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The apoptotic effect of simvastatin via the upregulation of BIM in nonsmall cell lung cancer cells

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ABSTRACT

Purpose: Statins are known to have pleiotropic effects that induce cell death in certain cancer cells. BIM is a member of the bcl-2 gene family, which promotes apoptotic cell death. This study investigated the hypothesis that simvastatin has pro-apoptotic effects in epidermal growth factor receptor (EGFR)-mutated lung cancer cell lines via the upregulation of the expression of the BIM protein.

Materials and methods: The cytotoxic effects of simvastatin on gefitinib-sensitive (HCC827, E716-A750del) and -resistant (H1975, T790M + L858R) nonsmall cell lung cancer (NSCLC) cells were compared. Cell proliferation and expression of apoptosis-related and EGFR downstream signaling proteins were evaluated. Expression of BIM was compared in H1975 cells after treatment with simvastatin or gefitinib. SiRNA-mediated BIM depletion was performed to confirm whether the cytotoxicity of simvastatin was mediated by the expression of BIM. Results: H1975 cells showed significantly reduced viability compared with HCC827 cells after treatment with simvastatin (2 μM) for 48 hours. In simvastatin-treated H1975 cells, expression of pro-apoptotic proteins was increased and the phosphorylation of ERK 1/2 (p-ERK 1/2) was reduced. Expression of BIM was suppressed by gefitinib (1 μM) treatment in H1975 cells, but it was significantly increased by treatment with simvastatin. BIM depletion by siRNA transfection enhanced the viability of H1975 cells that received simvastatin treatment and increased their expression of anti-apoptotic proteins. Conclusions: Simvastatin restored the expression of BIM to induce apoptotic cell death in NSCLC cells harboring an EGFR-resistant mutation. Our study suggests the potential utility of simvastatin as a BIM-targeted treatment for NSCLC.

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BIM: epidermal growth factor receptor; lung cancer; simvastatin

INTRODUCTION

The identification of epidermal growth factor receptor (EGFR) mutations has allowed patients with nonsmall cell lung cancer (NSCLC) who harbor activating EGFR mutations to benefit from tyrosine kinase inhibitors (TKIs), such as gefitinib or erlotinib.[1, 2] However, approximately 10% of patients have primary resistance to TKIs, and patients who have sensitizing mutations develop acquired resistance after using the TKIs for about 1 year.[3, 4] Therefore, molecular targets that can overcome resistance to TKIs have been continuously sought.

Bcl-2-like 11 (BIM) is a Bcl-2-homology domain 3-only (BH3-only) protein and a key modulator of apoptosis, which upregulates mitochondrial apoptosis.[5, 6] The role of BIM in tumorigenesis and cancer cell metastasis has been becoming increasingly clear recently, leading to the development of a druggable target for cancer therapy. Previous studies have demonstrated that BIM is involved in imatinib-induced apoptosis of BCR-ABL leukemic cells and gastric cancer cells.[7, 8] With regard to NSCLC, it has been suggested that there is a relationship between BIM and the EGFR pathway, in vitro studies have shown that BIM mediates EGFR-TKI-induced apoptosis.[9, 10] The BIM-deletion polymorphism independently predicted a poor clinical response to EGFR TKIs in patients with EGFR-sensitizing mutations.[11, 12] Therefore, EGFR-activated cells could have a resistance to EGFR-TKIs and EGFR-resistant cells could be rendered resistant.
to intrinsic apoptosis through the regulation of BIM protein expression.

Statins are well-known lipid-lowering agents that function in the mevalonate pathway as 3-hydroxy-3-methyl-gluteryl-CoA (HMG-CoA) reductase inhibitors. Statins have pleiotropic effects on signaling pathways by inhibiting the prenylation of small G-proteins, primarily Rho proteins. It has been reported that statins have shown synergistic cytotoxicity with EGFR-TKIs in NSCLC cell lines via interference with the function of cholesterol-rich lipid rafts and the Ras/RAF/MEK/ERK signaling pathways.\[13, 14\] The important mediator of cancer cell apoptosis, which is itself regulated by statins, is the RAF-mitogen-activated protein kinase 1 (MAP2K1, also known as MEK) -extracellular regulated kinase (ERK) pathway.\[15\] Recently, Zhu et al. reported that the anticancer effect of simvastatin in human colorectal carcinoma cells was regulated by the activation of BIM in a c-Jun N-terminal kinase (JNK)-dependent manner.\[16\] However, until now, the mechanisms underpinning the pro-apoptotic effect of statins related to BIM protein expression in NSCLC have not been clearly defined.

This study investigated the hypothesis that simvastatin has pro-apoptotic effects on EGFR-TKI-resistant lung cancer cell lines and that upregulation of BIM proteins is associated with the apoptotic effect of simvastatin.

Materials and methods

Cell culture

The human lung adenocarcinoma cell lines NCI-H1975 (American Type Culture Collection, Manassas, VA, USA) and HCC827 (Korean Cell Line Bank, Seoul, Korea) were cultured in RPMI-1640 medium (WELGENE, Daegu, Korea) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin solution (10,000 units/mL penicillin and 10 mg/mL streptomycin). Cells were incubated in a humidified incubator at 37°C with an atmosphere of 5% CO₂ and were split regularly before they attained approximately 80% confluence.

Drugs and reagents

Pure simvastatin powder was purchased from Sigma (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) forming a 50 mM stock solution. Gefitinib was purchased from Cell Signaling Technology (Beverly, MA, USA) and dissolved in DMSO forming a 100 mM stock solution. The final concentration of DMSO in all conditioned media did not exceed 0.1%. Antibodies specific for cleaved caspase-3 (Asp175), EGFR, phospho-EGFR (Tyr1068), AKT, phospho-Akt (Thr308), ERK1/2, phosphorylated ERK1/2 (Thr202/Tyr204), PARP, cleaved PARP (Asp214), BCL-2, BIM, and β-actin were purchased from Cell Signaling Technology and horseradish peroxidase-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell viability assay

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay. Cells were seeded into three replicate wells for each group, at a density of 1 × 10⁴ cells/well in 96-well culture plates. The cells were then treated with either the control solvent (0.1% DMSO) or various concentrations of simvastatin or gefitinib for 24 and 48 hours, followed by incubation with MTT for 4 hours. The formazan precipitate was dissolved in DMSO and the absorbance was measured at 570 nm using an ELISA reader. Cell viability was calculated according to the following equation: percentage cell viability = (absorbance of drug-treated cells)/(absorbance of control in the absence of simvastatin or gefitinib) × 100. All experiments were repeated at least three times.

Cell morphological observation

The cells (8 × 10⁴ cells/well) were seeded into 6-well culture plates. The cells were treated with simvastatin or gefitinib and cultured for 48 hours. After incubation, the cells were washed and morphologic changes were observed and photographed under a phase contrast inverted microscope.

RNA interference

RNAi knockdown studies were performed using small interfering RNA (siRNA) according to the manufacturer’s instructions (Invitrogen, Merelbeke, Belgium). The BIM-specific siRNA and β-actin positive control siRNA were designed by Bioneer (Daejeon, Korea). For transfection, 200 nM of siRNA was transfected into cells seeded in 6-well plates.
using Lipofectamine™2000 (Invitrogen, Merelbeke, Belgium). After 24 hours of transfection, cells were washed twice and treated with 2 μM of simvastatin for 48 hours in RPMI including 10% FBS. Efficiency of siRNA knockdown of the BIM expression was confirmed by real-time PCR and Western blot analysis. Experiments were performed in triplicate.

**Quantitative reverse transcription (qRT)-PCR**

Total RNA was extracted using the TRIzol reagent™ (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s recommendations. After extraction and quantification of total RNA, real-time PCR reactions were performed using a QuantiTect SYBR Green RT-PCR kit (Qiagen, Valencia, CA, USA). Each sample had a final volume of 25 μL containing approximately 100 ng of cDNA. The PCR conditions were 95°C (10 minutes), followed by 40 cycles of 95°C (15 seconds), 60°C (1 minutes), using a standard denaturation curve. The primer sequences used were: BIM, forward 5′-ACAGACAGGAGCCCAGCAC-3′, reverse 5′-TCTTGGGCATCCTCATCTC-3′; BAX, forward 5′-AAGCCTGAGGAGTGTCTCAAGCGC-3′, reverse 5′-TCGGCCACAAAGATGGTCACG-3′; Bcl-2, forward 5′-AGATGTCCAGCCAGCTGCACCTGAC-3′, reverse 5′-AGATAGGCACCCAGGGTGATGCAAGCT-3′; β-actin, forward 5′-CCTGCCTTGACATTGAGCAG-3′, reverse 5′-AGCATCTGCACTCTGGGTGAA-3′. β-actin expression was used to standardize the qRT-PCR results. The changes in target signal were expressed as ΔΔ CT = ΔCT; treatment − ΔCT, control and relative changes were calculated as 2−ΔΔ CT.

**DNA fragmentation analysis**

1 × 10⁵ cells were incubated with 100 μL of cell lysis buffer at 37°C for 1 hours and protein precipitation solution was added. After 15 minutes, the cells were centrifuged for 15 minutes at 12,000 rpm at 4°C followed by the addition of ice cold isopropanol to precipitate the DNA. This was incubated at room temperature for 30 minutes, followed by centrifugation at 12,000 rpm for 15 minutes. The pellet was washed with 70% ethanol and stored in 20–30 μL of TE buffer. The DNA samples were analyzed using a 3% agarose gel by electrophoresis at 50 V for 40 minutes.

**Western blot analysis**

The cells were washed twice with sterile phosphate-buffered saline (PBS), lysed using a radioimmunoprecipitation assay (RIPA) cell lysis buffer [150 mM NaCl, 1% triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 7.5, and 2 mM ethylenediamine tetra-acetic acid (EDTA) containing a mixture of protease inhibitors and phosphatase inhibitors (GenDEPOT, Barker, TX, USA), followed by centrifugation at 13,000 rpm for 30 min at 4°C. The protein concentration of each sample was determined using a BCA Protein Assay Kit (Thermo Scientific, Pierce Biotechnology, Rockford, IL, USA). After boiling the supernatants in a reducing SDS sample buffer, equal amounts of extracted protein (40 μg/sample) was loaded into lanes and the samples were electrophoresed on an 8% or 15% polyacrylamide SDS gel and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% (w/v) nonfat dried milk (Difco/Becton Dickinson, Atlanta, GA, USA) in Tris-buffered saline containing 0.05% Tween 20 (TBS-T). Primary antibodies were detected by horseradish peroxidase-conjugated secondary antibodies and, after five washes with TBS-T, the signal was visualized using an enhanced Western Blotting Luminol Reagent (Santa Cruz, CA, USA).

**Statistical analysis**

All statistical analyses were evaluated by one-way ANOVA followed by Dunnett’s multiple range testing using GraphPad Prism for Windows (ver. 5.0; Graphpad Prism Software, La Jolla, CA, USA). All experiments were repeated at least three times and the data were presented as means ± SD and a value of \( P < .05 \) was accepted as statistically significant.

**Results**

**Effects of simvastatin on the proliferation of EGFR-sensitive and -resistant NSCLC cell lines**

EGFR-sensitive (HCC827, E716-A750 del) and -resistant (H1975, T790M + L858R) NSCLC cell lines were used to compare the effects of simvastatin with those of gefitinib. Using a light microscope, H1975 cells were observed to be detached and shrunken with condensed nuclei, suggesting apoptotic cell death,
when treated by simvastatin (2 μM) (Figure 1). In the HCC827 cells, simvastatin treatment did not affect the proliferation and morphology of cells. With gefitinib (1 μM) treatment, apoptotic morphologic changes were observed in HCC827 cells, but not in HCC1975 cells. Figure 2 shows the cell proliferation determined by the MTT assay. Incubating with a 1 μM dose of gefitinib for 24 hours, growth was inhibited by 20% in HCC827 cells, but by < 5% in H1975 cells. Following 48 hours of gefitinib treatment, the IC_{50} values were 14.57 μM using the H1975 cells and 11.19 μM using the HCC827 cells. Consistent with the morphological changes, simvastatin significantly suppressed the growth of the H1975 cells, with IC_{50} values of 7.9 μM after 24 hours and 4.8 μM after 48 hours of treatment (Figure 2C).

**Simvastatin increased the pro-apoptotic and reduced the anti-apoptotic signals in H1975 cells**

To investigate the mechanism by which cell death was induced by simvastatin, the expression of apoptosis-related signaling proteins were examined, and DNA fragmentation assays were performed. H1975 cells were treated with 2 μM simvastatin for 12, 24, and 48 hours. Quantitative reverse transcription (qRT)-PCR results showed that the mRNA expression of BIM and pro-apoptotic protein Bcl-2-associated X protein (BAX) increased and that it reduced that of the anti-apoptotic protein B-cell lymphoma 2 (BCL-2) in a time-dependent manner with simvastatin treatment (Figure 3A). Reverse transcription (RT)-PCR of BIM mRNA was also increased in H1975 cells following simvastatin treatment (Figure 3B). These changes in the expression of signaling proteins associated with apoptotic cell death were also observed using Western blot analysis. Cleaved caspase 3, cleaved poly-ADP ribose polymerase (PARP), BAX, and BIM increased, whereas BCL-2 decreased (Figure 3C). The DNA fragmentation assay showed DNA laddering after 48 hours of treatment with simvastatin (Figure 3D).

**Effects of simvastatin on EGFR downstream protein signals in NSCLC cells**

Because simvastatin is known to affect the ERK pathway, we explored changes in the signaling pathways downstream of EGFR. Figure 4 shows the different effects of gefitinib on EGFR signaling in EGFR-resistant (H1975) and EGFR-sensitive (HCC827) cell lines according to Western blot analysis. Gefitinib efficiently reduced phosphorylated EGFR (p-EGFR) and phosphorylated ERK 1/2 (p-ERK 1/2) levels in HCC827 cells. P-EGFR and p-ERK 1/2 in H1975 cells were unchanged following gefitinib treatment (Figure 4A). Expression of EGFR and p-EGFR in H1975 cells was unchanged after simvastatin treatment, but p-ERK 1/2 was suppressed. Phosphorylation of Akt (p-Akt) was unchanged (Figure 4B).

**BIM depletion reversed the cell-death signals with simvastatin treatment in EGFR-resistant cells**

BIM is a BH3-only pro-apoptotic protein activator of BAX, which leads to apoptotic cell death. Based on our results showing that simvastatin induced the expression of BIM, resulting in apoptotic cell deaths in H1975 cells, we hypothesized that BIM may be a key mediator of apoptotic signals. For this reason, the effects of BIM knockdown on cellular apoptosis were investigated. H1975 cells were transfected with BIM siRNA and treated with simvastatin (2 μM) for 48 hours. Compared with si-control cells, cell proliferation was increased in BIM siRNA-transfected cells that received simvastatin treatment (P < .01) (Figure 5A). After transfection of BIM siRNA, mRNA expression of BIM was suppressed, but it increased with simvastatin treatment (P < .05) (Figure 5B). Following treatment with simvastatin, the level of BAX mRNA decreased and that of BCL-2 increased (Figure 5B) in BIM siRNA-transfected cells compared with si-control cells. This reflects cell survival signaling after simvastatin treatment in BIM-depleted cells. In addition, DNA laddering was not observed in the DNA fragmentation assay in BIM siRNA-transfected cells treated with simvastatin (Figure 5C). According to Western blot analysis, the expression of cleaved PARP and BIM was reduced in BIM siRNA-transfected cells compared with si-control cells (Figure 5D) following treatment with simvastatin.

**Discussion**

The current study showed that simvastatin effectively suppressed the proliferation of lung cancer cells that contain EGFR-resistant mutations and that this effect was potentially mediated by the induced expression of the BIM protein, a key signaling molecule for
**Figure 1.** Morphology of cells after treatment with gefitinib or simvastatin. H1975 and HCC827 cells were treated with gefitinib (1 μM) or simvastatin (2 μM) for the indicated time. The cells were seeded in six-well culture plates. After 24 hours, the cells were treated with the drugs for the indicated time. Cells were photographed under a phase contrast inverted microscope.

**Figure 2.** Effects of simvastatin or gefitinib on the proliferation of cells. H1975 and HCC827 cells were treated with gefitinib or simvastatin in a dose-dependent manner for 24 and 48 hours. The MTT assay was used to determine cell proliferation. (*) *P < .05, **P < .01, and ***P < .001 compared with the control after 24-hour treatment, #: *P < .05, ##: *P < .01 and ###: *P < .001 compared with the control after 48-hour treatment.*
apoptotic cell death. In addition, the induced expression of the BIM protein was associated with the suppressive effect of ERK signaling by simvastatin. This finding is clinically important for the treatment of patients with NSCLC, because therapeutic strategies to overcome acquired resistance are essential for controlling disease progression in such patients with EGFR mutations. In view of the diverse molecular

**Figure 3.** Effects of simvastatin on apoptosis of H1975 cells. Cells were treated with 2 μM simvastatin or 1 μM gefitinib for the indicated times, and the mRNA expression of BIM, BAX, and Bcl-2 was examined by quantitative reverse transcription (qRT)-PCR (A) and RT-PCR (B). (*: P < .05, **: P < .01, and ***: P < .001 compared with the control.) (C) Protein levels of apoptosis-related genes were examined using Western blot analysis. The three isoforms of BIM, BIMEL, BIML, and BIMS, are shown. (D) H1975 cells were treated with 2 μM simvastatin for the indicated times, and DNA fragmentation was examined using agarose gel electrophoresis. Lane 1: 1 kb DNA ladder.

**Figure 4.** Effect of gefitinib and simvastatin on EGFR downstream protein signaling in NSCLC cells. H1975 and HCC827 cells were treated with 1 μM gefitinib, and H1975 cells were treated with 2 μM simvastatin for the indicated times. Expression of EGFR, ERK, and apoptosis-related proteins was detected by Western blot analysis. The three isoforms of BIM, BIMEL, BIML, and BIMS, are shown.
mechanisms involved in the acquired resistance to EGFR-TKI,[19] a strategy that targets the molecular executioner that causes cell death, such as BIM, would seem to be beneficial.[5]

BIM-targeting therapies have emerged over the past few years. Given their strong apoptosis-inducing ability, imatinib, gefitinib, bortezomib, and the BIM protein itself have been identified as cancer-specific BIM-targeted therapeutic agents.[5] Recently, the relationship between EGFR signaling and the BIM protein has been extensively studied. Clinical studies conducted by Isobe et al. and Zhao et al. investigated progression-free survival (PFS) and the objective response rate (ORR) using EGFR-TKI in patients diagnosed with NSCLC. They reported that patients with a BIM-deletion mutation showed significantly shorter PFS and lower ORR in response to EGFR-TKI despite the fact that most of them had sensitive mutations to EGFR-TKI.[11, 12] These studies demonstrated that not only EGFR-TKI-resistant mutations, such as T790M, but also the BIM protein may be part of an important mechanism of chemoresistance and potential targets for overcoming acquired resistance to EGFR-TKI in the treatment of NSCLC.

At the molecular level, several studies have shown that BIM is involved in EGFR-TKI-induced apoptosis. In EGFR-sensitive lung cancer cell lines, BIM expression was up-regulated by EGFR-TKIs, and the knockdown of BIM by siRNA transfection attenuated the apoptosis by EGFR-TKIs.[9, 10] Our study is consistent with these reports; the BIM protein level increased with gefitinib treatment in EGFR-TKI-sensitive cells and with simvastatin treatment in EGFR-TKI-resistant cells, which was reversed by BIM siRNA transfection. It has also been suggested that expression of BIM is suppressed or BIM is phosphorylated to undergo proteosomal degradation through the activation of AKT and ERK signaling.[5, 20–23] Li et al. investigated the combined effect of phosphatidylinositol 3 kinase (PI3K)/AKT and MEK/ERK inhibitors on TKI-resistant NSCLC cells. The combination therapy induced a significant level of apoptosis and restored the sensitivity to TKI by increasing the expression of BIM.[24] This study supports previous reports confirming the association of BIM with the activation of EGFR downstream signaling and PI3K/AKT and MEK/ERK pathways. That is, blocking the PI3K/AKT and ERK signaling pathways may be a strategy for overcoming EGFR resistance, as it induces the expression of BIM.

Simvastatin is a commonly used anti-lipid agent, and its anti-cancer effects have been investigated for decades. Although its preventive role in human cancer remains controversial,[15] its inhibitory effects on
carcinogenesis have been confirmed in preclinical studies in colon, breast, and melanoma cancer. These effects are primarily attributable to the inhibition of Rho proteins and the regulation of signaling pathways, including MEK/ERK pathways, to a greater extent in malignant than in nonmalignant cells due to increased expression of HMG-CoA reductase in tumor cells.\[15\] In addition, the anti-cancer effects of statins could be explained by anti-inflammatory, immunomodulatory and anti-oxidant activities.\[15, 25, 26\] Simvastatin suppressed the proliferation of the mouse model of human gastric cancer by activation of the nuclear factor-\(\kappa\)B and inhibition of cyclooxygenase-2 (COX-2) protein.\[27\] Similar apoptotic effects were also reported in esophageal adenocarcinoma cells with simvastatin treatment by inhibition of COX-2.\[28\] In lung cancer cells, Park et al. demonstrated that lovastatin could overcome gefitinib resistance in K-Ras-mutated NSCLC cells by downregulating the RAF/ERK and AKT pathways.\[18\] Furthermore, Hwang et al. described the synergistic inhibitory effects of simvastatin with gefitinib in T790M EGFR-mutated NSCLC cells via the AKT/\(\beta\)-catenin-dependent downregulation of survivin and apoptosis induction.\[29\]

Our study is unique among the published investigations of the anti-cancer effects of simvastatin, as we suggest that simvastatin acts as an inducer of BIM, in addition to EGFR-TKI, in NSCLC. We observed significantly suppressed proliferation of H1975 cells with simvastatin treatment and investigated the potential molecular mechanisms underpinning this phenomenon. The suppressed proliferation was revealed to be mediated by apoptotic cell death. This was demonstrated by cellular morphology, DNA fragmentation assay, and changes in apoptosis-related proteins, such as cleaved caspase-3, cleaved PARP, BAX, and BCL-2. In addition, the pro-apoptotic effect of simvastatin differed depending on cell type, and it was observed in H1975 cells but not in HCC827 cells. This selectivity of simvastatin action is associated with the different characteristics of individual types of cancer cell. However, to date, few studies have investigated the associated mechanism. One randomized study investigated the effects of gefitinib plus simvastatin versus those of gefitinib alone in patients with NSCLC. The combination of gefitinib and simvastatin was associated with improved efficacy in patients with wild-type EGFR nonadenocarcinomas but not in an unselected population.\[30\] EGFR mutation status and pathologic cell type may affect the pleiotropic action of simvastatin, creating the need for further studies to investigate the connection.

Our results showed that the levels of the signaling proteins EGFR, p-EGFR, and p-AKT were not changed and that the level of p-ERK decreased with simvastatin treatment (Figure 4B). Song et al. reported that using the MEK inhibitor and EGFR-TKI in combination induced caspase-3-dependent apoptosis in H1975 cells. In this study, treatment with MEK inhibitor induced the expression of the BIM protein in only H1975 cells.\[31\] Li’s study showed that a MEK inhibitor increased the expression of BIM in H1975 cells.\[24\] Our data are consistent with these findings; both mRNA and protein levels of BIM were increased and ERK phosphorylation was inhibited when cells were treated with simvastatin. It is possible that the inhibition of ERK signaling by simvastatin affected the expression of BIM. However, it is well known that the homeostasis of the BIM protein is regulated not only by transcription but also by phosphorylation for proteosomal degradation.\[23\] Moreover, phosphorylation of the forkhead transcription factor 3 (FoxO3) upregulates transcription of the BIM protein in T cells and neurons.\[32, 33\] Besides FoxO3, several transcription factors, such as E2F1, c-Myc, NF-Y, Smad, RUNX and c-Jun upregulate its expression at the transcriptional level. Also, a series of microRNAs and RNA-binding proteins negatively regulate the translation of BIM mRNA.\[34\] The exact molecular mechanism will be investigated in further studies.

In conclusion, this study showed the apoptotic effect of simvastatin in EGFR-TKI-resistant NSCLC cells. Increased expression of the BIM protein was correlated with the pro-apoptotic effect of simvastatin. This result suggests simvastatin is a potential therapeutic agent targeting the BIM protein in NSCLC cells, and additional studies investigating the exact molecular mechanism by which simvastatin regulates BIM activity are needed.

Acknowledgment

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see http://www.textcheck.com/certificate/yDtl8l

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and preparation of this paper.
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