikon).

Statistical analysis. GraphPad 6.0 software was used for statistical analysis. Results are shown as mean ± standard deviation (SD) from three independent experiments. p-values were calculated using Student's t-test or one-way ANOVA with Tukey's post hoc test. A p-value <0.05 was considered as statistically significant.

Results

Matrine suppressed AML cell proliferation and inflammatory factor expression, but induced cell apoptosis. Firstly, the anti-tumor effect of matrine in AML was investigated. Base on the results of the pre-test (data not shown), 0.5, 1, or 2 g/l matrine exposure for 72 h was selected. Figure 1A showed that 1 or 2 g/ matrine obviously inhibited the viabilities of THP-1 and HL-60 cells ($p<0.05$). The effect of matrine on a non-AML cell line HS-5 was also analyzed and the results of Figure 1B displayed that these concentrations of matrine had no significant effect on the viability of HS-5 cells. The percentage of apoptotic THP-1 and HL-60 cells was significantly increased after 1 or 2 g/l matrine treatment (Figures 1C, 1D, $p<0.05$). However, matrine treatment did not change the apoptosis of HS-5 cells (Figure 1E). Moreover, the results of western blotting displayed that matrine treatment notably reduced the protein expression levels of PCNA and Bcl-2 both in THP-1 and HL-60 cells (Figures 1F, 1G, $p<0.05$). Pro-inflammatory cytokines are widely detected in AML patients [26]. Hence, we further analyzed the effect of matrine on the levels of IL-1β, IL-6, and TNF-α in AML cells. Figures 1I-1K illustrated that the concentrations of IL-1β, IL-6, and TNF-α in the culture supernatants of THP-1 and HL-60 cells were remarkably decreased after matrine treatment ($p<0.05$). Whereas matrine treatment did not affect the levels of PCNA, Bcl-2, and cytokine productions in HS-5 cells (Figures 1H–1K). These above outcomes suggested that matrine exerted anti-cancer activity in AML cells, but did not influence the non-AML cells.

Matrine functioned via reducing LINC01116 expression in AML cells. Studies have shown that LINC01116 played an oncogenic role in many cancers, and matrine could regulate lncRNA expression [19, 27, 28]. Hence, we analyzed the relationship between matrine and LINC01116 in AML. Analyzing LINC01116 expression in AML and normal samples, we found that compared with normal samples, LINC01116 was highly expressed in AML samples (Figure 2A, $p<0.05$). Matrine treatment decreased LINC01116 expression both in THP-1 and HL-60 cells ($p<0.05$; Figure 2B). These outcomes suggested that matrine inhibited LINC01116 expression in AML cells and implied that matrine might exert an anti-tumor effect in AML via reducing LINC01116 expression.

To explore whether LINC01116 took part in the anti-tumor effect of matrine on AML, LINC01116 was overexpressed in THP-1 and HL-60 cells. The transfection efficiency of LINC01116 is shown in Supplementary Figure S1A. The results of Figures 2C and 2D illustrated that compared to the matrine+vector group, the expression of LINC01116 in THP-1 and HL-60 cells was notably increased in the matrine+LINC01116 group ($p<0.05$). Moreover, Figures 2E and 2F showed that overexpression of LINC01116 promoted the viabilities of THP-1 and HL-60 cells, and it also significantly attenuated the loss of cell viabilities caused by the matrine treatment ($p<0.05$). Compared with the control group, LINC01116 upregulation declined the number of apoptotic cells, but without any significant difference, which may be due to the very low apoptotic number in the control (Figures 2G, 2H). As expected, matrine treatment-increased number of apoptotic THP-1 and HL-60 cells was reduced by LINC01116 overexpression (Figures 2G, 2H, $p<0.05$). Furthermore, the protein expression levels of PCNA and Bcl-2 in THP-1 and HL-60 cells were raised in the matrine+LINC01116 group compared to the matrine+vector group (Figures 2I, 2J, $p<0.05$). Besides, LINC01116 overexpression also weakened the inhibitory activity of matrine on the expression of IL-1β, IL-6, and TNF-α in AML cells (Figures 2K–2M, $p<0.05$). The above results suggested that matrine played an anti-tumor role in AML via downregulating LINC01116.

LINC01116 bound to miR-592 and downregulated its expression in AML cells. Then, the targeted regulatory relationship of LINC01116 on miR-592 was explored. Bioinformatics analysis using LncBase Predicted v.2 revealed that partial nucleotide sequences of LINC01116 could bind to miR-592 through complementary base pairing (Figure 3A). The results of the dual-luciferase reporter assay displayed that co-transfection of miR-592 and LINC01116-WT remarkably decreased the relative luciferase activities in THP-1 and HL-60 cells ($p<0.05$), while co-transfection of miR-592 and LINC01116-MUT had no significant influence on the relative luciferase activities (Figures 3B, 3C). In addition, LINC01116 gathered miR-592 in THP-1 and HL-60 cells, as evidenced by the increased relative enrichments of LINC01116 and miR-592 in Anti-Ago-2 complex when compared with IgG (Figures 3D, 3E, $p<0.05$). Furthermore, LINC01116 overexpression notably decreased miR-592 expression in AML cells (Figure 3F, $p<0.05$), while miR-592 upregulation did not affect LINC01116 expression in these cells (Supplementary Figure S1B, Figure 3G). Besides, matrine significantly raised the expression of miR-592 in THP-1 and HL-60 cells (Figures 3H, 3I, $p<0.05$). The above outcomes suggested that LINC01116 bound to miR-592 and downregulated its expression in AML cells. These data also hinted that matrine exerted an anti-tumor effect in AML via reducing LINC01116 expression and thereby enhancing miR-592 expression.

Knockdown of miR-592 weakened the effects of matrine in AML cells. Anti-miR-592 was transfected into THP-1 and HL-60 cells to knock down the miR-592 expression. The transfection efficiency is shown in Supplementary Figure S1C. The anti-miR-592 transfection partially reversed the increase of miR-592 expression by matrine in AML cells

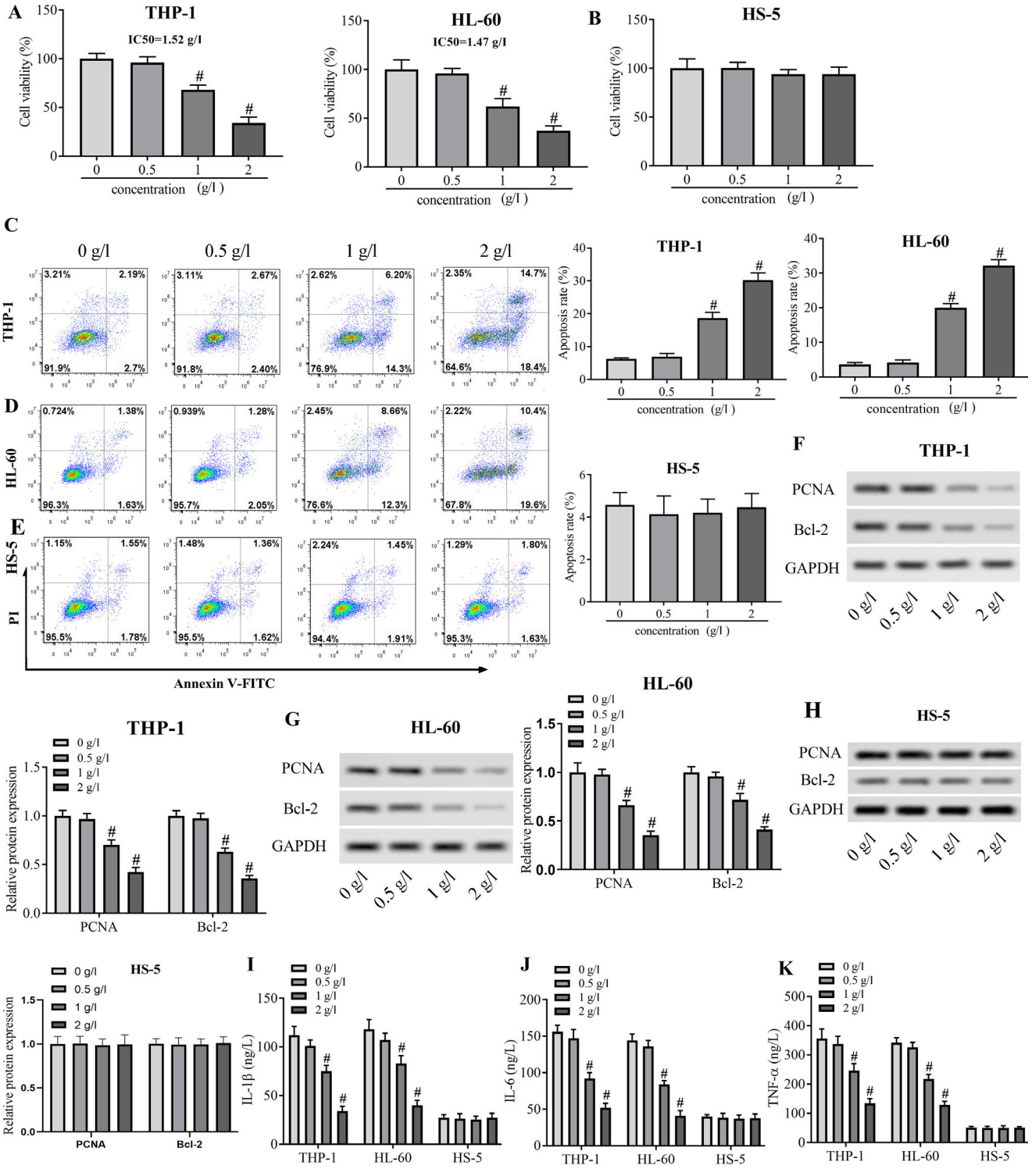

Figure 1. Matrine suppressed AML cell proliferation and inflammatory factor expression but induced cell apoptosis. Following 0, 0.5, 1, or 2 g/l matrine exposure for 72 h. A, B) The viabilities of THP-1, HL-60, and HS-5 cells were tested via MTT assay. C–E) Apoptosis of THP-1, HL-60, and HS-5 cells was examined by Annexin V-FITC/PI Apoptosis Detection kit. F–H) The protein expression levels of PCNA and Bcl-2 in THP-1, HL-60, and HS-5 cells were evaluated by western blotting. I–K) The concentrations of IL-1β, IL-6, and TNF-α in the culture supernatants of THP-1, HL-60, and HS-5 cells were measured by ELISA. N=3, #p<0.05 vs. 0 g/l matrine treatment group.

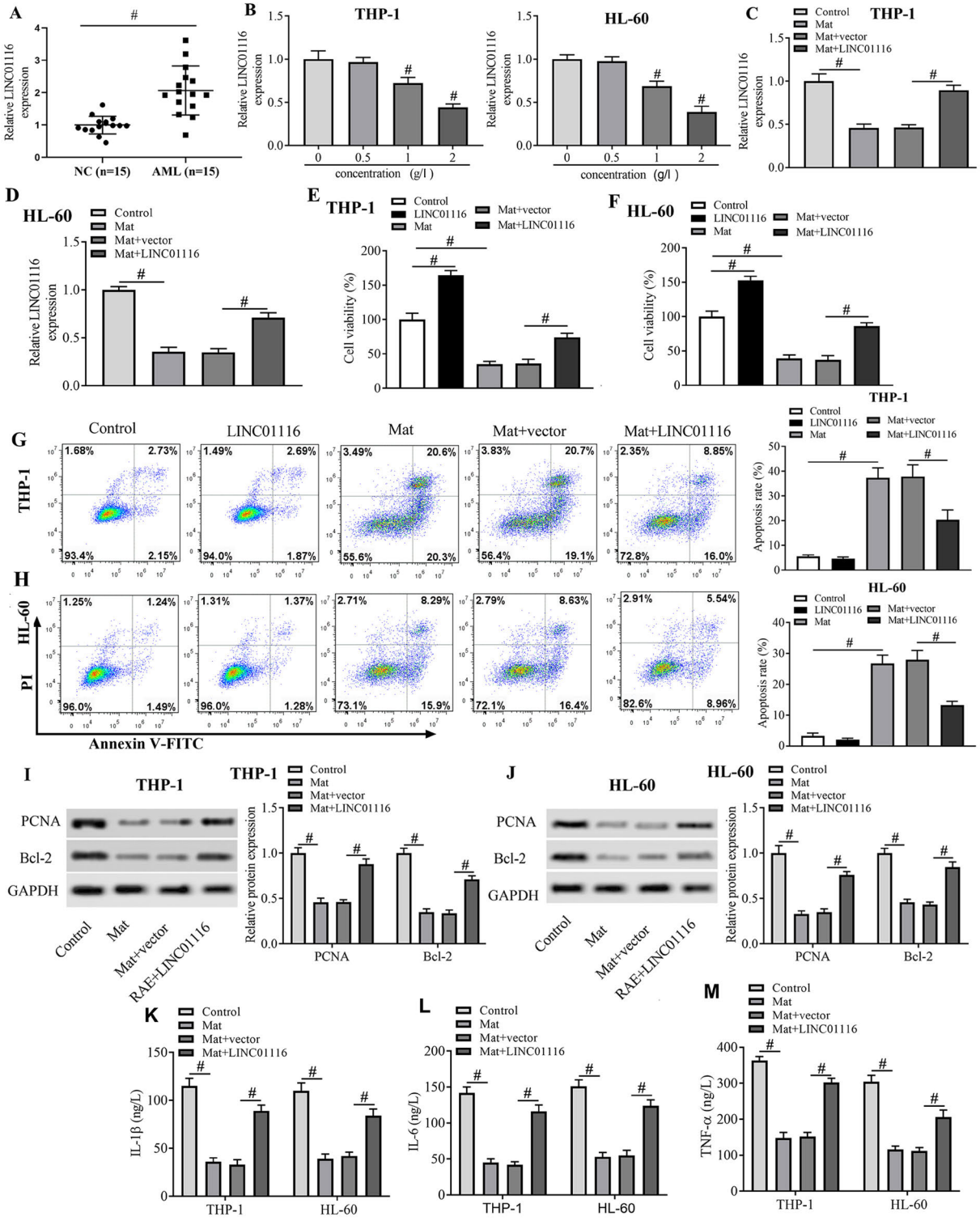

Figure 2. Matrine decreased LINC01116 expression and overexpression of LINC01116 attenuated the effects of matrine in AML cells. A) LINC01116 expression in AML and normal samples was tested by qRT-PCR. B) THP-1 and HL-60 cells were treated with 0, 0.5, 1, or 2 g/l matrine for 72 h. The expression of LINC01116 in cells was tested via qRT-PCR assay. After transfection with vector or LINC01116, THP-1 and HL-60 cells were treated with or without 2 g/l matrine for 72 h. C, D) LINC01116 expression in cells were detected by qRT-PCR assay. E, F) Cell viabilities were analyzed by MTT assay. G, H) Cell apoptosis was examined by Annexin V-FITC/PI Apoptosis Detection kit. I, J) The protein expression levels of PCNA and Bcl-2 in cells were evaluated by western blotting. K–M) IL-1β, IL-6, and TNF-α concentrations in cell culture supernatants were measured by ELISA. N=3, #p<0.05 vs. normal samples, control group, or matrine+vector group.

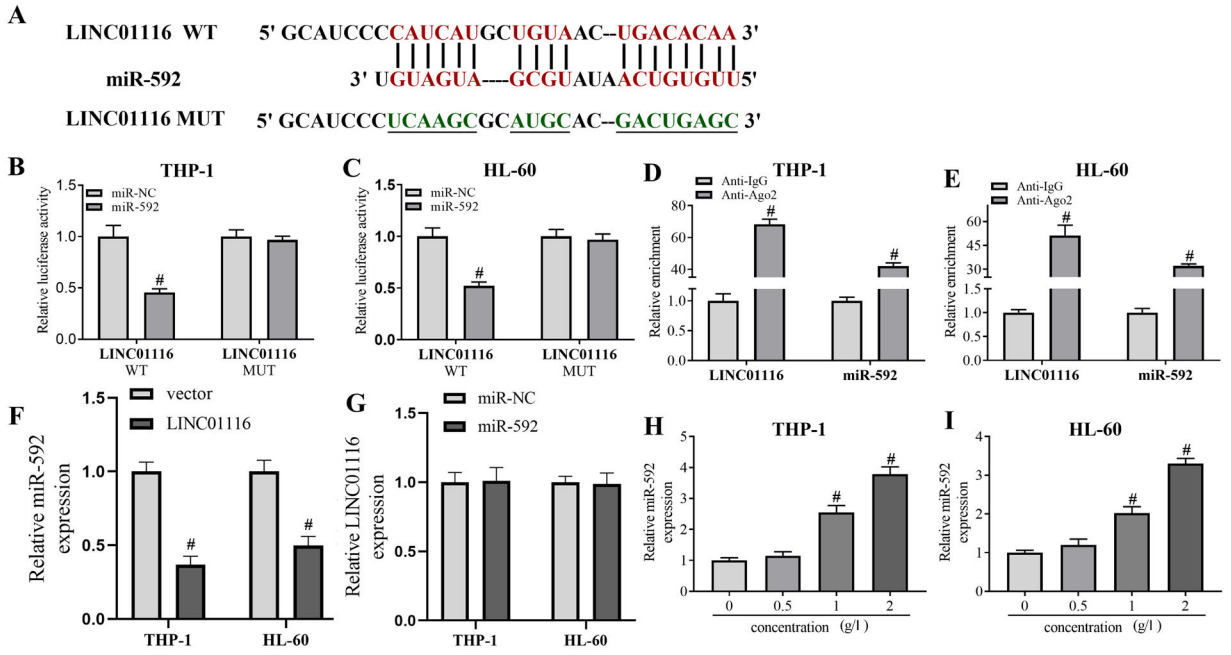

Figure 3. LINC01116 bound to miR-592 and downregulated expression in AML cells. A) The predicated binding sequences between LINC01116 and miR-592, as well as the sequence of LINC01116-MUT. B, C) After co-transfection with miR-592 and LINC01116-WT (or LINC01116-MUT), the relative luciferase activities in THP-1 and HL-60 cells were measured via the dual-luciferase reporter assay. D, E) The enrichments of LINC01116 and miR-592 were analyzed by the RIP assay using the anti-Ago2 antibody. F) After transfection with LINC01116, miR-592 expression in THP-1 and HL-60 cells was determined by qRT-PCR. G) The expression of LINC01116 was assayed in THP-1 and HL-60 cells after transfection with miR-592 mimic. H, I) THP-1 and HL-60 cells were treated with 0, 0.5, 1, or 2 g/l matrine for 72 h. The expression of miR-592 in THP-1 and HL-60 cells was detected by qRT-PCR. N=3, #p<0.05 vs. miR-NC group, anti-IgG group, vector group, or 0 g/l matrine treatment group.

(Figures 4A, 4B, p<0.05). miR-592 knockdown enhanced the viabilities of THP-1 and HL-60 cells and weakened the loss of cell viabilities induced by matrine treatment (Figures 4C, 4D, p<0.05). Moreover, miR-592 downregulation relieved cell apoptosis induced by matrine treatment, although it did not significantly decrease the apoptosis of THP-1 and HL-60 cells alone (Figures 4E, 4F, p<0.05). Compared to the matrine+miR-NC group, the protein expression levels of PCNA and Bcl-2 in THP-1 and HL-60 cells were notably increased in the matrine+miR-592 group (Figures 4G, 4H, p<0.05). Besides, miR-592 knockdown remarkably weakened the decreases of IL-1β, IL-6, and TNF-α caused by matrine in AML cells (Figures 4I–4K, p<0.05). The above outcomes suggested that the increase of miR-592 expression played a key role in the anti-tumor effect of matrine in AML.

miR-592 overexpression weakened the influences of LINC01116 overexpression on matrine-treated AML cells. After LINC01116 and miR-592 overexpression, THP-1 and HL-60 cells were subjected to matrine treatment, and the viabilities, apoptosis, and inflammatory factor expression of cells were tested. Results in Figures 5A and 5B showed that compared with the matrine+LINC01116+miR-NC group, the relative expression of miR-592 in THP-1 and HL-60 cells was notably raised in the matrine+LINC01116+miR-592 group (p<0.05). miR-592 overexpression significantly decreased the viability and induced the apoptosis of THP-1 and HL-60 cells. Also, its overexpression remarkably weakened the influences of LINC01116 overexpression on the viability and apoptosis in matrine-treated AML cells (Figures 5C–5F, p<0.05). Moreover, results of western blotting illustrated that the protein expression levels of PCNA and Bcl-2 in THP-1 and HL-60 cells were decreased in the matrine+LINC01116+miR-592 group in comparison to the matrine+LINC01116+miR-NC group (Figures 5G, 5H, p<0.05). Additionally, Figures 5I–5K show that miR-592 overexpression attenuated the influences of LINC01116 overexpression on matrine-induced decreases of IL-1β, IL-6, and TNF-α in AML cells (p<0.05). The above results further confirmed that matrine exerted an anti-tumor effect in AML via regulating the LINC01116/miR-592 axis.

Matrine inhibited tumor growth in an AML mouse xenograft model via modulating LINC01116/miR-592. An AML mouse xenograft model was established to further evaluate the anti-AML activity of matrine *in vivo*. Figures 6A–6C showed that matrine treatment notably reduced tumor volume and weight (p<0.05), which were weakened by the LINC01116 overexpression (p<0.05). Moreover, matrine lowered the LINC01116 expression, but raised the miR-592 expression in tumor samples (p<0.05), and LINC01116 overexpression increased LINC01116

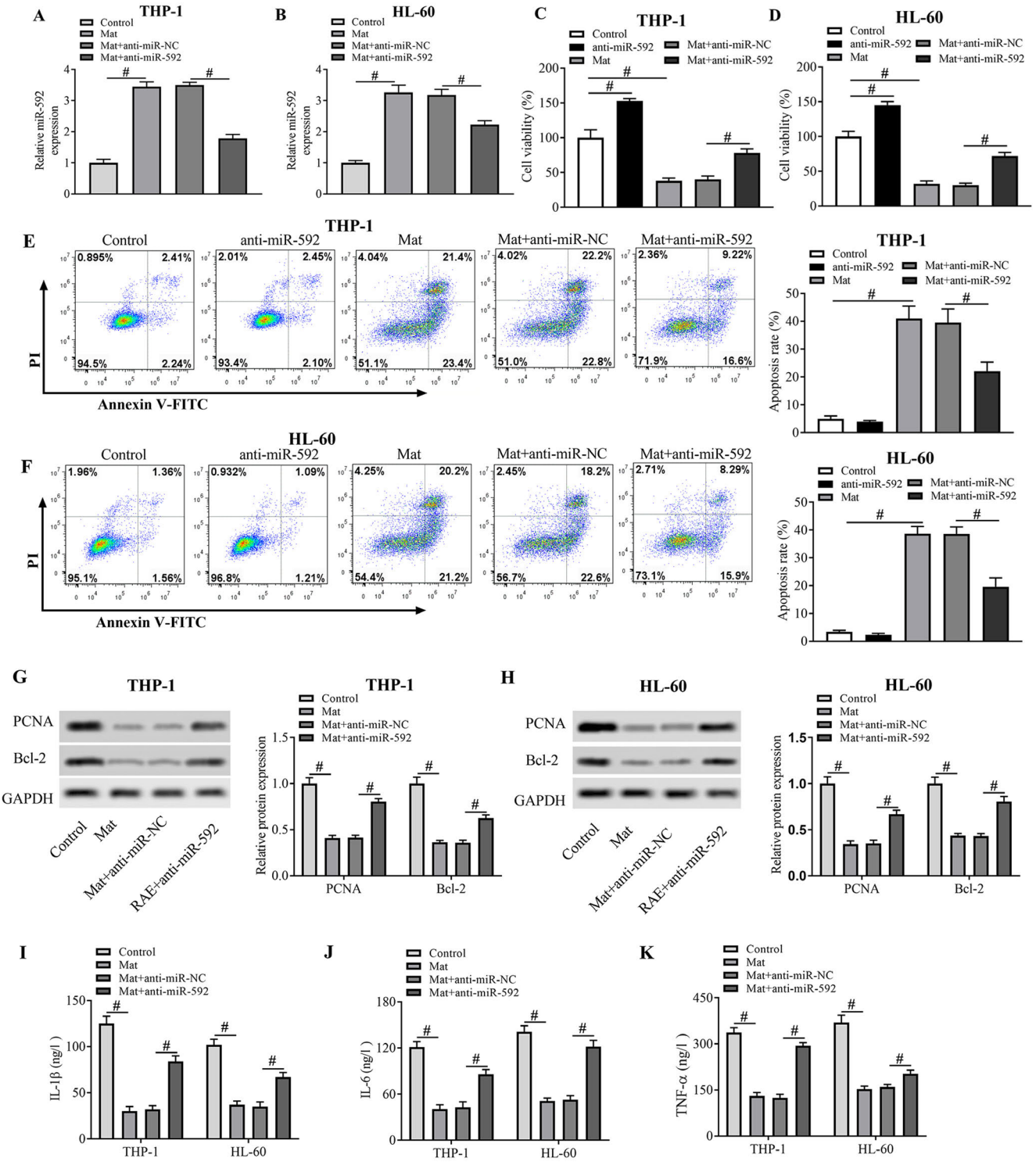

Figure 4. Knockdown of miR-592 weakened the effects of matrine in AML cells. After transfection with miR-NC or miR-592, THP-1 and HL-60 cells were treated with 2 g/l matrine for 72 h. A, B) miR-592 expression in cells was tested by qRT-PCR. C, D) Cell viabilities were detected by MTT assay. E, F) Cells apoptosis was examined by Annexin V-FITC/PI Apoptosis Detection kit. G, H) The protein expression levels of PCNA and Bcl-2 in cells were evaluated by western blotting. I–K) IL-1β, IL-6, and TNF-α concentrations in cell culture supernatants were measured by ELISA. N=3, #p<0.05 vs. control group or matrine+miR-NC group.

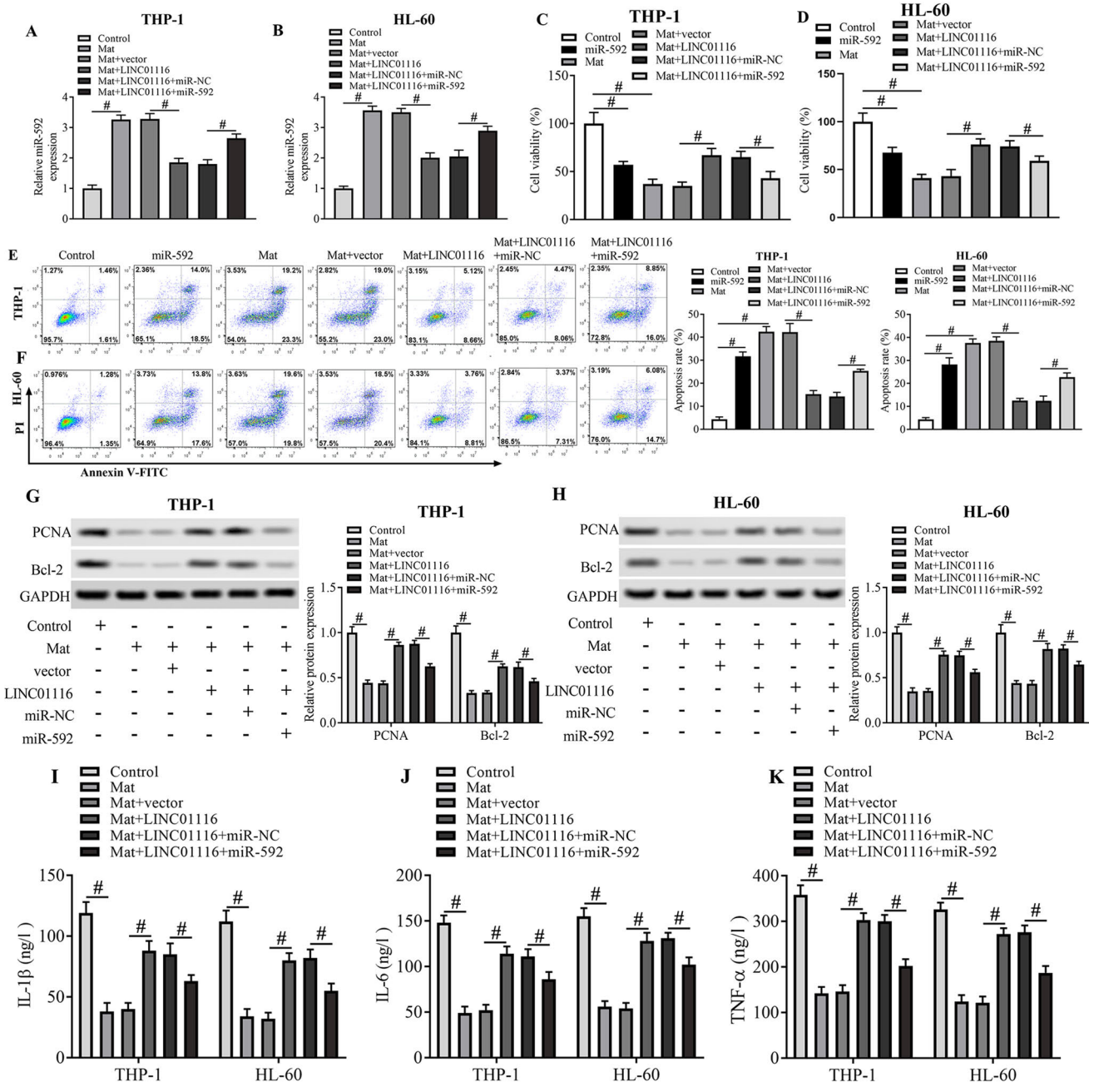

Figure 5. miR-592 weakened the influences of LINC01116 overexpression on matrine-treated AML cells. After transfection with LINC01116 and/or miR-592, THP-1 and HL-60 cells were treated with 2 g/l matrine for 72 h. A, B) miR-592 expression in cells was tested by qRT-PCR. C, D) Cell viabilities were measured by MTT assay. E, F) Cell apoptosis was examined by Annexin V-FITC/PI Apoptosis Detection kit. G, H) The protein expression levels of PCNA and Bcl-2 in cells were evaluated by western blotting. I–K) IL-1β, IL-6, and TNF-α concentrations in cell culture supernatants were detected by ELISA. N=3, #p<0.05 vs. control group, matrine+vector group, or matrine+LINC01116+miR-NC group.

expression but reduced miR-592 expression (Figures 6D, 6E, p<0.05). Additionally, matrine inhibited tumor cell proliferation, but promoted cell apoptosis, as evidenced by the decreased expression of PCNA and increased TUNEL positive cells. Compared with the matrine group, tumor cell proliferation was increased, while its apoptosis was decreased in the matrine+LINC01116 group (Figure 6F, p<0.05). The results indicated that matrine inhibited tumor growth via modulating LINC01116/miR-592 in the AML mouse xenograft model.

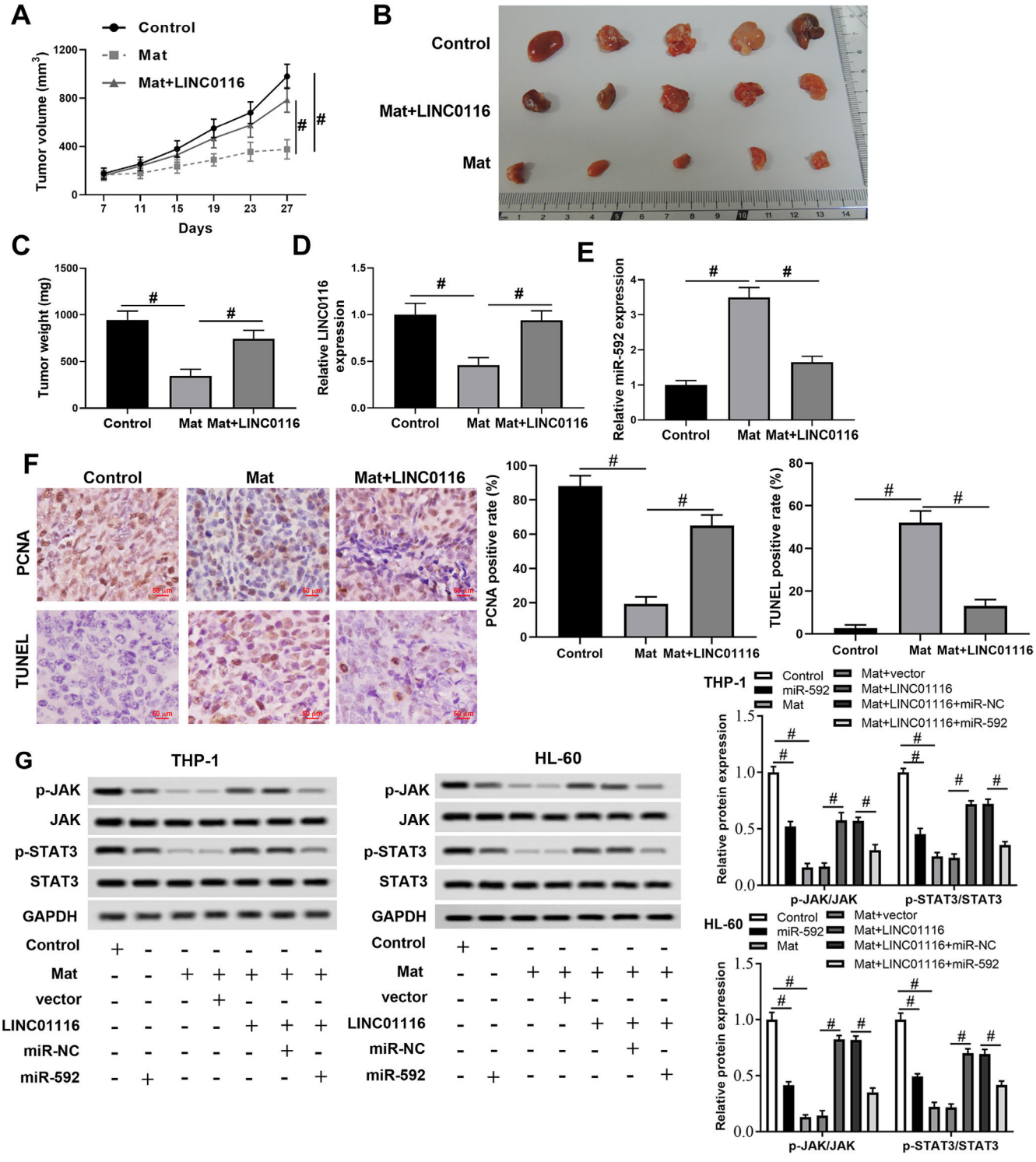

Figure 6. Matrine inhibited tumor growth via modulating LINC01116/miR-592-mediated inactivation of the JAK/STAT3 pathway. An AML mouse xenograft model was established and treated with matrine. A) Tumor volume was calculated on days 7, 11, 15, 19, 23, and 27. B) Tumor samples of each group. C) Tumor weight was measured. D, E) The expression levels of LINC01116 and miR-592 in tumor samples were detected by qRT-PCR. F) PCNA level in tumor samples was tested by IHC and tumor cell apoptosis was evaluated by TUNEL assay. G) The protein expression levels of p-JAK, JAK, p-STAT3, and STAT3 in THP-1 and HL-60 cells were detected by western blotting. N=3, #p<0.05

Matrine inactivated the JAK/STAT3 pathway in AML cells via modulating LINC01116/miR-592. Ma et al. have found that matrine inhibited the growth of chronic myeloid leukemia cells via suppressing the activation of the JAK/STAT3 signaling pathway [29]. Hence, we further evaluated the activity of the JAK/STAT3 pathway in THP-1 and HL-60 cells after relevant treatment and/or transfection. Results in Figure 6G show that both miR-592 overexpression and matrine treatment significantly reduced the levels of p-JAK/JAK and p-STAT3/STAT3 in these cells ($p<0.05$). LINC01116 overexpression notably attenuated the effects of matrine on the levels of p-JAK/JAK and p-STAT3/STAT3 in these cells ($p<0.05$). Moreover, compared with the matrine+LINC01116+miR-NC group, the levels of p-JAK/JAK and p-STAT3/STAT3 in AML cells were both decreased in the matrine+LINC01116+miR-592 group ($p<0.05$). The above results suggested that matrine inactivated the JAK/STAT3 pathway in AML cells via modulating the LINC01116/miR-592 axis.

Discussion

In recent years, more and more attention was given to the anti-cancer activity of plant-derived compounds, due to their high effectiveness and low side effects [30, 31]. Some plant-derived compounds have been used clinically for cancer treatment. For example, taxol, a natural secondary metabolite separated from the bark of *gymnosperm Taxus chinensis*, has been widely used to treat multiple human cancers, including breast cancer, ovarian cancer, and lung cancer [32]. As an alkaloid isolated from the legume plant *Sophora flavescens Ait*, matrine exerts outstanding anti-cancer activity in multiple human cancers [8]. AML affects the health of many people and threatens their lives [1]. Induction of AML cell apoptosis plays a key role in anti-AML therapy [33]. Earlier researches have demonstrated that matrine could induce AML cell apoptosis [9, 10]. Consistent with previous literature, we also confirmed that matrine promoted AML cell apoptosis, which was accompanied by the decreased protein levels of Bcl-2, a key anti-apoptotic molecule in cells. PCNA is closely related to DNA synthesis in cells, and it plays an important role in the initiation of cell proliferation [34]. In this research, we discovered that matrine also reduced PCNA protein level in AML cells. The overproduction of pro-inflammatory cytokines is widely detected in AML patients and decreased pro-inflammatory cytokines usually indicate a favorable phenomenon for the survival of AML patients [26]. We found that matrine notably suppressed the expression levels of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α in AML cells. These findings represented that the anti-AML activity of matrine could be achieved via inducing AML cell apoptosis, reducing AML cell proliferation, and inhibiting pro-inflammatory cytokine expression.

Earlier studies have reported that matrine could regulate the expression of lncRNAs [19, 35]. As a tumor-promoting lncRNA, LINC01116 was highly expressed in many human cancers [20, 22]. Fang et al. [27] indicated that the overexpression of LINC01116 promoted the progression of epithelial ovarian cancer through increasing cell proliferation and suppressing cell apoptosis. Chen et al. [28] demonstrated that LINC01116 overexpression promoted the proliferation but inhibited the apoptosis of gastric cancer cells. Besides, previous researches pointed out that LINC01116 could be induced in hypoxic vascular endothelial cells to regulate inflammatory reaction, and it also could modulate IL-1β expression in glioma cells [22]. In the present study, we discovered that LINC01116 was highly expressed in AML samples and matrine could reduce LINC01116 expression in AML cells. Overexpression of LINC01116 remarkably attenuated the anti-AML activity of matrine in AML both *in vitro* and *in vivo*. These findings represented that matrine exerted an anti-cancer effect in AML at least via regulating LINC01116.

Apart from lncRNAs, more and more miRNAs are also discovered to participate in the tumorigenesis and progression of human cancers. The mature miRNA forms a complex with the RNA induced silencing complex (RISC), which directs the complex to target mRNAs. miRNAs function via different regulatory networks in different cancers [36]. LncRNAs can modulate miRNA expression in cells through binding to it [13]. miR-592 is a tumor suppression-related miRNA, which is usually decreased in cancers [37]. Xu et al. [23] demonstrated that miR-592 was lowly expressed in the serum of AML patients and cells. It took part in regulating the growth and apoptosis of AML cells. In this research, we revealed that LINC01116 could bind to miR-592 and downregulate its expression in AML cells. Besides, matrine raised miR-592 expression in AML cells. Knockdown of miR-592 weakened the influences of matrine in AML cells. miR-592 overexpression attenuated the effects of LINC01116 overexpression on matrine-treated AML cells. Besides, we discovered that matrine inhibited tumor growth in an AML mouse xenograft model via regulating LINC01116/miR-592. These findings represented that matrine might play anti-AML activity through decreasing LINC01116 expression and thereby raising the miR-592 level. Considering that one lncRNA usually can regulate multiple miRNAs, while one miRNA is usually regulated by many lncRNAs, we proposed that other ncRNAs might be also involved in the anti-AML effect of matrine. Hence, more investigations are still needed.

The JAK/STAT3 pathway is one of the most important signaling pathways in cells, which plays a key role in regulating cell proliferation and apoptosis via affecting the activation of various downstream effector molecules [38]. Senga et al. [39] indicated that the JAK/STAT3 pathway was activated in M1 mouse myeloid leukemia cells. Han et al. [40] reported that the JAK/STAT3 pathway participated in the production of IL-17A, a cytokine secreted by Th17 cells, and IL-17A could promote the proliferation of IL-17 receptor (IL-17R)-positive AML cells. Ma et al. [41] found that matrine inhibited human

chronic myeloid leukemia cell growth by inactivating the JAK/STAT3 pathway. Besides, Zhang et al. [42] discovered that LINC01116 promoted osteosarcoma cell proliferation and migration via regulating the JAK/STAT3 pathway. In this study, we revealed that matrine inactivated the JAK/STAT3 pathway in AML cells. LINC01116 overexpression attenuated the inactivation of the JAK/STAT3 pathway in AML cells caused by matrine. Moreover, we found that miR-592 overexpression reversed the influence of LINC01116 overexpression on matrine-caused inactivation of the JAK/STAT3 pathway. These findings represented that matrine inactivated the JAK/STAT3 pathway in AML cells via modulating LINC01116 and miR-592.

Taken together, this research affirmed that matrine exhibited an anti-AML activity via regulating the LINC01116/miR-592 axis and thereby inactivating the JAK/STAT3 pathway. Chen et al. [43] reported that Kushen-based traditional Chinese medicine formulations exhibited good efficacy and low incidence of adverse events in the treatment of ulcerative colitis. Rong et al. [44] discovered that matrine could promote the efficacy and safety of platinum-based doublet chemotherapy for advanced non-small cell lung cancer. In this study, we found that matrine did not show any cytotoxicity to non-AML cells. However, more *in vitro* and *in vivo* studies are still needed to further test the efficacy and safety of matrine in AML in the future.

Supplementary information is available in the online version of the paper.

Acknowledgments: This research was supported by the Natural Science Key Project of Bengbu Medical College (No. BYKY2019114ZD).

References

[1] PELCOVITS A, NIROULA R. Acute Myeloid Leukemia: A Review. R I Med J (2013) 2020; 103: 38–40.

[2] DE KOUCHKOVSKY I, ABDUL-HAY M. 'Acute myeloid leukemia: a comprehensive review and 2016 update'. Blood Cancer J 2016; 6: e441. https://doi.org/10.1038/bcj.2016.50

[3] WEBSTER JA, PRATZ KW. Acute myeloid leukemia in the elderly: therapeutic options and choice. Leuk Lymphoma 2018; 59: 274–287. https://doi.org/10.1080/10428194.2017.1330956

[4] AU C, HO D, IP B, WAN T, NG M et al. Rapid detection of chromosomal translocation and precise breakpoint characterization in acute myeloid leukemia by nanopore long-read sequencing. Cancer Genet 2019; 239: 22–25. https://doi.org/10.1016/j.cancergen.2019.08.005

[5] SILL H, OLIPITZ W, ZEBISCH A, SCHULZ E, WOLFLER A. Therapy-related myeloid neoplasms: Pathobiology and clinical characteristics. Br J Pharmacol 2010; 162: 792–805. https://doi.org/10.1111/j.1476-5381.2010.01100.x

[6] BULLINGER L, D HNER K, DOHNER H. Genomics of Acute Myeloid Leukemia Diagnosis and Pathways. J Clin Oncol 2017; 35: 934–946. DOI: 10.1200/JCO.2016.71.2208

[7] WEI R, CAO J, YAO S. Matrine promotes liver cancer cell apoptosis by inhibiting mitophagy and PINK1/Parkin pathways. Cell Stress Chaperones 2018; 23: 1295–1309. https://doi.org/10.1007/s12192-018-0937-7

[8] LIU Y, XU Y, JI W, LI X, SUN B et al. Anti-tumor activities of matrine and oxymatrine: literature review. Tumor Biol 2014; 35: 5111–5119. DOI: 10.1007/s13277-014-1680-z

[9] ZHANG S, ZHANG Y, ZHUANG Y, WANG J, YE J et al. Matrine induces apoptosis in human acute myeloid leukemia cells via the mitochondrial pathway and Akt inactivation. PLoS One 2012; 7: e46853. https://doi.org/10.1371/journal.pone.0046853

[10] HAO Y, ZHANG N, WEI N, YIN H, ZHANG Y et al. Matrine induces apoptosis in acute myeloid leukemia cells by inhibiting the PI3K/Akt/mTOR signaling pathway. Oncol Lett 2019; 18: 2891–2896. https://doi.org/10.3892/ol.2019.10649

[11] WU J, HU G, DONG Y, MA R, YU Z et al. Matrine induces Akt/mTOR signalling inhibition-mediated autophagy and apoptosis in acute myeloid leukaemia cells. J Cell Mol Med 2017; 21: 1171–1181. https://doi.org/10.1111/jcmm.13049

[12] YAMAMURA S, IMAI-SUMIDA M, TANAKA Y, DAHIYA R. Interaction and cross-talk between non-coding RNAs. Cell Mol Life Sci 2018; 75: 467–484. https://doi.org/10.1007/s00018-017-2626-6

[13] LI Y, SHAN G, TENG Z-Q, WINGO TS. Editorial: Non-Coding RNAs and Human Diseases. Front Genet 2020; 11: 523–523. https://doi.org/10.3389/fgene.2020.00523

[14] TSAGAKIS I, DOUKA K, BIRDS I, ASPDEN JL. Long non-coding RNAs in development and disease: conservation to mechanisms. J Pathol 2020; 250: 480–495. https://doi.org/https://doi.org/10.1002/path.5405

[15] WURM AA, PINA C. Long Non-coding RNAs as Functional and Structural Chromatin Modulators in Acute Myeloid Leukemia. Front Oncol 2019; 9: 899–899. https://doi.org/10.3389/fonc.2019.00899

[16] CHEN L, WANG W, CAO L, LI Z, WANG X. Long Non-Coding RNA CCAT1 Acts as a Competing Endogenous RNA to Regulate Cell Growth and Differentiation in Acute Myeloid Leukemia. Mol Cells 2016; 39: 330–336. https://doi.org/10.14348/molcells.2016.2308

[17] GUO H, WU L, ZHAO P, FENG A. Overexpression of long non-coding RNA zinc finger antisense 1 in acute myeloid leukemia cell lines influences cell growth and apoptosis. Exp Ther Med 2017; 14: 647–651. https://doi.org/10.3892/etm.2017.4535

[18] ZEBISCH A, HATZL S, PICHLER M, W LFLER A, SILL H. Therapeutic Resistance in Acute Myeloid Leukemia: The Role of Non-Coding RNAs. Int J Mol Sci 2016; 17: 2080. https://doi.org/10.3390/ijms17122080

[19] LIU Y, LIU L, ZHANG J. Protective role of matrine in sepsis-associated cardiac dysfunction through regulating the lncRNA PTENP1/miR-106b-5p axis. Biomed Pharmacother 2021; 134: 111112. https://doi.org/10.1016/j.biopha.2020.111112

[20] FAN X, SUN Y, GUO X, HE C, HAN B et al. Long non-coding RNA LINC01116 regulated miR-744-5p/SCN1B axis to exacerbate lung squamous cell carcinoma. Cancer Biomark 2020; 28: 1–10. https://doi.org/10.3233/CBM-190945

[21] SU X, ZHANG J, LUO X, YANG W, LIU Y et al. LncRNA LINC01116 Promotes Cancer Cell Proliferation, Migration And Invasion In Gastric Cancer By Positively Interacting With lncRNA CASC11. Onco Targets Ther 2019; Volume 12: 8117–8123. https://doi.org/10.2147/OTT.S208133

[22] WANG T, CAO L, DONG X, WU F, DE W et al. LINC01116 promotes tumor proliferation and neutrophil recruitment via DDX5-mediated regulation of IL-1β in glioma cell. Cell Death Dis 2020; 11: 302. https://doi.org/10.1038/s41419-020-2506-0

[23] XU Y, LI K, WANG SB, YANG SG. MiR-592 functions as a tumor suppressor in acute myeloid leukemia by targeting ROCK1 and predicts patients' prognosis. Eur Rev Med Pharmacol Sci 2019; 23: 1610–1619. https://doi.org/10.26355/eurrev_201902_17120

[24] LIU ZM, YANG XL, JIANG F, PAN YC, ZHANG L. Matrine involves in the progression of gastric cancer through inhibiting miR-93-5p and upregulating the expression of target gene AHNAK. J Cell Biochem 2020; 121: 2467–2477. https://doi.org/10.1002/jcb.29469

[25] KSHEERASAGAR RL, KALIWAL BB. Temporal effects of mancozeb on testes, accessory reproductive organs and biochemical constituents in albino mice. Environ Toxicol Pharmacol 2003; 15: 9–17. https://doi.org/10.1016/j.etap.2003.08.006

[26] SANCHEZ-CORREA B, BERGUA JM, CAMPOS C, GAYOSO I, ARCOS MJ et al. Cytokine profiles in acute myeloid leukemia patients at diagnosis: survival is inversely correlated with IL-6 and directly correlated with IL-10 levels. Cytokine 2013; 61: 885–891. https://doi.org/10.1016/j.cyto.2012.12.023

[27] FANG YN, HUANG ZL, LI H, TAN WB, ZHANG QG et al. LINC01116 promotes the progression of epithelial ovarian cancer via regulating cell apoptosis. Eur Rev Med Pharmacol Sci 2018; 22: 5127–5133. https://doi.org/10.26355/eurrev_201808_15707

[28] CHEN J, YUAN ZH, HOU XH, SHI MH, JIANG R. LINC01116 promotes the proliferation and inhibits the apoptosis of gastric cancer cells. Eur Rev Med Pharmacol Sci 2020; 24: 1807–1814. https://doi.org/10.26355/eurrev_202002_20358

[29] MA L, ZHU Z, JIANG L, SUN X, LU X et al. Matrine suppresses cell growth of human chronic myeloid leukemia cells via its inhibition of the interleukin-6/Janus activated kinase/signal transducer and activator of transcription 3 signaling cohort. Leuk Lymphoma 2015; 56: 2923–2930. https://doi.org/10.3109/10428194.2015.1007507

[30] GUERRA AR, DUARTE MF, DUARTE IF. Targeting Tumor Metabolism with Plant-Derived Natural Products: Emerging Trends in Cancer Therapy. J Agric Food Chem 2018; 66: 10663–10685. https://doi.org/10.1021/acs.jafc.8b04104

[31] DHOLWANI K, SALUJA AK, GUPTA DA, SHAH DR. A review on plant-derived natural products and their analogs with anti-tumor activity. Indian J Pharmacol 2008; 40: 49–58. https://doi.org/10.4103/0253-7613.41038

[32] GALLEGO A, MALIK S, YOUSEFZADI M, MAKHZOUM A, TREMOUILLAUX-GUILLER J et al. Taxol from Corylus avellana: paving the way for a new source of this anti-cancer drug. Plant Cell, Tissue and Organ Culture (PCTOC) 2017; 129: 1–16. https://doi.org/10.1007/s11240-016-1164-5

[33] LIU J, LU W, LIU S, WANG Y, LI S et al. ZFP36L2, a novel AML1 target gene, induces AML cells apoptosis and inhibits cell proliferation. Leuk Res 2018; 68. https://doi.org/10.1016/j.leukres.2018.02.017

[34] GONZALEZ A, BLANCO F. Human PCNA Structure, Function and Interactions. Biomolecules 2020; 10: 570. https://doi.org/10.3390/biom10040570

[35] XU G, ZHANG W, WANG Z, CHEN M, SHI B. Matrine regulates H2O2-induced oxidative stress through long non-coding RNA HOTAIR/miR-106b-5p axis via AKT and STAT3 pathways. Biosci Rep 2020; 40. https://doi.org/10.1042/bsr20192560

[36] HAMMOND SM. An overview of microRNAs. Adv Drug Deliv Rev 2015; 87: 3–14. https://doi.org/10.1016/j.addr.2015.05.001

[37] HOU W, ZHANG H, BAI X, LIU X, YU Y et al. Suppressive role of miR-592 in breast cancer by repressing TGF-β2. Oncol Rep 2017; 38: 3447–3454. https://doi.org/10.3892/or.2017.6029

[38] JOHNSON DE, O'KEEFE RA, GRANDIS JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol 2018; 15: 234–248. https://doi.org/10.1038/nrclinonc.2018.8

[39] SENGA T, IWAMOTO T, KITAMURA T, MIYAKE Y, HAMAGUCHI M. JAK/STAT3-dependent activation of the RalGDS/Ral pathway in M1 mouse myeloid leukemia cells. J Biol Chem 2001; 276: 32678–32681. https://doi.org/10.1074/jbc.M105749200

[40] HAN Y, YE A, BI L, WU J, YU K et al. Th17 cells and interleukin-17 increase with poor prognosis in patients with acute myeloid leukemia. Cancer Sci 2014; 105: 933–942. https://doi.org/10.1111/cas.12459

[41] MA L, ZHU Z, JIANG L, SUN X, LU X et al. Matrine suppresses cell growth of human chronic myeloid leukemia cells via its inhibition of the IL-6/JAK/STAT3 signaling cohort. Leuk Lymphoma 2015; 56: 1–25. https://doi.org/10.3109/10428194.2015.1007507

[42] ZHANG B, YU L, HAN N, HU Z, WANG S et al. LINC01116 targets miR-520a-3p and affects IL6R to promote the proliferation and migration of osteosarcoma cells through the Jak-stat signaling pathway. Biomed Pharmacother 2018; 107: 270–282. https://doi.org/https://doi.org/10.1016/j.biopha.2018.07.119

[43] CHEN M, DING Y, TONG Z. Efficacy and Safety of Sophora flavescens (Kushen) Based Traditional Chinese Medicine in the Treatment of Ulcerative Colitis: Clinical Evidence and Potential Mechanisms. Front Pharmacol 2020; 11: 603476. https://doi.org/10.3389/fphar.2020.603476

[44] RONG B, ZHAO C, GAO W, YANG S. Matrine promotes the efficacy and safety of platinum-based doublet chemotherapy for advanced non-small cell lung cancer. Int J Clin Exp Med 2015; 8: 14701–14717.