Knockdown of the Bmi-1 oncogene inhibits cell proliferation and induces cell apoptosis and is involved in the decrease of Akt phosphorylation in the human breast carcinoma cell line MCF-7

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Abstract. It is well documented that B cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1), widely overexpressed in the vast majority of malignancies, plays an essential role in the occurrence and development of several different tumors. Here, we report Bmi-1 siRNA-mediated cell proliferation inhibition and cell apoptosis in vitro and in vivo in the human breast carcinoma cell line MCF-7. Our results demonstrated that Bmi-1 siRNA effectively down-regulated the expression of Bmi-1, inhibited cell proliferation in vitro and in vivo, evoked cell cycle arrest in the G0/G1 phase and induced cell apoptosis in MCF-7 cells, coupled with decrease in cyclin D1, cyclin E, cdk2, bcl-2 and Ki-67 expression and Akt phosphorylation levels and an increase of p21 and bax expression and activities of caspase-3/-9. Taken together, our results suggest that Bmi-1 may be a potential molecular target for the therapy of breast carcinoma.

Introduction

Breast carcinoma is the most common malignancy diagnosed among women and is the leading cause of cancer death worldwide (1). In 2000 alone, more than one million women were diagnosed and 373,000 women died of breast carcinoma (1). In the past few decades, the incidence of breast carcinoma has increased steadily, but the mortality of breast carcinoma seems to be declining (2), suggesting a benefit from the progression of early diagnosis and effective treatment. However, to date, the etiology of most breast carcinoma cases remains ill-defined, and the molecular mechanisms underlying the development and progression of breast carcinoma are complex and vary among individual tumors (3). Therefore, a better understanding of the molecular mechanisms in development and progression of breast carcinoma helps to improve the survival and prognosis of patients with breast carcinoma, providing the theoretical basis for molecular target therapy of the patients with breast carcinoma.

Polycomb group (PcG) proteins, as epigenetic gene silencers, play a pivotal role in the occurrence and development of tumors (4). Bmi-1, the first identified PcG gene, has been documented to be involved in multiple biological processes, such as embryonic development, organ formation, tumorigenesis, stem cell stabilization and differentiation (5). With the rapid development of molecular biology and oncology, more and more evidence has shown that Bmi-1 is tightly associated with the development of tumors, including non-small cell lung cancer (6), colorectal cancer (7), nasopharyngeal carcinoma (8), oral cancer (9), esophageal squamous cell carcinoma (10), breast carcinoma (11,12). However, detailed molecular mechanism of Bmi-1 in occurrence and development of breast carcinoma remains to be elucidated. Therefore, in the current study, Bmi-1 siRNA was used to down-regulate the expression of Bmi-1 in breast carcinoma, followed by analysis of cell proliferation and detection of cell apoptosis in vitro and in vivo. Further, expressions of cell cycle regulatory genes including cyclin D1, cyclin E, cdk2 and p21 and cell apoptosis related genes such as bcl-2 and bax were investigated by semi-quantitative RT-PCR and Western blotting methods, and activities of caspase-3/-9 and expression of Ki-67 were detected. The findings presented herein will provide the first new data for future gene therapy of breast carcinoma.

Materials and methods

Cell line and cell culture. Breast carcinoma cell line MCF-7 cells (human breast epithelial carcinoma) were purchased from the Institute of Medical Biotechnology, Chinese Academy of
Medical Sciences, and were maintained in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C in the presence of 5% CO2.

**Bmi-1 siRNA and control siRNA transfection.** Bmi-1 siRNA and control siRNA (Santa Cruz Co., CA, USA) at a concentration of 100 nM were transfected into MCF-7 cells, respectively. MCF-7 cells were cultured at 37˚C in a CO2 incubator until the cells are 60-80% confluent, subsequently, the cells were transfected with Lipofectamine™ 2000 (Invitrogen) according to manufacturer's instructions. The transfection reagent (10 μl) and Bmi-1 siRNA or control siRNA (6 μl each) were incubated with MCF-7 cells in RPMI-1640 culture (serum-free media) for 6 h, and then complete media were used to culture further 48 h for experiments below.

**CCK-8 for cell proliferation assay.** Cell proliferation was determined using WST-8 dye (Beyotime Institute of Biotechnology, China) according to manufacture's instructions. Briefly, 5x10^3 MCF-7 cells/well was seeded in a 96-well cell culture plate, grown at 37˚C for 24 h, and then placed in serum-starved conditions for a further 6 h. Subsequently, MCF-7 cells were transfected with Bmi-1 siRNA and control siRNA, respectively. On the day of measuring the growth rate of untreated and transfected cells (24, 48, 72 and 96 h, respectively), 100 μl of spent medium was replaced with an equal volume of fresh medium containing 10% CCK-8, then cells were continued to incubate at 37˚C for 3 h, and the absorbance was finally determined at 450 nm using Microplate reader.

**Cell cycle analysis by flow cytometry.** Cell cycle was performed using flow cytometry according to previous published methods (13). Briefly, 1x10^6 of untreated and transfected MCF-7 cells were harvested and washed in PBS, then fixed in 70% cold ethanol for 30 min at 4˚C. After washing in cold PBS thrice, cells were resuspended in 1 ml of PBS solution with 40 μg/ml of propidium iodide and 100 μg/ml of RNase A for 30 min at 37˚C. Finally, samples were subjected to analysis of their DNA contents by Becton-Dickinson FACScan Flow Cytometer (FAC Scan, Becton-Dickinson, Mountain View, USA). CellQuest software was used for acquisition and analysis of the data, and the percentage of cells in each phase was determined.

**Cell apoptosis detection.** MCF-7 cells untreated and transfected with control siRNA and Bmi-1 siRNA were trypsinized, washed with cold PBS buffer, and then resuspended in PBS buffer, respectively. Annexin V-FITC (BD Biosciences, USA) at a final concentration of 1 μg/ml and 250 ng of propidium iodide were added to a mixture containing 100 μl of cell resuspension and binding buffer (BD Biosciences) each. After cells were vortexed and incubated for 15 min at room temperature (RT) in the dark, 400 μl of binding buffer was added to the mixture for flow cytometric analysis. CellQuest software was used for acquisition and analysis of the data, and the percentage of cell apoptosis was determined.

**Measurement of caspases activity.** MCF-7 cells untreated and transfected with control siRNA and Bmi-1 siRNA were harvested and centrifuged at 1200 rpm for 10 min, respectively. Cells were washed two times with PBS (pH 7.4) and then resuspended with 50 μl lysis buffer at 4˚C and incubated on ice for 10 min. All the following steps were performed on ice. Cell extracts (30 μg) were diluted to 100 ml with 50 μl of 2x reaction buffer (40 mM PIPES, pH 7.2, 200 mM NaCl, 2 mM EDTA, 0.2% CHAPS, 20% sucrose and 20 mM DTT), and incubated for 30 min at 37˚C with 10 μM of fluorogenic caspase substrates of caspase-3 and -9 (Ac-DEVD-AMC and Ac-LEHD-AFC), respectively. After overnight incubation in dark at 37˚C, samples were read in a microplate reader at 405 nm. The data represent the mean ± SD from three separate experiments. The results are expressed as fold induction.

### Table I. Primers used for semi-quantitative RT-PCR analysis.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers</th>
<th>Size (bp)</th>
<th>Annealing temperature (˚C)</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cyclin D1</td>
<td>F: 5'-GCCCGAGGAGCTGCTGCAA-3' R: 5'-CCTGGGCGGACCTGCTGCTC-3'</td>
<td>386</td>
<td>55</td>
<td>NM_053056</td>
</tr>
<tr>
<td>cyclin E</td>
<td>F: 5'-GCCGGATTCGCTGCTGCTC-3' R: 5'-AGCGGGGACGCTGCTGCTC-3'</td>
<td>251</td>
<td>55</td>
<td>NM_001238</td>
</tr>
<tr>
<td>cdk2</td>
<td>F: 5'-TCCAGCTGCTCGAGGAC-3' R: 5'-CCGGGCCCACCTGGAACG-3'</td>
<td>309</td>
<td>54</td>
<td>NM_052827</td>
</tr>
<tr>
<td>bcl-2</td>
<td>F: 5'-CGGGTCCACTGCTGCTC-3' R: 5'-TCCCCAGTCCACCGCGG-3'</td>
<td>174</td>
<td>54</td>
<td>NM_000633</td>
</tr>
<tr>
<td>bax</td>
<td>F: 5'-CTGGACCGGCGTCGCAAGA-3' R: 5'-GCTGTCGTCGAGGCGGCCG-3'</td>
<td>122</td>
<td>55</td>
<td>NM_138764</td>
</tr>
<tr>
<td>β-actin</td>
<td>F: 5'-TATGGGCGGAGGAGCCGTC-3' R: 5'-GGAGCTGGAAGCAGCGCTG-3'</td>
<td>546</td>
<td>55</td>
<td>NM_001101</td>
</tr>
</tbody>
</table>
Semi-quantitative RT-PCR. Total RNAs were extracted from MCF-7 cells untreated and transfected with control siRNA and Bmi-1 siRNA by TRizol reagent (Invitrogen) according to the manufacturer’s instructions, and then subjected to first-strand cDNA synthesis with AMV first strand DNA synthesis kit (Biotech Co., Shanghai, P.R. China). Primers in Table I were used to amplify the specific band using the procedures described as follows: initial denaturation at 94°C for 2 min, followed by 30 sec at 94°C, 30 sec at different genes annealing temperatures (Table I), and 30 sec at 72°C for a total of 30 cycles and a terminal extension at 72°C for 6 min. After amplification, 10 μl of PCR products were resolved on a 1% agarose gel. DNA bands were visualized by UV light and documented with a Gene Tools (Model P67UA). Semi-quantitative analysis of Band intensity was performed with Gene Tools software (UVP, Inc., Upland, USA).

Western blot analysis. Western blotting was performed according to published methods (14). Briefly, MCF-7 cells untreated and transfected with control siRNA and Bmi-1 siRNA were harvested and lysed for 20 min in cold lysis buffer. Protein concentrations were determined with standard Bradford method. Equal amounts of protein (60 μg/lane) for each sample were electrophoresed through a 10% SDS-PAGE gel, and then electro-transferred to nitrocellulose membranes (Amersham, Uppsala, Sweden) by a semi-dry transferor. The membranes were blocked in 5% skimmed milk in PBS-T containing 0.05% Tween-20 at RT for 2 h, and then incubated at RT for 2 h with corresponding primary antibodies including anti-Bmi-1, anti-cyclin D1, anti-cyclin E, anti-cdk2, anti-p21, anti-bcl-2, anti-bax, anti-Ki-67, anti-Akt, anti-phospho-Akt at Ser308, anti-phospho-Akt at Ser473 and anti-ß-actin (all from Santa Cruz, Co.) diluted in 1% skimmed milk in PBS-T, respectively, followed by incubation with horse-radish peroxidase-conjugated secondary antibodies (anti-goat, anti-rabbit or anti-mouse) (Santa Cruz, Co.). For detection, DAB solution was used to develop the bands of specific proteins on the membranes according to manufacturer’s instructions. Quantification of band intensity was performed using Gene Tools software (UVP, Inc., Upland, USA).

Statistical analysis. For cell proliferation, cell cycle, cell apoptosis, RT-PCR, Western blotting and in vivo tumorigenic assays, values were obtained from three independent experiments as described above. The data were performed by one-way analysis of variance using SPSS version 13.0 (SPSS, Chicago, USA). Summary statistics are expressed as the means ± standard deviations. In the statistical analyses, a P<0.05 was considered statistically significant and all P-values were two-sided.

Results

Bmi-1 siRNA down-regulates the Bmi-1 expression and evokes decrease of Akt phosphorylation. It is well known that Akt phosphorylation status and p21 level are tightly associated with cell survival, cell cycle and cell apoptosis in multiple different tumors. In this study, total proteins were extracted at 48 h from untreated MCF-7 cells transfected with control siRNA and Bmi-1 siRNA, followed by Western blot analysis for expressions of Bmi-1 and p21 proteins and level of Akt phosphorylation. The results showed that expression of Bmi-1 protein in Bmi-1 siRNA group was obviously lower than that in untreated group or control siRNA group (P<0.05), while expression of p21 protein was contrary to expression of Bmi-1 protein (Fig. 1). In addition, the levels of Akt phosphorylation at Ser308 and Ser473 were both evidently reduced after Bmi-1 siRNA treatment, while there was no change in the total Akt protein level (Fig. 1). These results indicate that down-regulation of Bmi-1 expression results in the increase of p21 expression and decrease of Akt phosphorylation level. However, the precise mechanism for these events remains to be elucidated.

Bmi-1 siRNA inhibits proliferation of breast carcinoma cells in vitro. Recently, p21 was perceived mainly as an anticancer protein because of its antiproliferative effects (18). The down-regulation of Bmi-1 expression mediated the up-regulation of...
Bmi-1 siRNA group was markedly lower than that in untreated percentage of cell numbers in S phase (14.85±2.65%) in comparison could arrest cell cycle at G0/G1 phase. In addition, the (Fig. 3A), suggesting that down-regulation of Bmi-1 expres- or control siRNA group (45.41±1.37%) (F=72.199, P=0.000) was obviously higher than that in untreated (46.40±1.85%) (30.62±1.86%) or control siRNA group (30.69±2.35%) (F=46.852, P=0.000), indicating that Bmi-1 siRNA resulted in the blockage of DNA synthesis (Fig. 3A). Moreover, in order to explore the possible molecular mechanism of changes of cell cycle distributions, a large number of genes involved in cell cycle kinetics were investigated by RT-PCR and Western blotting methods. The results revealed that expressions of cyclin D1, cyclin E and cdk2 mRNA and proteins were obviously down-regulated compared with untreated group and control siRNA group (Fig. 3B-E). These findings suggest that cell cycle changes may be tightly correlated with aberrant changes of cell cycle related genes, such as cyclin D1, cyclin E and cdk2.

Flow cytometry analysis for cell apoptosis. It is well known that cell apoptosis play a pivotal role in the occurrence and development of tumors. Whether down-regulation of Bmi-1 expression is involved in the apoptosis of MCF-7 cells. In this study, flow cytometry was utilized to analyze the apoptosis of MCF-7 cells mediated by Bmi-1 siRNA. The results demonstrated that the proportions of early cell apoptosis (region II) (21.28±1.71%) in Bmi-1 siRNA group were evidently higher than that in untreated group (3.33±0.20%), and the tendency of total cell apoptosis rates (region II plus III) was consistent with that of early cell apoptosis rate (Fig. 4). However, there was no difference in the early apoptosis rate and total apoptosis rate between MCF-7 cells untreated and transfected with control siRNA (P>0.05), suggesting the introduction of Bmi-1 siRNA has an obvious inductive effect on apoptosis of MCF-7 cells. Further, to elucidate the possible molecular mechanism of cell apoptosis mediated by Bmi-1 siRNA, we then investigated expressions of bcl-2 and bax tightly associated with cell apoptosis after Bmi-1 siRNA treatment. The results revealed that the expressions of apoptosis inhibitor bcl-2 mRNA and protein in Bmi-1 siRNA group were significantly lower than that in untreated group or control siRNA group, while bax expression was in contrast with bcl-2 expression. Furthermore, Akt has been verified to be a large number of downstream substrates that contribute to cell cycle arrest. Cell cycle analysis was carried out using Flow cytometry, the result demonstrated that the percentage of cell numbers in G1/G phase (67.52±3.76%) in Bmi-1 siRNA group was obviously higher than that in untreated (46.40±1.85%) or control siRNA group (45.41±1.37%) (F=72.199, P=0.000) (Fig. 3A), suggesting that down-regulation of Bmi-1 expression could arrest cell cycle at G0/G1 phase. In addition, the percentage of cell numbers in S phase (14.85±2.65%) in Bmi-1 siRNA group was markedly lower than that in untreated
cell apoptosis (19), therefore, down-regulation of bcl-2 expression and elevation of bax expression evoked by Bmi-1 siRNA was regulated at least in part by the decrease of Akt phosphorylation level in MCF-7 cells, but the precise mechanism remains to be elucidated. In a word, Bmi-1 mediated cell apoptosis in MCF-7 cells may be tightly associated with the status of Akt phosphorylation and changes of bcl-2 and bax expressions.

Caspase-3/9 activity analysis. An important component of the apoptotic machinery is the proteolytic system of caspases. Therefore, we investigated the activation of caspase-3 and caspase-9 48 h after transfection with Bmi-1 siRNA and control siRNA. The results demonstrated that caspase-3/9 activity significantly increased in Bmi-1 siRNA group compared to that in MCF-7 cells untreated or transfected with control siRNA, there were no differences in the activities of caspase-3/9 in MCF-7 cells untreated and transfected with control siRNA (Fig. 5), suggesting that Bmi-1 siRNA mediated cell apoptosis at least in part contribute to the increase of activities of caspase-3/9.

The inhibitory effect of down-regulation of Bmi-1 expression on tumor formation in nude mice. To further verify the down-regulation of Bmi-1 expression mediated the proliferation inhibition in vivo, MCF-7 cells implanted into the right flank of nude mice developed palpable tumors after 2 weeks (Fig. 6A), and the volumes of the animals were monitored for tumor inspection every other day after Bmi-1 siRNA treatment. The results revealed that there was an obviously decrease in the volumes and weights of tumors in Bmi-1 siRNA group, compared to that in the untreated or control siRNA groups (Fig. 6B-D) (P<0.05). However, there was no difference between untreated and control siRNA group in
the volumes and weights of tumors (Fig. 6B-D) \((P>0.05)\), suggesting Bmi-1 down-regulation could significantly inhibit the tumor formation. Combined with the results \textit{in vitro}, our data presented here demonstrate that Bmi-1 may be a potential molecular target for the therapy of breast carcinoma.
Bmi-1 siRNA down-regulates the expression of Bmi-1 and decreases proliferation but increases apoptosis of breast carcinoma cells in vivo. To verify whether the Bmi-1 siRNA could down-regulate the Bmi-1 expression in vivo, immunohistochemistry was used to analyze the Bmi-1 expression in the tissues of nude mice. The results demonstrated that Bmi-1 expression was obviously down-regulated compared with untreated group and control siRNA group (P<0.05) (Fig. 7A).

Further, to quantitatively compare the proliferation and apoptotic index of tumors treated with Bmi-1 siRNA, the tumor sections were stained for expression of Ki-67 and TUNEL assay, respectively. As shown in Fig. 7A, Bmi-1 siRNA treatment significantly decreases the number of Ki-67-positive cells in tumors compared with untreated group and control siRNA. Based on the counting of 40 randomly selected microscopic fields, the proliferation index was decreased from 86.35±3.47% in the untreated group, 83.89±4.39% in control siRNA group to 43.71±3.16% in Bmi-1 siRNA-treated tumors (Fig. 7B). In contrast to the proliferation index, Bmi-1 siRNA-treated tumors compared with untreated group and control siRNA group showed increased numbers of apoptotic cells with condensed and irregularly shaped nuclei, staining positively for TUNEL (Fig. 7A). Based on the counting of randomly selected microscopic fields, the number of apoptotic cells was increased from 9±3 per 10 high-power microscopic fields in untreated group, 11±4 to 93±11 in Bmi-1 siRNA-treated tumors (Fig. 7C). The changes in both proliferation and apoptosis indexes were statistically significant among three groups (both P<0.05).

Discussion

Bmi-1 was originally discovered as an oncogene that cooperated with c-Myc in the initiation of lymphoma in murine models (20,21). Bmi-1 may be new diagnostic and prognostic marker in multiple different tumors including medulloblastoma (22), tongue squamous cell carcinoma (23), colon carcinoma (24), breast carcinoma (11), bladder carcinoma (25). However, precise molecular regulation mechanism of Bmi-1 in breast carcinoma remains elusive, prompting the authors to investigate the possible molecular...
mechanism of Bmi-1 in occurrence and development of breast carcinoma.

It is well documented that Bmi-1 plays important roles in cell cycle regulation, cell immortalization and cell senescence (26). Previous studies have suggested that Bmi-1 may enhance cell survival by regulating the level of cell cycle regulatory genes (7,27,28). Lee et al found overexpression of Bmi-1 was associated with increased levels of cyclin D1, cyclin-dependent kinases such as cdk2 and cdk4 (29). The absence of modulation of Bmi-1 level is consistent with a recent report in SCC-4 oral cancer cells, where cell growth was slowed by siRNA knockdown of Bmi-1 expression (9).

All the studies mentioned above have suggested that Bmi-1 was closely correlated with cell proliferation and cell cycle of tumors. However, to date, effect of Bmi-1 siRNA on cell proliferation and cell cycle in breast carcinoma cells remains ill-defined. In the present study, our data showed that Bmi-1 siRNA could markedly inhibit cell proliferation in MCF-7 cells. The results of cell cycle analysis suggested that Bmi-1 siRNA could arrest cell cycle at G0/G1 phases and inhibit DNA synthesis, however, to clarify whether Bmi-1 mediated proliferation inhibition and cell cycle arrest of MCF-7 cells were involved in changes of a series of cell cycle regulatory genes, we investigated the expressions of a large number of genes involved with cell cycle kinetics including p21, cyclin D1, cyclin E and cdk2, we found that Bmi-1 siRNA resulted in a decrease in cyclin D1, cyclin E and cdk2 levels, whereas a increase in p21 level, which might have a direct relevance to cell proliferation inhibition and G0/G1 phase arrest observed in MCF-7 cells.

Apoptosis, the process of programmed cell death, is governed by complex, gene-directed pathways, and is involved in expressions changes of a series of genes (30). Dysregulation of apoptosis plays a key role in tumorigenesis and
can allow tumor cells to become resistant to anticancer treatments (30). Akt/protein kinase B (PKB) plays a critical role in controlling the balance between cell survival and apoptosis (31). Akt regulates cellular survival and metabolism by binding and regulating many downstream effectors, e.g. nuclear factor-xB, bcl-2 family proteins and murine double minute 2 (MDM2) (32). In addition, the high level of expression of phosphorylated Akt correlated with Ki-67 cell proliferation activity and inhibitors of apoptosis, suggesting decrease of Akt phosphorylation level induces cell apoptosis. In the current study, the results of flow cytometry revealed that Bmi-1 siRNA could induce cell apoptosis in MCF-7 cells, meanwhile, to elucidate the possible mechanism of MCF-7 cell apoptosis, the levels of Akt phosphorylation at Ser308 and Ser473 were detected using Western blotting method. The results demonstrated that down-regulation of Bmi-1 expression obviously gave rise to the decrease of the levels of Akt phosphorylation at Ser308 and Ser473, suggesting Bmi-1 siRNA mediated MCF-7 cell apoptosis may be tightly associated with reduced level of Akt phosphorylation. Additionally, caspase-3 works downstream of Akt in the cell signaling pathway, subsequently leading cells to undergo apoptosis (33), once phosphorylated, Akt is released from the cell membrane and migrates to intracellular locations where Akt can activate other proteins, resulting in the suppression of cellular apoptosis. Activated Akt leads to cellular survival by several mechanisms, including suppressing caspase-9 and caspase-3 activation (34). To further verify the decrease of Akt phosphorylation level-mediated cell apoptosis of breast carcinoma, the activities of caspase-3 and caspase-9 and expressions of bcl-2 and bax were analyzed, the results suggested that Bmi-1 siRNA markedly increased the activities of caspase-3 and caspase-9 and expression of bax, but reduced the expression of bcl-2. These findings suggest that down-regulation of Bmi-1 expression mediated cell apoptosis of breast carcinoma was tightly associated with the decrease of Akt phosphorylation level, followed by increase of the activities of caspase-3 and caspase-9 and expression of bax, and decrease of bcl-2.

Given the role of Bmi-1 in breast carcinoma in vitro, we investigated whether the same effect appears in vivo. Our results demonstrated that Bmi-1 effectively inhibited the proliferation of tumors in nude mice. The expression of the human Ki-67 protein is strictly associated with cell proliferation. The Ki-67 index is the best available marker of proliferation because it is not involved in DNA repair process as proliferation cell nuclear antigen (PCNA). High Ki-67 expression was also significantly and independently associated with decreased survival, in accordance with the findings of other studies (35). In this study, we evaluated the Ki-67 expression representing the fraction of cellular proliferation to prove Bmi-1 siRNA mediated proliferation inhibition. Our results demonstrated that Bmi-1 siRNA treatment significantly decreases the number of Ki-67-positive cells in tumors of nude mice compared with untreated group and control siRNA, and the proliferation index was obviously decreased after treatment of Bmi-1 siRNA. In contrast to the proliferation index, Bmi-1 siRNA-treated tumors compared with untreated group and control siRNA group showed increased numbers of apoptotic cells. These results mentioned above suggest that down-regulation of Bmi-1 expression evokes cell proliferation inhibition closely associated with decrease of Ki-67 expression and cell apoptosis in vivo.

In conclusion, we found the down-regulation of Bmi-1 expression inhibits the proliferation of breast carcinoma cells in vitro and in vivo, followed by cell cycle arrest and cell apoptosis, which may be tightly associated with changes of cell cycle and apoptosis-related genes, which will provide important insights into the role of Bmi-1 in occurrence and development of breast carcinoma, in the hope that manipulation of Bmi-1 will provide the potential molecular target for therapy of breast carcinoma.

References