

# Effects of SMYD3 over-expression on cell cycle acceleration and cell proliferation in MDA-MB-231 human breast cancer cells

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**Abstract** SET and MYND domain-containing protein 3 (SMYD3) is a histone methyltransferase that plays an important role in transcriptional regulation in human carcinogenesis. It can specifically methylate histone H3 at lysine 4 and activate the transcription of a set of downstream genes, including several oncogenes (e.g., *N-myc*, *Crkl*, *Wnt10b*, *RIZ* and *hTERT*) and genes involved in the control of cell cycle (e.g., *CyclinG1* and *CDK2*) and signal transduction (e.g., *STAT1*, *MAP3K11* and *PIK3CB*). To determine the effects of SMYD3 over-expression on cell proliferation, we transfected SMYD3 into MDA-MB-231 cells and found that these cells showed several transformed phenotypes as demonstrated by colony growth in soft agar. Besides, we show here that down-regulation of SMYD3 could induce G1-phase cell cycle arrest, indicating the potent induction of apoptosis by SMYD3 knockdown. These results suggest the regulatory mechanisms of SMYD3 on the acceleration of cell cycle and facilitate the development of strategies that may inhibit the progression of cell cycle in breast cancer cells.

**Keywords** SMYD3 · Cell proliferation · Cell cycle · Apoptosis

## Introduction

Recent evidence has accumulated that modifications of histone tails play a key role in the regulation of chromatin structure, transcription, telomere maintenance and DNA replication. Within the past 2 years, great progress has been made in understanding the functional implications of histone modifications. Histone modifications, which include histone methylation, histone demethylation and so on [1], are one of the two major epigenetic pathways that interplay to regulate transcriptional activity and other genome functions [2]. There have been some researches, which indicate that histone methylation can affect proliferation and differentiation in eukaryotic cells [3–5]. Indeed, a growing number of histone methyltransferase have been shown to promote or inhibit tumorigenesis through their histone methyltransferase activity [6, 7].

SMYD3, which encodes a histone methyltransferase involved in cancer cells proliferation [7], is a subfamily of SET domain-containing proteins with unique domain architecture [8]. Several trials have been designed to explore the oncogenic activity of SMYD3, and the results suggest it is a histone H3-K4 specific di- and tri-methyltransferase, which elicits its oncogenic effect via activating transcription of its downstream target genes. Enhanced expression of SMYD3 was essential for the growth of many cancer cells [7]. Moreover, in our previous study, we clarified that over-expression of SMYD3 gene affects cell viability, adhesion and migration [9–12], indicating that SMYD3 might be a novel target for therapeutic strategy against the metastasis of human cancers.

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Although the previous study has shown that introduction of SMYD3 into mouse fibroblast NIH3T3 cells can significantly promote the cell growth and foci formation [13], more details of the effects of SMYD3 over-expression on the acceleration of cell cycle and cell proliferation are still not very clear. To determine these, here, we transfected SMYD3 into MDA-MB-231 cells showed that these cells had several transformed phenotypes as demonstrated by colony growth in soft agar. Besides, we show here that down-regulation of SMYD3 could induce G1-phase cell cycle arrest, indicating the potent induction of apoptosis by SMYD3 knockdown. These findings will help to better understand the regulatory mechanisms of SMYD3 about the acceleration of cell cycle and facilitate the development of strategies that may inhibit the progression of cell cycle in breast cancer cells.

## Materials and methods

### Cell lines and culture conditions

MDA-MB-231 tumor cell line was cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM Gibco, Paisley, UK), containing 10% fetal bovine serum (FBS, Gibco), 100 units/ml penicillin and 100 mg/L streptomycin.

### Reagents and antibodies

Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA). Low-gelling point SeaPlaque agarose was purchased from Sigma (St Louis, MO). Anti-human CyclinD1, anti-human p53, anti-human p21 and anti-human  $\beta$ -actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-human SMYD3 was obtained from Proteintech Group (Chicago, IL, USA), and anti-human CDK4 was purchased from Biologend (San Diego, CA, USA).

### Plasmids and transfection

Cells were transfected with either the SMYD3 expression plasmid (pcSMYD3) or empty control vector (pcDNA3.1) using Lipofectamine 2000 according to manufacturer's protocol, and the cells were then harvested for analysis after 48 h. The targeted sequences used for SMYD3 shRNAs (S920) were 5'-AACATCTACCAGCTGAAGGTG-3'. The negative control shRNAs (Scr) with targeted sequences 5'-GTAGATGGTTCGACCTTCAC-3' had no significant homology with any known gene. Forty-eight hours after SMYD3 gene silenced by RNAi, pcSMYD3 was re-transfected into the SMYD3 knocked down cells,

and the cells were then harvested for analysis another 48 h after transfection.

### MTT assay

For cell proliferation assay, the cells were seeded at a density of  $3 \times 10^3$  cells/well in 96-well plates after transfection, each group of cells was treated for methyl thiazolyl tetrazolium (MTT) (50  $\mu$ g/well) assay. When the cells were incubated at 37°C for 4 h, the reaction was stopped by the addition of 150  $\mu$ l/well of DMSO, lysing for 10 min. The color reaction was quantified using an automatic plate reader (Bio Rad) at 570 nm with a reference filter of 620 nm. The absorbance value (A) was directly proportional to the number of viable cell. All assays were performed in triplicate.

### Soft agar cloning assay

To determine the transformation activity of SMYD3, the soft agar colony assay was performed. A total of 1.5 ml of 0.5% agar prepared in DMEM supplemented with 10% FBS were poured into six-well plates (35 mm) and let solidify. Then,  $6.0 \times 10^4$  cells mixed with 1 ml 0.3% agar in complete medium were layered over bottom agar. The cultures were incubated at 37°C for 3 weeks. The colony number was counted under an inverted phase-contrast microscope. Each treatment of cells was done in triplicate.

### Cell cycle and apoptosis analysis (facial action coding system)

Cells were seeded onto 6-cm dishes and treated with or without the pcDNA-SMYD3 expression plasmid for 24 h, and then cells were harvested, fixed in 70% ethanol and stored at 20°C. Cells were then washed twice with ice-cold PBS and incubated with RNase and propidium iodide, a DNA-intercalating dye. Cell cycle phase analysis was performed using a Becton–Dickinson Facstar flow cytometer equipped with Becton–Dickinson cell-fit software.

Cells were seeded onto 6-cm dishes and treated with or without the pcDNA-SMYD3 expression plasmid for 24 h, and then cells were harvested, washed twice with PBS, and then suspended in a binding buffer, stained with annexin V incubation reagent and incubated in the dark for 15 min at room temperature. Then, propidium iodide was added, and the cells were immediately processed with a Facstar flow cytometer (Becton–Dickinson).

### RNA extraction and reverse transcription PCR

Total cellular RNA was extracted, and then potential residual genomic DNA was eliminated with RNase-free

DNase I (Bio Basic Inc, Ontario, Canada). For each sample, 2 µg of total RNA was reverse transcribed using oligo dT 18 primer and MMLV (Promega, Madison, WI, USA) to synthesize complementary DNA (cDNA) following standard protocols. Two microliter newly synthesized cDNA was used as a template for PCR, which was performed with 1.5 mM MgCl<sub>2</sub>, 2.5 U Taq polymerase and 0.5 µM primers. The primer sequences were as follows: for SMYD3, 5'-CCCAGTATCTCTTTGCTCAATCAC-3' (forward) and 5'-ACTTCCAGTGTGCCTTCAGTTC-3' (reverse); for internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-ATTCAACGGCACAGTCAAGG-3' (forward) and 5'-GCAGAAGGGGCGGAGATGA-3' (reverse); for CyclinD1, 5'-CGTCCATGCGGAAGATCGTC-3' (forward) and 5'-CGCGTGTTCGCGATGATC-3' (reverse); for CDK4, 5'-ACGGGTGTAAGTGCATCTG-3' (forward) and 5'-TGGTGTTCGCTGCCTATGGGA-3' (reverse); for p53, 5'-AGCGATGGTCTGGCCCC TCC-3' (forward) and 5'-GCGCCGGTCTCTCCAGGA-3' (reverse); for p21, 5'-ATGTCAGAACCGGCTGGGGA-3' (forward) and 5'-GCCGTTTTTCGACCCTGAGAG-3' (reverse); for Bcl-2, 5'-TGTGTGTGGAGAGCGTCAAC C-3' (forward) and 5'-TTCAGAGACAGCCAGGAGAAA TC-3' (reverse); for Bax, 5'-TCAGGATGCGTCCACCAAGAA-3' (forward) and 5'-TCCCGGAGGAAGTCCAATG TC-3' (reverse); for CyclinE, 5'-TGCCTTGAATTCCTT ATGG-3' (forward) and 5'-CCGCTGCTCTGCTTCTTAC-3' (reverse); for CDK2, 5'-GCTTTCTGCCATTCTCATC G-3' (forward) and 5'-CTTGTTAGGGTCGTAGTGC-3' (reverse). PCR was performed at 94°C for 5 min, then 26 cycles at 94°C for 45 s, at 54°C for 45 s and at 72°C for 1 min; extension was carried out at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gels, and fragments were visualized by ethidium bromide staining.

#### Western blot analysis

Total cellular proteins were extracted with lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% deoxycholic phenylmethyl-sulfonyl fluoride, 1 µg/ml of aprotinin, 5.0 mM sodium pyrophosphate, 1.0 g/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride and 1 mM/l of DTT). Proteins (40 µg) were separated on a 12% SDS-polyacrylamide gel and transferred electrophoretically onto a nitrocellulose membrane. Blots were blocked for 1 h in blocking buffer (10% non-fat dried milk, 0.5% Tween in TBS) and incubated with anti-human SMYD3 rabbit polyclonal antibody (1:500) overnight at 4°C, anti-human CyclinD1 mouse polyclonal antibody (1:500), anti-human CDK4 rabbit polyclonal antibody (1:500), anti-human p53 mouse polyclonal antibody (1:500), anti-human p21 rabbit polyclonal antibody (1:500), with anti-human β-actin rabbit polyclonal antibody (1:200) as control. Blots were

washed, incubated according to standard procedures and developed with ECL system (Amersham Pharmacia).

#### Statistical analysis

The data from the above-mentioned experiments were summarized by mean and standard deviation. The Student's t test was used to compare the means. Differences were considered significant at  $P < 0.01$ .

## Results

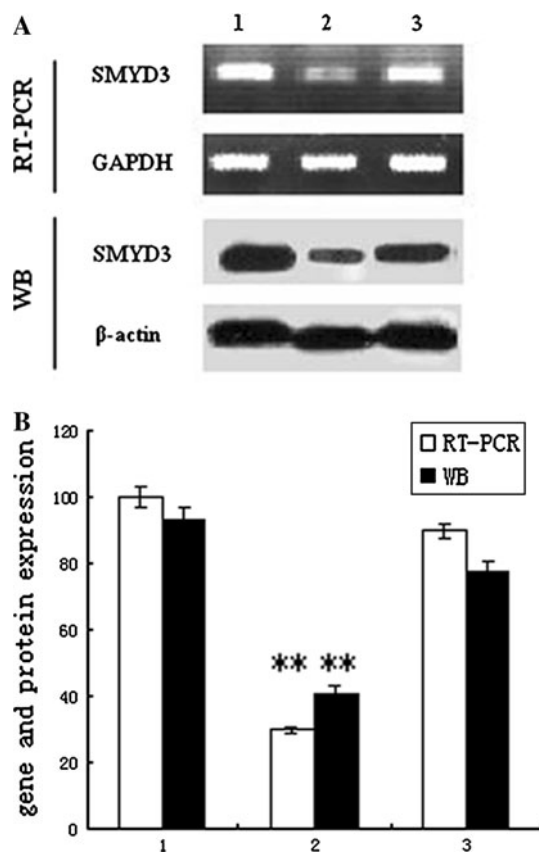
#### Effects of shRNA-SMYD3 on the expression of SMYD3

To detect the effect of ShRNA-SMYD3 on SMYD3 mRNA expression, RT-PCR and Western Blot analysis were performed. As shown in Fig. 1a, a strong SMYD3 band was detected in lane 1, suggesting that SMYD3 mRNA was expressed at a high level in MDA-MB-231 cells. However, the band of cells treated with shRNA-SMYD3 was much weaker than the band of control pshRNA (Scr) cells, indicating that shRNA-SMYD3 could suppress the mRNA expression of SMYD3. Furthermore, the changes of SMYD3 protein levels after treated with shRNA-SMYD3 were detected using the method of Western Blot (WB), and the results also showed that shRNA-SMYD3 could decrease the protein expression of SMYD3 (as shown in Fig. 1b).

#### Effects of SMYD3 on cell proliferation

To determine the effect of SMYD3 on cell proliferation, the 3-day growth curves of cells transfected with different plasmids were detected by MTT assay. The cell growth curve showed that the proliferation of MDA-MB-231 cells transfected with ShRNA-SMYD3 was inhibited notably in a time-dependent manner compared with control (Fig. 1a). The growth rate of MDA-MB-231 cells transfected with pcSMYD3 was significantly higher than that of ShRNA-SMYD3 and control cells.

In another, colony formation assay in soft agar was also performed to investigate the effect of SMYD3 on contact inhibition and anchorage dependence of cell growth. As seen in Fig. 1b and c, cells transfected with pcSMYD3 formed colonies in an agar suspension. In contrast, cells transfected with ShRNA-SMYD3 and control cells still remained as single cells in the agar suspension. The rate of colony formation of cells transfected with pcSMYD3 was  $49.35\% \pm 2.57\%$  ( $P < 0.01$ ). This result suggested that MDA-MB-231 cells transfected with SMYD3 could



**Fig. 1** Effects of ShRNA-SMYD3 on the expression of SMYD3 MDA-MB-231 cells were transfected by pcDNA-SMYD3, SMYD3 pshRNA (S920) and the control pshRNA (Scr) for 48 h. SMYD3 suppression at the mRNA level was determined by RT-PCR and Western blot analysis. Lanes 1–3 show the results of the mRNA and protein levels for pcDNA-SMYD3, SMYD3 pshRNA (S920) and the control pshRNA (Scr) cells, respectively. The experiments were performed in triplicate

overcome contact inhibition and gain the ability of anchorage-independent growth.

Down-regulation of SMYD3 induced G1-phase cell cycle arrest

In an effort to investigate the effect of SMYD3 on cell cycle, we down-regulated SMYD3 gene expression by shRNA in MDA-MB-231 cells, which are shown to over-express SMYD3 gene. Based on flow cytometric analysis, we found a significant increase in the number of cells in the G1-phase (Fig. 2a). In G1-phase, 70.43% of the cells transfected with ShRNA-SMYD3 were blocked. However, in control cells, only 54.64% of the cells were blocked. This result suggests that down-regulation of SMYD3 inhibits cell growth by causing cell cycle arrest in G1-phase. Next, the effect on cell cycle regulatory molecules that affect the G1- and S-phases of the cell cycle was examined. Down-regulation of SMYD3 by RNAi

decreased the expression of CyclinD1 as well as CDK4 and CDK2, which are the cell cycle regulatory molecules that affect G1- and S-phases. (Fig. 2b,c,d,e). These results suggest that down-regulation of SMYD3 induced G1-phase cell cycle arrest, followed by inhibition of CyclinD1 and CDK4.

Effects of SMYD3 on the expression of apoptosis-related genes

To investigate the effect of SMYD3 on the sensitivity of cells to apoptosis, we down-regulated SMYD3 gene expression by shRNA in MDA-MB-231 cells and then stained with fluorescein isothiocyanate (FITC)-conjugated annexin V and PI. Thereafter, samples were analyzed by flow cytometry. In this experiment, after treated with shRNA, 25.31% of MDA-MB-231 cells were in early apoptosis compared with control cells (Fig. 3a). Next, the effect on the expression of p53 and p21 was examined. We found that down-regulation of SMYD3 increased the expression of p53 and p21 remarkably. These results suggest that ShRNA-SMYD3 is able to induce cell apoptosis by regulating the expression of genes associated with G1- and S-phases, including up-regulation of p53/p21 and down-regulation of Bcl-2/Bax.

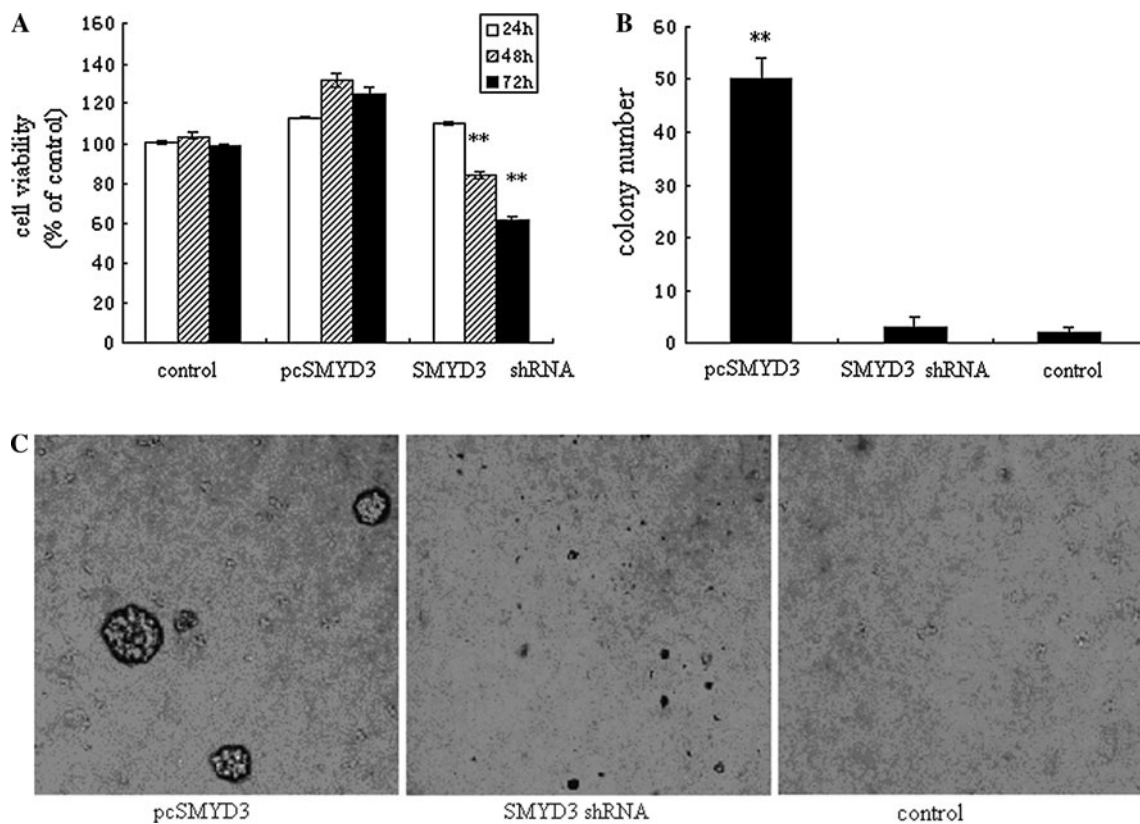
## Discussion

The abnormal DNA methylation and histone modification, which are two important kinds of epigenetic, often lead to aberrant epigenetic alterations. DNA methylation is associated with the formation of nuclease-resistant chromatin and methyl-CpG-binding proteins while DNA methyltransferases (DNMTs) are associated with histone deacetylases and histone methyltransferases, two key regulators of histone modification [14–17]. DNA methylation also occurs in the context of other epigenetic modifications. SMYD3, which is one kind of histone methyltransferases, may affect some important processes in tumor progression.

Histone modification is a crucial step in transcriptional regulation while deregulation of this modification process is important in human carcinogenesis [14, 16]. We previously showed that SMYD3 functioned as a specific histone H3-K4 di- and tri-methyltransferase. SMYD3 can significantly promote cell proliferation while the suppression of SMYD3 by siRNA can induce cell death as well as apoptosis in cancer cells, which might be a promising therapeutic strategy to treat human breast cancer.

Cell proliferation and cell death are two sides of the same coin. Prior studies have revealed that SMYD3 can significantly promote cell proliferation and that the





**Fig. 2** Effects of SMYD3 knockdown on the growth of MDA-MB-231 cells and soft agar cloning assay. **a** SMYD3 knockdown led to a reduced cell growth rate, as detected by MTT assay. **b** Colony numbers in soft agar of the cells (compared with the result of control

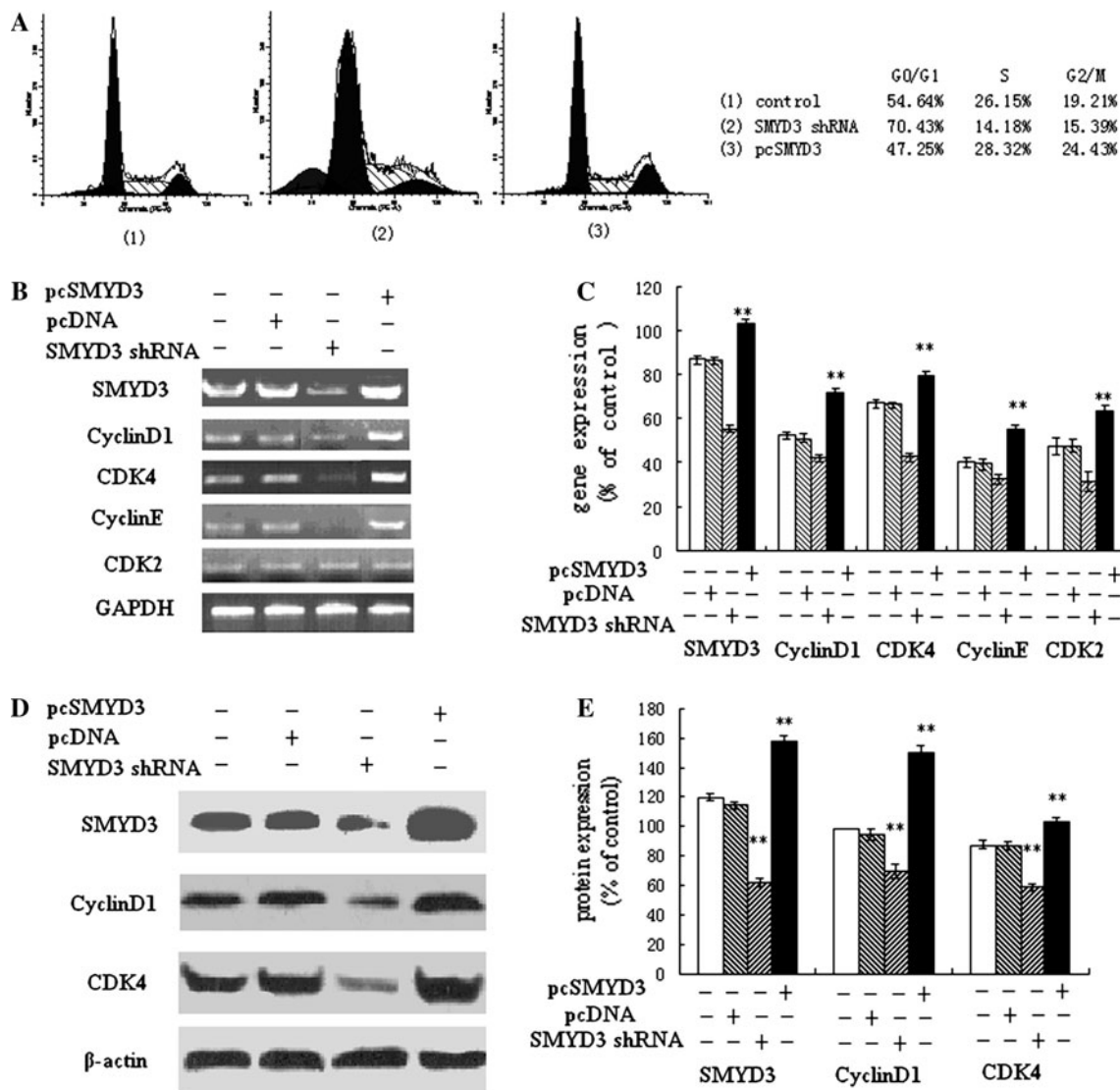
cells). **c** Photomicrograph of a typical colony in soft agar of the cells (3100 magnification). All experiments were performed in triplicate. \*  $P < 0.05$  and \*\*  $P < 0.01$  compared with control

suppression of SMYD3 can induce cell death or apoptosis [7, 8]. In this study, the results showed that MDA-MB-231 cells transfected with SMYD3 could develop several characteristics, including the morphological changes, enhancement on cell proliferation and colony formation in soft agar. These results confirmed our expectation that over-expression of SMYD3 can enhance cell viability and stimulate cell proliferation. Moreover, as we know, there are four major cyclins (D, E, A, and B) activated sequentially during the cell cycle. They activate several cyclin-dependent kinases, which are complexes able to catalyze successive stages of cell cycle progression. Of these modulators, CyclinD1 regulates cyclin-dependent kinases CDK4 and CDK6, thus plays an important role in the early to mid G1-phase of cell cycle [18–21]. This study also found that down-regulation of SMYD3 could induce G1-phase cell cycle arrest. This effect might be associated with its transcriptional regulation on CyclinD1 and CDK4. Besides, the Bcl-2 family of proteins is key regulators of apoptosis [22]. The Bcl-2 family is subdivided into two classes: antiapoptotic members, such as Bcl-2 and Bcl-xL, and proapoptotic members, Bax and Bak [23]. The results

showed that ShRNA-SMYD3 probably induced cell apoptosis by regulating the expression of genes related, such as up-regulating the expression of p53/p21 and reducing the rates of Bcl-2/Bax.

According to the report of Hamamoto, SMYD3 plays an important role in transcriptional regulation and can significantly alter the expression of downstream genes [7, 13]. Moreover, prior studies have revealed that SMYD3 significantly reduced cell susceptibility to cell death caused by DEX, a commonly used chemotherapeutic agent [12]. Consistent with the observations that down-regulation of SMYD3 inhibited cell cycle and modulated cell apoptosis-associated proteins, these data provided the first evidence that implies the mechanisms behind SMYD3-mediated oncogenic activity in cancer cell proliferation (Fig. 4).

Obviously, the biological function of SMYD3 depends to a large extent on the activity of these downstream genes. The previous studies have confirmed that SMYD3 interacts with its binding motif 5' -CCCTCC-3' in the promoter region of its target genes and dimethylates or trimethylates H3-K4. The growing evidence have showed that these downstream genes included several oncogenes



**Fig. 3** Down-regulation of SMYD3 induced G1-phase cell cycle arrest in MDA-MB-231 cells. **a** MDA-MB-231 cells were transfected by pcDNA-SMYD3, pcDNA or SMYD3 pshRNA for 48 h. Cells were subjected to flow cytometric analysis to determine the effect of SMYD3 on cell cycle distribution. RT-PCR and Western blot

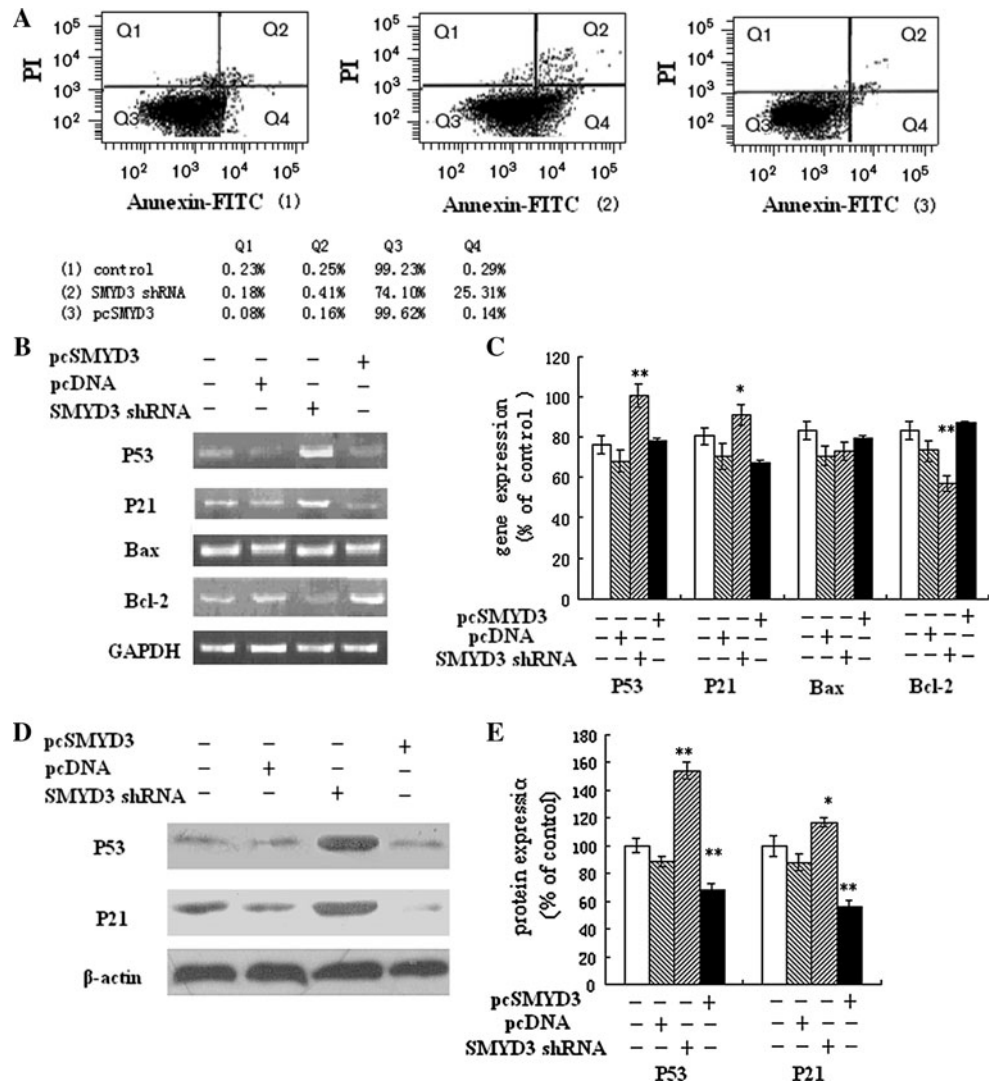
assessments were performed with antibodies specific for SMYD3, CyclinD1, CDK4, CyclinE, CDK2. The results from representative experiments were normalized to GAPDH and β-actin expression. **b–e** All experiments were performed in triplicate. \*  $P < 0.05$  and \*\*  $P < 0.01$  compared with control

(e.g., *N-myc*, *Crkl*, *Wnt10b*, *RIZ* and *hTERT*), genes involved in cell cycle regulation (e.g., *CyclinG1* and *CDK2*) and signal transduction (e.g., *STAT1*, *MAP3K11*, and *PIK3CB*). As we know, the functions of such downstream genes were involved in many aspects of the process of cell growth and apoptosis. Although the mechanism remains poorly understood, much evidence indicated that Bcl-2 could inhibit cell death induced by a wide variety of apoptotic signals in many cell types. But the mechanism underlying its protective effects still remains unclear. Bcl-2 has been shown to bind to a multifunctional chaperone protein BAG-1 [24]. In turn, BAG-1 binds to and activates the protein kinase Raf-1 [25]. Raf-1 kinase plays a central

role in the conserved Ras-Raf-mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK)-ERK pathway, acting to relay signals from activated Ras proteins via MEK1/2 to the ERK1/2 [26]. Further studies might be necessary to provide a full understanding of the relationships between SMYD3 and the signaling pathway of nuclear hormone receptors.

In summary, we provided for the first time the in vitro evidence about the mechanisms of SMYD3-mediated oncogenic activity in cancer cell proliferation. We showed here that ShRNA-SMYD3 arrested cell cycle, and SMYD3 could stimulate cell proliferation. This study provided a useful tool for further investigating the potential role of

**Fig. 4** Sensitivity of the cells to apoptosis induced by down-regulation of SMYD3. **a** MDA-MB-231 cells were transfected by pcDNA-SMYD3, pcDNA or SMYD3 pshRNA for 48 h. The apoptosis level of the cells was measured with annexin V staining method. RT-PCR and Western blot assessments were performed with antibodies specific for p53, p21, Bax, Bcl-2. The results from representative experiments were normalized to GAPDH and  $\beta$ -actin expression. **b–e** All experiments were performed in triplicate. \*  $P < 0.05$  and \*\*  $P < 0.01$  compared with control



SMYD3 in physiological or pathological processes and the chemosensitivity of cancer cells in response to clinically chemotherapeutic agents.

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**References**

- Lennartsson A, Ekwall K. Histone modification patterns and epigenetic codes. *Biochim Biophys Acta*. 2009;1790(9):863–8.
- Hu JL, Zhou BO, Zhang RR, Zhang KL, Zhou JQ, Xu GL. The N-terminus of histone H3 is required for de novo DNA methylation in chromatin. *Proc Natl Acad Sci USA*. 2009;106(52):22187–92.
- Hamon MA, Batsche E, Regnault B, Tham TN, Seveau S, Muchardt C, et al. Histone modifications induced by a family of bacterial toxins. *Proc Natl Acad Sci USA*. 2007;104(33):13467–72.
- Schmeck B, Beermann W, van Laak V, Zahlten J, Opitz B, Witzernath M, et al. Intracellular bacteria differentially regulated

- endothelial cytokine release by MAPK-dependent histone modification. *J Immunol*. 2005;175(5):2843–50.
- Kim JM, To TK, Ishida J, Morosawa T, Kawashima M, Matsui A, et al. Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. *Plant Cell Physiol*. 2008;49(10):1580–8.
- Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*. 2002; 419(6907):624–9.
- Hamamoto R, Furukawa Y, Morita M, Iimura Y, Silva FP, Li M, et al. SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nat Cell Biol*. 2004;6(8):731–40.
- Hamamoto R, Silva FP, Tsuge M, Nishidate T, Katagiri T, Nakamura Y, et al. Enhanced SMYD3 expression is essential for the growth of breast cancer cells. *Cancer Sci*. 2006;97(2):113–8.
- Luo XG, Zou JN, Wang SZ, Zhang TC, Xi T. Novobiocin decreases SMYD3 expression and inhibits the migration of MDA-MB-231 human breast cancer cells. *IUBMB Life*. 2010; 62(3):194–9.
- Zou JN, Wang SZ, Yang JS, Luo XG, Xie JH, Xi T. Knockdown of SMYD3 by RNA interference down-regulates c-Met expression and inhibits cells migration and invasion induced by HGF. *Cancer Lett*. 2009;280(1):78–85.

11. Wang SZ, Luo XG, Shen J, Zou JN, Lu YH, Xi T. Knockdown of SMYD3 by RNA interference inhibits cervical carcinoma cell growth and invasion in vitro. *BMB Rep*. 2008;41(4):294–9.
12. Luo XG, Ding Y, Zhou QF, Ye L, Wang SZ, Xi T. SET and MYND domain-containing protein 3 decreases sensitivity to dexamethasone and stimulates cell adhesion and migration in NIH3T3 cells. *J Biosci Bioeng*. 2007;103(5):444–50.
13. Sims RJ 3rd, Reinberg D. From chromatin to cancer: a new histone lysine methyltransferase enters the mix. *Nat Cell Biol*. 2004;6(8):685–7.
14. Miremadi A, Oestergaard MZ, Pharoah PD, Caldas C. Cancer genetics of epigenetic genes. *Hum Mol Genet*. 2007;16 Spec No 1:R28–49.
15. Wade PA. Methyl CpG-binding proteins and transcriptional repression. *Bioessays*. 2001;23(12):1131–7.
16. Dobosy JR, Selker EU. Emerging connections between DNA methylation and histone acetylation. *Cell Mol Life Sci*. 2001; 58(5–6):721–7.
17. Wang Y, Fischle W, Cheung W, Jacobs S, Khorasanizadeh S, Allis CD. Beyond the double helix: writing and reading the histone code. *Novartis Found Symp*. 2004;259:3–17 (discussion -21, 163–9).
18. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet*. 2007;8(4):286–98.
19. Suzuki M, Shigematsu H, Shames DS, Sunaga N, Takahashi T, Shivapurkar N, et al. Methylation and gene silencing of the Ras-related GTPase gene in lung and breast cancers. *Ann Surg Oncol*. 2007;14(4):1397–404.
20. Zhao P, Zhong W, Ying X, Yuan Z, Fu J, Zhou Z. Manganese chloride-induced G0/G1 and S phase arrest in A549 cells. *Toxicology*. 2008;250(1):39–46.
21. Obaya AJ, Sedivy JM. Regulation of cyclin-Cdk activity in mammalian cells. *Cell Mol Life Sci*. 2002;59(1):126–42.
22. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*. 2002;2(9):647–56.
23. Antonsson B. Bax and other pro-apoptotic Bcl-2 family “killer-proteins” and their victim the mitochondrion. *Cell Tissue Res*. 2001;306(3):347–61.
24. Takayama S, Sato T, Krajewski S, Kochel K, Irie S, Millan JA, et al. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell*. 1995;80(2): 279–84.
25. Wang HG, Takayama S, Rapp UR, Reed JC. Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1. *Proc Natl Acad Sci USA*. 1996;93(14):7063–8.
26. Downward J. Ras signalling and apoptosis. *Curr Opin Genet Dev*. 1998;8(1):49–54.

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