miR-196b/miR-1290 participate in the antitumor effect of resveratrol via regulation of IGFBP3 expression in acute lymphoblastic leukemia

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Abstract. MicroRNAs play critical roles in the progression of acute lymphoblastic leukemia (ALL). Previous studies have indicated that miR-196b and miR-1290 play critical roles in T-cell ALL (T-ALL) and B-cell ALL (B-ALL), respectively. Resveratrol, a natural edible polyphenolic phytoalexin, possesses certain anticancer activities. Nevertheless, the mechanism involved in the regulation of ALL by resveratrol is still poorly understood. The present study aimed to reveal the potential mechanism underlying the antitumor effect of resveratrol in ALL focusing on miRNAs. Research indicates that insulin-like growth factor binding protein 3 (IGFBP3) plays a critical role in the aetiology of ALL. In the present study, we first demonstrated that the expression of IGFBP3 was decreased in ALL patients. We further identified that miR-196b and miR-1290 were overexpressed in T-ALL TALL-104 and B-ALL SUP-B15 cell lines, respectively. Moreover, resveratrol markedly decreased the overexpression of miR-196b/miR-1290 and elevated IGFBP3 expression in the ALL cell lines. As an miR-196b/miR-1290 inhibitor, resveratrol was further demonstrated to exert antitumor effects on ALL cells including antiproliferation, cell cycle arrest, apoptosis and inhibition of migration. Dual-luciferase reporter assay revealed that miR-196b/miR-1290 directly bound to the 3'-untranslated (3'-UTR) region of IGFBP3 mRNA. Moreover, we observed that IGFBP3 short interfering RNA reversed the antitumor activity of resveratrol against ALL cells. Taken together, the present study provides evidence that resveratrol targets miR-196b and miR-1290 for its antitumor activity in T-ALL and B-ALL, respectively. The present study also confirms that both miR-196b and miR-1290 target the IGFBP3 3'-UTR and are potential therapeutic targets for ALL.

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Introduction

Acute lymphoblastic leukemia (ALL) is one of the most common childhood (0-15 years of age) hematologic malignancies (1,2). In recent years, with more and more in-depth studies of ALL, the survival rate of ALL patients has been significantly improved (3). Complete remission (CR) has been attained in more than 95% of cases and the 5-year event-free survival (EFS) has reached 63-83% in pediatric ALL patients (4), while, the CR and 3-5-year EFS of adult ALL patients have reached 75-89% and 40%, respectively (5). Nevertheless, numerous patients still suffer from the adverse events caused by conventional treatment and die from relapse (6). Therefore, a better understanding of the mechanism underlying ALL and development of new strategies for improving efficiency of ALL therapy are required. Emerging evidence indicates that insulin-like growth factor binding protein 3 (IGFBP3) is inversely associated with leukemia (7). Low IGFBP3 is related to the high-risk of events such as recurrence and decreased remission at the time of diagnosis (6), suggesting that the downregulation of expression of IGFBP3 plays an important role in the development of ALL.

MicroRNAs (miRNAs) are a family of endogenous, conserved, small non-coding RNAs (20-25 nucleotides in length). The complementary messenger RNAs (mRNAs) can be directly targeted on the 3'-untranslated regions (3'-UTRs) and suppressed by miRNAs in eukaryotes (8,9). Altered expression of miRNAs participates in a variety of biological processes such as carcinogenesis, immunity, infection, endocrine homeostasis, differentiation and apoptosis (10,11). By targeting complementary genes to control the expression of tumor-suppressor or oncogenic proteins, miRNAs are considered to play a significant role in the biology of cancers and to regulate cell proliferation, migration, invasion and apoptosis in cancers (12), thereby suggesting that a promising alternative novel approach for cancer treatment may be provided by miRNAs. It was reported that miR-196b is one of the most upregulated miRNAs in T-cell ALL (T-ALL) (13,14). In addition, regardless of treatment protocol, miR-1290 is capable to

serve as a new biomarker in childhood B-cell ALL (B-ALL) patients for outcome (3). However, the detailed regulatory mechanism of miR-196b or miR-1290 in ALL is still not well understood.

Numerous chemotherapeutic and chemopreventive compounds have been developed from natural sources and offer potential new alternatives to treat cancers (15). Resveratrol (3,5,40-trihydroxy-*trans*-stilbene), a natural polyphenol, is widely used in Traditional Chinese medicines (TCMs; such as *Polygonum cuspidatum* and *Rheum officinale* Baill.) and is found in peanuts, blueberries, cranberries, red wine and grape skin (16,17). Accumulating research suggests that resveratrol has a number of important pharmacological properties such as antiproliferative, antioxidant, cardio-protective and anti-inflammatory activities (18-20). Resveratrol also displays anticancer activities by disturbing the three stages of carcinogenesis: initiation, promotion and progression (21). Previous studies have demonstrated that resveratrol inhibited the cell growth and induced apoptosis in several ALL cell lines, suggesting the anti-ALL effect of this agent (22-25). Nevertheless, the molecular mechanism of resveratrol-mediated anti-ALL activity has not been fully elucidated.

The present study aimed to ascertain whether miR-196b and miR-1290 serve as novel targets involved in the antitumor effect of resveratrol in ALL and to explore the probable common regulatory mechanism focusing on IGFBP3.

Materials and methods

Clinical samples. Peripheral blood and bone marrow samples were collected from 15 pairs of ALL patients and healthy volunteers at the Department of Hematology, Guangzhou First People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China. Density gradient separation was used to isolate the human peripheral blood mononuclear cells (PBMCs) from whole blood by Ficoll-Paque Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and the samples were then cryopreserved in liquid nitrogen with 90% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA) and 10% dimethyl sulfoxide (DMSO) until analyzed.

Ethics statements. Permission to use the human bone marrow and peripheral blood samples for the present study was approved by the Ethics Committee of Guangzhou First People's Hospital (Guangdong, China).

Cell lines and cell culture. American Type Culture Collection (ATCC) (Manassas, VA, USA) provided the human embryonic kidney 293T, T-ALL TALL-104 and B-ALL SUP-B15 cells. 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and 2 mM L-glutamine (Invitrogen Life Technologies, Carlsbad, CA, USA). TALL-104 cells were cultured in complete medium [ATCC-formulated Iscove's modified Dulbecco's medium (IMDM) with 20% FBS, supplemented with 2.5 mg/ml human albumin, 0.5 mg/ml D-mannitol and 100 U recombinant human IL-2 (all from Sigma-Aldrich, St. Louis, MO, USA)]. SUP-B15 cells were cultured in IMDM, supplemented with 10% FBS, 2 mM L-glutamine, 0.05 μM 2-β-mercaptoethanol (Sigma-Aldrich), 100 μg/ml streptomycin and 100 U/ml penicillin (Gibco). The cells were maintained at 37˚C in a humidified atmosphere containing 5% CO_2.

Reagents. Resveratrol was obtained from Sigma-Aldrich. IGFBP3 siRNA and negative control siRNA were purchased from GenePharma (Shanghai, China). The antibody against IGFBP3, caspase-3 and GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (Kumamoto, Japan).

Cell proliferation assay. The cells were seeded into 96-well cell culture plates, and were incubated at 37˚C for 0, 24, 48 or 72 h in 5% CO_2. Cell proliferation was assessed via the CCK-8 assay. The numerical values obtained on an enzyme-labeled instrument (Thermo Fisher Scientific, Germany) with 450 nm wavelength were used to compare the cell viability.

Flow cytometry. Cells were collected, washed in ice-cold phosphate-buffered saline (PBS) and fixed in ice-cold 70% ethanol (4˚C, overnight). After centrifugation (1,000 rpm, 5 min), the cells were diluted with PBS and re-centrifuged. For the cell cycle assay, the cells were stained using a cell cycle kit (LiankeBio, Zhejiang, China) and incubated in the dark at 37˚C for 30 min. For analysis of apoptosis, the cells were stained using the Annexin V-FITC apoptosis detection kit (LiankeBio) and incubated in the dark at room temperature for 15 min. Stained cells were detected via flow cytometry with a BD FACSCalibur (BD Biosciences, Heidelberg, Germany).

Cell migration assay. The migration of cells was performed in a Boyden Transwell chamber (Millipore, Bedford, MA, USA) containing a polycarbonate filter with a pore size of 8-μm. A cell suspension (0.2 ml) ($1x10^5$ cells/ml) was added to the upper compartment of each chamber lined with an uncoated membrane. The bottom chamber was filled with 0.6 ml IMDM containing 10% FBS as a chemoattractant. After incubation for 48 h at 37˚C with 5% CO_2, the non-filtered cells were gently removed with a cotton swab and fixed with 4% paraformaldehyde. Filtered cells on the lower surface of the chamber were stained with 0.1% crystal violet (Sigma-Aldrich) and quantified manually in five random fields under a microscope (Olympus, Tokyo, Japan).

Quantitative real-time RT-PCR (qRT-PCR). Total RNA was extracted from the cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The mRNA expression of IGFBP3, miR-196b or miR-1290 was detected by qRT-PCR using the standard SYBR-Green RT-PCR kit (Takara, Tokyo, Japan) following the manufacturer's manual. Real-time RT-PCR was performed using a sequence detector (Sigma-Aldrich). Specific primers were obtained from Genepharma: IGFBP3 forward, 5'-ATAA TCATCATCAAGAAAGGGCA-3' and reverse, 5'-AGTTCTG GGTATCTGTGCTCTGA-3'; miR-196b forward, 5'-ACAC TCCAGCTGGGTAGGTAGTTTCATG-3' and reverse, 5'-CT CAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCC CAACAA-3'; miR-1290 forward, 5'-ACACTCCAGCTGGGT GGATTTTTGGATC-3' and reverse, 5'-CTCAACTGGTGTC

GTGGAGTCGGCAATTCAGTTGAGTCCCTG-3'. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting. Protein was extracted from the peripheral blood or cells using RIPA lysis buffer with a proteinase inhibitor. The protein concentration in the lysates was quantitated with the BCA Protein Assay kit (Bio-Rad, Hercules, CA, USA). Proteins were resolved on 10% SDS-PAGE gels under reducing conditions, followed by electrophoretic transfer onto polyvinylidene difluoride membranes (Millipore). Immunoblots were incubated with primary antibodies against IGFBP3 (1:2,000) or caspase-3 (1:1,000) (both from Abcam, Cambridge, USA) at 4˚C overnight. Immunoreactive bands were detected using horseradish peroxidase (HRP)-conjugated secondary antibodies (1:20,000; Boster, Wuhan, China) with the Western Lightning Chemiluminescence Plus reagent (Perkin-Elmer Life Sciences, Boston, MA, USA). GAPDH was selected as the reference protein.

Dual-luciferase reporter assay. Cells were co-transfected with psiCHECK2-IGFBP3 3'-UTR or psiCHECK2-IGFBP3 3'-UTR mutant and miR-196b/miR-1290 mimics. Cells were lysed and the firefly luciferase activity was detected. *Renilla* luciferase activity was used for normalization. The lysate was detected using Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) with a luminometer (Turner Designs, Sunnyvale, CA, USA).

Immunohistochemistry. Specimens were embedded in paraffin and a rotary microtome was used (HM355; Microm, Walldorf, Germany) to prepare serial sections with 3-μm thickness. Some sections were stained with hematoxylin and eosin (H&E) according to the manufacturer's protocol (Sigma-Aldrich). Before immunostaining, antigen retrieval was carried out via the treatment of 0.1% pepsin with 10 mM HCl at 37˚C for 10 min. The slides were incubated with the monoclonal mouse anti-human IGFBP3 (1:500; Sigma-Aldrich), and then anti-mouse IgG conjugated to HRP (Santa Cruz Biotechnology) for immunohistochemistry. The slides were exoposed to diaminobenzidine for 5 min and counterstained with hematoxylin (both from Sigma-Aldrich). A microscope (Olympus) was used to obtain the images.

Short interfering (si)RNA transfection. Synthetic IGFBP3 siRNA (20 ng) (Ambion, Austin, TX, USA) and the respective negative control were delivered into TALL-104 or SUP-B15 cells using Lipofectamine™ RNAiMAX (Life Technologies Corp., Carlsbad, CA, USA). Briefly, the cells were seeded into 6-well plates at 30% confluency. On the following day, IGFBP3 siRNA and the negative control were diluted in serum-free medium, and incubated with Lipofectamine™ RNAiMAX transfection reagent for 20 min at room temperature. The plates were gently swirled when adding the transfection complexes to the cell cultures. Fresh media were used to replace the culture media after 6 h and then the cells were incubated for 48 h.

Statistical analysis. All data are expressed as the mean ± SD. Student's t-test was used to evaluate the differences between two groups. For multiple comparisons, statistically significant differences were assessed via one-way ANOVA. P-value <0.05 was considered to indicate a statistically significant.

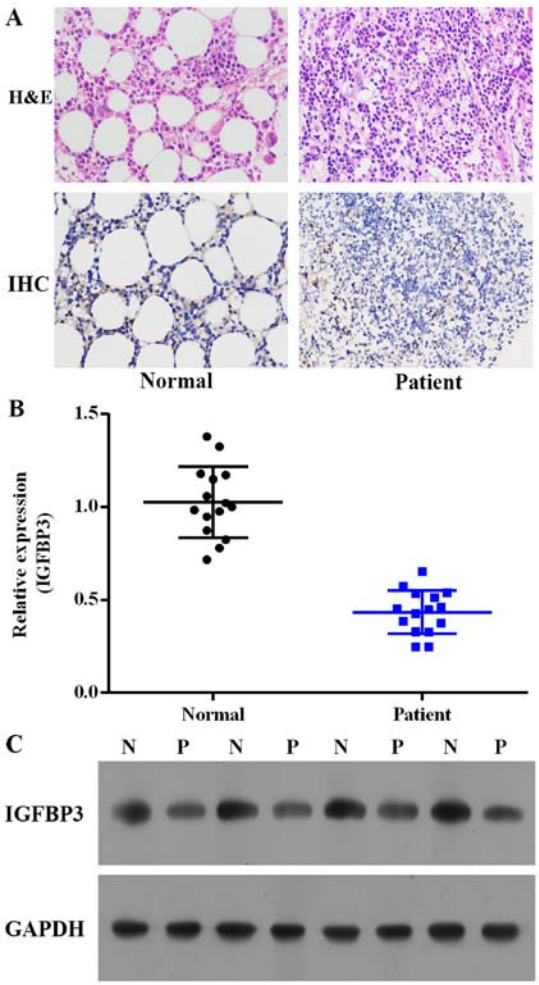

Figure 1. IGFBP3 expression is decreased in ALL patient samples. (A) Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) of bone marrow. The protein expression of IGFBP3 in samples from ALL patients (patient) was notably decreased compared with samples from the healthy volunteers (normal). (B) The mRNA expression levels of IGFBP3 in 15 pairs of peripheral blood samples from ALL patients and healthy volunteers were detected by qRT-PCR. (C) The protein expression of IGFBP3 was detected in ALL patients (P) or the healthy volunteers (N) by western blotting.

Results

IGFBP3 expression is decreased in ALL patients. To explore the role of IGFBP3 in ALL, we initially examined the protein expression of IGFBP3 in 15 pairs of bone marrow from ALL patients and the healthy volunteers by immunohistochemistry. As depicted in Fig. 1A, the IGFBP3 expression in ALL patients was notably decreased compared with the level in the healthy volunteers. We further investigated the expression levels of IGFBP3 mRNA and protein in peripheral blood from the ALL patients and the healthy volunteers. As depicted in Fig. 1B and C, the mRNA and protein expression levels of IGFBP3 were decreased in the ALL patients compared with these levels in the healthy volunteers.

Resveratrol exerts an antitumor effect by the regulation of miR-196b/miR-1290 in ALL cells. Resveratrol

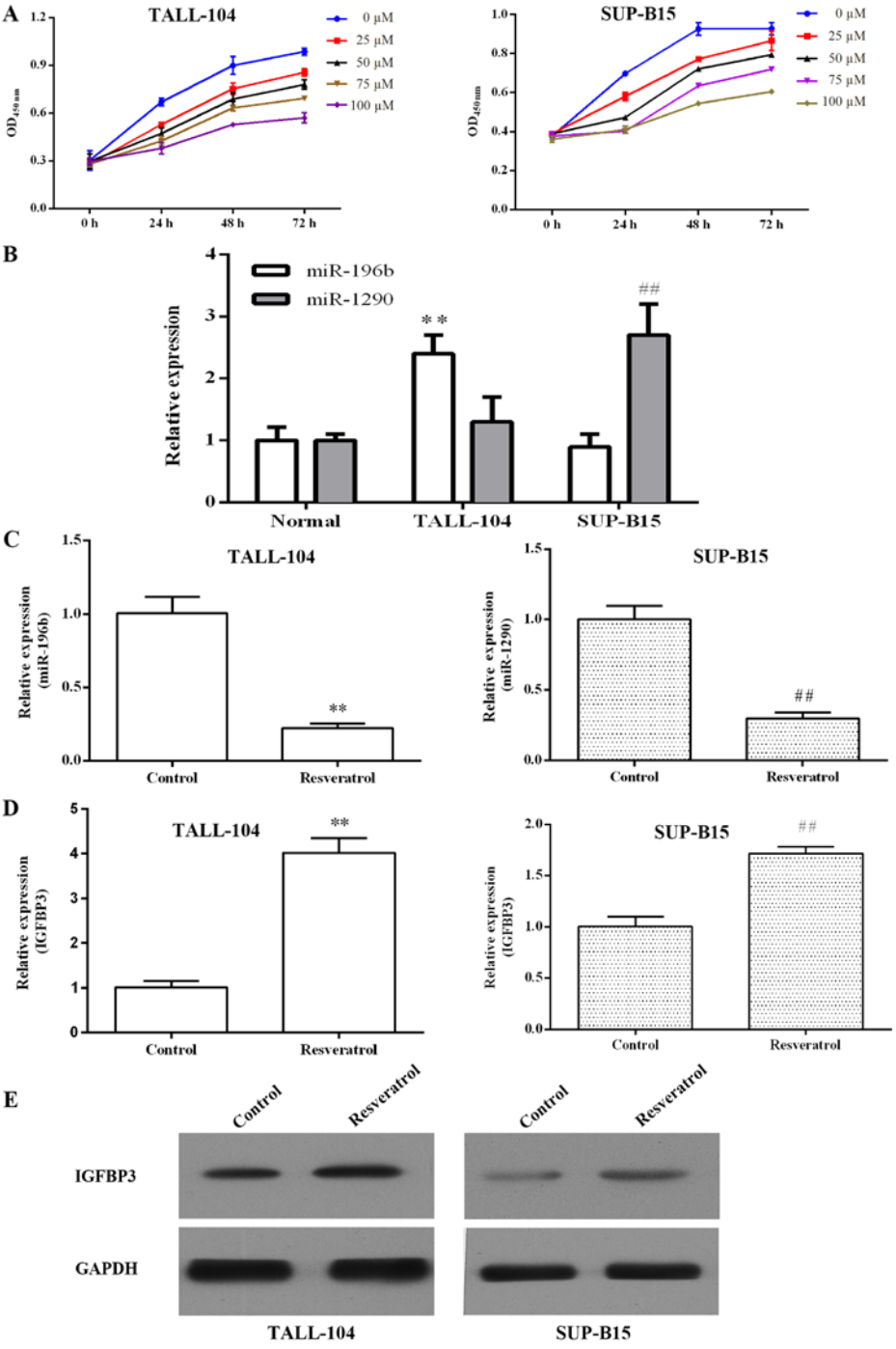

Figure 2. Resveratrol exerts an antiproliferation effect by regulating miR-196b/miR-1290 in ALL cells. (A) Effect of resveratrol on the proliferation of TALL-104 and SUP-B15 cells. Cells were incubated overnight, exposed to resveratrol (25, 50, 75 and 100 μM) and then cultured for 0, 24, 48 and 72 h. Inhibition of proliferation was measured via the CCK-8 assay. (B) PBMCs (normal), TALL-104 or SUP-B15 cells were collected and the relative expression of miR-196b or miR-1290 was quantitated by qRT-PCR. (C) TALL-104 and SUP-B15 cells were treated with vehicle (control) or resveratrol at 75 μM for 48 h. Total RNA was extracted and the relative expression of miR-196b or miR-1290 was quantitated by qRT-PCR. (D) The mRNA expression of IGFBP3 in TALL-104 or SUP-B15 cells after the treatment of vehicle (control) or 75 μM resveratrol for 48 h. (E) The protein expression of IGFBP3 in TALL-104 or SUP-B15 cells after the treatment of vehicle (control) or 75 μM resveratrol for 48 h was detected by western blotting. The results are expressed as the mean ± SD of three independent experiments; *P<0.01 and $^{\#\#}$P<0.01, statistically significant difference from the normal/control group.

dose- and time-dependently inhibited the proliferation of TALL-104 and SUP-B15 cells (Fig. 2A). Previous miRNA microarray profiling indicated that miR-196b was upregulated in T-ALL and miR-1290 was upregulated in B-ALL. As shown in Fig. 2B, we confirmed that the miR-196b expression level was significantly increased in TALL-104 cells compared with the level in the PBMCs (P<0.01), and miR-1290 was overexpressed in the SUP-B15 cells (P<0.01). qRT-PCR was performed to investigate whether resveratrol regulates miR-196b/miR-1290 in ALL cells. As shown in Fig. 2C, resveratrol markedly inhibited miR-196b/miR-1290 expression in TALL-104/SUP-B15 cells, respectively. Furthermore, we found that resveratrol elevated IGFBP3 mRNA and protein expression in both TALL-104 and SUP-B15 cells (Fig. 2D and E).

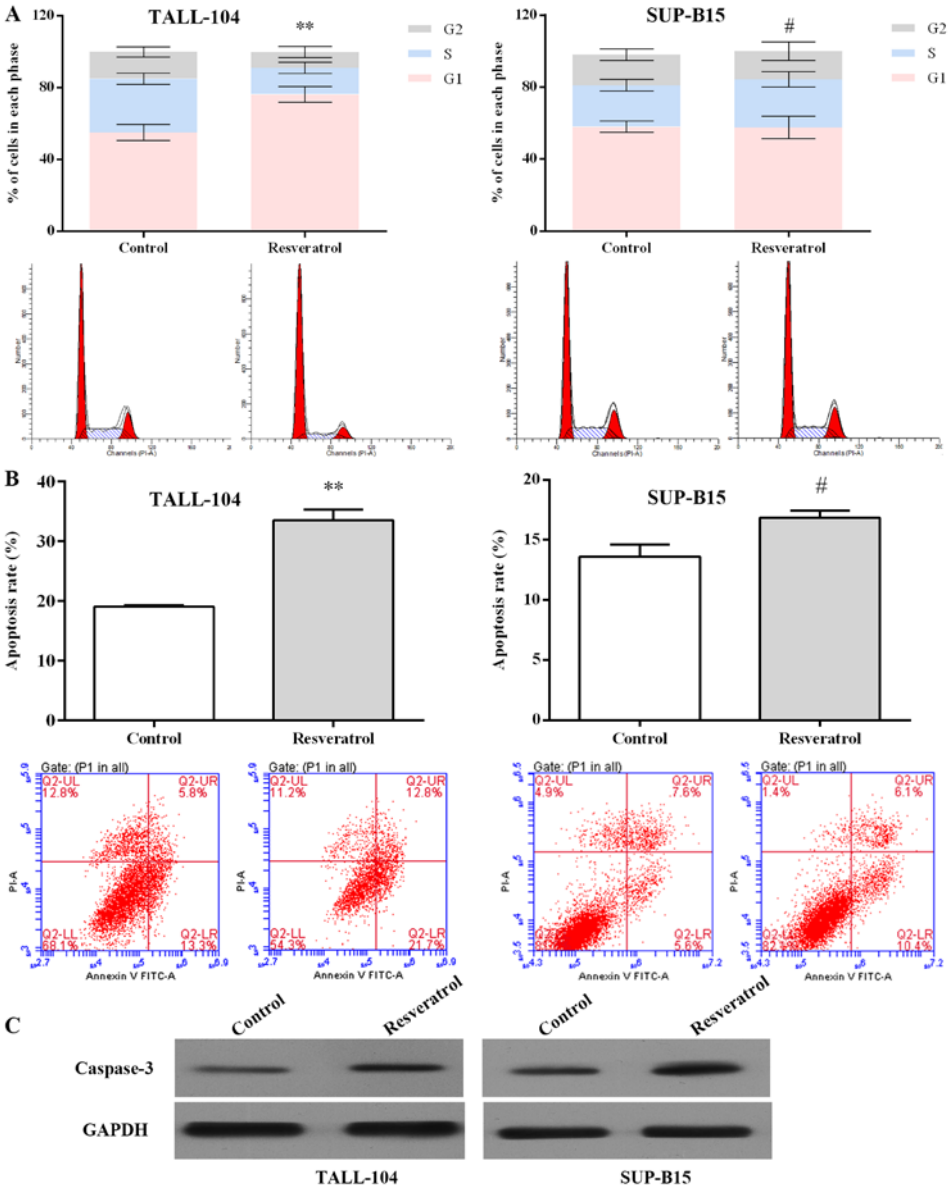

Figure 3. Resveratrol induces cell cycle arrest and apoptosis in ALL cells. Cells were treated with vehicle (control) or 75 μM resveratrol for 48 h. (A) Resveratrol treatment caused cell cycle arrest at the G1 phase in TALL-104 cells and S phase in SUP-B15 cells, as analyzed for propidium iodide stained-DNA content via flow cytometry. (B) Resveratrol treatment significantly increased the apoptosis of both TALL-104 and SUP-B15 cells, as determined by Annexin V-FITC staining using flow cytometry. (C) Resveratrol elevated the protein expression of caspase-3 in both TALL-104 and SUP-B15 cells, as detected by western blotting. Data in the bar graphs are expressed as mean ± SD; n=3; **P<0.01, $^{\#}$P<0.05.

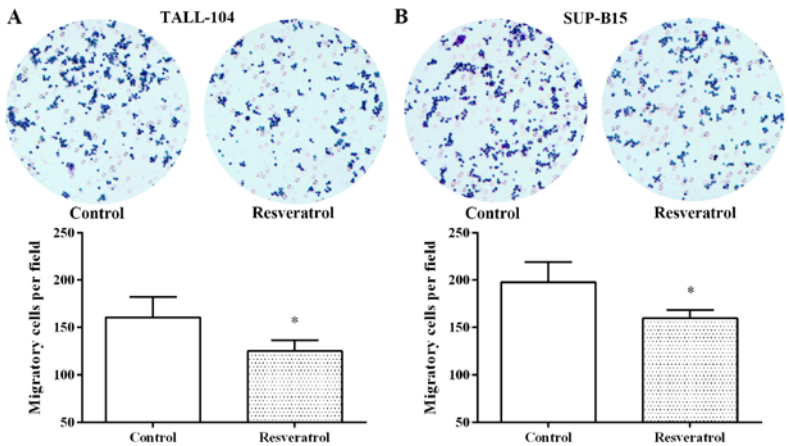

Figure 4. Resveratrol suppresses the migration capability of ALL cells. Cells were treated with vehicle (control) or 75 μM resveratrol for 48 h. Resveratrol notably inhibited cell migration in both the (A) TALL-104 and (B) SUP-B15 cells, as examined by Transwell assay. Data in the bar graphs are expressed as mean ± SD; n=3; *P<0.05.

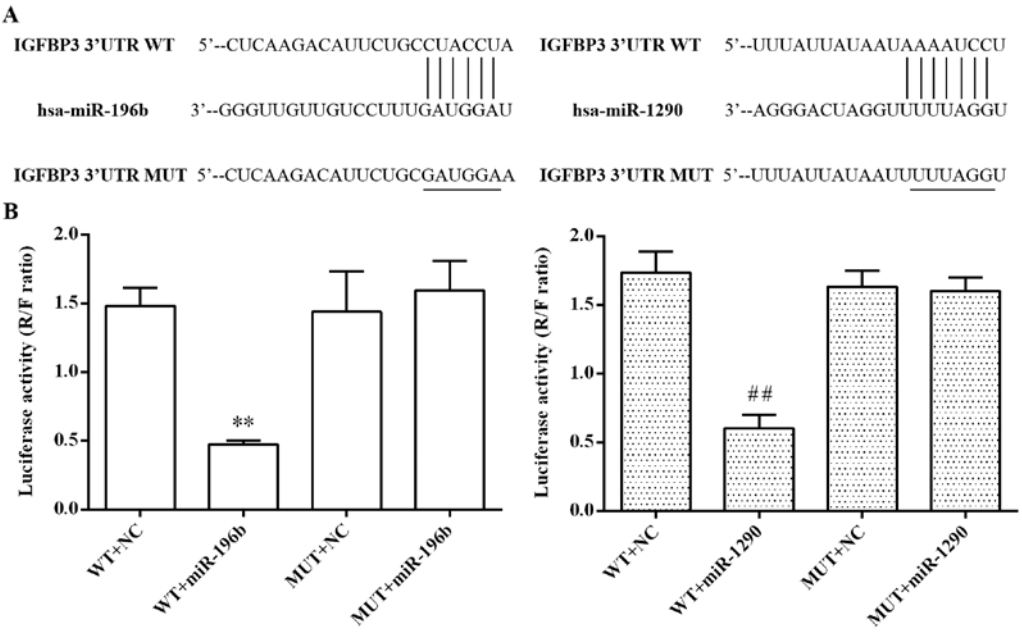

Figure 5. miR-196b and miR-1290 directly target IGFBP3 in ALL cells. (A) The putative binding sites of miR-196b and miR-1290 within the IGFBP3 3'-UTR WT (wild-type) are indicated. (B) Relative luciferase activity of the indicated IGFBP3 reporter constructs. The luciferase activity was suppressed in the cells co-transfected with IGFBP3-WT and miR-196b/miR-1290. Each value represents the mean ± SD; n=3. (**) and (##) indicate statistically significant difference (P<0.01) from the WT + NC (IGFBP3-WT, miR-196b/miR-1290) group.

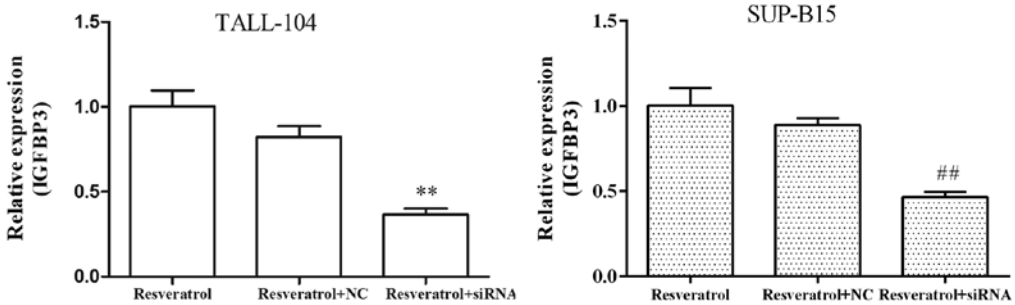

Figure 6. IGFBP3 siRNA reduces the upregulation of IGFBP3 expression by resveratrol. TALL-104 and SUP-B15 cells were transfected with either scramble (NC) or IGFBP3 siRNA and then exposed to 75 μM resveratrol for 48 h. mRNA expression level of IGFBP3 was detected by qRT-PCR. Each point represents the mean ± SD; n=3; *P<0.01 and ##P<0.01, statistically significant difference from the resveratrol + NC group.

As an miR-196b/miR-1290 inhibitor, resveratrol was further examined in regards to its antitumor effect. As displayed in Fig. 3A, resveratrol arrested the cell cycle at the G1 phase in TALL-104 cells (P<0.01), and arrested the cell cycle at S phase in SUP-B15 cells (P<0.05). Resveratrol increased the apoptotic rate in the TALL-104 and SUP-B15 cells notably when compared with the rate in the control group (Fig. 3B). Activation of caspase-3 is significant in apoptosis (26). As shown in Fig. 3C, resveratrol markedly upregulated the caspase-3 expression in both TALL-104 and SUP-B15 cells. Furthermore, resveratrol also notably inhibited cell migration in the TALL-104 and SUP-B15 cells (Fig. 4). These findings suggest that resveratrol exerted an anti-ALL effect by regulating miR-196b/miR-1290.

Both miR-196b and miR-1290 target IGFBP3 in ALL cells. As shown in Fig. 5A, the predicted binding sites of miR-196b and miR-1290 within the 3'-UTR of the IGFBP3 gene are indicated. To confirm their relationship, we further performed dual-luciferase reporter assay. As shown in Fig. 5B, the relative luciferase activity was markedly decreased after co-transfection with the wild-type 3'-UTR of IGFBP3 and miR-196b or miR-1290 in 293T cells (P<0.01, respectively), while the mutant 3'-UTR of IGFBP3 showed slight inhibitory function on the luciferase activity, suggesting that both miR-196b and miR-1290 suppressed the transcription activity of the IGFBP3 gene by directly targeting the binding site in the 3'-UTR of IGFBP3 mRNA.

siRNA against IGFBP3 attenuates the antitumor effect of resveratrol on ALL cells. Resveratrol markedly upregulated the expression levels of IGFBP3 in both TALL-104 and SUP-B15 cells (Fig. 2D and E), indicating that resveratrol exhibited a common response in the different types of ALL

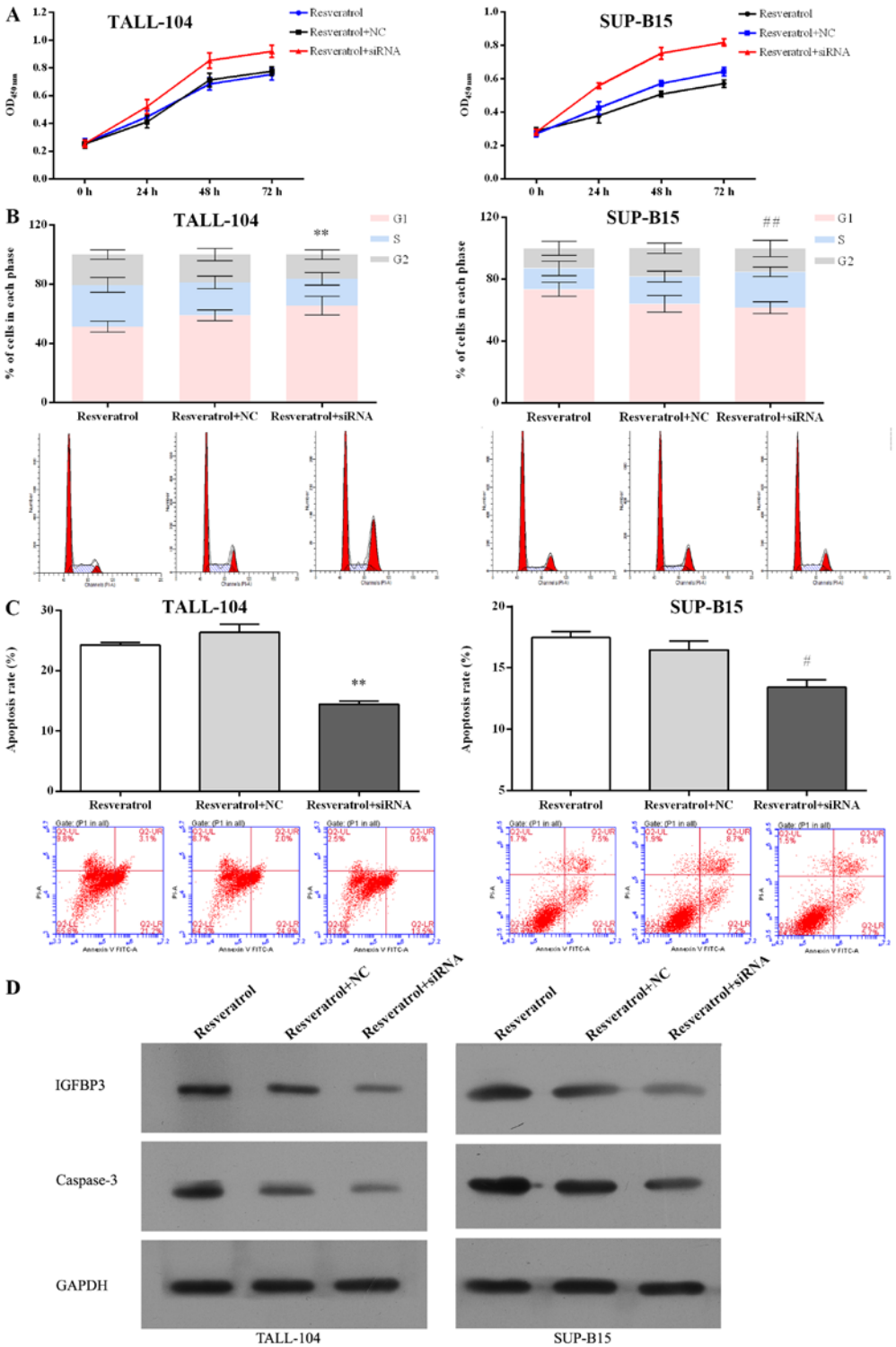

Figure 7. Downregulation of IGFBP3 attenuates the antitumor effect of resveratrol in ALL cells. TALL-104 and SUP-B15 cells were transfected with either scramble (NC) or IGFBP3 siRNA. (A) Cells were exposed to 75 μM resveratrol for 0, 24, 48 or 72 h. Cell viability was evaluated via the CCK-8 assay. (B) Cells were exposed to 75 μM resveratrol for 48 h. The cell cycle progression was analyzed for propidium iodide stained-DNA content via flow cytometry. (C) Cell apoptosis assay was carried out by Annexin V-FITC staining using flow cytometry. (D) The protein expression of IGFBP3 and caspase-3 was detected by western blotting. Data in the bar graphs are expressed as mean ± SD; n=3; **P<0.01, $^{\#\#}$P<0.01, $^{\#}$P<0.05 vs. the resveratrol + NC group.

cell lines. To determine the role of miR-196b/miR-1290 in the antitumor efficacy of resveratrol against ALL, we first transfected both TALL-104 and SUP-B15 cells with IGFBP3 siRNA, and then examined whether IGFBP3 siRNA affects the antitumor actions of resveratrol in ALL cells. As shown in Fig. 6, targeting IGFBP3 by siRNA resulted in marked attenuation of the absolute induction of mRNA expression levels of IGFBP3 observed following treatment of resveratrol. Furthermore, IGFBP3 siRNA blocked the inhibitory effect pf proliferation mediated by resveratrol (Fig. 7A) in both TALL-104 and SUP-B15 cells. IGFBP3 siRNA also attenuated the ability of resveratrol to induce cell cycle arrest (Fig. 7B)

and cell apoptosis (Fig. 7C and D) in both TALL-104 and SUP-B15 cells. These data indicate that downregulation of IGFBP3 attenuated the anti-ALL effect of resveratrol, suggesting that miR-196b/miR-1290 play a pivotal role in the antitumor effect of resveratrol in ALL cells.

Discussion

Accumulating evidence suggests that miRNAs may function as oncogenes or tumor suppressors in human cancer development (27,28). In acute lymphoblastic leukemia (ALL), different miRNAs have been reported to play critical roles in T-ALL and B-ALL (29). For example, miRNA-193b-3p was reported to be a potential tumor-suppressor in T-ALL (30) and miRNA-17-92 was found to play a critical role in B-ALL (31). In the present study, we identified that miR-196b and miR-1290 were overexpressed in T-ALL and B-ALL cells, respectively. However, the function of the two cellular miRNAs in ALL and their potential contribution to ALL therapy are still not well clarified.

It is well known that miRNAs function by regulating the expression of complementary genes. We hypothesized whether there is a key target co-regulated by the different miRNAs in T-ALL and B-ALL. We found various studies concerning IGFBP3, which is downregulated and acts as a key target in ALL (7,32). In the present study, we validated that the expression of IGFBP3 was decreased in both bone marrow and peripheral blood of the 15 ALL patients, which was in accordance with previous studies. Then, we further explored whether IGFBP3 can be co-regulated by different miRNAs and its role in T-ALL and B-ALL. The results indicate that both miR-196b and miR-1290 directly bind to the 3'-UTR of IGFBP3, suggesting the negative regulation of IGFBP3 expression in T-ALL and B-ALL cells by miR-196b and miR-1290, respectively.

Resveratrol has been reported to possess antitumor effects via regulation of specific miRNAs and alteration of the crucial gene expression they target in colorectal (33), pancreatic (27) and bladder cancer (34), and glioma (15). However, the regulation of miRNAs by resveratrol in ALL warrants further investigation. We initially used two ALL cell lines: T-ALL TALL-104 and B-ALL SUP-B15 to examine the potential antiproliferation effect of resveratrol. Resveratrol exhibited similar antiproliferative activities in TALL-104 and SUP-B15 cells. Moreover, resveratrol markedly decreased the overexpression of miR-196b and miR-1290. Numerous published studies in recent years have demonstrated the fundamental roles of miRNAs in carcinogenesis, cell proliferation, migration, invasion and apoptosis (35). As an miR-196b/miR-1290 inhibitor, resveratrol was further found to induce cell cycle arrest, apoptosis, and inhibit migration in ALL cells. The data suggest that miR-196b and miR-1290 may participate in the anti-ALL effect of resveratrol, which needs more confirmation.

By applying IGFBP3 siRNA, we found that knockdown of IGFBP3 reversed the antiproliferation, cell cycle arrest, apoptosis induction abilities of resveratrol in both T-ALL and B-ALL cells. According to the pieces of evidence, we conclude that resveratrol exhibits anticancer activity in T-ALL and B-ALL by targeting miR-196b and miR-1290, respectively. However, it should be noted that each miRNA targets a diversity of genes. Li *et al* reported that miR-196b directly targets both FAS tumor-suppressor and HOXA9/MEIS1 oncogenes in MLL-rearranged leukemia (36). Endo *et al* revealed that miR-1290 decreased the expression of forkhead box A1 and N-acetyltransferase-1 in ER-positive breast cancer (37). The complex functions of miR-196b (36,38) and miR-1290 (37,39) in tumors indicate that resveratrol may exert antitumor activity against other cancers as an miR-196b/miR-1290 inhibitor and the modulating mechanism of miR-196b/miR-1290 in ALL warrants further exploration.

In summary, miR-196b and miR-1290 were identified as new targets of resveratrol. miR-196b and miR-1290 mediated the inhibition of T-ALL and B-ALL cell growth, survival and migration achieved by resveratrol. The findings also support that both miR-196b and miR-1290 target the IGFBP3 3'-UTR and may be potential therapeutic targets for ALL.

Acknowledgements

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