# Suppression of HSP27 increases the anti-tumor effects of quercetin in human leukemia U937 cells

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**Abstract.** Quercetin, a natural flavonoid, inhibits the growth of leukemia cells and induces apoptosis. Heat shock protein 27 (HSP27) has been reported to promote the development of leukemia by protecting tumor cells from apoptosis through various mechanisms. The present study investigated the effects of small hairpin (sh)RNA-mediated HSP27 knockdown on the anti-cancer effects of quercetin in U937 human leukemia cells. Cells were transfected with recombinant lentiviral vector pCMV-G-NR-U6-shHSP27 (shHSP27), which expressed shRNA specifically targeting the HSP27 gene, alone or in combination with quercetin. The results showed that shHSP27 and quercetin synergistically inhibited U937 cell proliferation and induced apoptosis by decreasing the Bcl2-to-Bax ratio. Furthermore, this combined treatment significantly suppressed the infiltration of tumor cells and the expression of angiogenesis-associated proteins HIF1α and VEGF. Compared with shHSP27 or quercetin alone, shHSP27 plus quercetin markedly decreased the protein expression of cyclinD1 and thus blocked the cell cycle at G1 phase. The Notch/AKT/mTOR signaling pathway is important in tumor aggressiveness; quercetin plus shHSP27 significantly decreased Notch 1 expression and the phosphorylation levels of the downstream signaling proteins AKT and mTOR. The inhibitory effects of quercetin plus shHSP27 on this pathway may thus have been responsible for the cell cycle arrest, inhibition of proliferations and infiltration as well as enhancement of apoptosis. Therefore, these findings collectively suggested that suppression of HSP27 expression amplified the anti-cancer effects of quercetin in U937 human leukemia cells, and that quercetin in combination with shHSP27 represents a promising therapeutic strategy for human leukemia.

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*Key words:* quercetin, heat shock protein 27, AKT/mammalian target of rapamycin, leukemia

## Introduction

Medicinal herbs are a tremendous source of natural products with anti-cancer activity. Administration of natural products has become an integral part in the prevention and treatment of cancer. These phytochemicals provide potential novel leads for the development of anti-angiogenic drugs (1,2).

Quercetin (3,3',4',5,7-pentahydroxy-flavone) is a polyphenolic substance, which has been shown to have beneficial biological properties due to its anti-oxidative activity as well as the capacity to modify eicosanoid biosynthesis, prevent atherosclerotic plaque formation and platelet aggregation, and to have relaxant effects on cardiovascular smooth muscles (3-5). Of note, quercetin has been demonstrated to induce apoptosis in a variety of tumor cell types, while preventing the apoptosis of certain non-tumorous cell types (6,7). Furthermore, quercetin inhibits angiogenesis of tumors as well as the expression of enzymes that activate carcinogens (2,8). In particular, quercetin has been indicated to inhibit the growth and to induce apoptosis of human leukemia cells by inhibiting the Wnt protein β-catenin (9,10). Leukemia is among the most common types of cancer worldwide; furthermore, its prevalence has been increasing due to the extended human life, radiation (11,12) and pollution (13,14), as a result of the decrease in the incidence of infectious diseases in developed countries. The incidence of leukemia is higher in developed countries than in developing countries (15). In developing countries, the use of natural plant extracts as remedies against leukemia is common (15,16).

Heat shock proteins (HSPs) are a class of functionally associated proteins with regulatory roles comprising protein synthesis and degradation, prevention of stress-associated injury, apoptosis and the generation of immune responses (17,18). Five types of HSP have been identified in mammalian cells, among which HSP27 has the important biological function of protecting cells from damage arising from various stress factors with phosphorylated HSP27 being considered as a potential diagnostic marker for cancer (19). In leukemia cells, HSP27 is closely associated with proliferation, differentiation and heat resistance (20). Overexpression of

HSP27 increases the tumorigenicity and protects malignant cells against apoptotic cell death through several mechanisms (21-24).

Previous studies have reported that quercetin down-regulates the mRNA and protein expression of HSP27 and increases the sensitivity of tumors to hyperthermia (25-27); however, to the best of our knowledge, the anti-cancer effects of quercetin together with HSP27 interference have not been studied in leukemia cells. The present study aimed to investigate the effects of small hairpin (sh)RNA with specificity against HSP27 (shHSP27) on the anti-tumor effects of quercetin, including the inhibition of cell proliferation and adhesion, induction of apoptosis and cell cycle arrest, in U937 human leukemia cells.

The mammalian target of rapamycin (mTOR) is a central regulator of cell growth, proliferation, differentiation and survival. Studies have shown that mTOR is frequently hyper-activated in cancer and is a crucial regulator of cancer cell motility, invasion and metastasis (28,29). Quercetin has been found to inhibit mTOR signaling during cancer treatment and prevention (30,31). Therefore, the present study also evaluated the regulatory activity of quercetin plus shHSP27 on mTOR signaling in human leukemia U937 cells.

## Materials and methods

*Cell culture and reagents*. The U937 human leukemia cell line was purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI-1640 medium (Hyclone Corp., Logan, UT, USA), supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Quercetin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide to a concentration of 50 $\mu$M and stored at -20˚C for use. A Cell Counting Kit 8 (CCK-8) was obtained from JRDUN Biotechnology Co. Ltd (Shanghai, China). An Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit and Matrigel were purchased from BD Biosciences (Franklin Lakes, NJ, USA).

*Lentiviral vector construction*. For knockdown of HSP27 expression, the pCMV-G-NR-U6-shRNA vector (Genechem, Shanghai, China) was used. Three sequences of the human HSP27 gene *HSPB1* (GenBank accession no. NM001540) were selected as targets for RNA interference: shHSP27-1 (start, 585 bp); 5'-GCTGCAAAATCCGATGAGA-3'; shHSP27-2 (start, 293 bp), 5'-CCTGGATGTCAACCACTTC-3'; and shHSP27-3 (start, 322 bp), 5'-AGCTGACGGTCAAGA CCAA-3'. In all subsequent experiments, shHSP27-3 was used if not indicated otherwise.

For construction of the lentiviruses, 293T cells (Enzyme-Linked Biological Technology Co., Ltd., Shanghai, China) were transfected using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific) with plasmids expressing retroviral proteins Gag-Pol and VSV-G (Addgene, Cambridge, MA, USA). At 48 h after transfection, supernatants containing the retrovirus were collected and frozen at -70˚C until use.

*RNA quantification by reverse-transcription quantitative polymerase chain reaction (RT-qPCR)*. The expression of HSP27 in U937 cells was quantified by real-time PCR. Total RNA was extracted using the TRIzol reagent (Invitrogen) and 1 $\mu$g total RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase in the presence of oligo (dT)12-18. RT-PCR was performed in triplicate with SYBR Green master mix (Toyobo, Osaka, Japan) for 10 min at 95˚C for initial denaturation, followed by 40 cycles of 95˚C for 15 sec, 58˚C for 30 sec and 72˚C for 30 sec in the StepOne™ Real-Time PCR System (Genuine Biosystem, Chennai, India). The following primers (Generay Biotech Co., Ltd., Shanghai, China) were used: HSP27 forward, 5'-CCAGAGCAGAGT CAGCCAGCAT-3' and reverse, 5'-CGAAGGTGACTGGG ATGGTGA-3'; GAPDH forward, 5'-ACCACAGTCCATGCC ATCAC-3' and reverse, 5'-TCCACCACCCTGTTGCTGTA-3'. GAPDH was used as a reference gene.

*Cell proliferation assay*. U937 cells were seeded in triplicate into 96-well plates at a density of $2x10^4$ cells/well. Cells were treated with medium (control group), empty vector (50 $\mu$M/l), shHSP27 vector (50 $\mu$M/l), quercetin (50 $\mu$M/l), empty vector plus quercetin or shHSP27 vector plus quercetin. Following incubation at 37˚C for 0, 24 or 48 h, 10 $\mu$l CCK-8 solution was added to each well and subsequent to further incubation for 3 h, the absorbance was detected at a wavelength of 450 nm. The cell proliferation rate (relative to control) of the five treated groups was calculated as follows: Proliferation rate (relative to control) = (absorbance of experimental group/absorbance of control group) x100%. All experiments were performed in triplicate.

*Flow cytometry*. U937 cells at $2x10^6$ per well were seeded into a six-well plate and treated with medium, empty vector, shHSP27 vector, quercetin, empty vector + quercetin or shHSP27 vector + quercetin and then cultured for 48 h. Cells were then collected, washed twice with phosphate-buffered saline (PBS) and prepared for analysis of apoptosis and the cell cycle. For the apoptosis assay, cell suspensions were adjusted to a density of $1x10^6$/ml with binding buffer (Thermo Fisher Scientific, Inc.). Annexin V-FITC and propidium iodide (PI) were added to the cell suspension according to the manufacturer's instructions and apoptosis was detected using a BD C6 flow cytometer (BD Biosciences). For cell cycle analysis, the cells were fixed in 2 ml pre-cooled 70% ethanol at 4˚C overnight. The cells were washed twice, stained with 400 $\mu$l PI (50 $\mu$g/ml) and 50 $\mu$g/ml RNase, and then subjected to flow cytometric analysis.

*Cell adhesion assay*. An adhesive artificial basement membrane was prepared by adding a mixture of serum-free RPMI-1640 medium and Matrigel at a density of 100 $\mu$g/2,500 $\mu$l into a 12-well plate at 250 $\mu$l/well and drying overnight. U937 cells were seeded into the 12-well plate at a density of $2x10^4$ cells/well and treated with the respective vectors and/or quercetin as described above. Following 48 h of incubation at 37˚C, the culture medium was discarded and non-adherent cells were removed by rinsing with PBS. Adherent cells were then fixed with methanol for 15 min at 37˚C and then stained with crystal violet (JRDUN Biotechnology Co., Ltd., Shanghai, China) for 20 min. The numbers of adherent cells were counted under a microscope (BX51; Olympus, Tokyo, Japan).

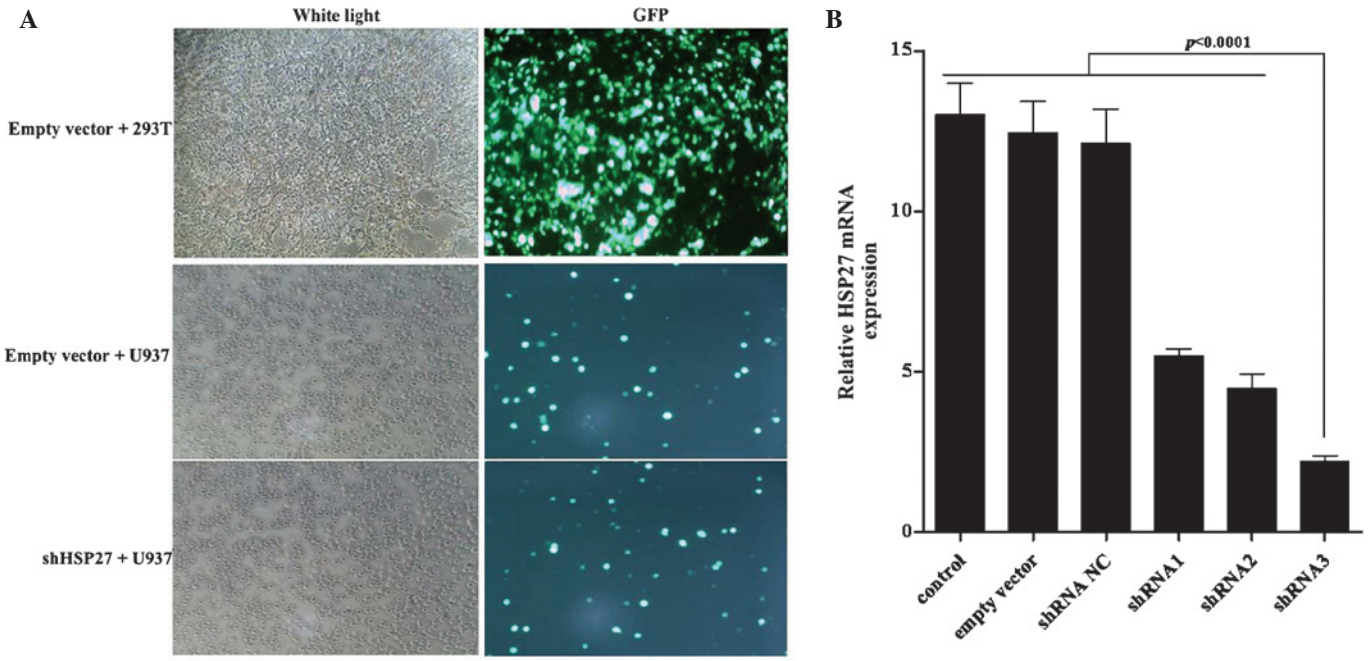


Figure 1. Assessment of HSP27 knockdown in U937 cells. (A) GFP was detected in cells to assess the transfection efficiency (magnification, x100). (B) Relative mRNA expression of HSP27 was analyzed. Values are expressed as the mean ± standard deviation. P<0.0001 vs. the control group. shRNA, small hairpin RNA; NC, negative control; HSP, heat shock protein; GFP, green fluorescent protein.

*Western blot analysis*. Cells were lysed using radioimmunoprecipitation assay lysis buffer (Keygentec, Nanjing, China) to extract total proteins. The protein concentration was determined using a bicinchoninic acid assay (Keygentec). Protein samples (30 $\mu$g) were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat milk at room temperature for 1 h and incubated overnight at 4˚C with the following antibodies: B-cell lymphoma 2 (Bcl-2; cat. no. sc-492; 1:100) and Bcl-2-associated X protein (Bax; cat. no. sc492; 1:150), from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); cyclin B1 (cat. no. 12231; 1:1,000), cyclin D1 (cat. no. 2978; 1:1,000), AKT (cat. no. 9272S; 1:1,000), phosphorylated (p)-AKT (cat. no. 4058S; 1:1,000), mammalian target of rapamycin (mTOR; cat. no. 2983; 1:1,000), p-mTOR (cat. no. 5536; 1:800) and hypoxia-inducible factor (HIF)1α (cat. no. 3176; 1:1,000) from Cell Signaling Technology, Inc. (Danvers, MA, USA); vascular endothelial growth factor (VEGF; cat. no. ab46154; 1:1,000) and Notch1 (cat. no. ab52627; 1:1,000), from Abcam (Cambridge, UK); and GAPDH (cat. no. 5471; 1:1,500; Cell Signaling Technology, Inc.). Membranes were washed three times with Tris-HCl (pH 7.6; 20 mM) containing 137 mM NaCl and 0.01% Tween-20 and then incubated with horseradish peroxidase-conjugated goat anti-rabbit/mouse secondary antibody (cat. no. A0208/A0216; 1:1,000; Beyotime Institute of Biotechnology, Inc., Shanghai, China) at 25˚C for 2 h. Membranes were visualized using enhanced chemiluminescence solution (Millipore) and x-ray film. Protein levels were determined by densitometric analysis with normalization to GAPDH.

*Statistical analysis*. Values are expressed as the mean ± standard deviation. All statistical analyses were performed using GraphPad Prism 5.00 software (GraphPad Software, La Jolla, CA, USA). Differences among groups were tested by one-way analysis of variance followed by the Neuman-Keuls post-hoc test. A two-sided P-value <0.05 was considered to indicate a statistically significant difference.

## Results

*shHSP27 decreases HSP27 expression in U937 cells*. Numerous types of cancer cell constitutively express HSP27 at elevated levels, which is associated with drug resistance (32). To inhibit HSP27 expression in U937 human leukemia cells, three pairs of shRNA were designed to specifically target HSP27 and then ligated into the pCMV-G-NR-U6-shRNA vector, which contains a green fluorescent protein (GFP) expression gene for identification. Lentiviruses for the expression of shHSP27 were harvested from the supernatant of 293T packaging cells. U937 cells were transfected with this supernatant containing shHSP27 lentivirus and mRNA expression of HSP27 was detected following 48 h of incubation. As shown in Fig. 1A, a proportion of the U937 cells were successfully transfected with shHSP27-3 lentivirus and HSP27 mRNA expression was considerably decreased by shHSP27-3 (Fig. 1B).

*shHSP27 and quercetin jointly inhibit U937-cell proliferation*. Quercetin has been reported to inhibit the proliferation of human leukemia cell lines, including U937 (33,34). At a concentration of 2 $\mu$M, quercetin inhibited the proliferation of U937 cells by 15% following 24 h of incubation, which was consistent the findings of a previous study (34). When cells were simultaneously treated with shHSP27, cell proliferation further decreased by ~10% (Fig. 2A).

mTOR signaling has been reported to stimulate cellular proliferation and aggressive tumor growth in numerous

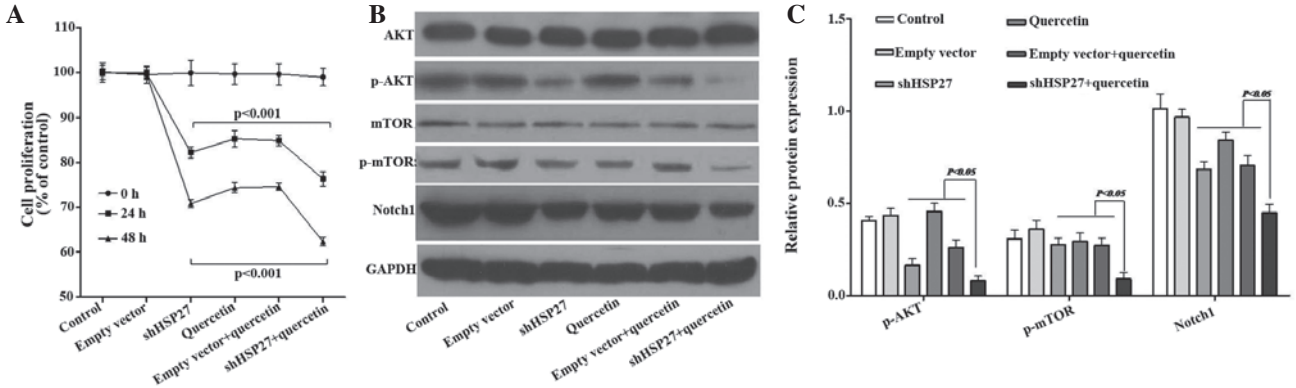


Figure 2. Effect of shHSP27 and quercetin on cell proliferation of U937 cells. (A) Cell viability was determined using a Cell Counting Kit-8 assay. (B) The expression of cell proliferation-associated proteins was assessed using western blot analysis. (C) Values are expressed as the mean ± standard deviation of three experiments. shHSP27, small hairpin RNA specific for heat shock protein 27; p-mTOR, phosphorylated mammalian target of rapamycin.

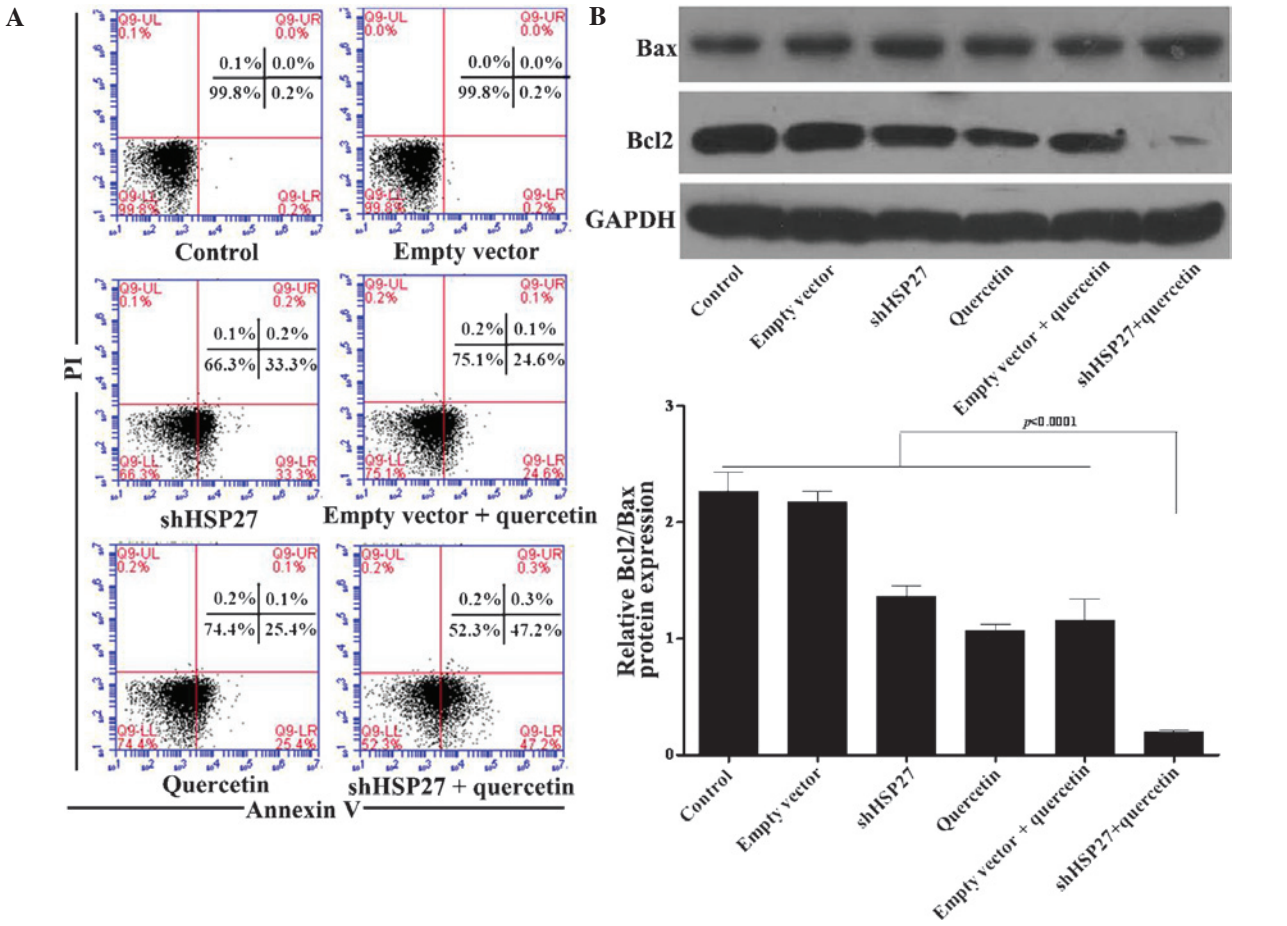


Figure 3. Effect of shHSP27 and quercetin on apoptotic rates were analyzed in U937 cells. (A) The apoptotic rate of U937 cells was analyzed by Annexin V/PI double staining and flow cytometric analysis. Cell populations in the quadrants were defined as follows: LL, viable cells; LR, early apoptotic cells; UR, late apoptotic cells; UL, necrotic cells. (B) Expression of Bcl2 and Bax in the experimental groups was assessed using western blot analysis. A representative blot is shown and the Bcl2/Bax ratio relative to GAPDH was determined by densitometric analysis. Values are expressed as the mean ± standard deviation. P<0.001 vs. the control group. PI, propidium iodide; shHSP27, small hairpin RNA specific for heat shock protein 27; Bcl2, B-cell lymphoma 2; Bax, Bcl2-associated X protein; UL, upper left; LR, lower right.

cancer models (35). To investigate the potential role of mTOR in the mechanism of action of quercetin and shHSP27, the expression of mTOR signaling proteins in U937 cells was assessed following treatment with quercetin and/or shHSP27. Western blot analysis showed that following treatment with quercetin, shHSP27 or their combination, the protein levels of p-AKT and p-mTOR were markedly decreased, while levels of total AKT were not obviously affected and those of total mTOR were only marginally reduced; furthermore, the protein expression of Notch1 was significantly decreased compared with that in the control or empty vector-treated groups (Fig. 2B and C).

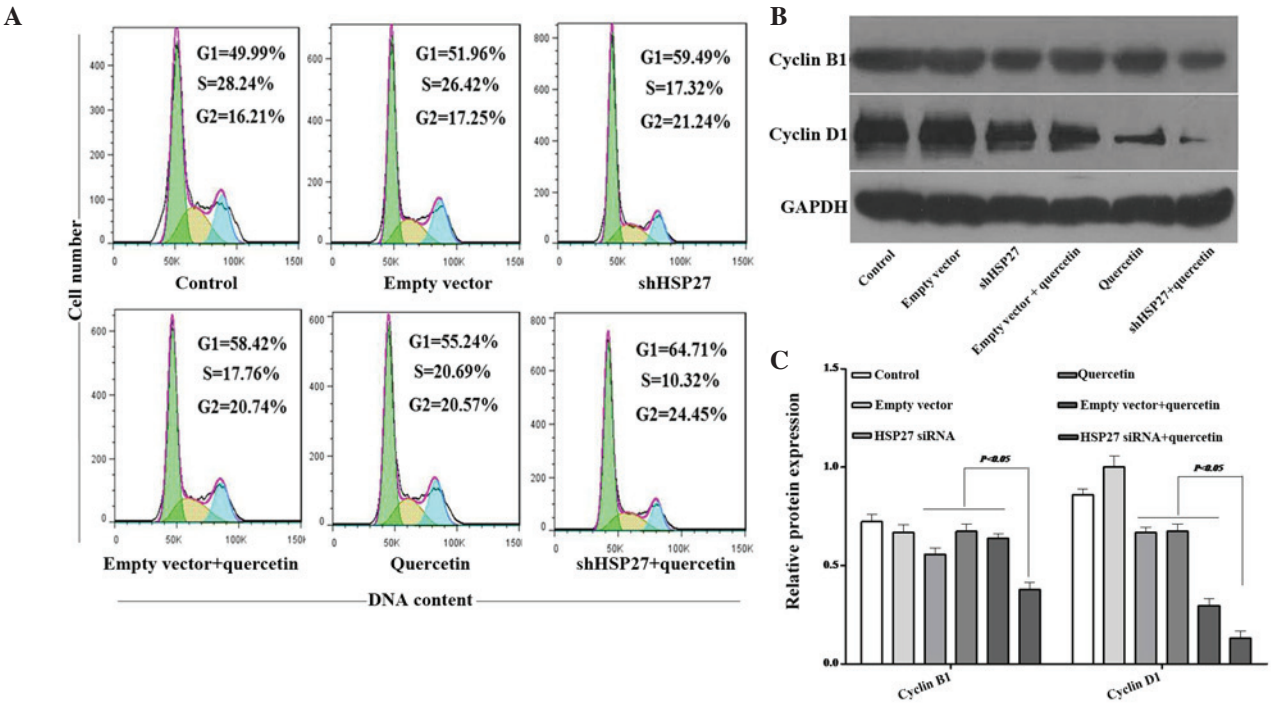


Figure 4. Effects of shHSP27 and quercetin on the cell cycle distribution. U937 cells were treated with quercetin for 24 h. (A) Propidium iodide staining and flow cytometric analysis were performed to determine the percentages of cells in G1, S and G2 phases. Representative images are shown. (B and C) The expression of the cell cycle-associated proteins cyclin B1 and cyclin D1 was assessed by western blot analysis. shHSP27, small hairpin RNA specific for heat shock protein 27.

*HSP27 knockdown enhances quercetin-induced apoptosis of U937 cells*. Following incubation of U937 cells with quercetin or shHSP27 plus quercetin for 48 h, the percentage of apoptotic cells significantly increased from 25.4 to 47.2% (Fig. 3A). To investigate the underlying mechanism of this phenomenon, the protein expression of Bcl-2 and Bax, two genes that regulate cell apoptosis, was assessed. Bcl-2 is known inhibit apoptosis and to increase resistance to various apoptosis-stimulating factors, while not being associated with cell division and proliferation (36). Bax, a homologous gene of the Bcl-2 family, antagonizes the anti-apoptotic effect of the Bcl-2 by forming a heterodimer with Bcl-2. It has been reported that the Bcl-2/Bax ratio is negatively correlated with the apoptotic rate (37). While treatment with quercetin or shHSP7 alone significantly reduced the Bcl-2/Bax ratio, their combination synergistically decreased the Bcl-2/Bax ratio by ~90% and almost totally blocked Bcl-2 expression in U937 cells (Fig. 3B).

*Quecetin plus shHSP27 induces U937-cell accumulation in G1 phase*. Cell proliferation is controlled by the progression of the cell cycle (38). After treatment with quercetin alone or shHSP27 with quercetin for 48 h, the fraction of cells in G1 phase significantly increased from 49.99 to 55.24 and 64.71%, and the fraction of cells in G2 phase significantly increased from 16.21 to 20.57 and 24.45%, however the fraction of cells in S phase decreased from 28.24 to 20.69 and 10.32%, respectively (Fig. 4A). This result indicated that shHSP27 further enhanced the G1-phase arrest mediated by quercetin. Cyclins have an essential role in the regulation of the cell cycle (38). Cyclin B1 was the first cell cycle-associated protein to be identified (39); overexpression of cyclin B1 promotes cell cycle progression to G2/M-phase and may lead to uncontrolled cell proliferation and malignant transformation (40,41). It has been reported that inhibition of cyclin B1 expression decreases the G2/M-phase population, thereby suppressing cell growth and inducing apoptosis (42). Cyclin D1 is closely associated with the proliferation of cancer cells (43). By contrast to cyclin B1, cyclin D1 has an important role in the G1/S-phase transition, which may, however, promote the occurrence of tumors; it is therefore considered to be an oncoprotein (44). Quercetin significantly downregulated the protein expression of cyclin D1; furthermore, when combined with shHSP27, cyclinD1 expression was almost totally diminished. In addition, quercetin and shHSP27 reduced the expression of cyclin B1 (Fig. 4B and C). These results may explain for the reduced cell-cycle progression observed by flow cytometry.

*Suppression of HSP27 enhances quercetin-induced inhibition of U937-cell adhesion*. Adhesion of acute myeloid leukemia cells is linked with resistance to chemotherapy (45,46), and adhesion of U937 cells to fibronectin via β1 integrins has been shown to inhibit etoposide- and mitoxantrone-induced apoptosis (47).

Following treatment with quercetin, the adhesive capacity of U937 cells dropped by ~30%, while simultaneous transfection with shHSP27 vector further reduced cell adhesion to ~80% (Fig. 5A and B). This result indicated that quercetin may serve as an adjuvant for chemotherapeutics.

Besides cellular adhesion, angiogenesis is required for invasive tumor-cell metastasis and represents an important target in the control of cancer progression (48). The HIF1α/VEGF signaling pathway, which regulates angiogenesis (49), is activated by upstream mTOR signaling. As shown in Fig. 5C, shHSP27 and quercetin markedly decreased the protein expression of HIF1α and VEGF in U937 cells.

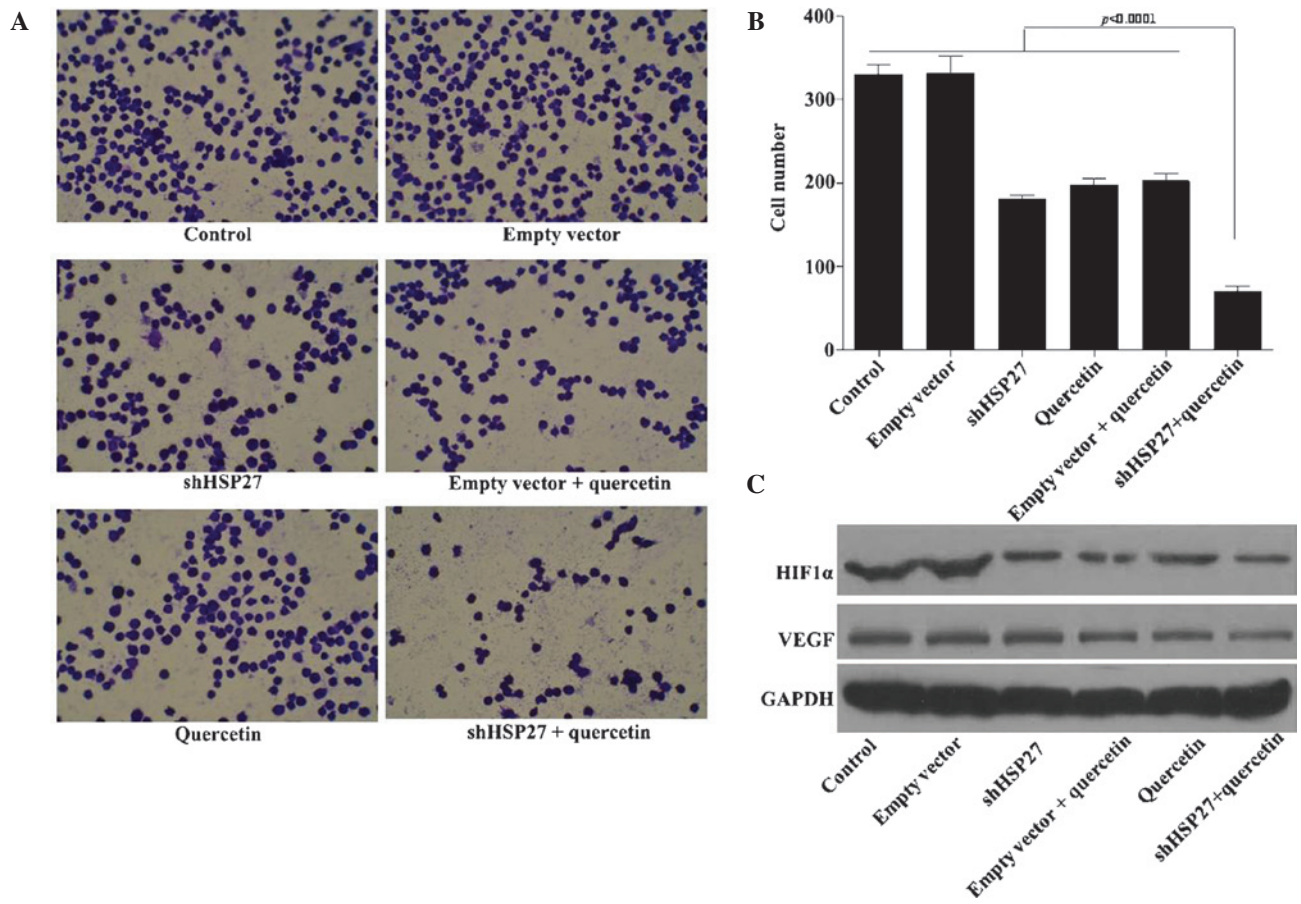


Figure 5. Effect of shHSP27 and quercetin on the adhesive properties of U937 cells. (A) Representative images of U937 cells subjected to the adhesion assay (magnification, x200). (B) The number of adhesive cells in A was quantified. Values are expressed as the mean ± standard deviation of three experiments. (C) The expression of adhesion-associated proteins was determined using western blot analysis. Blots and cell images are representative of three separate experiments. shHSP27, small hairpin RNA specific for heat shock protein 27; VEGF, vascular endothelial growth factor; HIF, hypoxia-inducible factor.

Previous studies have indicated that HIF1α increases the expression of anti-apoptotic Bcl-2 and reduces the expression of Bax (50); furthermore, in ovarian cancer cells, phosphoinositide-3 kinase (PI3K) was reported to mediate G1-to-S-phase progression and cyclin D1 expression through activation of AKT/mTOR/p70S6K1 signaling (51). In line with this, the results of the present study suggested that quercetin suppresses leukemia progression by inhibiting mTOR signaling and that this suppressive function can be further enhanced by shHSP27.

## Discussion

Quercetin a major flavonoid contained in foods including apples, onions, tea, red wine. Several *in vitro* studies have shown that quercetin has activity against certain types of cancer cell (27,52,53). Furthermore, a clinical study on patients with an inherited tendency to develop colorectal cancer found that combined dietary intake of quercetin and curcumin decreased the number and size of pre-cancerous rectal tumors (54). Leukemia is a common condition worldwide and affects all age groups; it is also the most common cancer type in children (55) and adolescents. In recent years, the incidence of leukemia has significantly increased (56).

Studies have found that quercetin inhibits the proliferation and induces apoptosis in human leukemia cells (9,34); however, to the best of our knowledge, the anti-tumor effects of quercetin combined with shHSP27, as well as the underlying molecular mechanisms, have not been reported. The present study evaluated the anti-tumor effects of quercetin on the U937 acute myeloid leukemia cell line with HSP27 knockdown.

The results showed that the time-dependent inhibition of the proliferation of U937 cells by quercetin was enhanced with simultaneous transfection of shHSP27. Furthermore, cell cycle analysis showed that quercetin plus shHSP27 significantly induced the accumulation of U937 cells in G1 phase with a minor increase in G2 phase, which indicated that quercetin plus shHSP27 may inhibit the proliferation of U937 cells by blocking the cell cycle. In addition, the expression of cell cycle-associated proteins cyclin D1 and cyclin B1 in U937 cells treated with quercetin plus shHSP27 was decreased following treatment with quercetin plus shHSP27. Cyclin D1 is mainly involved in G1/S-phase transition (44), while cyclin B1 is associated with progression to G2/M phase. These results indicated that shHSP27 plus quercetin blocked cell cycle progression by inhibiting the expression of the cell-cycle proteins cyclin D1 and -B1.

Evasion of apoptosis is a key factor during carcinogenesis, cancer progression and drug resistance, while induction of apoptosis is a desirable property of anti-cancer treatments (57). The present study found that the percentage

of apoptotic U937 cells significantly increased after treatment with quercetin plus shHSP27. Furthermore, the expression of apoptotic signaling proteins was detected, which revealed that the expression of anti-apoptotic protein Bcl-2 decreased, while that of pro-apoptotic protein Bax in U937 cells increased when treated with shHSP27 and quercetin. This result indicated that shHSP27 plus quercetin induced cell apoptosis by reducing the Bcl-2/Bax ratio in U937 cells.

Cell adhesion has a vital role in tumor metastasis and the adhesion assay performed in the present study revealed a significant inhibitory effect of shHSP27 plus quercetin compared with that of quercetin only. In addition, angiogenesis is associated with the occurrence and prognosis of leukemia (58,59). VEGF, the primary factor stimulating blood-vessel growth, has been confirmed to be elevated in leukemia and is vital for its pathogenesis and progression (60). The present study revealed that quercetin plus shHSP27 inhibited VEGF expression in U937 cells.

The Akt and mTOR (PI3K/Akt/mTOR) signaling network is important in leukemia, where it regulates cell growth and survival (61). Proteins demonstrated to be involved in regulating apoptosis, cell cycle and angiogenesis of U937 cells in the present study have all been confirmed as parts of the mTOR signaling network in other diseases (28,30,35,51,61,62), while they have not been previously determined in leukemia. Levels of p-AKT and p-mTOR were decreased following treatment with shHSP27 plus quercetin; furthermore, Notch1, Bcl-2, cyclin D1, HIF1α and VEGF were decreased. These proteins constitute a huge network regulated by mTOR expression, and mTOR signaling has a critical role in the regulation of tumor-cell motility, invasion and cancer metastasis (63). In order to further determine the therapeutic potential of quercetin plus shHSP27 in leukemia, the effects of quercetin on the mTOR-regulated signaling network require further elucidation.

In conclusion, the present study demonstrated that, compared with quercetin or shHSP27 alone, combined treatment with quercetin plus shHSP27 significantly inhibited the proliferation and adhesion as well as induced apoptosis and cell-cycle arrest in U937 human leukemia cells. These anti-tumor effects may mainly depend on the inhibition of the mTOR signal network. Quercetin plus shHSP27 synergistically decreased the cellular adhesion capacity of leukemia cells and HSP27 knockdown may therefore potentiate the efficiency of chemotherapies of human leukemia.

## Acknowledgements

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