Construction of SET overexpression vector and its effects on the proliferation and apoptosis of 293T cells

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Abstract. The expression of SET nuclear proto-oncogene (SET) is commonly associated with cell proliferation and tumorigenesis. In the present study, a eukaryotic SET expression plasmid (pEGFP-N1-SET) was constructed and transiently transfected into 293T human embryonic kidney cells. Transfection led to expression of the SET oncoprotein at high levels, as indicated by polymerase chain reaction and western blot analysis. In addition, the relative mRNA and protein expression of protein phosphatase 2A in pEGFP-N1-SET-transfected 293T cells was downregulated compared with that in empty vector-transfected cells. Furthermore, overexpression of SET increased the percentage of 293T cells in S and G_2/M phases compared with the control transfectants. An increase in B-cell lymphoma 2 (Bcl-2) and a decrease in Bcl-2-associated X (Bax) protein expression was observed in the pEGFP-N1-SET-transfected cells compared with that in the controls, and their susceptibility to As_4S_4-induced apoptosis was decreased. The protein SET is involved in a number of cellular processes, including DNA replication, chromatin remodeling, gene transcription, differentiation, migration and cell cycle regulation. SET is overexpressed in several neoplasms, particularly in acute myeloid leukemia. The findings of the present study suggested that the SET gene may contribute to tumorigenesis and may be a potential novel effective therapeutic target for leukemia and other cancer types.

Introduction

SET nuclear proto-oncogene (SET), also known as protein phosphatase type 2A inhibitor (I2PP2A) or template-activating

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factor-1β, is involved in a number of cell processes, including the replication of DNA, chromatin remodeling, gene transcription, differentiation, migration and cell cycle regulation (1). The SET protein was initially extracted from the bone marrow of a leukemia patient (2). Originally identified as a potent PP2A inhibitor, SET is overexpressed in several neoplasms (3,4), particularly acute myeloid leukemia (5). N-terminal and internal amino acid sequencing indicated that I2PP2A was a truncated form of SET (5) Clinical data have indicated that patients with SET overexpression exhibit worse overall survival and event-free survival, suggesting an oncogenic role of SET in tumorigenesis (1).

In our previous proteomics study, the ATRA-resistant acute promyelocytic leukemia (APL) NB4-R1 cell line was treated with 25 μmol/l As_4S_4 and an As_4S_4-induced differential protein expression profile was obtained (6). There was a significant decrease in the expression of the SET oncoprotein after the treatment with As_4S_4 for 24 h, which was completely abolished after treatment for 48 h compared with the vector control; this indicated the importance of SET in the regulation of signal transduction pathways and in the mechanism of APL cell apoptosis. Next, we further investigated the possible cellular and molecular mechanisms of As_4S_4 treatment and the role of SET in the apoptosis of NB4-R1 cells. Consistent with previous studies, it was found that As_4S_4-induced apoptosis was accompanied by reduced mRNA and protein expression levels of SET in the NB4-R1 cells (Wang *et al*, unpublished data). These results indicated that As_4S_4 may induce the apoptosis of NB4-R1 cells through the downregulation of SET protein expression.

In the present study the SET gene was selected for further investigation. To avoid interference and possible compensation by other genes, SET was transfected into human embryonic kidney 293T cells and it was observed whether SET expression led to a change in the expression of PP2A or the apoptosis-related genes Bcl-2 and Bax in 293T cells treated with As_4S_4.

Materials and methods

Cell culture and reagents. The human embryonic kidney 293T cell line and the APL NB4-R1 cell line were stored in in the Oncology Research Laboratory, The First Affiated Hospital of Xi'an Jiaotong University (Xi'an, China). The 293T cells were

maintained in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific Inc., Waltham, MA, USA) at 37°C in a 5% CO_2 atmosphere in air. The growth medium was supplemented with 10% heat-inactivated (at 56°C for 30 min) fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml). The NB4-R1 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific Inc.) supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, and maintained in a 5% CO_2 humidified atmosphere at 37°C.

Extraction of total RNA and reverse transcription-polymerase chain reaction (RT-PCR). The total RNA was extracted from the NB4-R1 cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific Inc.) and was precisely quantified using an ultraviolet spectrophotometer. The sequences of the primers for SET amplification are listed below: Forward, 5'-CCGCTCGAGATGTCGGCGCCGGCG GCCAAAG-3' (the underlined section represents the *Xho*I cleavage site); and reverse, 5'-GGGGTACCGTGTCATCTT CTCCTTCATCC-3' (the underlined section indicates the *Kpn*I cleavage site). The RT reaction system included 2 μg RNA, 2 μl 5X PrimeScript RT Master Mix (Takara Bio Inc., Otsu, Japan) and RNase-free dH$_2$O to a final volume of 10 μl. The reaction program was as follows: 37°C for 15 min, followed by 85°C for 5 sec. The PCR was performed in a total volume of 20 μl, which included 1 μl (10 ng/μl) cDNA, 4 μl 5X Taq buffer; 1.6 μl (2.5 mM) dNTPs, 0.4 μl (10 μM) forward and reverse primer; 0.2 μl Taq polymerase; and 12.4 μl ddH$_2$O. The reaction program was performed in a PCR Thermocycle Instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as follows: 94°C for 5 min, and then 30 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 50 sec and an extension step of 72°C for 10 min. The amplified PCR products were electrophoresed on a 1% agarose gel (Beijing SBS Genetech Co., Ltd., Beijing, China) and were visualized under ultraviolet light using a Chemidox XRS and Quantity One gel image analysis system (Bio-Rad Laboratories, Inc.). The length of the amplified PCR segment was 850 bp and the experiment was replicated 3 times.

Construction of pEGFP-N1-SET plasmid. The gel-purified PCR segment was cut with *Xho*I and *Kpn*I restriction enzymes. The length of the restriction enzyme digestion product was 835 bp. Next, the segment was introduced into the pEGFP-N1 plasmid (Shanghai Genechem Co., Ltd., Shanghai, China). The pEGFP-N1-SET construct was transformed into DH5α cells (stored in our laboratory), and then the correct fragment was identified as a mini-plasmid by digestion with *Xho*I and *Kpn*I, and was verified as the correct clone through sequence analysis.

Transfection of 293T cells. For transient transfection, the 293T cells were plated at a density of 8x10⁴/ml cells per well in 6-well plates and transfected with 2 μg pEGFP-N1-SET or pEGFP-N1 (vector group) plasmids using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific Inc.). After 48 h of transfection, the cells were observed with an inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan) and used for further assays.

SET and PP2A mRNA analyses. The total RNA was extracted from each sample of 293T cells in TRIzol reagent. Each RNA sample (1 μg) was reverse-transcribed into cDNA using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific Inc.) according to the manufacturer's protocols, and then the cDNA was used for RT-quantitative PCR (RT-qPCR). The RT-qPCR amplification reaction contained 12.5 μl 2X SYBR-Green I, 1 μl forward (0.4 μM) and 1 μl reverse (0.4 μM) primer and 10.5 μl cDNA diluted with RNase-free water. The RT-qPCR included control samples with RNase-free water instead of the cDNA template. All samples were analyzed in three independent biological replicates. The PCR conditions were as follows: Initial denaturation at 95°C for 30 sec, denaturing at 95°C for 5 sec, and annealing at 60°C for 30 sec, where the last two steps of the reaction were repeated for 40 cycles. The primer sequences used to amplify the target genes are listed as follows: SET forward, 5'-CAT CTTCGAAGTCCACCGAAATC-3' and reverse; 5'-TGCATC AGAATGGTCAGTAAACCAG-3'; PP2A forward, 5'-GTA ACCAAGCTGCAATCATGGAA-3' and reverse, 5'-CTCTAC GAGGTGCTGGGTCAAAC-3'; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5'-CACCCTGTT GCTGTAGCCAAA-3' and reverse, 5'-CACCCTGTTGCT GTAGCCAAA-3'. RT-qPCR was conducted using an iCycler iQ Real-Time PCR Detection system (Bio-Rad Laboratories Inc.) with SYBR-Green I fluorescent dye (Takara Bio Inc.). The relative mRNA levels of the target genes were expressed in terms of the rates that were based on the cycle threshold (Cq) (7). The mRNA expression levels of each group were compared through $2^{-\Delta\Delta Cq}$. The GAPDH gene was used for inter-sample normalization and three independent experiments were performed in triplicate.

SET and PP2A protein analysis. The cultured cells were harvested and washed three times with cold phosphate-buffered saline (PBS). Next, the cells were solubilized in radioimmunoprecipitation assay buffer containing a protease inhibitor cocktail containing 104 mM AEBSF, 80 μM aprotinin, 4 mM bestatin, 1.4 mM E-64, 2 mM leupeptin and 1.5 mM pepstatin A (Sigma-Aldrich, St. Louis, MO, USA). Subsequent to being maintained on ice for 10 min, the cell suspension was centrifuged for protein at 15,500 x g for 15 min at 4°C. The protein (30 μg) was separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and transferred to a nitrocellulose membrane at 110 V for 2 h. The non-specific binding sites on the membranes were blocked with 5% (w/v) skimmed milk in Tris-buffered saline [TBS: 20 mM Tris-HCl and 200 mM NaCl (pH 7.6)] for 2 h under gentle rocking at room temperature. Next, the membranes were incubated with the following primary antibodies: Rabbit polyclonal anti-SET antibody (cat. no. ab92872) and rabbit monoclonal anti-PP2A (cat. no. ab32141) antibodies (1:10,000; Abcam, Cambridge, MA, USA); and mouse monoclonal anti-GAPDH antibody (1:10,000; cat. no. sc-47724; Santa Cruz Biotechnology Inc., Dallas, TX, USA) directed against the protein or enzyme of interest for 1 h at room temperature and then at 4°C overnight. The membranes were then incubated with goat anti-rabbit (cat. no. sc-2004) or goat anti-mouse (cat. no. sc-2005) horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Subsequent to being washed extensively with TBS

plus Tween 20, the membranes were incubated under chemiluminescence and wrapped in clear plastic wrap for film exposure. The bands on the immunoblots were quantified using Quantity One version 4.6.2 software (Bio-Rad Laboratories Inc.). The protein expression of each sample was internally normalized to GAPDH, and the quantity was compared with the expression of the transfected control groups.

Cell cycle analysis. The cell cycle distribution was analyzed via flow cytometry (Becton Dickinson FACSCalibur double laser flow cytometer; BD Biosciences, Franklin Lakes, NJ, USA). The 293T cells ($1x10^6$/ml) were harvested and washed twice with ice-cold PBS. The cells were suspended gently in 70% chilled ethanol at -20°C overnight. After washing with PBS, the cells were re-suspended in 500 μl of PBS containing propidium iodide (50 μg/ml) and RNase (50 μg/ml), and incubated for 30 min at 37°C in the dark. The cell cycle phase distribution of each experiment was analyzed using 10,000 cells per sample. The proportion of cells in the G_0/G_1, S and G_2/M phases were represented as DNA histograms.

Bcl-2 and Bax protein analyses. At 48 h post-transfection, the transfected cell medium was changed to new medium that contained 5 μmol/l As_4S_4 for 24 h. The method was similar to that used for western blotting analysis. The relevant primary antibodies were mouse monoclonal anti-Bcl-2 (cat. no. sc-7382) or rabbit polyclonal anti-Bax (cat. no. sc-493) antibodies (1:1,000 dilutions; Santa Cruz Biotechnology Inc.). The protein expression of each sample was internally normalized to GAPDH.

Statistical analysis. Experiments were performed in duplicates or triplicates over three or more independent experiments and the results are presented as the mean ± standard deviation. Statistical significance was calculated via a t-test using SPSS software, version 19.0 (IBM SPSS, Armonk, NY, USA). P<0.05 was used to indicate a statistically significant difference.

Results

Construction and package of plasmid. Using PCR-based techniques, an 850-bp human SET gene product of the correct size was generated, and then the gel-purified PCR segment was cut with the *Xho*I and *Kpn*I restriction enzymes. The length of the restriction enzyme digestion product was 835 bp. Next, the segment was inserted into the pEGFP-N1 expression vector.

The SET gene product and the positive recombinant clones were identified with PCR (Fig. 1). DNA sequencing analysis confirmed that the sequences were correct.

SET expression analysis. As the recombinant plasmid pEGFP-N1-SET was labeled with enhanced green florescent protein (EGFP), EGFP expression could be observed by laser confocal microscopy, indirectly reflecting the transfection rate of the SET gene. At 48 h post-transfection, the pEGFP-N1 empty vector and pEGFP-N1-SET construct transfection yielded efficiencies of >90%, as visualized by fluorescence microscopy for EGFP expression (Fig. 2).

To confirm that the gene had been transfected into the cells, RT-qPCR and western blotting was used to detect SET expression in the recombinant cells. The results showed that the SET
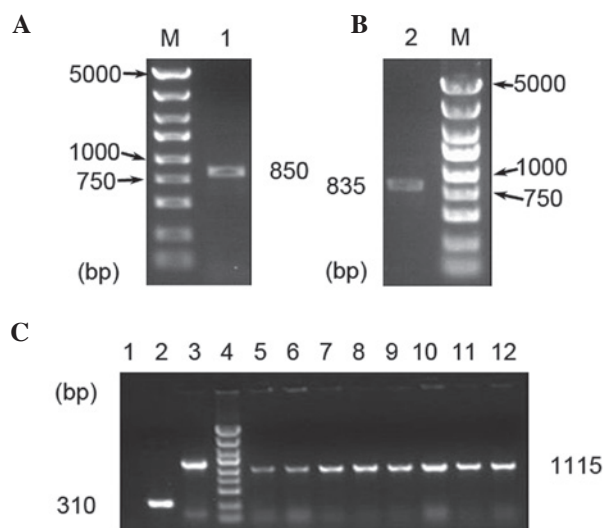

Figure 1. Gels showing products of (A) PCR and (B) restriction enzyme analysis. Lanes: M, DNA marker (5,000, 3,000, 2,000, 1,500, 1,000, 750, 500, 250 and 100 bp); 1, PCR-amplified SET gene; 2, PCR-amplified product of double-digestion by *Xho*I and *Kpn*I. (C) Gel electrophoretic analysis of vectors. Lanes: 1, double-distilled H_2O; 2, recombinant negative vector pEGFP-N1; 3, GAPDH; 4, DNA marker (as above); 5-12, recombinant positive vector of pEGFP-N1-SET. PCR, polymerase chain reaction; EGFP, enhanced green fluorescent protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
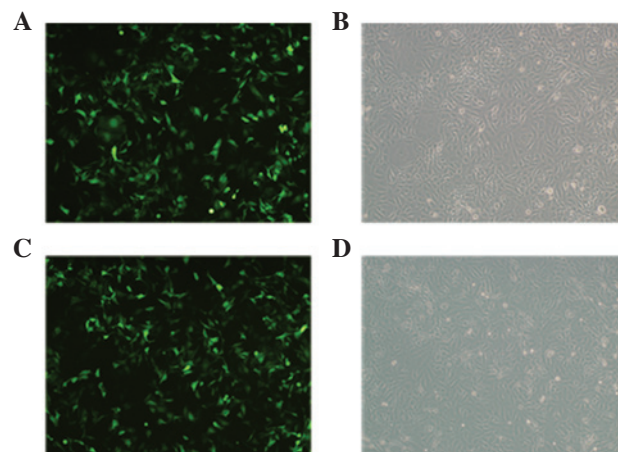

Figure 2. Analysis of 293T cells by inverted phase-contrast microscopy and fluorescence microscopy (magnification, x200). (A) Fluorescence and (B) light microscopy images of 293T cells transfected with pEGFP-N1 plasmid; and (C) fluorescence and (D) light microscopy images of 293T cells transfected with pEGFP-N1-SET plasmid. EGFP, enhanced green fluorescent protein.

expression of the construct transfection group was markedly increased at the mRNA and protein levels compared with that of the empty vector group. Both real-time quantitative PCR (P=2.1x10-7) and western blotting (P=0.001) demonstrated that pEGFP-N1-SET were effective for SET overexpression. These results demonstrated that the target gene had been successfully transfected (Fig. 3).

PP2A expression analyses. PP2A expression was detected by RT-qPCR and western blotting to ascertain if the PP2A gene was involved in the study. PP2A expression was decreased by ~92% (mRNA; P=0.0002) and ~47% (protein; P=0.002) in
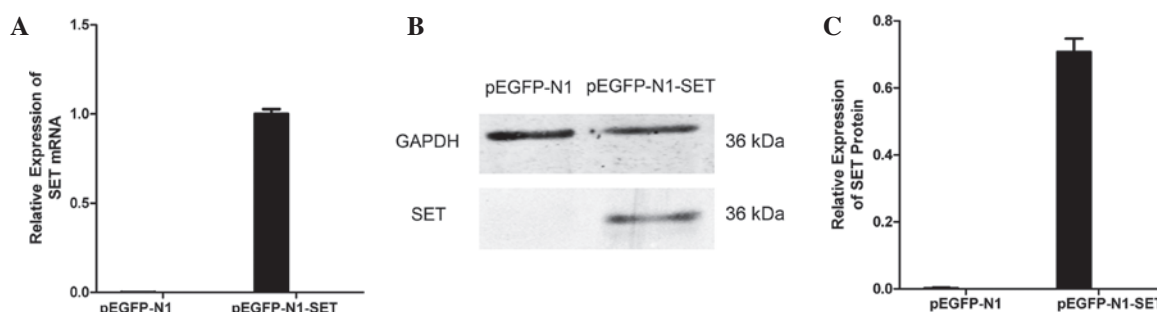
Figure 3. Vector-mediated SET expression in 293T cells. (A) mRNA expression of SET was detected by reverse transcription-quantitative polymerase chain reaction analysis. (B) Representative western blot for the detection of the protein expression of SET. GAPDH expression was used as a loading control. (C) Relative expression of SET protein as determined by densitometric analysis of protein bands. Values are expressed as the mean ± standard deviation from three individual experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFP, enhanced green fluorescent protein.
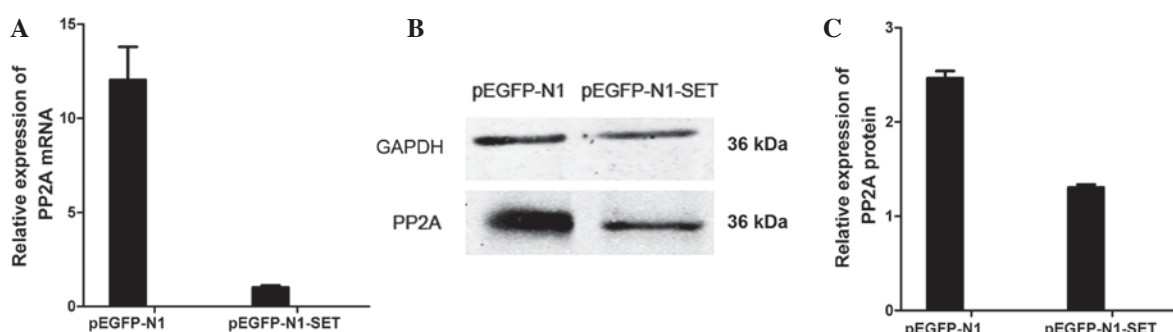

Figure 4. PP2A is expressed in 293T cells following upregulation of SET. (A) mRNA expression of PP2A was detected by reverse transcription-quantitative polymerase chain reaction analysis. (B) Representative western blot for the detection of the protein expression of PP2A. GAPDH expression was used as loading control. (C) Relative expression of PP2A protein as determined by densitometric analysis of protein bands. Values are expressed as the mean ± standard deviation from three individual experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFP, enhanced green fluorescent protein; PP2A, protein phosphatase 2A.

the pEGFP-N1-SET-transfected 293T cells compared with the pEGFP-N1-transfected control group (Fig. 4).

SET increases the percentage of transfected cells in S and G_2/M phases. To monitor the stimulatory effect of SET on cell growth, the effect of SET overexpression on cell cycle progression was investigated by flow cytometric analysis. The result demonstrated that the overexpression of SET significantly increased the cell percentage in the S (P=0.025) and G_2/M (P=0.045) phases in the 293T cells compared with that in the control transfectants (Fig. 5).

Bcl-2 and Bax expression analyses. Anti-apoptotic Bcl-2 expression and pro-apoptotic Bax expression was detected by western blotting to ascertain if Bcl-2 and Bax were involved in the apoptosis in this study. Bcl-2 expression was increased by ~84% (P=0.001) in the pEGFP-N1-SET-transfected 293T cells compared with the pEGFP-N1-transfected control group. The incubation with 5 μmol/l As_4S_4 for 24 h decreased the relative Bcl-2 expression level by 86% (P=0.001) in the pEGFP-N1-transfected 293T cells and by 91% (P=0.001) in the pEGFP-N1-SET-transfected cells, when compared with the untreated control cells (Fig. 6).

Bax expression was decreased significantly by ~54% (P=0.004) in the pEGFP-N1-SET-transfected group compared with the pEGFP-N1-transfected control group. As_4S_4-treatment increased Bax expression by 14% (P>0.05)
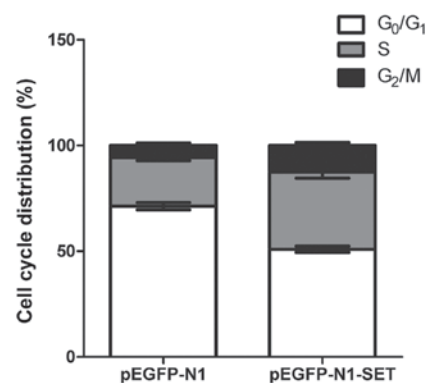

Figure 5. Cell cycle analysis by flow cytometry. Overexpression of SET increased the percentage of 293T cells compared with the control transfectants in the S phase (36.6 vs. 23.0%, respectively) and G_2/M phase (12.5 vs. 5.7%, respectively) (P<0.05). Values are expressed as the mean ± standard deviation from three individual experiments. EGFP, enhanced green fluorescence protein.

in the pEGFP-N1-transfected cells. In comparison, As_4S_4 addition increased Bax expression by 66% (P=0.009) in the pEGFP-N1-SET-transfected cells (Fig. 6).

Discussion

Abnormally expressed genes associated with signal transduction play a role in the development of carcinoma. The
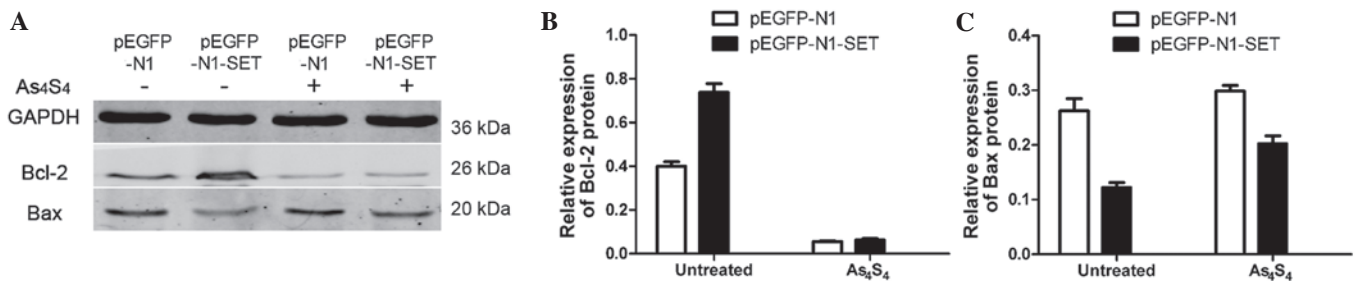
Figure 6. As$_4$S$_4$-induced Bcl-2 or Bax protein expression was detected in 293T cells transfected with pEGFP-N1 or pEGFP-N1-SET. Cells in the As$_4$S$_4$ groups were treated with 5 μmol/l As$_4$S$_4$ for 24 h. (A) Representative western blots showing Bcl-2 or Bax protein levels. GAPDH was used as the loading control. (B and C) Relative protein levels of Bcl-2 or Bax were determined by densitometric analysis of protein bands. Values are expressed as the mean ± standard deviation from three individual experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFP, enhanced green fluorescent protein; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein.

investigation of the abnormal expression of genes in cancer can not only enhance our understanding of the pathogenesis of the tumor, but can also provide reliable therapeutic targets. Our previous proteomics study showed a total of 21 proteins that were differentially regulated after treatment with As$_4$S$_4$ in NB4-R1 cells, including 5 downregulated proteins and 16 upregulated proteins (6). It was suggested that either the loss of tumor suppressor genes or oncogene overexpression would be involved in the neoplastic cell transcription of normal cells. Among these differential displayed proteins, the expression of the SET oncoprotein showed a significant decrease after the treatment with As$_4$S$_4$, which indicated the importance of SET in the regulation of signal transduction pathways (6).

SET oncoprotein expression is often associated with cell proliferation and tumorigenesis (8,9). Therefore, in the present study, a eukaryotic cell expression plasmid was constructed and transfected into human 293T cells by lipofection. Transient cell transfection resulted in a high level of SET oncoprotein expression, which was not regulated by natural promoters. A fluorescence marker was included in the expression cassette in order to indicate the expression and localization of the target protein in the transfected cells. SET expression in the pEGFP-N1-SET-transfected group compared with the control group was monitored by RT-qPCR and western blotting. EGFP was expressed in the pEGFP-N1- and pEGFP-N1-SET-transfected groups, but only pEGFP-N1-SET effectively exhibited SET overexpression. This result demonstrated that the target gene had been successfully transfected.

As a nuclear protein, SET has been identified to play a key role in the inhibition of normal histone acetylation and the demethylation of ectopically methylated DNA, ultimately leading to the inhibition of gene silencing and transcriptional activation (4). It has been reported that SET is overexpressed in chronic myeloid leukemia and acute myeloid leukemia, in which it is correlated with BCR/ABL expression and activity, resulting in the inhibition of PP2A (5,10). In addition, clinical data have indicated that the increasing expression of the SET gene corresponds to higher degrees of tumor malignancy (11), suggesting an oncogenic role of SET in tumorigenesis. In in vitro experiments, SET was identified to promote the progression of the cell cycle by modulating the activities of CDKs, including CDK1, CDK2 and CDK5, by interacting with the activators and/or inhibitors of the CDKs (12). In the present

study, the functional activity of SET was shown to be present in the 293T cell line. The accumulation of cells in the S and G$_2$/M phases was observed for the 293T cells, which may be associated with the anti-apoptosis function of SET, suggesting that the overexpression of SET may exert its carcinogenesis through cell cycle arrest. Therefore, SET may be a potential target of gene therapy for leukemia and other cancers in the future.

It has been demonstrated that SET overexpression is a key contributing mechanism involved in the PP2A inhibition observed in tumors (1). Therefore, the change in PP2A level was detected in the following experiments of the present study. The relative PP2A mRNA and protein expression levels in the pEGFP-N1-SET-transfected 293T cells were downregulated compared with those in the pEGFP-N1-transfected cells. The results showed that SET mediated the decreased expression of PP2A may be of greater importance in light of the potential role of PP2A in leukemogenesis and other cancers. PP2A is a major serine/threonine phosphatase in eukaryotic cells, which consists of a 36-kD catalytic subunit C (PP2Ac), a regulatory subunit A with a molecular mass of 65 kD, and a third regulatory subunit B (13). As a tumor suppressor, PP2A plays an irreplaceable role in a number of cellular processes, such as cellular metabolism, DNA replication, transcription, RNA splicing, translation, cell cycle progression and cell transformation (14). Furthermore, PP2A exhibits a positive regulatory function in apoptosis, mediating the dephosphorylation and inactivation of the anti-apoptotic Bcl-2 and activation of the pro-apoptotic factor Bad (15). As a result, PP2A has been noted as a potential therapeutic target in leukemia patients (9,16,17). SET may promote the suppression of PP2A by binding directly to the catalytic subunits of PP2A and causing hyperphosphorylation of its τ unit (18). Another study has shown that SET could activate the mitogen-activated protein kinases (MAPK) kinase/extracellular signal-regulated kinases/MAPK signaling pathway, which is involved with cell spreading, motility and death receptor-elicited apoptosis. Moreover, ERK has interactions with PP2A, suggesting that cell migration may be regulated through a SET-PP2A-ERK signaling complex (19). Therefore, the overexpression of the SET oncoprotein may result in the inactivation of PP2A and thus the deactivation of PP2A target gene dephosphorylation, leading to apoptotic resistance in the tumor cells.

Our previous study showed that As_4S_4-induced apoptosis was associated with a reduced level of SET mRNA and protein expression in NB4-R1 cells (Wang *et al*, unpublished data). Therefore, the present study analyzed Bcl-2 (an anti-apoptotic protein) and Bax (a proapoptotic protein) expression in transfected 293T cells in order to determine if these Bcl-2 family members played a role in As_4S_4-induced apoptosis. The Bcl-2 expression level was increased in the pEGFP-N1-SET-transfected 293T cells compared with the expression level in the pEGFP-N1-transfected control group. Additionally, the Bcl-2 expression level was decreased in the pEGFP-N1-transfected 293T cells treated with As_4S_4 and was further decreased in the pEGFP-N1-SET-transfected cells. Bax expression was increased slightly in the pEGFP-N1-transfected 293T cells treated with As_4S_4 and was further increased in the pEGFP-N1-SET-transfected 293T cells. These results demonstrated that transfection with SET led to upregulated Bcl-2 expression and downregulated Bax expression; these changes in expression may be associated with the SET carcinogenic effect observed in previous studies (8,9). The susceptibility of cells to apoptosis may be determined by the ratio between anti-apoptotic and proapoptotic members of the Bcl-2 family. The decrease in the Bcl-2/Bax ratio leads to the translocation of Bax from the cytoplasm to the mitochondria, promoting the release of cytochrome c and the activation of caspases (20,21). The present results also suggested that SET has suppressive effects on apoptosis. However, further studied are required.

In conclusion, the present results showed that the SET gene could lead to the promotion of 293T cell proliferation and the inhibition of PP2A expression. Overexpression of SET increased the protein expression of Bcl-2 and decreased the expression of Bax in the cells, and their susceptibility to As_4S_4-induced apoptosis was decreased. As a key factor in carcinogenesis, this study identified SET as a critical gene in As_4S_4-induced apoptosis and a potential novel effective therapeutic target for leukemia and other cancers. Further studies will be focused on defining the mechanism through which SET regulates PP2A levels and the role of such regulation in cell differentiation, growth and carcinogenesis.

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