MicroRNA-325 inhibits the proliferation and induces the apoptosis of T cell acute lymphoblastic leukemia cells in a BAG2-dependent manner

FENGYU WANG[1], FENGLI WANG[2], SHENGYU ZHANG[3] and XIAOGANG XU[4]

Departments of [1]Pediatrics, [2]Radiology and [3]Rehabilitation, Zibo Central Hospital, Zibo, Shandong 255036; [4]Department of Oncology, Chongqing University Three Gorges Hospital, Chongqing 404100, P.R. China

Received September 3, 2020; Accepted January 18, 2021

DOI: 10.3892/etm.2021.10063

Abstract. The inhibitory effect of microRNA (miR)-325 in multiple different types of cancer cell has been identified; however, its biological function in T cell acute lymphoblastic leukemia (T-ALL) remains unknown. Moreover, Bcl-2-associated athanogene (BAG)2 is highly expressed in a various types of tumors and is regarded as an anti-apoptotic gene. In the present study, the roles of miR-325 and BAG2 in a T-ALL cell line (Jurkat cells) were investigated. BAG2 and miR-325 expression levels in clinical blood samples from healthy donors and pediatric patients with T-ALL, as well as in T-ALL cell lines was detected using western blot analysis and/or reverse transcription-quantitative PCR. Dual-luciferase reporter gene assays and TargetScan were used to evaluate the interaction between BAG2 and miR-325. Small interfering RNA technology was applied to knockdown BAG2 expression in Jurkat cells. The effects of miR-325 mimic and BAG2 downregulation on the proliferation and apoptosis were assessed by an MTT assay, flow cytometry and western blot analysis. The results revealed that the expression of miR-325 was downregulated in blood samples from pediatric patients and in T-ALL cell lines, and its expression was lowest in Jurkat cells. The expression levels of BAG2 exhibited the opposite results. The knockdown of BAG2 markedly induced the apoptosis and inhibited the proliferation of Jurkat cells. In addition, the overexpression of miR-325 significantly inhibited the growth and promoted the apoptosis of Jurkat cells, with these effects being eliminated by BAG2 overexpression. In conclusion, the findings of the present study demonstrated that miR-325 directly targets the BAG2 gene and that the introduction of miR-325 can accelerate apoptosis and suppress the proliferation of Jurkat cells by silencing BAG2 expression.

Correspondence to: Dr Xiaogang Xu, Department of Oncology, Chongqing University Three Gorges Hospital, 165 Xincheng Street, Wanzhou, Chongqing 404100, P.R. China
E-mail: kf47342@163.com

Key words: microRNA-325, Bcl-2-associated athanogene 2, proliferation, apoptosis, T cell acute lymphoblastic leukemia

Introduction

T cell acute lymphoblastic leukemia (T-ALL) is a malignant hematological disease involving the infinite expansion of defective naive T cells (1,2). Children diagnosed with T-ALL account for ~15% of all cases of ALL (3). Currently, chemoradiotherapy and hematopoietic stem cell transplantation (HSCT) are the main therapeutic regimens used for ALL (1,4). With regards to the use of chemoradiotherapy, drug resistance and the tolerance of patients to the drugs used in the later stages of treatment are the main drawbacks for its use (5). On the other hand, HSCT, is associated with high costs and severe complications, such as graft-versus-host disease, which greatly limits its use and effectiveness (6-8). Even in cases in which initial treatment may seem effective, relapses can often occur unexpectedly (9). As such, effective treatment regimens for T-ALL are urgently required, particularly for children who are diagnosed with early T cell progenitor ALL (10). Currently, the development of novel strategies which can overcome the current obstacles is a major challenge to effectively treat the disease (11).

MicroRNAs (miRNAs/miRs), as non-coding RNAs, play an important role in regulating mRNA expression (12,13). Researchers have found that miRNAs are involved in various cellular processes, such as cell cycle progression and determining cell fate, through affecting proliferation, differentiation, metabolism and apoptosis (14). Consequently, miRNAs are regarded as targets for cancer therapeutic intervention, rendering them promising candidates (15,16). The focus of research on miRNAs is increasing. Previous studies have demonstrated that miR-325 can potently inhibit cell growth, such as that of hepatocellular carcinoma and non-small cell lung cancer (NSCLC), by targeting high mobility group box1 and aquaporin 5 (17-19).

Bcl-2-associated athanogene (BAG)2 is an anti-apoptotic oncogene, which plays a pivotal role in various diseases, such as numerous types of cancer, Alzheimer's disease, Parkinson's disease and spinocerebellar ataxia type-3 (20). Previous studies have demonstrated that BAG2 is highly expressed in a number of tumor types, such as multiple myeloma, colorectal cancer, ovarian cancer and lung cancer (21-24). However, the roles of miR-325 and BAG2 in T-ALL, as well as the interaction between the two, remain to be determined. As such, the present study aimed to investigate the roles of miR-325 and

BAG2 in a T-ALL cell line (Jurkat cells) and to further explore the underlying mechanisms of action.

Materials and methods

Cell lines and clinical samples. In the present study, human T-ALL cell lines, including TALL-1, KOPTK1, Jurkat, CCRF-CEM and Molt16, were purchased from the American Type Culture Collection (ATCC). The cells mentioned above were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, (NanJing SunShine Biotechnology Co., Ltd.) and 100 μg/ml streptomycin (Sunshine Biotechnology, Nanjing, China). The cells were maintained in a humidified atmosphere with 5% CO_2 at 37˚C.

Clinical samples were obtained from Zibo Central Hospital (Zibo, China) from February 2018 to April 2019. Blood samples were obtained from 20 pediatric patients (age range, 3.6-14 years; 10 males, 10 females) who were diagnosed with T-ALL and 20 healthy donors (age range, 3-15.2 years; 10 males, 10 females). All fresh blood samples were immediately separated into several portions, snap-frozen in liquid nitrogen and stored at -80˚C prior to protein and RNA extraction. The present study was approved by the Ethical Review Committee of Zibo Central Hospital. All participants and their legal guardians agreed to the use of their samples in the present study and written informed consents were obtained from all of the legal guardians of all participants.

Transient transfection. 0.2 μM BAG2-specific small interfering RNA (BAG2-siRNA; 5'-GGGAAGAACUCUCACCGUUTT-3'; Santa Cruz Biotechnology, Inc.), 0.2 μM control-siRNA (5'-UUCUCCGAACGUGUCACGUTT-3'; Santa Cruz Biotechnology, Inc.), 1 μg control-plasmid (cat. no. sc-437275; Santa Cruz Biotechnology, Inc.), 1 μg BAG2-plasmid (cat. no. sc-404540-ACT; Santa Cruz Biotechnology, Inc.), 100 nM mimic control (Shanghai GenePharma Co., Ltd.) and 100 nM miR-325 mimic (Shanghai GenePharma Co., Ltd.) were transfected into the Jurkat cell line, separately, using Lipofectamine® 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol at 37˚C. Immediately following 48 h of transfection, the cells were collected for protein and RNA extraction.

Bioinformatics analysis. Bioinformatics analysis software TargetScan version 7.2 (http://www.targetscan.org/vert_72/) was used to predict the potential targets of miR-325.

Luciferase reporter assay. The pGL-3 plasmid vector (Promega Corporation) was employed in the luciferase reporter assay, which contained the wild-type (BAG2-WT) and mutant-type BAG2-mutant, containing mutations in the binding region of miR-325 with the BAG2 gene. The BAG2 gene was designed to contain the predicted miR-325 binding site. For the luciferase assay, 293T cells were cultured in a 24-well plate at a density of $5x10^4$ cells/well overnight at 37˚C prior to transfection. The cells were co-cultured with the pGL-3 plasmid vectors and miR-325 mimic using a Lipofectamine® 3000 reagent kit at 37˚C for 48 h. After 48 h of transfection, the luciferase activity was measured using the Dual-luciferase Reporter Assay system (Promega Corporation) according to the manufacturer's protocol, and the results were normalized to *Renilla* luciferase activity.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). To further confirm the expression levels of miR-325 and BAG2, RT-qPCR was performed. Total RNA was extracted from the cell lines using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and RNA was extracted from clinical samples using the miRNeasy Mini kit (Qiagen, Inc.), according to the manufacturer's instructions. A NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, Inc.) was applied to quantify the extracted RNA. RNA was reverse transcribed into cDNA using Superscript III Reverse Transcriptase (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The reaction conditions for RT were as follows: 70˚C for 5 min, 37˚C for 5 min and 42˚C for 60 min. The quantitative expression of each gene was determined using SYBR-Green I (Thermo Fisher Scientific, Inc.). Thermocycling conditions used for the qPCR were as follows: Initial denaturation at 95˚C for 5 min; followed by 38 cycles of 15 sec at 95˚C, 1 min at 60˚C and 30 sec at 72˚C; and a final extension for 10 min at 72˚C. The primers were synthesized and purified by Sangon Biotech (Shanghai) Co., Ltd. Primer sequences were listed as following: miR-325 forward, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACACUUAC-3' and reverse, 5'-ACACTCCAGCTGGGCCUAGUAGGUGUCCAGU-3'; BAG2 forward, 5'-CTTTGAGAGAAGCAGCAACTG-3' and reverse, 5'-TGACACTTCAACGGTGAGAG-3'; U6 forward, 5'-ATACAGAGAAAGTTAGCACGG-3' and reverse, 5'-GGAATGCTTCAAAGAGTTGTG-3'; GAPDH forward, 5'-TTTGGTATCGTGGAAGGACTC-3' and reverse, 5'-GTAGAGGCAGGGATGATGTTCT-3'. GAPDH (for mRNA) and U6 (for miRNA) were used as the internal controls. Gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (25).

Western blot analysis. Following transfection, the Jurkat cells were harvested and lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology) containing protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA). The clinical samples were lysed using a Total Protein Extraction kit (Beijing Solarbio Science & Technology Co., Ltd.). The protein concentration was then determined using a BCA kit (Beyotime Institute of Biotechnology). Individual samples (15 μg/lane) were separated by 12% SDS-PAGE and transferred electrophoretically onto PVDF membranes (EMD Millipore). The membranes were blocked using 5% non-fat milk contained in TBST (0.1% Tween 20) for 1 h at room temperature, followed by incubation with primary antibodies against BAG2 (cat. no. ab79406; Abcam; working dilution, 1:1,000), Bcl-2 (cat. no. ab182858; Abcam; working dilution, 1:1,000), Bax (cat. no. ab32503; Abcam; working dilution, 1:1,000) and GAPDH (cat. no. ab9485; Abcam; working dilution, 1:1,000) overnight at 4˚C. The following day, the membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (cat. no. 7074; Cell Signaling Technology, Inc.; working dilution, 1:2,000) at room temperature for 1 h. To observe the protein bands, an ECL kit (Amersham Pharmacia Biotech) was used for staining and the membranes were then photographed immediately. Band densities were quantified using Gel-Pro Analyzer densitometry software (version 6.3; Media Cybernetics, Inc.).

In vitro proliferation assay. To assess the effects of BAG2-siRNA and miR-325 on proliferation *in vitro*, a standard

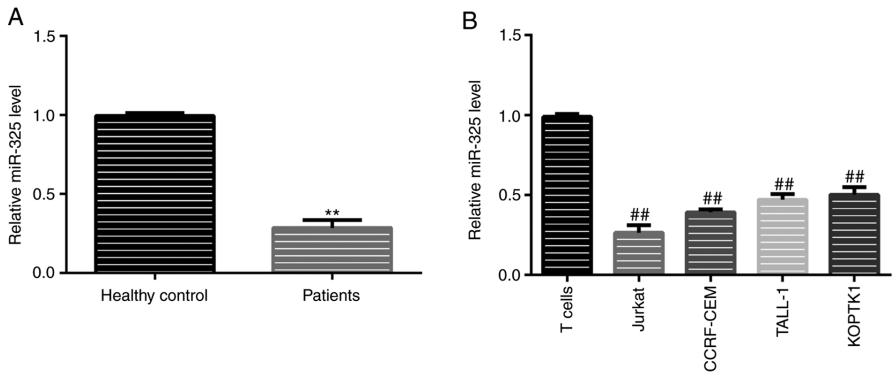

Figure 1. Expression of miR-325 in clinical samples and T-ALL cell lines. Expression of miR-325 in blood samples of patients and healthy donors was detected using RT-qPCR (n=20). (B) Expression of miR-325 in T-ALL cell lines was detected using RT-qPCR. **P<0.01 vs. healthy controls; ##P<0.01 vs. T cells. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; T-ALL, T cell acute lymphoblastic leukemia.

MTT assay was performed. Briefly, the Jurkat cells were seeded in 96-well plates at a density of 4,000 cells/well in complete medium, as described above, and cultured overnight. The following day, the cells were transfected with control-siRNA, BAG2-specific siRNA, control-plasmid, mimic control, miR-325 mimic, miR-325 mimic + control-plasmid, or miR-325 mimic + BAG2-plasmid in fresh medium at 37˚C for 48 h following the same conditions as aforementioned. The Jurkat cells were separately centrifuged and examined at 0, 12, 24 and 48 h immediately following the end of the transfection. A total of 150 μl DMSO (Sigma-Aldrich; Thermo Fisher Scientific, Inc.) was added to dissolve the purple formazan. Untreated cells were used as controls and regarded to have 100% viability. The data were measured using a BioTek microplate reader (BioTek Instruments, Inc.) at the absorbance of 570 nm.

Flow cytometry (FCM) for apoptosis. To verify the effects on apoptosis *in vitro*, Jurkat cells were harvested following transfection at the concentration of 1x10^6 cells/tube. The cells were then washed, pelleted and stained with Annexin V-FITC (Beyotime Institute of Biotechnology) and PI on ice in the dark for 15 min. The samples were then analyzed using a flow cytometer (FACSCalibur; BD Biosciences) and quadrants 2 and 3 (Q2 + Q3) were used for calculating the extent of apoptosis. FlowJo software (version 7.6.1; FlowJo LLC) was applied to analyze the data.

Statistical analysis. Unless otherwise stated, the data in the present study are expressed as the means ± SD, with n=3 or more replicates. GraphPad Prism 6.0 (GraphPad Software) was used for statistical analysis. The statistical significance of differences between groups was determined using unpaired Student's t-tests or one-way ANOVAs followed by Tukey's post hoc tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miR-325 in clinical samples and human T-ALL cell lines. Previous studies have demonstrated that miR-325 can suppress cancer cell proliferation and accelerate apoptosis (17-19); however, to the best of our knowledge, there are no previous studies investigating the role of miR-325 in T-AL. In the present study, miR-325 expression levels in clinical samples from healthy donors and pediatric patients diagnosed with T-ALL, as well as in T-ALL cell lines (Jurkat, CCRF-CEM, TALL-1 and KOPTK1) were detected using RT-qPCR. The results revealed that, compared with the healthy samples, the levels of miR-325 in the patient samples were significantly lower (Fig. 1A). A similar trend was observed in the cell lines, with the Jurkat cells exhibiting the lowest expression levels of miR-325 (Fig. 1B). For this reason, Jurkat cells were selected for use in further *in vitro* experiments.

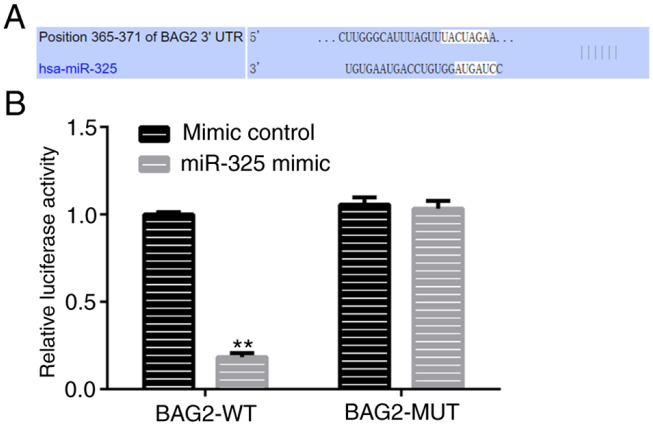

Figure 2. miR-325 is directly targeted by the BAG2 gene. (A) Prediction of the binding site between miR-325 and BAG2 using TargetScan software. (B) Interaction between miR-325 and BAG2 assessed by a luciferase report gene assay. **P<0.01 vs. mimic control. BAG2, Bcl-2-associated athanogene; miR, microRNA; MUT, mutant; UTR, untranslated region; WT, wild-type.

Verification of the interaction between miR-325 and BAG2. To date, the experimental approach available for the verification of miRNAs and their target gene include the dual-luciferase report assay, which can detect the interaction between miRNAs and their corresponding target genes (26). In the present study, TargetScan, which is a popular miRNA target predication algorithm, was used to identify the target of miR-325. The results revealed that miR-325 shared a binding site with BAG2 (Fig. 2A). In addition, a dual-luciferase report assay was performed and the results further verified the interaction between miR-325 and BAG2 (Fig. 2B). In view of this hypothesis, it was confirmed that BAG2 was a direct target gene of miR-325.

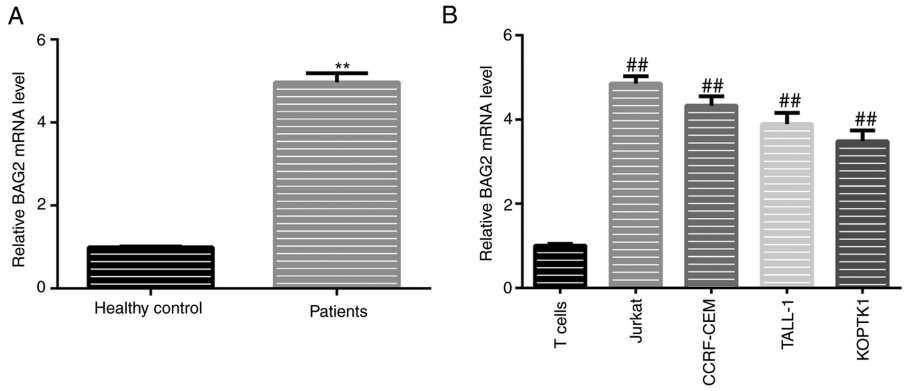

Figure 3. Expression of BAG2 in clinical samples and T-ALL cell lines. (A) Expression of BAG2 mRNA in the blood samples of patients and healthy donors was detected using RT-qPCR (n=20). (B) Expression of BAG2 mRNA in T-ALL cell lines was detected using RT-qPCR. **P<0.01 vs. healthy controls; $^{\#\#}$P<0.01 vs. T cells. BAG2, Bcl-2-associated athanogene; RT-qPCR, reverse transcription-quantitative PCR; T-ALL, T cell acute lymphoblastic leukemia.

Expression of BAG2 in clinical samples and human T-ALL cell lines. As it was found that BAG2 was a target of miR-325, as mentioned above, it was hypothesized that the expression levels of BAG2 in patient samples and T-ALL cell lines would exhibit an opposite trend to that of miR-325. To verify this hypothesis, the mRNA expression levels of BAG2 in patient samples and T-ALL cell lines was detected using RT-qPCR. The results revealed that the expression of BAG2 in patients diagnosed with T-ALL was significantly higher compared with the healthy donors (Fig. 3A). It was also found the trend for the expression levels of miR-325 in T-ALL cell lines was in accordance with that in patient samples, with significantly higher levels in all T-ALL cell lines (Fig. 3B). Moreover, the BAG2 expression levels were highest in the Jurkat cells compared with all other T-ALL cell lines (Fig. 3B).

BAG2 knockdown inhibits the proliferation and promotes the apoptosis of Jurkat cells. To explore the role of BAG2 in cell proliferation and apoptosis, siRNA technology was applied in the present study. BAG2-specific siRNA and control-siRNA were generated and co-cultured with Jurkat cells for 48 h, respectively. The RT-qPCR and western blotting data revealed that the expression of BAG2 in Jurkat cells was notably decreased following transfection with BAG2-siRNA, compared with the control-siRNA transfected group, demonstrating a successful transfection (Fig. 4A and B). Subsequently, the biological behaviors, namely the proliferation and apoptosis of Jurkat cells, following BAG2 knockdown were assessed. Firstly, an MTT assay was performed at 0, 24, 48 and 72 h. The analysis revealed that the knockdown of BAG2 markedly inhibited the proliferation of Jurkat cells (Fig. 4C). Secondly, using Annexin V-FITC/PI double staining, the cells were analyzed using FCM. It was found that the percentage of apoptotic cells was markedly increased in the group transfected with BAG2-siRNA (Fig. 4D and E). Finally, the expression levels of the apoptosis-related proteins, Bcl-2 and Bax, were examined using western blot analysis. The data demonstrated that the protein expression levels of Bcl-2 were decreased and the Bax protein expression levels were increased (Fig. 4F), with the ratio of Bcl-2/Bax being significantly decreased (Fig. 4G) compared with the control-siRNA group. Taken together, these data showed that BAG2 is a key target which can directly influence the cell proliferation and apoptosis of Jurkat cells.

Overexpression of miR-325 can partially inhibit cell proliferation and induce apoptosis by downregulating the expression of BAG2. Based on the previous results, it was hypothesized that miR-325 might protect Jurkat cells from proliferation and accelerate apoptosis in a BAG2-dependent manner. Then, to determine whether the overexpression of miR-325 can protect Jurkat cells from proliferation and accelerate apoptosis in a BAG2-dependent manner, Jurkat cells were first transfected with various plasmids, miRNA mimics or a combination of these for 48 h. RT-qPCR and western blot analyses were carried out to identify the transfection efficiency. It was found that, compared with the control group, the expression levels of BAG2 and miR-325 were significantly increased following transfection with BAG2-plasmid and miR-325 mimic alone (Fig. 5A-C), demonstrating the successful transfections. It was also found that the expression levels of BAG2 were notably decreased in the group transfected with miR-325 mimic (Fig. 5D and E), as hypothesized.

The analysis revealed that the overexpression of miR-325 reduced the expression levels of BAG2 in Jurkat cells and the downregulatory effect was reversed by the introduction of the BAG2-plasmid (Fig. 5D and E). To better understand the biological effects of miR-325 mimic on cell proliferation and apoptosis following knocking down of the expression levels of BAG2, an MTT assay, western blot analysis and FCM were performed. In the group transfected with the miR-325 mimic alone, the cell proliferation of the Jurkat cells was inhibited (Fig. 6A) and the percentage of apoptotic cells was significantly increased (Fig. 6B and C). In addition, the effect on the protein expression levels of Bcl-2 and Bax, was investigated. A decreased Bcl-2 expression and an increased Bax expression were observed (Fig. 6D and E). Moreover, the data also demonstrated that the overexpression of BAG2 reversed the biological behaviors caused by miR-325 mimic transfection. These results demonstrated that expression of BAG2 was necessary for the induction of apoptosis of Jurkat cells by miR-325.

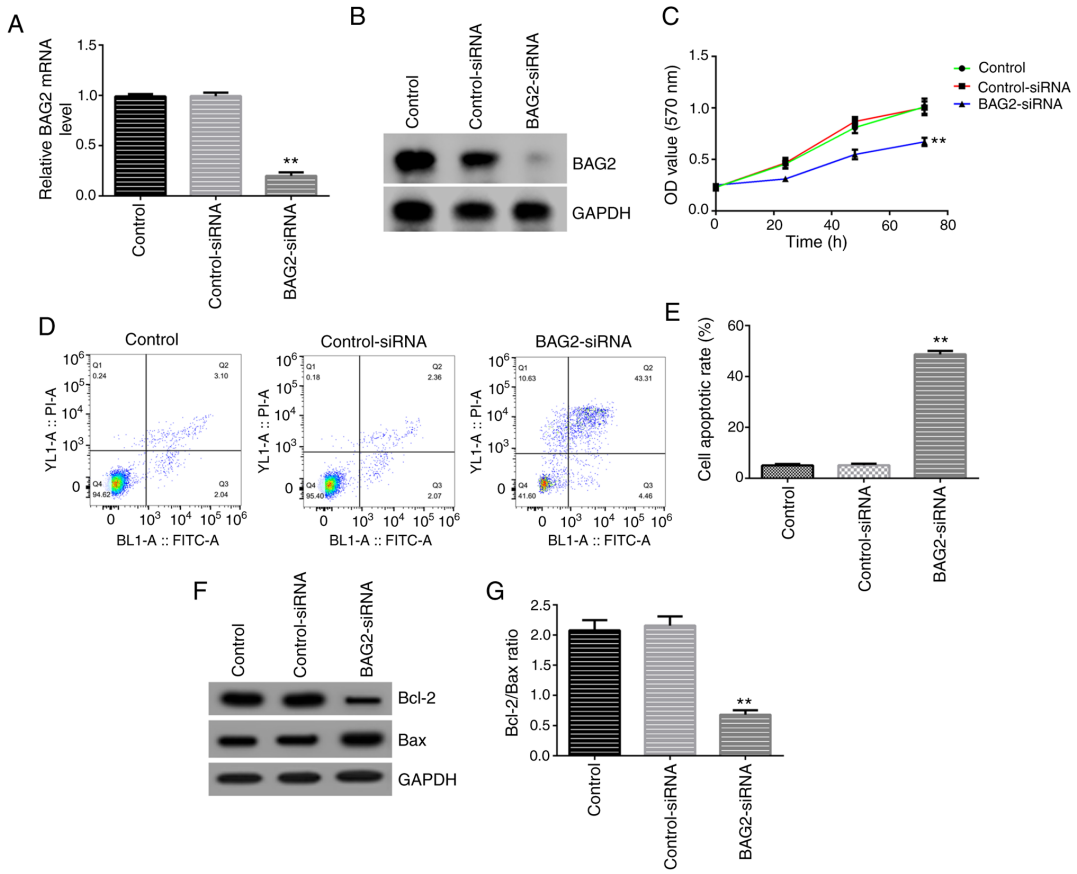

Figure 4. Biological functions of BAG2 in Jurkat cells. Transfection efficiency of BAG2-siRNA, detected using (A) reverse transcription-quantitative PCR and (B) western blotting. Effect of BAG2 downregulation on (C) cell proliferation was determined using MTT assays. (D) Apoptosis was determined and (E) quantified using flow cytometry. (F) The protein expression levels of Bcl-2 and Bax proteins were determined using and western blot analysis. (G) The ratio of Bcl-2/Bax was calculated. **P<0.01 vs. control-siRNA. BAG2, Bcl-2-associated athanogene; OD, optical density; siRNA, small interfering RNA.

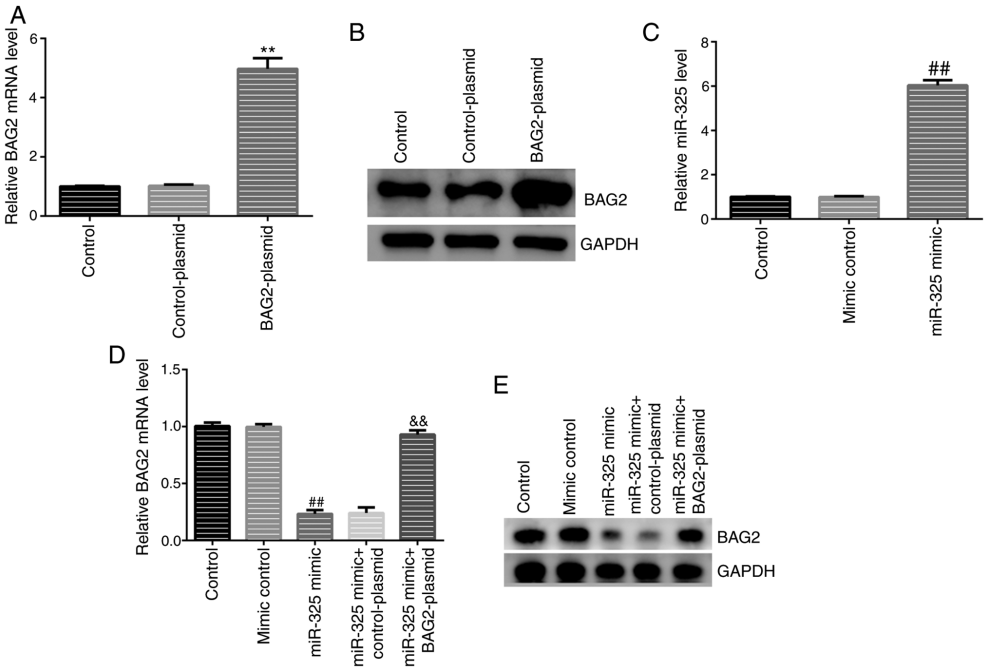

Figure 5. miR-325 negatively regulates BAG2 expression in Jurkat cells. The expression level of (A) BAG2 mRNA and (B) BAG2 protein in Jurkat cells transfected with BAG2-plasmid was determined using RT-qPCR and western blot analyses, respectively. (C) miR-325 mimic increased the expression levels of miR-325 in Jurkat cells transfected with miR-325 mimic, as determined using RT-qPCR. The (D) mRNA and (E) protein expression levels of BAG2 in Jurkat cells transfected with mimic control, miR-325 mimic, miR-325 mimic + control-plasmid or miR-325 mimic + BAG2-plasmid was determined using RT-qPCR and western blot analyses, respectively. **P<0.01 vs. control-plasmid; $^{\#\#}$P<0.01 vs. mimic control; $^{\&\&}$P<0.01 vs. miR-325 mimic + control-plasmid. BAG2, Bcl-2-associated athanogene; miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR.

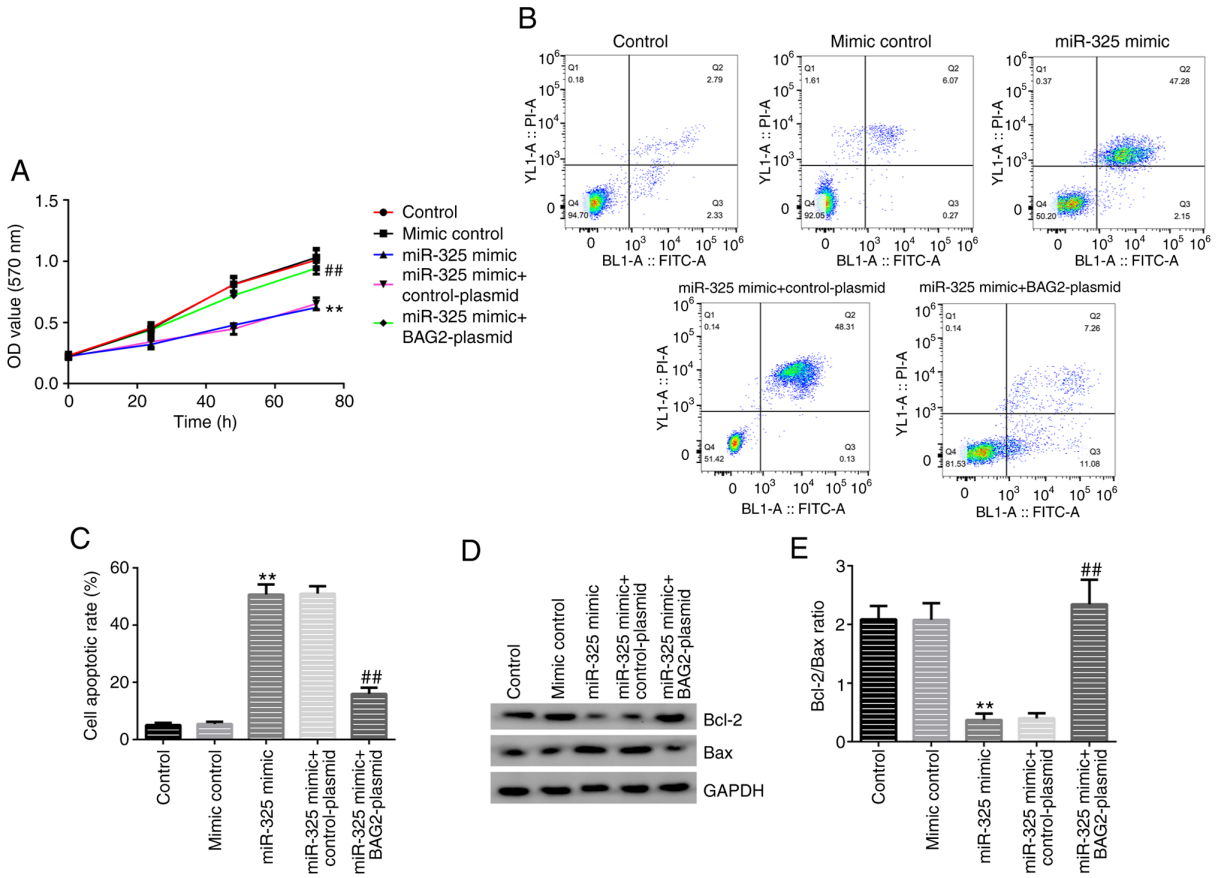

Figure 6. Biological functions of miR-325 in Jurkat cells. Jurkat cells were transfected with mimic control, miR-325 mimic, miR-325 mimic + control-plasmid or miR-325 mimic + BAG2-plasmid for 48 h. Subsequently, (A) cell proliferation, (B and C) cell apoptosis and (D) the protein expression levels of Bcl-2 and Bax were determined using an MTT assay, flow cytometry and western blot analysis, respectively. (E) The ratio of Bcl-2 to Bax was calculated and presented. **P<0.01 vs. mimic control; $^{\#\#}$P<0.01 vs. miR-325 mimic + control-plasmid. BAG2, Bcl-2-associated athanogene; miR, microRNA; OD, optical density.

Discussion

It has previously been demonstrated that BAG2 plays a crucial role in the progression of tumor cell growth (27). BAG2, as an anti-apoptotic gene, can promote cell proliferation, inhibit cell apoptosis and arrest the cell cycle, and a raised BAG2 expression is found in a number of tumor types, such as thyroid cancer (28) and breast cancer (22). Ge *et al* (29) found that upregulation of BAG2 might be associated with underlying TNF-related apoptosis-inducing ligand (TRAIL)-resistance mechanisms in NSCLC. However, its biological function in T-ALL is not yet well understood.

In the present study, preliminary analysis with RT-qPCR and western blot analysis suggested that the mRNA and protein expression levels of BAG2 were higher in the blood samples of patients diagnosed with T-ALL and in T-ALL cell lines compared with those in healthy donor samples. To discover the role of BAG2 on proliferation and apoptosis, BAG2 expression was silenced in Jurkat cells using siRNA technology. The data displayed a significant decrease in cell expansion and an increased portion of apoptotic cells in the group transfected with BAG2-siRNA. This suggested that BAG2 indeed affects cell growth and survival.

miRNAs, as non-coding small RNAs, are regulators of gene expression at the mRNA level (28,30). An increasing number of studies have focused on oncogenic miRNAs, which determine the tumor cell fate and oncogenic miRNAs have been shown to be promising targets for therapy (31,32). T-ALL is a refractory and relapsing malignant cancer which has been the focus of numerous clinicians and studies in recent years (33,34). In recent years, the research of miRNA in T-ALL has attracted more and more attention (1). In 2004, Chen *et al* (35) identified three miRNAs that are specifically expressed in hematopoietic cells, which were dynamically regulated in the period of early hematopoiesis and lineage commitment. miR-142-3p (36), miR-155 (37), miR-146a (38) and miR-150 (39) have been reported to play roles in T-ALL. The present study found that miR-325 shared a binding site with BAG2 using TargetScan and the result was in accordance with the results of the dual-luciferase reporter assay. As previously reported, miRNAs are small RNAs which play a significant role in regulating gene expression by interfering with mRNA translation or promoting mRNA degradation (40). The results described above demonstrated the following 3 points: i) Compared with healthy donor samples, the expression of miR-325 was markedly lower and the level of BAG2 was markedly higher in patients with T-ALL and in T-ALL cell lines; ii) BAG2 knockdown can influence cell proliferation and apoptosis; and iii) miR-325 shares a binding site with BAG2. Taken together, it was hypothesized that BAG2 may be targeted by miR-325 in T-ALL. To verify this hypothesis, Jurkat cells were transfected with miR-325 mimics *in vitro*

and cell proliferation was significantly inhibited compared with the control group. This was consistent with previous studies (17-19), demonstrating that miR-325 has an inhibitory effect on the proliferation of cancer cells. Additionally, the biological effects of miR-325 on the proliferation and apoptosis of Jurkat cells were reversed by the introduction of BAG2.

The BAG family was first identified as a group of proteins that prevent cell death through their interaction with Bcl-2 (20). The Bcl-2 family genes (Bcl-2 and Bax) play critical roles in the apoptosis of T-ALL cells (41,42). In the present study, the results also demonstrated that the protein expression levels of Bcl-2 were decreased and the levels of Bax were increased simultaneously following the downregulation of the levels of BAG2. This indicated that Bcl-2/Bax may be the downstream genes of BAG2 regulated by miR-325. The antitumor biological effects may be dependent on the miR-325/BAG2/Bcl-2/Bax pathway; however, this requires verification in future studies.

In conclusion, the findings of the present study demonstrated that the miR-325/BAG2 axis may be a promising therapeutic target for the treatment of T-ALL. miR-325 inhibited the proliferation and promoted the apoptosis of T-ALL cells in a BAG2-dependent manner.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

FYW contributed to study design, data collection, statistical analysis, data interpretation and manuscript preparation. FLW and SZ contributed to data collection and statistical analysis. XX contributed to data collection, statistical analysis and manuscript preparation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethical Review Committee of Zibo Central Hospital. All participants and their legal guardians agreed to the use of their samples in the present study and written informed consents were obtained from all of the legal guardians of all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Correia NC and Barata JT: MicroRNAs and their involvement in T-ALL: A brief overview. Adv Biol Regul 74: 100650, 2019.
2. Litzow MR and Ferrando AA: How i treat T-cell acute lymphoblastic leukemia in adults? Blood 126: 833-841, 2015.
3. Hounjet J, Habets R, Schaaf MB, Hendrickx TC, Barbeau LM, Yahyanejad S, Rouschop KM, Groot AJ and Vooijs M: The anti-malarial drug chloroquine sensitizes oncogenic NOTCH1 driven human T-ALL to γ-secretase inhibition. Oncogene 38: 5457-5468, 2019.
4. Lange A, Dlubek D, Zdziarski R, Chodorowska A, Mordak-Domagala M, Klimczak A, Lange J and Jaskula E: Donor lymphocyte infusions to leukemic bone lesions are therapeutically effective in a ph^+ all patient with post-HSCT relapse. J Immunotoxico 11: 347-352, 2014.
5. Follini E, Marchesini M and Roti G: Strategies to overcome resistance mechanisms in T-cell acute lymphoblastic leukemia. Int J Mol Sci 20: 3021, 2019.
6. Ali N, Flutter B, Rodriguez RS, Paghaleh ES, Barber LD, Lombardi G and Nestle FO: Xenogeneic graft-versus-host-disease in NOD-scid IL-2rγnull mice display a T-effector memory phenotype. PLoS One 7: e44219, 2012.
7. Gill S, Tasian SK, Ruella M, Shestova O, Li Y, Porter DL, Carroll M, Desnoyers GD, Scholler J, Grupp SA, *et al*: Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. Blood 123: 2343-2354, 2014.
8. Riegel C, Boeld TJ, Doser K, Huber E, Hoffmann P and Edinger M: Efficient treatment of murine acute GvHD by in vitro expanded donor regulatory T cells. Leukemia 34: 895-908, 2020.
9. Kuhlen M, Willasch AM, Dalle JH, Wachowiak J, Yaniv I, Ifversen M, Sedlacek P, Guengoer T, Lang P, Bader P, *et al*: Outcome of relapse after allogeneic HSCT in children with ALL enrolled in the ALL-SCT 2003/2007 trial. Br J Haematol 180: 82-89, 2018.
10. Pui CH, Pei DQ, Cheng C, Tomchuck SL, Evans SN, Inaba H, Jeha S, Raimondi SC, Choi JK, Thomas Paul G and Dallas MH: Treatment response and outcome of children with T-cell acute lymphoblastic leukemia expressing the gamma-delta T-cell receptor. Oncoimmunology 8: 1599637, 2019.
11. Hefazi M and Litzow MR: Recent advances in the biology and treatment of T cell acute lymphoblastic leukemia. Curr Hematol Malig Rep 13: 265-274, 2018.
12. Drury RE, O'Connor D and Pollard AJ: The clinical application of microRNAs in infectious disease. Front Immunol 8: 1182, 2017.
13. Lin T, Zhou SM, Gao H, Li YQ and Sun LJ: MicroRNA-325 is a potential biomarker and tumor regulator in human bladder cancer. Technol Cancer Res Treat 17: 1533033818790536, 2018.
14. Yooa B, Ghosha SK, Kumar M, Moore A, Yigit MV and Medarova Z: Design of nanodrugs for miRNA targeting in tumor cells. J Biomed Nanotechnol 10: 1114-1122, 2014.
15. Ingenito F, Roscigno G, Affinito A, Nuzzo S, Scognamiglio I, Quintavalle C and Condorelli G: The Role of Exo-miRNAs in cancer: A focus on therapeutic and diagnostic applications. Int J Mol Sci 20: 4687, 2019.
16. Rupaimoole R and Slack FJ: MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 16: 203-222, 2017.
17. Li H, Huang W and Luo R: The microRNA-325 inhibits hepatocellular carcinoma progression by targeting high mobility group box 1. Diagn Pathol 10: 117, 2015.
18. Zhang Z, Han Y, Sun G, Liu X, Jia X and Yu X: MicroRNA-325-3p inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma by down-regulation of aquaporin 5. Cell Mol Biol Lett 24: 13, 2019.
19. Yao SH, Zhao TJ and Jin H: Expression of microRNA-325-3p and its potential functions by targeting HMGB1 in non-small cell lung cancer. Biomed Pharmacother 70: 72-79, 2015.

20. Qin L, Guo J, Zheng Q and Zhang H: BAG2 structure, function and involvement in disease. Cell Mol Biol Lett 21: 18, 2016.
21. Ge F, Zhang L, Tao SC, Kitazato K, Zhang ZP, Zhang XE and Bi LJ: Quantitative proteomic analysis of tumor reversion in multiple myeloma cells. J Proteome Res 10: 845-855, 2011.
22. Yang KM, Bae E, Ahn SG, Pang K, Park Y, Park J, Lee J, Ooshima A, Park B, Kim J,*et al*: Co-chaperone BAG2 determines the pro-oncogenic role of cathepsin B in triple-negative breast cancer cells. Cell Rep 21: 2952-2964, 2017.
23. Zhang XY, Hong SS, Zhang M, Cai QQ, Zhang MX and Xu CJ: Proteomic alterations of fibroblasts induced by ovarian cancer cells reveal potential cancer targets. Neoplasma 65: 104-112, 2018.
24. Yue X, Zhao Y, Liu J, Zhang C, Yu H, Wang J, Zheng T, Liu L, Li J, Feng Z and Hu W: BAG2 promotes tumorigenesis through enhancing mutant p53 protein levels and function. Elife 4: e08401, 2015.
25. 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.
26. Xia W, Cao G and Shao N: Progress in miRNA target prediction and identification. Sci China C Life Sc 52: 1123-1130, 2009.
27. Sun L, Chen G, Sun AQ Wang Z, Huang H, Gao Z, Liang W, Liu C and Li K: BAG2 promotes proliferation and metastasis of gastric cancer via ERK1/2 signaling and partially regulated by miR186. Front Onco 10: 31, 2020.
28. Selmansberger M, Feuchtinger A, Zurnadzhy L, Michna A, Kaiser JC, Abend M, Brenner A, Bogdanova T, Walch A, Unger K, *et al*: CLIP2 as radiation biomarker in papillary thyroid carcinoma. Oncogene 34: 3917-3925, 2015.
29. Ge Y, Yan D, Deng H, Chen W and An G: Novel molecular regulators of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in NSCLC cells. Clin Lab 61: 1855-1863, 2015.
30. Jamali L, Tofigh R, Tutunchi S, Panahi G, Borhani F, Akhavan S, Nourmohammadi P, Ghaderian SMH, Rasouli M and Mirzaei H: Circulating microRNAs as diagnostic and therapeutic biomarkers in gastric and esophageal cancers. J Cell Physiol 233: 8538-8550, 2018.
31. Kwan JY, Psarianos P, Bruce JP, Yip KW and Liu FF: The complexity of microRNAs in human cancer. J Radiat Res 57 (Suppl): 106-111, 2016.
32. D'Angelo B, Benedetti E, Cimini A and Giordano A: MicroRNAs: A puzzling tool in cancer diagnostics and therapy. Anticancer Res 36: 5571-5575, 2016.
33. Pehlivan KC, Duncan BB and Lee DW: CAR-T cell therapy for acute lymphoblastic leukemia: Transforming the treatment of relapsed and refractory disease. Curr Hematol Malig Rep 13: 396-406, 2018.
34. Vairy S, Garcia JL, Teira P and Bittencourt H: CTL019 (tisagenlecleucel): CAR-T therapy for relapsed and refractory B-cell acute lymphoblastic leukemia. Drug Des Devel Ther 12: 3885-3898, 2018.
35. Chen CZ, Li L, Lodish HF and Bartel DP: MicroRNAs modulate hematopoietic lineage differentiation. Science 303: 83-86, 2004.
36. Lv M, Zhang X, Jia H, Li D, Zhang B, Zhang H, Hong M, Jiang T, Jiang Q, Lu J, *et al*: An oncogenic role of miR-142-3p in human T-cell acute lymphoblastic leukemia (T-ALL) by targeting glucocorticoid receptor-a and cAMP/PKA pathways. Leukemia 26: 769-777, 2012.
37. Zhang H, Ji W, Huang R, Li L, Wang X, Li L, Fu X, Sun Z, Li Z, Chen Q and Zhang M: MicroRNA-155 is a potential molecular marker of natural killer/T-cell lymphoma. Oncotarget 7: 53808-53819, 2016.
38. Saki N, Abroun S, Soleimani M, Mortazavi Y, Kaviani S and Arefian E: The roles of miR-146a in the differentiation of Jurkat T-lymphoblasts. Hematology 19: 141-147, 2014.
39. Abe F, Kitadate A, Ikeda S, Yamashita J, Nakanishi H, Takahashi N, Asaka C, Teshima K, Miyagaki T, Sugaya M and Tagawa H: Histone deacetylase inhibitors inhibit metastasis by restoring a tumor suppressive microRNA-150 in advanced cutaneous T-cell lymphoma. Oncotarget 8: 7572-7585, 2016.
40. Takahashi RU, Prieto-Vila M, Hironaka A and Ochiya T: The role of extracellular vesicle microRNAs in cancer biology. Clin Chem Lab Med 55: 648-656, 2017.
41. Senkevitch E, Li W, Hixon JA, Andrews C, Cramer SD, Pauly GT, Back T, Czarra K and Durum SK: Inhibiting Janus Kinase 1 and BCL-2 to treat T cell acute lymphoblastic leukemia with IL7-Rα mutations. Oncotarget 9: 22605-22617, 2018.
42. Zadi Heydarabad M, Vatanmakanian M, Abdolalizadeh J, Mohammadi H, Azimi A, Mousavi Ardehaie R, Movasaghpour A and Farshdousti Hagh M: Apoptotic effect of resveratrol on human T-ALL cell line CCRF-CEM is unlikely exerted through alteration of BAX and BCL2 promoter methylation. J Cell Biochem 119: 10033-10040, 2018.