Molecular mechanism underlying the anticancer effect of simvastatin on MDA-MB-231 human breast cancer cells

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Abstract. Breast carcinoma is the leading cause of cancer-associated mortality in female individuals worldwide. Previous studies have investigated the pro-apoptotic and antimetastatic effects of statins, and have demonstrated that simvastatin exhibits antitumor activity and potent chemopreventive effects. However, the mechanism underlying the effects of simvastatin in breast cancer remains to be elucidated. The present study demonstrated that simvastatin inhibited the proliferation of MDA-MB-231 human breast cancer cells in a dose-dependent manner, decreased the protein expression of B cell lymphoma 2 (Bcl-2) and increased the protein expression of Bcl-2-associated X protein in time- and dose-dependent manners. In addition, simvastatin arrested cells in the G_0/G_1 phase of the cell cycle, downregulated the protein expression levels of cyclin D1 and cyclin-dependent kinase (CDK)2, mediated the mitochondria-dependent caspase cascade by increasing the protein expression levels of caspase-3, -8 and -9, and downregulated the protein expression of X-linked inhibitor of apoptosis, which induced cell apoptosis. In addition, simvastatin decreased the protein expression of matrix metalloproteinase (MMP)-2 and suppressed the activation of nuclear factor (NF)-κB in the MDA-MB-231 cells. Taken together, these results demonstrated that the antitumor effect of simvastatin in the human MDA-MB-231 breast cancer cell line was via the inhibition of cell proliferation, affecting the cell cycle, downregulating the expression levels of cyclin D1 and CDKs, inducing apoptosis and decreasing the expression of MMP-2, possibly by inhibiting the activation of NF-κB. Statin treatment may provide a novel therapeutic approach for the treatment of breast cancer.

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Introduction

Breast cancer is the most common type of cancer in females and is the predominant cause of female cancer-associated mortality worldwide, accounting for 23% of the cases of newly diagnosed cancer and 14% of the total cancer-associated mortality (1). Current therapies using endocrine agents, particularly selective estrogen receptor (ER) modulators and, more recently, aromatase inhibitors, have successfully prevented or treated ER-positive breast cancer by interfering with estrogen signaling or production (2). However, these drugs have been observed to reduce the incidence of breast cancer by only 50% and had no effect in preventing ER-negative breast cancer, which accounts for 30% of all cases of breast cancer in North America (3,4). ER negativity is frequently combined with high grade tumors and the proliferation and overexpression of human epidermal growth factor receptor (HER)2/neu, resulting in a poor prognosis (5,6). Therefore, effective novel drugs with different molecular structures from conventional chemotherapeutic agents, which may aid in the prevention or treatment of ER-negative breast cancer require development.

Statins lower serum cholesterol levels by inhibiting 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in the mevalonate pathway (7). This pathway produces various end products, including cholesterol, dolichol, ubiquinone, isopentenyladenine, geranylgeranyl pyrophosphate and farnesyl pyrophosphate, which are critical for normal cellular functions, including cell proliferation, differentiation and survival, in normal and cancerous cells (8,9). Statins are currently used as cholesterol-lowering medications and exhibit effectiveness in the primary and secondary prevention of heart disease and stroke (10). In addition, statins interest for their use in cancer prevention has increased. The anticancer function of statins is based on preclinical evidence of their antiproliferative, pro-apoptotic and anti-angiogenic properties. A growing body of evidence suggests that various statins possess antiproliferative, anti-invasive, antimetastatic and pro-apoptotic properties in various types of cancer cell (11-14). Simvastatin, one of the HMG-CoA reductase inhibitors, is currently used as a safe and well-tolerated therapeutic agent for the treatment of hypercholesterolemia, atherosclerosis and stroke (15). Simvastatin demonstrates *in vitro* and *in vivo* antitumor

actions in several human malignancies including those of the breast, colon and prostate, which has been attributed to cell cycle arrest, thereby inhibiting cell proliferation and inducing apoptotic and necrotic cell death (11,12,16). A previous study revealed that the use of simvastatin, a highly lipophilic statin, reduced the risk of recurrence in Danish females with Stage I-III breast cancer, with 10 fewer cases per 100 females over 10 years (17). In addition, patients with breast cancer on long-term statin treatment have proportionately fewer ER/progesterone (PR)-negative tumors, which are of a lower grade and stage compared with patients who have never received statin treatment (18). By contrast, Bonovas *et al* concluded from a meta-analysis of seven randomized and nine observational breast cancer trials, that treatment with statins failed to significantly affect the risk of breast cancer (19). However, the efficacy and the molecular mechanism underlying the effects of simvastatin on breast cancer progression remain to be elucidated.

The requirement for alternative therapeutic strategies is increasing and these findings, in parallel with limited knowledge of the affect of simvastatin on breast cancer, led to the present study evaluating its potential for therapeutic effects in breast cancer, as the antitumor and cancer chemopreventive effects of statins on breast cancer require further investigation. The present study hypothesized that statin therapy may reduce the progress of breast carcinoma by inhibiting cell proliferation, altering the cell cycle, inducing apoptosis, downregulating the protein expression levels of cyclin D1 and cyclin dependent kinases (CDKs) and decreasing the expression of matrix metalloproteinase (MMP)-9. MDA-MB-231 cells were used *in vitro* to confirm this.

Materials and methods

Cell culture. The human MDA-MB-231 breast cancer cell line was kindly provided by the Laboratory of Molecular Biology of Anhui Medical University (Anhui, China). The cells ($1x10^5$/ml) were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA), supplemented with 10% heat inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine, which were all purchased from Sijiqing Biological Engineering Materials (Hangzhou, China), and maintained in a humidified atmosphere of 5% CO_2 at 37˚C.

Drugs. Simvastatin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich) at a stock concentration of 1 mM and stored at -20˚C. The final concentrations of simvastatin were 3.125, 6.25, 12.5, 25, 50, 100 μM. The final concentration of DMSO in the DMEM was maintained at <0.1%. An equal volume of solvent was added to cells as a control.

Cell proliferation assay. The proliferation rate of the MDA-MB-231 cells was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma-Aldrich) assay. The exponentially growing cells were plated at a density of $1x10^4$ cells/well into 96-well plates, cultured overnight at 37˚C and subsequently treated with various concentrations of simvastatin for 72 h. Following incubation with simvastatin for 72 h, 20 μl MTT solution (5 mg/ml) was added to each well and the plates were incubated for a further 4 h at 37˚C. The colored formazan product was dissolved using 150 μl DMSO. The 96-well plates were then placed on a shaker for 10 min at room temperature to thoroughly dissolve the MTT product. The half maximal inhibitory concentration (IC_{50}) value was determined as the concentration resulting in 50% cell growth inhibition following 72 h exposure to simvastatin compared with the untreated control cells. Six replicate wells were used for each drug concentration and the assessment was performed independently in triplicate.

Cell cycle analysis by flow cytometry. The cells ($2x10^5$/well) were plated into 6-well dishes and treated with simvastatin at the IC_{50} concentration (7.979±0.201 μM) for 72 h. The adherent cells were harvested by trypsinization (Sijiqing Biological Engineering Materials), washed twice with phosphate-buffered saline (PBS; Sijiqing Biological Engineering Materials) and fixed overnight in 70% ethanol (Sijiqing Biological Engineering Materials) at 4˚C. The ethanol was removed and the cells were washed twice in PBS, prior to being resuspended in 1 ml propidium iodide (PI; Sigma-Aldrich)/Triton X-100 (Sigma-Aldrich) staining solution, containing PBS, 0.1% Triton X-100, 200 μg/ml RNAse A (Sigma-Aldrich) and 50 μg/ml PI in the dark for 30 min at 37˚C. The cell cycle was measured by flow cytometry (BD Biosciences, San Jose, CA, USA) and analyzed using Cell Quest WinMDI 2.9 software (BD Biosciences). The cell cycle profiles, including the G_0/G_1, S and G_2/M phases, were calculated using ModFit LT^{TM} 4.0 software.

Flow cytometric analysis of apoptosis. The cells were plated in the exponential growth phase into six-well plates, allowed to attach overnight at 37˚C and treated with simvastatin at the IC_{50} concentration for 72 h. Following treatment, the adherent and floating cells were collected, washed twice with precooled (4˚C) PBS and resuspended in 400 μl binding buffer (10 mM HEPES/NaOH pH 7.4; 140 mM NaCl; KCl; $MgCl_2$; and 2.5 mM $CaCl_2$). The cells were incubated with 5 μl annexin V-fluorescein isothiocyanate (BestBio, Shanghai, China) at room temperature in the dark for 15 min and then with 10μl PI (40 μg/ml) at room temperature in the dark for 5 min. The cell suspensions were transferred to flow cytometric analysis tubes and detected using flow cytometry. Cells without drug treatment were used as a control.

Western blotting. The MDA-MB-231 cells, growth with or without simvastatin, were washed with ice-cold PBS solution and scraped in lysis buffer (50 mM Tris-HCl pH7.4; 250 mM NaCl; 0.5% Triton X100; 10% glycerol; 1 mM dichlorodiphenyltrichloroethane; and 1 mM phenylmethylsulfonyl fluoride). The lysates were centrifuged at 16,853 x g for 30 min at 4˚C and the supernatant was collected. Briefly, the protein concentration of each sample was determined using a Bicinchoninic Acid Protein Assay kit (Beyotime Institute of Biotechnology, Inc., Shanghai, China). Equal quantities of protein from each sample were loaded onto 10% SDS-polyacrylamide minigels (HyClone Laboratories, Inc.) and electrophoresed. The proteins

were transblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) and then blocked with a solution of PBS, containing 5% non-fat milk and 0.1% Tween 20 (HyClone Laboratories, Inc.) for 2 h. The PVDF membranes were probed with specific primary antibodies against anti-B cell lymphoma-2 (Bcl-2; rabbit monoclonal; 1:2,000; Cell Signaling Technologies, Inc., Danvers, MA, USA), anti-Bcl-2 associated X protein (Bax; rabbit monoclonal; 1:2,000; Cell Signaling Technologies, Inc.), rabbit X-linked inhibitor of the apoptosis protein antibody (Xiap; rabbit monoclonal; 1:500; Santa Cruz Biotechnology, Inc., Carlsbad, CA, USA), anti-cyclin D1 (1:1,000; rabbit monoclonal; Abcam, Cambridge, MA, USA), rabbit CDK2 (1:1,000; rabbit monoclonal; Abcam), anti-caspase-3 (1:1,000; mouse monoclonal; Abcam), caspase-8 (1:1,000; mouse monoclonal; Abcam) and caspase-9 (1:1,000; mouse monoclonal; Abcam), rabbit MMP-2 antibody (1:1,500; rabbit monoclonal; Cell Signaling Technologies, Inc.), rabbit nuclear factor-κB antibody (NF-κB p65; mouse monoclonal; 1:1,500; Cell Signaling Technologies, Inc.) and anti-β-actin (1:1,500; rabbit monoclonal; Cell Signaling Technologies, Inc.). Following washing with Tris-buffered saline (Cell Signaling Technologies, Inc.), containing 0.1% Tween-20 three times, the PVDF membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5,000) at room temperature for 1 h. Positive bands were detected using enhanced chemilluminescence reagents (Millipore) and β-actin was used as a loading control.

Statistical analysis. The data were analyzed from three independent experiments and are expressed as the mean ± standard deviation. One-way analysis of variance and Student's t-test were performed to determine the statistical significance of any differences between the control and treatment groups. All statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Dose-dependent antiproliferative effects of simvastatin in the human breast cancer cell line. The effects of simvastatin on the proliferation of MDA-MB-231 cells were determined using an MTT assay. The MDA-MB-231 cells were treated with different doses of simvastatin (3.125-100 μM) for 72 h. A dose-dependent decrease in the cell viability was observed following treatment with simvastatin, exhibiting an IC_{50} value of 7.979±0.201 μM following exposure for 72 h (Fig. 1).

Effects of simvastatin treatment on Bcl-2 and Bax. The Bcl-2 and Bax family are important in the regulation of apoptosis, proliferation and invasion of tumor cells (20). The present study examined the protein expression levels of Bcl-2 and Bax using western blot analysis. To assess the effects of time and dose on response, the MDA-MB-231 cells were cultured with different concentrations of simvastatin for different durations. The most marked effects were observed following treatment with simvastatin at the IC_{50} for 72 h (P<0.05). In addition, treatment with 20 μm simvastatin significantly downregulated the protein expression of Bcl-2 and upregulated the protein expression of Bax in the MDA-MB-231 cells compared with the other concentrations (P<0.05). These results demonstrated that the effect of simvastatin on MDA-MB-231 cells occurred in a time- and dose-dependent manner (Fig. 2).

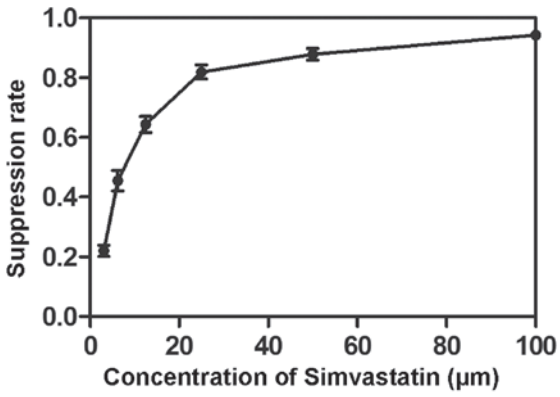

Figure 1. *In vitro* effects of simvastatin on the proliferation of breast cancer cells. An MTT assay was performed to examine the inhibitory activities of simvastatin on cell proliferation. The cells were exposed to varying concentrations of simvastatin for 72 h. Values are expressed as the mean ± standard deviation and each data point was repeated in at least three independent experiments.

Cell cycle effects of simvastatin. The cell cycle distribution of the cells exposed to simvastatin at the IC_{50} concentration for 72 h was assessed by flow cytometry. The percentage of G_0/G_1 phase cells was markedly increased following simvastatin treatment compared with the control group (P<0.05; Fig. 3A). This finding suggested that simvastatin arrested the cells at the G_0/G_1 phase of the cell cycle, which may be a mechanism underlying its antitumor effect. In addition, the cell cycle checkpoint proteins, cyclin D1 and CDK2, which are associated with distributional change, were also assessed. The protein expression levels of cyclin D1 and CDK were markedly decreased following pretreatment with simvastatin for 72 h, and occurred in a dose-dependent manner (P<0.05; Fig. 3B).

Effects of simvastatin on cell apoptosis. To examine whether the observed growth inhibition was caused by increased apoptosis, the present study investigated the apoptotic response of the MDA-MD-231 cell line treated with the IC_{50} of simvastatin using an annexin V/PI assay. As shown in Fig. 4A, the apoptotic rates induced by simvastatin in the MDA-MB-231 cells after 72 h were 9.54%. Furthermore, protein expression of caspase-3, -8 and -9 was detected following treatment with the IC_{50} concentration of simvastatin for 72 h. Notably, the protein expression levels of caspase-3, -8 and -9 were significantly increased in the simvastatin-treated MDA-MB-231 cells compared with the control group (P<0.05; Fig. 4B). These results demonstrated that simvastatin activated the caspase cascade reaction and was, therefore, important in the apoptotic response of MDA-MB-231 cells. In addition, Xiap is important in the regulation of tumor cell apoptosis. The present study measured the protein expression of Xiap in the MDA-MB-231 cells and found that simvastatin significantly downregulated the protein expression of Xiap (P<0.05; Fig. 4C).

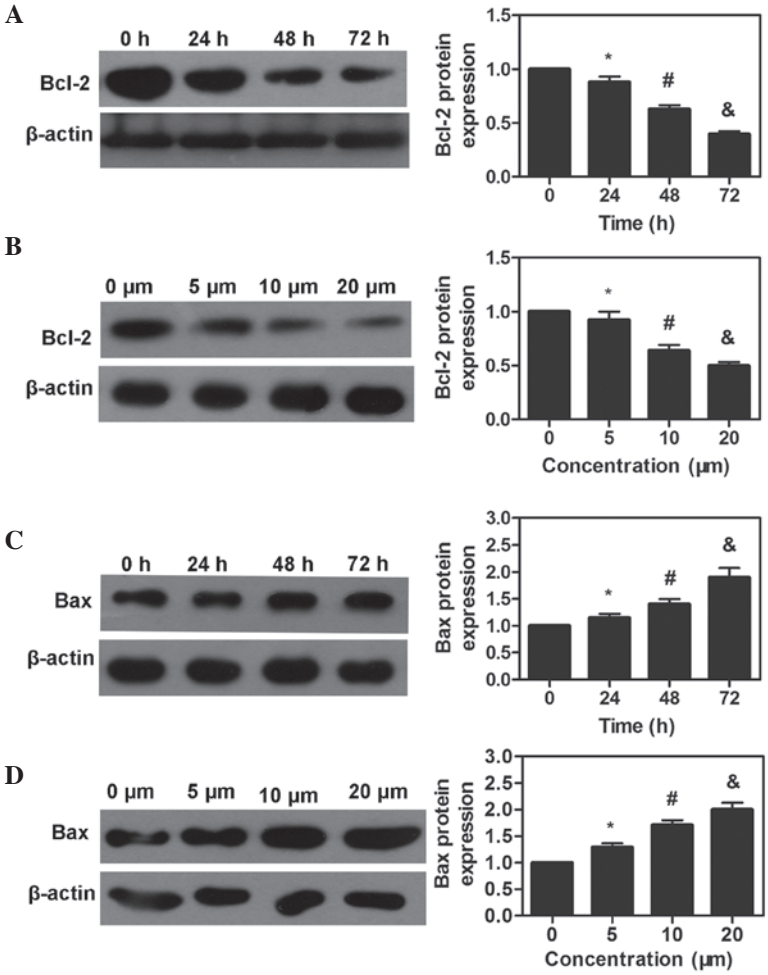

Figure 2. Protein expression levels of Bcl-2 and Bax were detected by western blotting in the MDA-MB-231 cells. (A) Protein expression of Bcl-2 following treatment with simvastatin for different durations (24, 48 and 72 h) at the IC_{50} concentration, with quantitative analysis (*P<0.05, vs. control group; $^{\#}$P<0.05, vs. 24 h group; $^{\&}$P<0.05, vs. 48 h group). (B) Protein expression of Bcl-2 protein following treatment with simvastatin at different concentrations (5, 10 and 20 μm) for 72 h, with quantitative analysis (*P<0.05, vs. control group; $^{\#}$P<0.05, vs. 5 μm group; $^{\&}$P<0.05, vs. 10 μm group). (C) Protein expression of Bax following treatment with simvastatin for different durations at its IC_{50} concentration, with quantitative analysis (*P<0.05, vs. control group; $^{\#}$P<0.05, vs. 48 h group; $^{\&}$P<0.05, vs. 48 h group). (D) Protein expression of Bax following treatment with simvastatin at different concentrations (5, 10 and 20 μm) for 72 h, with quantitative analysis (*P<0.05, vs. control group; $^{\#}$P<0.05, vs. 5 μm group; $^{\&}$P<0.05, vs. 10 μm group). Values are expressed as the mean ± standard deviation. Bcl, B-cell lymphoma; Bax, Bcl-2 associated X protien; IC_{50}, half maximal inhibitory concentration.

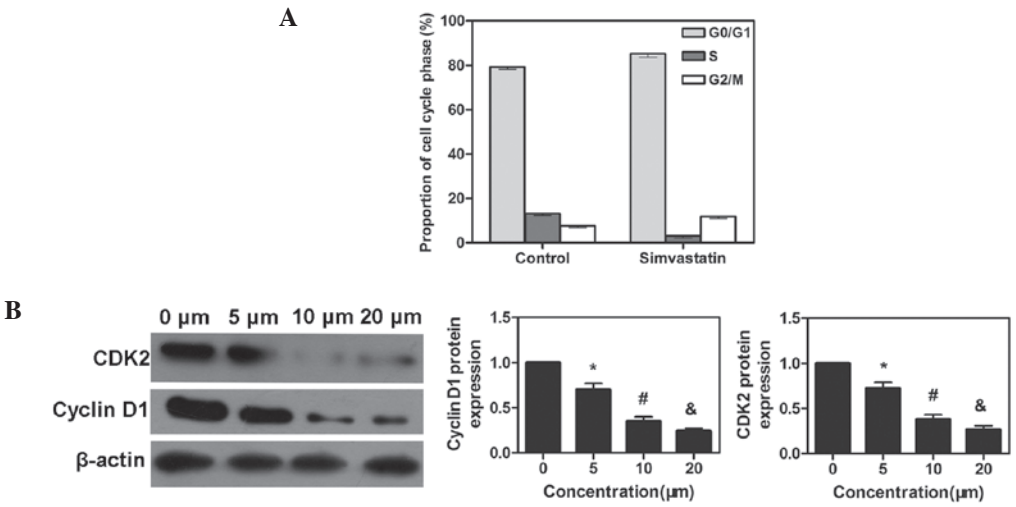

Figure 3. Effect of simvastatin on the cell cycle. (A) Flow cytometric analysis was performed to determine alterations in cell cycle distribution in MDA-MB-231 cells following treatment with the half maximal inhibitory concentration of simvastatin for 72 h. The MDA-MB-231 cells treated with simvastatin arrested the cell cycle at the G_0/G_1-phase (P<0.05). (B) Protein expression levels of cyclin D1 and CDK2 following treatment with simvastatin at different concentrations (5, 10 and 20 μm) for 72 h, with quantitative analysis (*P<0.05, vs. control group; $^{\#}$P<0.05, vs. 5 μm group; $^{\&}$P<0.05, vs. 10 μm group). Values are expressed as the mean ± standard deviation. CDK, cyclin-dependent kinase.

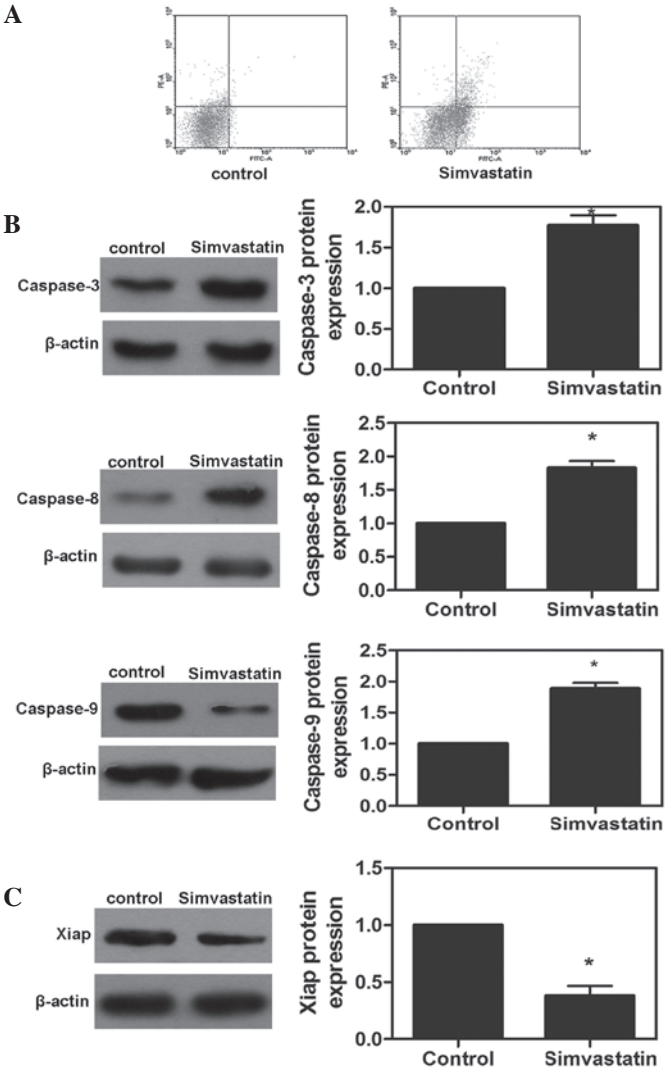

Figure 4. Effect of simvastatin on apoptosis. (A) Effects of treatment with simvastatin on apoptosis were analyzed in MDA-MB-231 cells using flow cytometry. Following treatment with the half maximal inhibitory concentration of simvastatin for 72 h, adherent and floating cells were collected and incubated with annexin V and propidium iodide. Apoptotic rates induced by simvastatin in the MDA- MB-231 cells after 72h were 9.54% which increased by 7.58% compared with the control group, as determined using WinMDI 2.9 software. (B) Protein expression levels of caspase-3, -8 and -9 were determined to evaluate the effect of caspase in simvastatin-induced apoptosis. Notably, the protein expression levels of caspase-3, -8 and -9 were significantly increased in the simvastatin-treated MDA-MB-231 cells compared with the control group (P<0.05). These results demonstrated that simvastatin activated the caspase cascade reaction and was involved in MDA-MB-231 cell apoptosis. (C) Xiap is important in the regulation of tumor cell apoptosis. The protein expression of Xiap in MDA-MB-231 cells was measured and simvastatin significantly downregulated the protein expression of Xiap (P<0.05) and induced the apoptosis of MDA-MB-231 cells. Values are expressed as the mean ± standard deviation. Xiap, X-linked inhibitor of apoptosis protein.

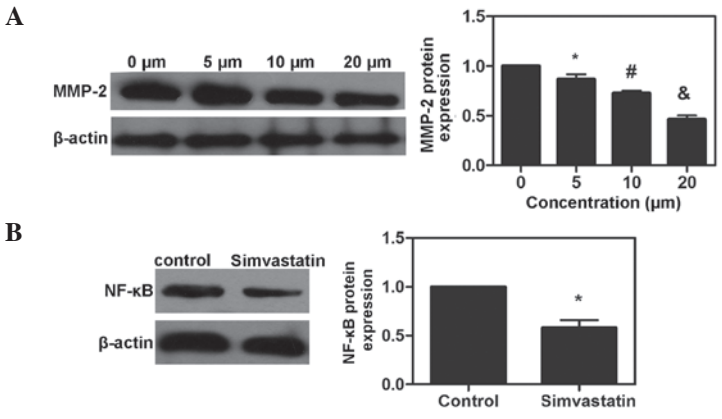

Figure 5. Protein expression levels of MMP-2 and NF-κB p65 in MDA-MB-231 cells following treatment with simvastatin. (A) Protein expression of MMP-2 following treatment with simvastatin at different concentrations (5, 10 and 20 μm) for 72 h, with quantitative analysis (*P<0.05, vs. control group; $^{\#}$P<0.05, vs. 5 μm group; $^{\&}$P<0.05, vs. 10 μm group). (B) Protein expression of NF-κB p65 following treatment with the half maximal inhibitory concentration of simvastatin for 72 h, with quantitative analysis (*P<0.05, vs. control group). Values are expressed as the mean ± standard deviation. NF, nuclear factor.

Simvastatin suppresses the expression of MMP-2 and the activation of NF-κB in MDA-MB-231 cells. The protein expression of MMP-2 was examined by western blot analysis, which revealed that treatment with simvastatin decreased the protein expression of MMP-2 in a dose-dependent manner ($P<0.05$). p65 is a major component of NF-κB, and the levels of NF-κB p65 were also examined. Following co-culture with simvastatin at the IC_{50} for 72 h, the expression of NF-κB p65 was significantly suppressed in the simvastatin-treated group ($P<0.05$). The results demonstrated that simvastatin suppressed the expression of MMP-2 and the activation of NF-κB in the MDA-MB-231 cells (Fig. 5).

Discussion

Breast cancer is one of the most life-threatening types of cancer among female individuals worldwide (1). Statins are widely used cholesterol-lowering drugs, and the use of statins has been observed to significantly lower the risk of cancer (7,17). Although an increasing quantity of evidence suggests that statins may have useful activity in breast cancer prevention and/or therapy (21), the molecular mechanisms underlying the neoplastic development and progression of statins in the breast remain to be elucidated. In the present study, the effect of simvastatin on MDA-MB-231 breast cancer cells was observed and the underlying mechanisms were investigated.

The present study demonstrated that simvastatin significantly inhibited the proliferation of the breast cancer cells. The acceleration of the cell cycle is an initial factor in tumor growth, and control of cell cycle progression in cancer cells is a potentially effective strategy for the control of tumor growth (22,23). The results revealed that simvastatin arrested the cells at the G_1/S cell cycle transition and directly induced G_1/S phase arrest in the MDA-MB-231 cells. Cell cycle progression is regulated by CDKs and cyclin-dependent kinase inhibitors, whose activity is highly controlled and coordinated by their association with cyclins (24). CDK inhibitors interact with active CDK-cyclin complexes and exert tumor-suppressive functions that downregulate cell cycle progression (25,26). A previous study demonstrated that simvastatin induced cell cycle arrest at G_0/G_1 by downregulating the expression of CDKs and cyclins, which was accompanied by apoptosis and reduced cell proliferation (16). The present study demonstrated that simvastatin significantly decreased the protein expression levels of cyclin D1 and CDK2 in breast tumor cells, which revealed that simvastatin-induced cell cycle arrest at G_0/G_1 was associated with downregulation in the protein expression levels of cyclin D1 and CDK2. This may be a direct mechanism of simvastatin against the growth of breast cancer cells.

Apoptosis is a fundamental cellular activity and is crucial for eliminating genetically damaged cells, which is key in the pathogenesis of cancer, and the proteins associated with this process have been a focus of interest in investigations of cancer onset and progression (27). The upregulation of pro-apoptotic gene expression and downregulation of anti-apoptotic gene expression induce the initiation of apoptosis, and the progression of cancer depends predominantly on the balance between pro-apoptotic proteins, including Bax, and anti-apoptotic proteins, including Bcl-2 (20). Several studies have suggested that the survival or death of human breast cancer cells is determined by an altered balance between pro-apoptotic and anti-apoptotic proteins, including the ratio of Bcl-2 to Bax (28,29). Increased expression levels of mitochondrial anti-apoptotic proteins contribute to augmented survival of several types of cancer cells, including breast cancer (30). The present study demonstrated that simvastatin increased the expression of Bax and downregulated the expression of Bcl-2, suggesting that the simvastatin-induced apoptosis of MDA-MB-231 cells was associated with modulation of the expression levels of Bax and Bcl-2.

Caspases are also involved in the execution of apoptosis associated with these two signaling pathways. Bcl-2 and Bax activate the caspase cascade reaction and are important in the regulation of the intrinsic pathway of apoptosis (31). As demonstrated in previous studies, Bcl-2 prevents the activation of caspase-3 in response to a variety of apoptotic signals (32,33). In the caspase family, either caspase-8 or -9 and the subsequent effector, caspase-3, are crucial in the apoptotic process and initiation of a caspase cascade triggers the proteolytic activation of executioner caspases, including caspase-3, to perform the final steps in the apoptotic process (34). Inhibition of the expression of Xiap promotes the development of apoptosis, therefore, Xiap suppresses apoptosis through the inhibition of caspases (35,36). In the present study, simvastatin increased the expression levels of caspase-3, -8 and -9, and downregulated the expression of Xiap. This suggested that the simvastatin-induced MDA-MB-231 apoptosis was also associated with activation of the caspase signaling pathway and modulation of the expression of Xiap.

The MMP family consists of 23 zinc-dependent endopeptidases, which are all involved in the degradation of the extracellular matrix. MMPs are upregulated in almost every type of cancer and their expression is often associated with a poor prognosis for patients (37). Based on their unique ability to degrade gelatinases, a major constituent of the basement membrane, MMP-2 and MMP-9, are the most important MMPs involved in tumor invasion and metastasis (38). It has been reported that the expression levels and activities of MMPs are associated with an advanced stage of breast cancer, increased invasion of tumor cells and building of metastatic formations (39). Atorvastatin, a member of the statin drug family, suppresses the expression levels of MMP-2 and MMP-9 in human endothelial cells (40). In addition, it also inhibits the RhoA-JNK-c-Jun-MMP2 cascade, resulting in a decrease in osteosarcoma cell invasion (41). The present study demonstrated that simvastatin inhibited the expression levels of MMPs, potentially inhibiting the invasion and metastasis of breast cancer.

The NF-κB complex, an essential cell mediator, is composed of a family of inducible transcription factors, expressed in almost all cell types (42). The overexpression of NF-κB implies an aggressive tumor in breast cancer and can predict tumors, which are likely to have a poor prognosis (43). A previous study revealed that the expression of NF-κB is necessary for the maintenance of the malignant phenotype, and provides a therapeutic approach for the treatment of cancer (44). The activation of NF-κB upregulates the

expression of the anti-apoptotic protein, Bcl-2, and regulates the expression levels of cyclin D1 and MMPs (45,46). In the present study, simvastatin inhibited the expression of NF-κB, and this may be an important mechanism underlying the anticancer effects of simvastatin in breast cancer.

There is increasing interest in cancer prevention and in the drugs that, used in low doses, either alone or in combination, which have different modes of action and low toxicity, act as chemopreventive agents (47). Therefore, the present study investigated other molecules, which are used for the treatment of well-known pathological diseases and have effects on cancer cell proliferation. Statins sensitize cancer cells to chemotherapeutic drugs, and evidence indicates that treatment with simvastatin increases the antitumor activity of cisplatin and docetaxel, common chemotherapeutic agents used against a wide range of types of cancer (48). Therefore, the present study demonstrated the antiproliferative and anticarcinogenic effects of simvastatin in a breast cancer cell line, the results of which suggested that simvastatin may be promising as a therapeutic approach for the treatment of cancer.

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