Technology in Cancer Research &
Treatment
Volume 19: 1-9
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1533033820934130
journals.sagepub.com/home/tct
SAGE

MiR-7 Functions as a Tumor Suppressor by Targeting the Oncogenes TAL1 in T-Cell Acute Lymphoblastic Leukemia

Hongbo Sun, MD[1], Zhifu Zhang, MD[1], Wei Luo, MD[1], Junmin Liu, MD[1], Ye Lou, MD[2], and Shengmei Xia, MD[3]

Abstract

Background: T-cell acute lymphoblastic leukemia is a hematologic malignancy characterized by T-cell proliferation, and in many cases, the ectopic expression of the oncogenic transcription factor T-cell acute lymphocytic leukemia protein 1 (TAL1). MicroRNA-7 has been shown to play a critical role in proliferation, migration, and treatment sensitivity in a diverse array of cancers. In this study, we sought to establish a novel link between microRNA-7 and T-cell acute lymphoblastic leukemia oncogenesis. **Material and Method:** To do so, we characterized gene expression of microRNA-7 as well as *TAL1* in both T-cell acute lymphoblastic leukemia patient-derived tissue and cell lines, as well as performing functional luciferase assays to assess microRNA-7 binding to the *TAL1* 3′-untranslated region. We also performed growth, apoptosis, and migration experiments using 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide, Annexin V, and transwell assays in the context of microRNA-7 overexpression. **Results:** We found that microRNA-7 expression is attenuated and inversely correlated with *TAL1* expression in TAL1 + T-cell acute lymphoblastic leukemia cells. Additionally, microRNA-7 directly targets and suppresses TAL1 levels. Finally, microRNA-7 overexpression reduces growth, motility, and migration while inducing apoptosis in T-cell acute lymphoblastic leukemia cells, phenotypes that can be rescued by concomitant overexpression of TAL1. **Conclusions:** These results indicate that microRNA-7 functions as a potent tumor suppressor by inhibiting the oncogene TAL1 and suggest microRNA-7 could function as a prognostic biomarker and possible therapeutic in the clinical management of T-cell acute lymphoblastic leukemia.

Keywords

miR-7, TAL1, T-cell acute lymphoblastic leukemia

Abbreviations

ALL, acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; BM, bone marrow; FBS, fetal bovine serum; mRNA, messenger RNA; miRNA, microRNA; miR-7, MicroRNA-7; MTT, 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide; NC, negative control; RT-PCR, Real-time polymerase chain reaction; T-ALL, T-cell acute lymphoblastic leukemia; 3′-UTR, 3′-untranslated region.

Received: December 10, 2019; Revised: May 11, 2020; Accepted: May 22, 2020.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, comprising close to 30% of new-onset pediatric cancers. Due to significant advances in treatment regimens over the last few decades, 5-year survival is now estimated to exceed 85%.[1-3] Acute lymphoblastic leukemia can be broadly subclassified into T-cell acute lymphoblastic leukemia (T-ALL) or B-cell ALL (B-ALL) depending on the cancer's cellular origin, with T-ALL representing 12% to 15% of pediatric and 25% of adult-onset ALL cases, respectively.[1,4]

---

[1] Department of Hematology, Shenzhen Longhua People's Hospital, Shenzhen, China
[2] Department of Hematology, Daqing Oilfield General Hospital, Daqing, China
[3] Department of Neurology, Shenzhen Longhua People's Hospital, Shenzhen, China

Corresponding Author:
Hongbo Sun, Department of Hematology, Shenzhen Longhua People's Hospital, 38 Jinglong Construction Road, Shenzhen 518109, China.
Email: sunhongboto@outlook.com

T-cell acute lymphoblastic leukemia has remained relatively understudied when compared with B-ALL despite the observation that it arises from biologically separate molecular pathways and has distinct disease kinetics and treatment responses. In particular, existing salvage therapy for recurrent disease is notoriously poor.[5-7] Therefore, much work remains to be done to elucidate disease pathogenesis and identify novel therapeutic targets in T-ALL.

TAL1 is a basic helix-loop-helix transcription factor that functions as a master regulator of hematopoiesis and cardiovascular development.[8-10] Significantly, in hematopoietic stem cells, TAL1 complexes with a number of important transcription factors including GATA and RUNX1 to regulate a complex transcriptional cascade critical for differentiation, cell-fate determination, and population maintenance.[11,12] Furthermore, TAL1 expression has been observed in endothelial precursors, multipotent progenitor cells, and megakaryocyte and erythrocyte lineages.[12,13] Given its pivotal role in normal hematopoiesis, it is unsurprising that misregulation of TAL1 has been implicated in leukemia. Indeed, ectopic expression of TAL1 due to various chromosomal aberrations is present in 40% to 60% of T-ALL cases. In particular, translocation of the *TCL* promoter *t*(1;14) upstream of the *TAL1* gene locus as well as an intergenic deletion leading to a SIL-TAL1 fusion protein are commonly observed in addition to a variety of other alterations and point mutations.[14-16] Thus, TAL1 has emerged as a critical target in understanding T-ALL biology and for the development of novel therapeutics.

Like in several other cancers, the role of epigenetic modifications, microRNAs (miRNAs) has been explored in T-ALL as well. Studies have elucidated the impact of transcription factors such as TAL-1 on miRNA expression profiles.[17-19] However, regulation of these oncogenic genes by miRNA has received little attention in T-ALL.

Using an in silico approach, we identified that TAL1 was a target gene for miR-7. Although miR-7 is involved in the development of multiple organs and biological function of cells, growing evidence indicates the role of miR-7 in growth, migration, and invasion of multiple cancers.[19,20] Further, the expression of miR-7 in ALL has been associated with a poor prognosis. However, the role of miR-7 in the molecular subset of pediatric T-ALL has not been explored so far. We thus investigated the role of miR-7 in mediating pathogenesis of T-ALL.

Materials and Methods

Patient Samples and T-ALL Cell Lines

Primary T-ALL cells were collected from pediatric patients following acquisition of informed consent from their guardians in accordance with the Declaration of Helsinki and national ethics guidelines. This study was approved by the institutional review board of Daqing Oilfield General Hospital (approval no. DQM-yan-2019101). Age-matched participants with no manifestations of any hematological malignancy were used as control. All patients were younger than 12 years. Only cases with bone marrow (BM) samples containing 70% leukemic cells were enrolled in this study. All T cells were collected from BM prior to treatment initiation. The human T-ALL cell lines (JURKAT, PF-382, MOLT-4, LOUCY SUPT-11, All-SIL, SUP-1, and CCRF-CEM) were maintained in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. Cells were cultured at 37 °C with 5% CO_2. All patients were provided the informed consent for using of their tissues in this research.

Oligonucleotides, Cell Transfection, and Real-Time Polymerase Chain Reaction

The sequences of miRNAs used in this study were as follows: negative control (NC) miRNA; 5′-UUCUCCGAACGUGUCACGUTT-3′, miR-7; 5′-UGGAAGACUAGUGAUUUUGUUGU-3′. Control and TAL1 expression plasmids were obtained from Addgene. Negative control and miR-7 were transfected into cells at 70% to 80% confluence using Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific). Cells were cotransfected with miRNA, luciferase reporter, or TAL1 overexpression plasmids using Lipofectamine 3000 Reagent. All transfections were performed according to the manufacturer's instructions. At 6 hours posttransfection, the medium was replaced with fresh medium containing 10% FBS. Real-time polymerase chain reaction (RT-PCR) was performed as previously described.[21] MicroRNA and messenger RNA (mRNA) quantification was performed using the SYBR Green method (Applied Biosystems) on an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems) in accordance with the manufacturer's instructions. U6 snRNA was used as an internal control for miRNA quantification and GAPDH was used for mRNA quantification. Relative levels of gene expression were represented using the $2^{-\Delta\Delta Ct}$ method. The following primer sequences were used: *U6 snRNA* forward 5′-CTCGCTTCGGCAGCACATATACT-3′; reverse 5′-ACGCTTCACGAATTTGCGTGTC-3′; *TAL1* forward 5′-GTTCTTTG-GGGAGCCGGATG-3′ and reverse 5′-ACATTCTGCTGCCGCCATCG-3′; *TRAF3* forward 5′-ACAAGTGCAGCGTCCAGACTCT-3′ and reverse 5′-GCCTTGATCTGCTGGT-TTGTCC-3′; *RAB40B* forward 5′-GGGCATCGACTACAAGACG-3′ and reverse 5′-AATCTTCCCTGGCCTGAAGT-3′; *EPHB1* forward 5′-CTTTGACCCTCCAGAAGTGG-3′ and reverse 5′-CTCCACATTGTCGTCACAGC-3′; *GAPHD* forward 5′-GGGTGTGAACCATGAGAAGT-3′ and reverse 5′-TGAGTCCTTCCACGATACCAA-3′. The experiments were repeated in 3 times.

Luciferase Assays

The wild-type full-length *TAL1* 3′-untranslated region (3′-UTR) was amplified and ligated into the psi-CHECKTM luciferase reporter vector (Promega). The mutated *TAL1* 3′-UTR was generated utilizing the *TAL1* 3′-UTR plasmid as a template and mutating the miR-7 seed binding site using the

QuikChange Multi Site-Directed Mutagenesis kit (Stratagene). JURKAT and CCRF-CEM cells were cultured for 24 hours and transfected with a mixture of 2 μl Lipofectamine 2000 (Life Technologies), 200 μL of OPTIMEM medium (Thermo Fisher Scientific), 100 ng of the reporter vector, and 100 nM of miR-7 or NC. After 48 hours, cells were lysed and processed according to the Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase and Renilla luciferase activity were measured and calculated as described previously.[22] The experiments were repeated in 3 times.

Western Blot

Western blot was performed as previously described.[23] Antibodies including anti-TAL1, anticleaved caspase-3, anti-Bcl-2, and anti-β-actin were obtained from Santa Cruz Biotechnology. The experiments were repeated in 3 times.

3-(4,5-dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide Assays

3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide assays were performed to examine cell growth. 5×10^3 JURKAT or CCRF-CEM cells per well were seeded into a 96-well plate and maintained as described above. The MTT reagent (20 μL/well) was added and the plate was incubated for 3 to 4 hours at 37 °C. The MTT reagent was then removed and 150 μL of dimethyl sulfoxide was added to each well to solubilize the formazan. Absorbance was measured at 590 nm using a microplate reader. The experiments were repeated in 3 times.

Colony Formation

Colony formation was performed as previously described.[21] The experiments were repeated in 3 times.

Annexin-V FITC Apoptosis Assay

The Annexin-V Fluorescein isothiocyanate (FITC) apoptosis assay was performed using the Annexin-V: FITC apoptosis detection kit I (BD Biosciences). Cells (5×10^5) were centrifuged at 1100 rpm for 5 minutes and resuspended in 0.5 mL binding buffer. Annexin-V FITC (5 μL) and propidium iodide (50 μg/mL, 5 μL) were then added into the cell suspension. Cells were incubated at room temperature for 5 minutes in the dark and analyzed by flow cytometry within 30 minutes. The experiments were repeated in 3 times.

TUNEL Assay

Apoptotic cells on coverslips were stained with DeadEnd Fluorometric Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) System (Promega) according to the manufacturer's protocol. Coverslips were mounted on glass slides with VECTRASHIELD (Vector Laboratories). Detection of free 3′-OH ends was performed through the addition of digoxigenin-conjugated nucleotides or fluorescein-12-deoxyuridine triphosphate (dUTP) by terminal deoxynucleotidyl transferase and visualized by fluorescent microscopy. In each sample, at least 100 transfected cells were counted. The experiments were repeated in 3 times.

Cell Migration and Invasion Assays

Cell migration and invasion assays were performed using 8 μM-pore membrane Transwell inserts (BD Biosciences). For the migration assay, JURKAT or CCRF-CEM cells (1×10^5 cells per chamber) were seeded into the upper chambers in serum-free RPMI-1640 medium supplemented with 0.1% bovine serum albumin (BSA). Complete RPMI-1640 medium with 10% FBS was placed into the lower chambers. After 24 hours, cells on the upper surface of the chamber were removed with a cotton swab, and the inserts were fixed with 4% paraformaldehyde and stained with crystal violet. Cells attached to the lower surface of the filter were counted in 6 randomly selected areas using a microscope. For invasion assays, the Transwell inserts were coated with a layer of Matrigel Growth Factor Reduced Matrix (BD Biosciences). For time-lapse video assessment of cell movement, phase-contrast images were obtained every 60 seconds for 30 minutes at 37 °C with 5% CO_2. The velocities and accumulated distances of 20 randomly selected T-ALL cells were determined by manually tracking individual cells using ImageJ (NIH) software and a manual tracking plugin. The experiments were repeated in 3 times.

Statistics

Data are expressed as mean ± standard deviation of 3 independent experiments. Differences among groups were analyzed using 1-way analysis of variances or paired *t* tests. A *P* value <.05 was considered statistically significant. All statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software).

Results

MicroRNA-7 Expression Is Suppressed in T-ALL

To investigate the role of miR-7 in T-ALL, we first analyzed the expression levels of miR-7 in 2 publicly available data sets. We found that T-ALL primary cells and cell lines express lower levels of miR-7 than thymocytes, BM precursors, and $CD34^+$ hematopoietic progenitor cells (Figure 1A). MicroRNA-7 (miR-7) levels were also significantly lower in T-ALL primary cells and cell lines compared with normal hematopoietic T-cells (Figure 1B). These results indicate that miR-7 is downregulated in human T-ALL, suggesting a functional role in disease pathogenesis.

MicroRNA-7 Directly Targets TAL1

Next, we assessed the expression of miR-7 in various genetic subtypes of human T-ALL. We found miR-7 expression negatively correlated with *TAL1* expression in 40 primary T-ALL patient samples (Figure 2A and B). Consistent with our

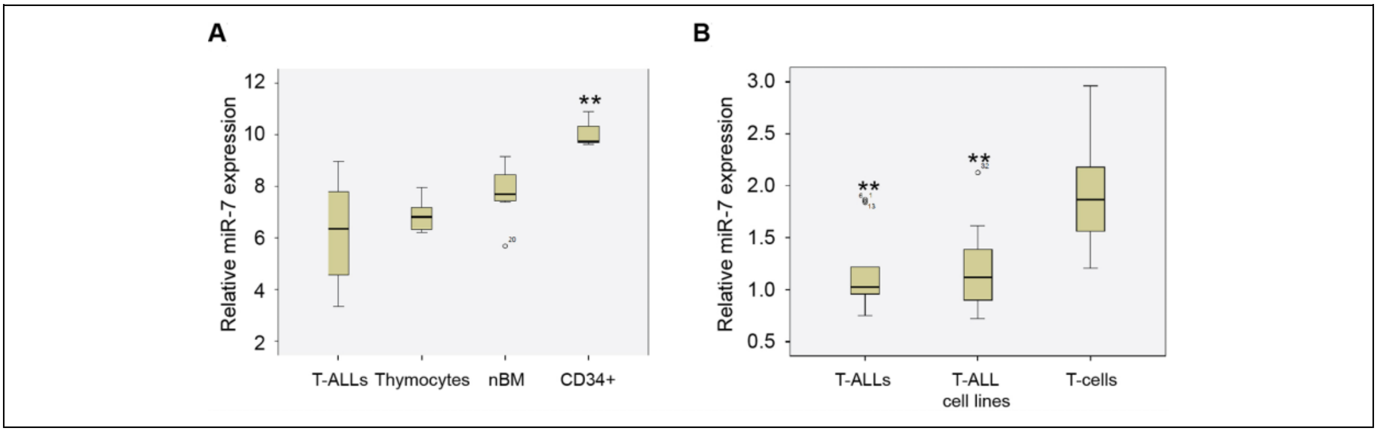

Figure 1. Human T-cell acute lymphoblastic leukemia (T-ALL) cells express lower levels of miR-7 than normal cells. (A) MicroRNA-7 (miR-7) expression in cells harvested from T-ALL patients was compared to thymocytes, bone marrow (nBM), and CD34$^+$ peripheral blood cells of pediatric samples from a publically available data set (GSE23024). $^{**}P < .001$. (B) MiR-7 expression in T-ALL patient and cell lines was compared to normal T-cells from an additional publically available data set (GSE51908). $^{**}P < .001$.

findings in clinical samples, miR-7 was highly expressed in *TAL1*-negative cell lines as compared to the *TAL1*-positive cell lines (Figure 2C). To identify potential miRNAs, targeting TAL1, we undertook a bioinformatic analysis using TargetScan. Through our analysis, we found that miR7 targets TAL1 at sites depicted in Figure 2D. To experimentally confirm, whether TAL1 is a target gene of miR-7, we fused firefly luciferase to the 3′-UTR of wild-type or mutant *TAL1* to form reporter constructs. These were then cotransfected into JURKAT or CCRF-CEM cells along with either a miRNA NC or miR-7. Using a luciferase assay, we then demonstrated that the reporter harboring the wild-type *TAL1*-3′-UTR was significantly reduced to 30% of baseline ($P < .01$) by miR-7 as compared to the NC. However, mutant *TAL1*-3′-UTR was unaffected by miR-7 (Figure 2E). Furthermore, RT-PCR and Western blot analysis revealed that the expression of *TAL1* was significantly downregulated in miR-7-transfected JURKAT and CCRF-CEM cells (Figure 2F and G). MicroRNA-7 overexpression suppressed TAL1 downstream genes including *TRAF3*, *RAB40B*, and *EPHB1* (Figure 2H). Finally, we examined miR-7 expression levels in LOUCY and SUPT-1 cells overexpressing TAL1 and identified significantly decreased expression of miR-7 (Figure 2I). Taken together, these data suggest that TAL1 is a direct target of miR-7 and that TAL1 also inhibits miR-7 expression through negative feedback.

MicroRNA-7 Impairs Growth and Induces Apoptosis of T-ALL Cells by Regulating TAL1

We therefore investigated the functional impact of miR-7 on the growth of T-ALL cells. Both JURKAT and CCRF-CEM cells transfected with miR-7 demonstrated significant reductions in growth compared with those transfected with NC (Figure 3A and B). Anchorage-independent growth, as assessed by soft agar assays, was significantly reduced in miR-7-transfected cells to about 60% of that of NC-transfected cells (Figure 3C and D). These effects were corroborated by investigations of cell apoptosis, in which we found that miR-7 overexpression led to an increased number of Annexin V positive cells, increased caspase-3 levels, and decreased Bcl-2 expression (Figure 3E and F). Additionally, the number of TUNEL-positive cells was significantly higher in T-ALL cells transfected with miR-7 as compared to control cells (Figure 3G and H). We then tested whether the decrease in *TAL1* expression observed after miR-7 transfection was responsible, at least in part, for growth inhibition and apoptosis. We therefore cotransfected TAL1 and miR-7 or NC into T-ALL cells. As expected, anchorage-independent cell growth and apoptosis were rescued in the context of TAL1 overexpression (Figure 3A-H).

MicroRNA-7 Suppressed Migration, Invasion, and Cell Motility of T-ALL Cells by Regulating TAL1

Using the transwell migration assay, we found that overexpression of miR-7 resulted in decreased directional cell migration and invasion in control JURKAT and CCRF-CEM cells, but not in cells overexpressing TAL1 (Figure 4A and B). This suggests that miR-7 negatively regulates cell migration and invasion via inhibition of TAL1. Using time-lapse microscopy, we found that miR-7 overexpression resulted in slower migration velocity and shorter migration distance over a span of 30 minutes (Figure 4C-E). By contrast, the inhibition of cell motility by miR-7 was not detected in *TAL1*-overexpressed T-ALL cells, which indicates that miR-7 reduces random movement of T-All cells via TAL1 as well. Together, these data indicate that miR-7 negatively regulates the migration, invasion, and motility of T-ALL cells. This phenotype is likely to be underpinned mechanistically by a reduction of TAL1 via miRNA-mediated decay.

Discussion

In this study, we have identified a critical and novel role for miR-7 in the pathogenesis of T-ALL. We found that the miR-7

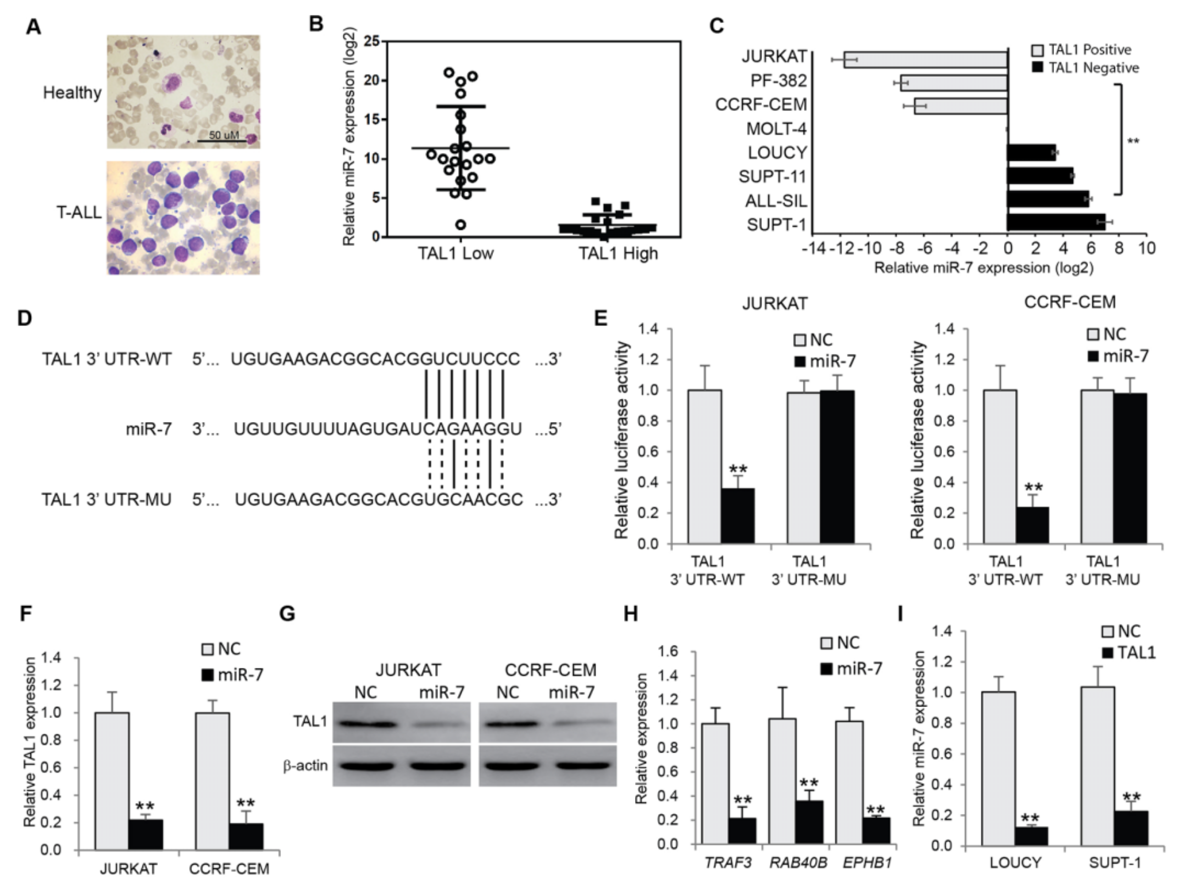

Figure 2. MicroRNA-7 (miR-7) expression inversely correlates with *TAL1* expression in human T-cell acute lymphoblastic leukemia (T-ALL). (A) Morphological images of healthy or T-ALL patient bone marrow samples. (B) miR-7 expression in 40 pediatric T-ALL patients categorized by high or low *TAL1* expression (n = 20 TAL1-positive; n = 20 TAL1-negative). $^{**}P < .001$ compared to *TAL1* low expression group. (C) Comparison of miR-7 expression as determined by quantitative real-time polymerase chain reaction (qRT-PCR) in T-ALL cells positive (gray) or negative (black) for TAL1. $^{**}P < .001$. (D) Wild-type (WT) or mutant (MU) sequences of the predicted miR-7 binding region in the *TAL1* 3′-untranslated region (3′-UTR). (E) Luciferase activity in JURKAT (left) or CCRF-CEM (right) cells cotransfected with either negative control (NC) or miR-7 and wild-type or mutant *TAL1* 3′-UTR reporters. $^{**}P < .001$ compared to NC transfected cells. (F) Messenger RNA (mRNA) levels of *TAL1* as determined by qRT-PCR in JURKAT or CCRF-CEM cells transfected with either NC or miR-7. $^{**}P < .001$ compared to NC transfected cells. (G) TAL1 levels as determined by Western blot in JURKAT or CCRF-CEM cells transfected with either NC or miR-7. $^{**}P < .001$ compared to NC transfected cells. (H) Expression of TAL1-downstream genes *TRAF3*, *RAB40B,* and *EPHB1* in JURKAT or CCRF-CEM cells transfected with either NC or miR-7. $^{**}P < .001$ compared to NC transfected cells. (I) MiR-7 levels determined by RT-PCR in NC or TAL1 transfected LOUCY or SUPT-1 cells. $^{**}P < .001$ compared to NC transfected cells.

expression levels are decreased in T-ALL cells compared with healthy controls and that the expression of miR-7 controls important cellular process including cell growth, apoptosis, motility, and migration. Furthermore, we identified the T-ALL-associated transcription factor TAL1 as a target of miR-7, showing an inverse correlation between miR-7 and *TAL1* expression, and showing evidence for direct binding of miR-7 to the *TAL1* 3′-UTR. Furthermore, concomitant TAL1 overexpression attenuates the proapoptotic and migration-inhibitory effects of miR-7 expression, providing functional insight into the tumor-suppressive ability of miR-7 as well as mechanistic understanding concerning the role of TAL1 in T-ALL oncogenesis.

T-cell acute lymphoblastic leukemia is an important yet understudied subtype of ALL. Because of this, treatment regimens are typically like those used in B-ALL, despite abundant evidence indicating that T-ALL is governed by distinct biological processes. Induction usually entails use of an anthracycline (doxorubicin or daunorubicin), vincristine, a steroid such as dexamethasone, and intrathecal methotrexate. It has also been shown that asparaginase improves efficacy in conjunction with methotrexate, especially for pediatric patients. Despite great success with these treatment modalities, nearing 85% for new-onset disease, much work remains.[6,7,24-26] For instance, ideal consolidation therapy in T-ALL is an area of active research. More saliently, outcomes in recurrent disease are notoriously poor. Although the purine nucleoside analog Nelarabine has had promising results in recent clinical trials, new therapeutics are needed.[27,28] Targeting specific molecular pathways has been

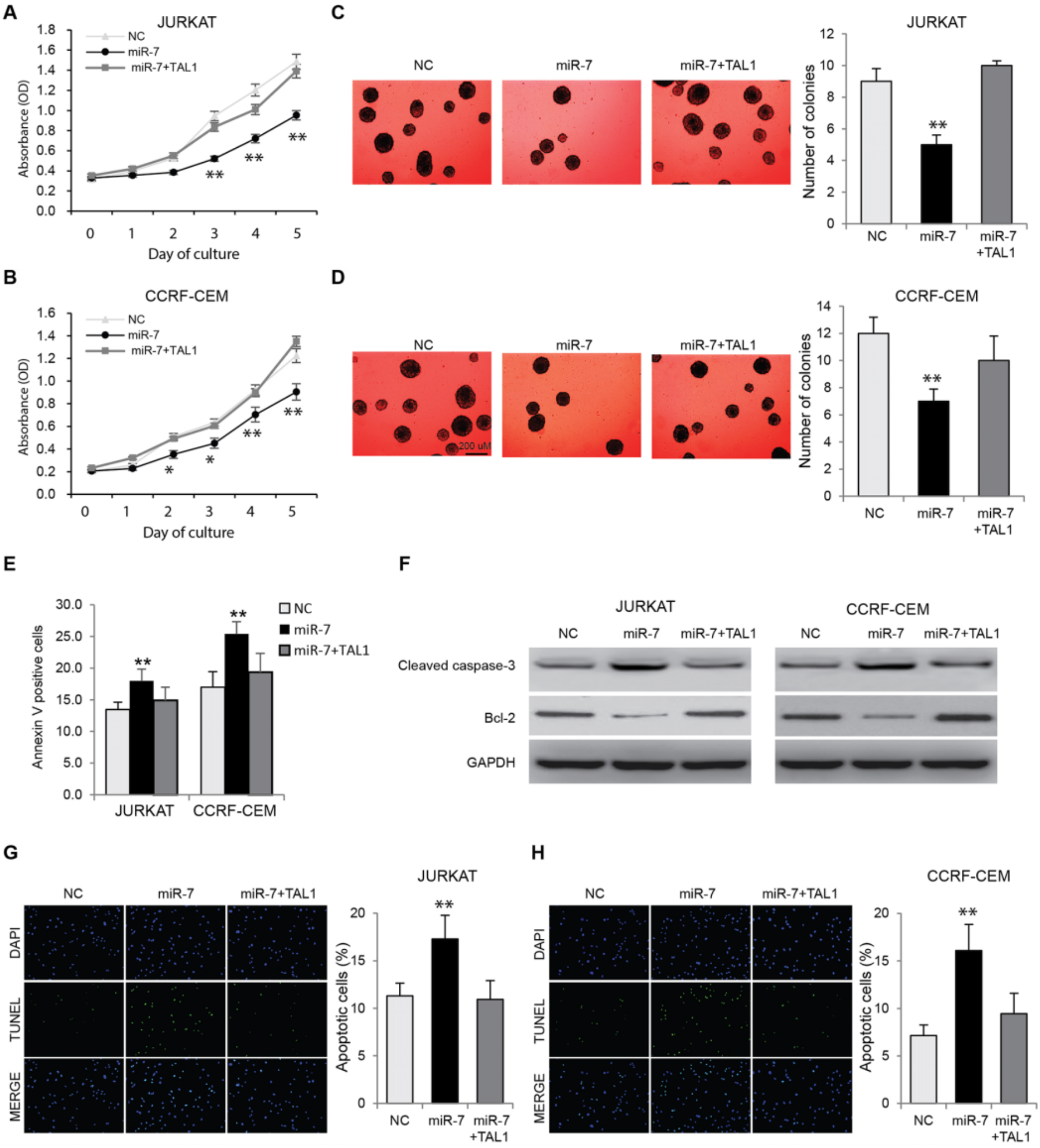

Figure 3. MicroRNA-7 (miR-7) impairs growth and induces apoptosis of T-cell acute lymphoblastic leukemia (T-ALL) cells, which is reversed by TAL1 overexpression. 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay of JURKAT (A) or CCRF-CEM (B) cells transfected with negative control (NC), miR-7, or miR-7 + TAL1 plasmid at day 0, 1, 2, 3, 4, and 5. $^{**}P < .001$ compared to NC-transfected cells. Soft agar assay of JURKAT (C) or CCRF-CEM (D) Cells transfected with NC, miR-7, or miR-7 + TAL1 plasmid. Representative images are shown (left panel) as well as colony numbers (right panel). $^{**}P < .001$ compared to NC-transfected cells. Cell apoptosis characterized by Annexin V positive cells (E), cleaved caspase-3, and Bcl-2 level (F) and TUNEL staining (G, H) in JURKAT or CCRF-CEM cells transfected with NC, miR-7, or miR-7 + TAL1 plasmid. $^{**}P < .001$ compared to NC-transfected cells.

successful for other hematologic cancer subtypes[29,30] and may therefore be effective here.

Although transcription factor TAL1 plays an important role in normal hematopoiesis, its expression is strictly regulated by several tumor-suppressive E-box binding proteins including E2A and HEB. With ectopic expression of TAL1 due to translocation events, it cooperates with several other complex factors including GATA3 and RUNX1 to both increase their own expression in an autoregulatory feedback loop as well as inhibit expression of tumor suppressor E-box proteins. This leads to a differentiation failure, allowing for mutations in NOTCH1 to accumulate that ultimately result in a proliferative phenotype and oncogenesis.[12] Similar to other cancers, several miRNA genes have been identified in the context of

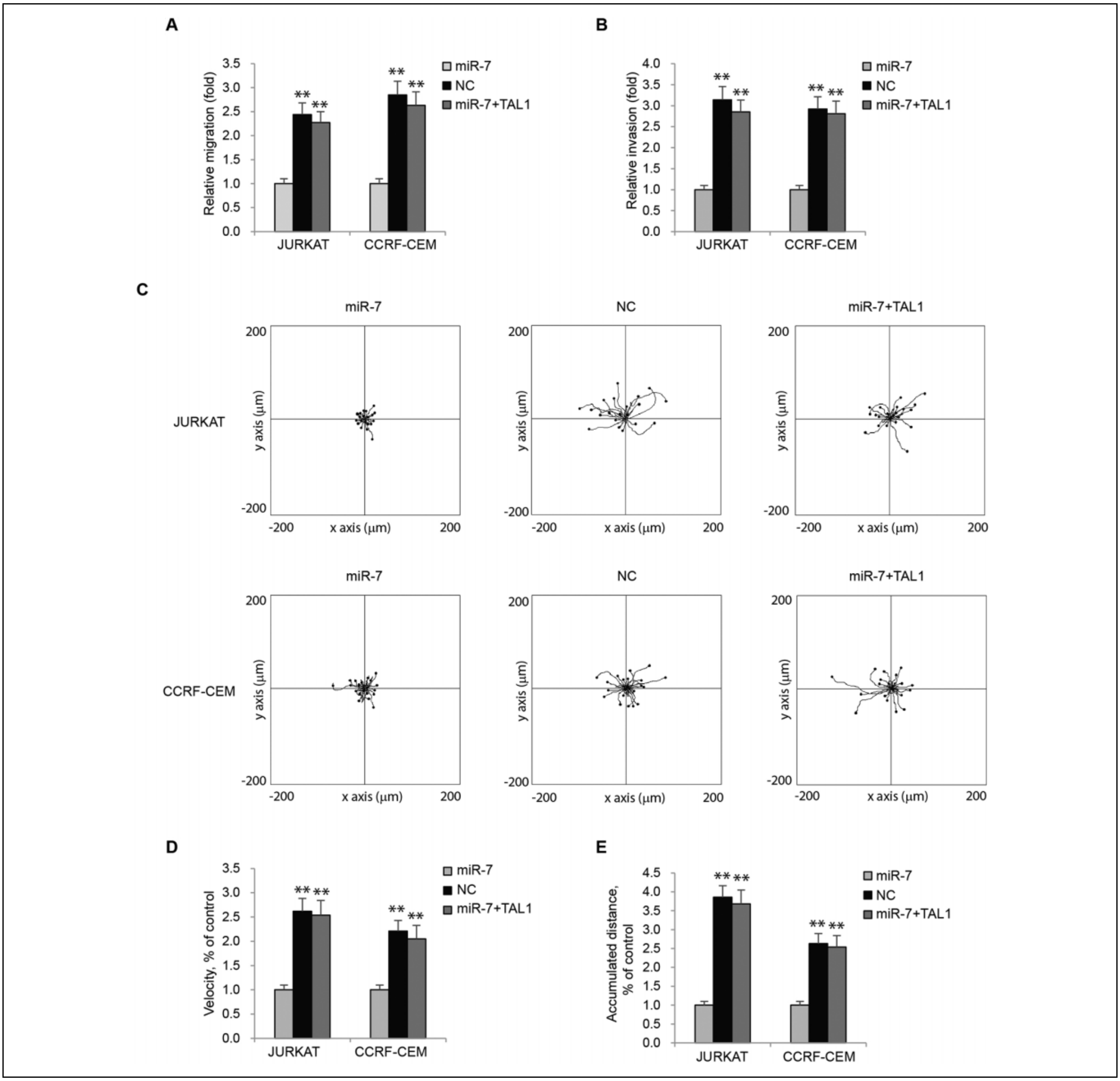

Figure 4. MicroRNA-7 (miR-7) suppresses migration, invasion, and cell motility in T-cell acute lymphoblastic leukemia (T-ALL) cells, which can be rescued by TAL1. JURKAT or CCRF-CEM cells transfected with NC, miR-7, or miR-7 + TAL1 plasmid. (A) Transwell migration analysis of JURKAT or CCRF-CEM cells transfected with NC, miR-7, or miR-7 + TAL1. (B) Transwell invasion analysis of JURKAT or CCRF-CEM cells transfected with NC, miR-7, or miR-7 + TAL1. (C) Representative migration images of individual JURKAT (top panel) or CCRF-CEM (bottom panel) cells recorded for 30 minutes by time-lapse video microscopy (n = 20). Mean velocity (D), accumulated, and traveled distance (E) of 20 individual cells transfected with NC, miR-7, and miR-7 + TAL1 assessed in (C). $^{**}P < .001$ compared to miR-7 transfected cells.

T-ALL.[17,18] Some of the genes found to be significantly regulated upon overexpression or silencing specifically of TAL1 were miR-135a, miR-223, miR-330-3p, miR-146b-5p, miR-545.[19] Although most of the studies were identifying regulation of miRNAs by TAL, we evaluated the regulation of TAL1 through epigenetic regulation mediated by miRNA. Through our bioinformatic analysis, we confirmed miR-7 as the miRNA with target sites on TAL1. Consistent with other reports, our *in vitro* data suggest that miR-7 negatively regulates TAL1.[31] miR-7, by directly targeting TAL1 for degradation, likely functions as a tumor suppressor by inhibiting this tumorigenic molecular cascade.

MicroRNA-7 is well known as tumor regulator in various malignant tumors besides T-ALL.[32] For instance, Jiang and colleagues provided evidence that miR-7 regulates proliferation in chronic myeloid leukemia.[33] The expression of miR-7 has been related to cellular migration in several cancer subtypes. It has also been shown to have prognostic value in breast cancer and to regulate migration and metastasis.[32] Furthermore, miR-7 regulates sensitivity and modulates resistance to a number of chemotherapeutic agents in a variety of tumor types.[33-35] Our findings concerning the role of miR-7 in T-ALL proliferation and migration are consistent with these studies, which in turn suggest that miR-7 may play an important role in drug sensitivity and resistance in T-ALL. This should be an avenue of further research.

Conclusions

Although we have uncovered evidence that miR-7 functions as a tumor suppressor by acting through TAL1, much further work remains. For instance, it would be important to elucidate the mechanism whereby TAL1 inhibits miR-7 expression and whether this process can be modified. Additionally, it is currently unclear what role, if any, that miR-7 plays in TAL1-negative T-ALL. Although the *in vitro* findings need an *in vivo* validation, it would be of interest to explore potential therapeutic avenues for miR-7 in the treatment of T-ALL and to identify further miRNAs that regulate other members of the TAL1 signaling cascade.

Authors' Note

The data used to support the findings of this study are available from the corresponding author upon request. H.S. and Z.Z. contributed equally to this work. H.S. designed the manuscript; H.S., Z.Z., and W.L. performed experiments, H.S. and S.X. collected the data; H.S., Z.Z., and J.L. analyzed the results; Z.Z. and Y.L. wrote the manuscript.

Acknowledgments

The authors are grateful to all patients participated in this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Hongbo Sun https://orcid.org/0000-0002-8404-4996

References

1. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. *N Eng J Med*. 2015;373(16):1541-1552.
2. Pui CH, JSandlund JT, Pei D, et al. Improved outcome for children with acute lymphoblastic leukemia: results of total therapy study XIIIB at St Jude Children's Research Hospital. *Blood*. 2004;104(9):2690-2696.
3. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. 2011;61(4):212-236.
4. Raetz EA, Teachey DT. T-cell acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1) 580-588.
5. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. *J Clin Oncol*. 2011;29(5):532-543.
6. Gökbuget N, Kneba M, Raff T, et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. *Blood*. 2012;120(9):1868-1876.
7. Marks DI, Rowntree C. Management of adults with T-cell lymphoblastic leukemia. *Blood*. 2017;129(9):1134-1142.
8. Souza SL, Elefanty AG, Keller G. SCL/Tal-1 is essential for hematopoietic commitment of the hemangioblast but not for its development. *Blood*. 2005;105(10):3862-3870.
9. Porcher C, Chagraoui H, Kristiansen MS. SCL/TAL1: a multifaceted regulator from blood development to disease. *Blood*. 2017;129(15):2051.
10. Patterson LJ, Gering M, Patient R. Scl is required for dorsal aorta as well as blood formation in zebrafish embryos. *Blood*. 2005;105(9):3502-3511.
11. Hoang T, Lambert JA, Martin R. SCL/TAL1 in hematopoiesis and cellular reprogramming. *Curr Top Dev Biol*. 2016;118:163-204.
12. Sanda T, Leong WZ. TAL1 as a master oncogenic transcription factor in T-cell acute lymphoblastic leukemia. *Exp Hematol*. 2017;53:7-15.
13. Mouthon MA, Bernard O, Mitjavila MT, Romeo PH, Vainchenker W, Mahul DM. Expression of tal-1 and GATA-binding proteins during human hematopoiesis. *Blood*. 1993;81(3):647-655.
14. Mansour MR, Abraham BJ, Anders L, et al. Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. *Science*. 2014;346(6215):1373-1377.
15. Breit TM, Mol EJ, Wolvers Tettero IL, Ludwig WD, van Wering ER, van Dongen JJ. Site-specific deletions involving the tal-1 and sil genes are restricted to cells of the T cell receptor alpha/beta lineage: T cell receptor delta gene deletion mechanism affects multiple genes. *J Exp Med*. 1993;177(4):965-977.
16. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. *J Clin Oncol*. 2005;23(26):6306-6315.
17. Correia NC, Barata JT. MicroRNAs and their involvement in T-ALL: a brief overview. *Adv Biol Regul*. 2019;74:100650.
18. Wallaert A, Van Loocke W, Hernandez L, Taghon T, Speleman F, Van Vlierberghe P. Comprehensive miRNA expression profiling in human T-cell acute lymphoblastic leukemia by small RNA-sequencing. *Sci Rep*. 2017;7(1):7901.
19. Correia NC, Durinck K, Leite AP, et al. Novel TAL1 targets beyond protein-coding genes: identification of TAL1-regulated microRNAs in T-cell acute lymphoblastic leukemia. *Leukemia*. 2013;27(7):1603-1606.

20. Vera O, Jimenez J, Pernia O, et al. DNA methylation of miR-7 is a mechanism involved in platinum response through MAFG overexpression in cancer cells. *Theranostics*. 2017;7(17):4118-4134.
21. Lou Y, Liu L, Zhan L, Wang X, Fan H. miR-187-5p regulates cell growth and apoptosis in acute lymphoblastic leukemia via DKK2. *Oncol Res*. 2016;24(2):89-97.
22. Guo M, Zhang X, Wang G, et al. miR-603 promotes glioma cell growth via Wnt/beta-catenin pathway by inhibiting WIF1 and CTNNBIP1. *Cancer Lett*. 2015;360(1):76-86.
23. Guo M, Jiang Z, Zhang X, et al. miR-656 inhibits glioma tumorigenesis through repression of BMPR1A. *Carcinogenesis*. 2014; 35(8):1698-1706.
24. Litzow MR, Ferrando AA. How I treat T-cell acute lymphoblastic leukemia in adults. *Blood*. 2015;126(7):833-841.
25. Durinck K, Goossens S, Peirs S, et al. Novel biological insights in T-cell acute lymphoblastic leukemia. *Exp Hematol*. 2015;43(8): 625-639.
26. Patrick K, Vora A. Update on biology and treatment of T-cell acute lymphoblastic leukaemia. *Curr Opin Pediatr*. 2015;27(1):44-49.
27. Luskin MR, Ganetsky A, Landsburg DJ, et al. Nelarabine, cyclosphosphamide and etoposide for adults with relapsed T-cell acute lymphoblastic leukaemia and lymphoma. *Br J Haematol*. 2016; 174(2):332-334.
28. Lonetti A, Cappellini A, Bertaina A, et al. Improving nelarabine efficacy in T cell acute lymphoblastic leukemia by targeting aberrant PI3K/AKT/mTOR signaling pathway. *J Hematol Oncol*. 2016;9(1):114.
29. Kayser S, Levis MJ. Advances in targeted therapy for acute myeloid leukaemia. *Br J Haematol*. 2018;180(4):484-500.
30. Jabbour E, Cortes JE, Ghanem H, O'Brien S, Kantarjian HM. Targeted therapy in chronic myeloid leukemia. *Expert Rev Anticancer Ther*. 2008;8(1):99-110.
31. Mansour MR, Sanda T, Lawton LN, et al. The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. *J Exp Med*. 2013; 210(8):1545-1557.
32. Zhao J, Tao Y, Zhou Y, et al. MicroRNA-7: a promising new target in cancer therapy. *Cancer Cell Int*. 2015;15:103.
33. Jiang MJ, Dai JJ, Gu DN, Huang Q, Tian L. MicroRNA-7 inhibits cell proliferation of chronic myeloid leukemia and sensitizes it to imatinib in vitro. *Biochem Biophys Res Commun*. 2017;494(1-2): 372-378.
34. Cheng MW, Shen ZT, Hu GY, Luo LG. Prognostic significance of microRNA-7 and its roles in the regulation of cisplatin resistance in lung adenocarcinoma. *Cell Physiol Biochem*. 2017;42(2): 660-672.
35. Cui YX, Bradbury R, Flamini V, Wu B, Jordan N, Jiang WG. MicroRNA-7 suppresses the homing and migration potential of human endothelial cells to highly metastatic human breast cancer cells. *Br J Cancer*. 2017;117(1):89-101.