

MCM2 is a therapeutic target of lovastatin in human non-small cell lung carcinomas

XU ZHANG^{1*}, YANG TENG^{2*}, FANG YANG³, MENG WANG³, XUAN HONG³,
LEI-GUANG YE³, YI-NA GAO³ and GONG-YAN CHEN³

¹Department of Stomatology, The Fourth Affiliated Hospital of Harbin Medical University;

²The Fourth Affiliated Hospital of Harbin Medical University; ³Department of Medical Oncology,
The Affiliated Tumor Hospital of Harbin Medical University, Harbin, Heilongjiang, P.R. China

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Abstract. Human non-small cell lung carcinoma (NSCLC) is one of the most common cancer worldwide. In previous studies, lovastatin, acting as an inhibitor of 3-hydroxy-3-methylglutaryl Co A (HMG-CoA) reductase, exhibited significant antitumor activity during tumorigenesis. However, whether or not this effect is mediated through changes in minichromosome maintenance (MCM) 2 expression remains unclear. The present study investigated whether lovastatin inhibits proliferation due to MCM2 in NSCLCs. We first assessed the effects of lovastatin on cell anti-proliferation, cell cycle progression and apoptosis in NSCLC cells. We found, by quantitative RT-PCR and western blot analysis, that lovastatin treatment markedly and consistently inhibited the expression of MCM2. Then, to further explore the anticancer mechanism of lovastatin involving MCM2, we silenced MCM2 by siRNA in two cell lines (A549 and GLC-82). Silencing of MCM2 triggered G1/S arrest. Following further examination of cell cycle-related factors, MCM2 knockdown inhibited protein retinoblastoma (Rb), cyclin D1 and CDK4 expression, but increased p21 and p53 expression, suggesting that siMCM2 indeed triggered cell cycle arrest. In addition, siMCM2 induced apoptosis. Finally, lovastatin treatment increased p-JNK, which is involved in the downregulation of MCM2. In conclusion, our data suggest that MCM2 may be a novel therapeutic target of lovastatin treatment in NSCLCs.

Introduction

Lung cancer is a common malignant tumor. Patients particularly with human non-small cell lung carcinomas (NSCLCs), which

constitute 80% of total lung cancer cases worldwide, present with an extremely low survival rate due to the poor sensitivity of NSCLCs to traditional chemotherapy and radiotherapy. Thus, new antitumor chemicals and therapeutic targets must be urgently developed. Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are chemotherapeutic agents for lowering plasma cholesterol. Extensive studies, however, have demonstrated the role of statins in cancer therapies, particularly the inhibition of HMG-CoA reductase by statins based on the suppression of tumor growth, stimulation of cell cycle arrest, and induction of cancer cell apoptosis *in vivo* and *in vitro*. Moreover, it was reported that patients who were treated with statins prior to cancer diagnosis exhibited a reduced cancer-related mortality of up to 15% (1).

Lovastatin, a first generation statin drug, has been suggested as a promising potential therapeutic agent in cancer. Brummer *et al* reported that atorvastatin inhibits vascular smooth muscle cell DNA synthesis by blocking E2F and thereby decreasing minichromosome maintenance (MCM) 6 and MCM7 expression (2). Recent studies have also shown that MCM2, MCM4 and MCM7 are potential therapeutic targets in NSCLCs (3-5). Although the effects of lovastatin on the anti-proliferation in cancer cells have been confirmed, lovastatin's effects on MCM2 have not yet been fully elucidated.

DNA replication licensing in eukaryotic cells is a strictly regulated process that ensures proper genome replication and inheritance. MCM complexes, including MCM2, MCM3, MCM4, MCM5, MCM6 and MCM7, bind to replication origins in the G1 phase of the cell cycle and are the key regulatory components for DNA replication licensing. Deregulation of these MCM proteins have been linked to tumor formation, progression and malignant transformation. Moreover, MCM2 has been considered a valuable proliferation marker in many types of cancers, including cervical progressive disease (6), colonic adenoma and adenocarcinoma (7), meningiomas (8), gingival fibromatosis (9), non-melanoma epithelial skin cancers (10), and gastric cardiac cancer (11). Thus, inhibiting MCM2 may present an attractive opportunity for the development of effective anticancer drugs with few side effects. Indeed, the silencing of MCM2 has resulted in anti-proliferation, cell cycle arrest, and apoptosis in several types of cancers, such as colon cancer (12), yet the anti-proliferative mechanism

Correspondence to: Professor Gong-Yan Chen, Department of Medical Oncology, The Affiliated Tumor Hospital of Harbin Medical University, Harbin, Heilongjiang, P.R. China
E-mail: gongyanchenteach@126.com

*Contributed equally

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of MCM2 under the conditions of lovastatin treatment for NSCLCs remains unknown. In the present study, we aimed to explore the possible molecular mechanism of lovastatin related to MCM2 as a therapeutic target in human non-small cell lung carcinomas.

Materials and methods

Antibodies and reagents. Lovastatin, purchased from Sigma Chemical Co. (St. Louis, MO, USA) was dissolved into a refrigerated absolute alcohol stock solution. The rabbit anti-MCM2 monoclonal antibody, the anti- β -actin antibody, and secondary antibodies were purchased from Abcam (Cambridge, UK). CDK4, cyclin D1, protein retinoblastoma (Rb), Bax, Bcl-2, p21 and p53 antibodies were purchased from Bioworld Technology Inc. (Visalia, CA, USA). SP60012 was purchased from Beyotime Biotechnology Inc. (Nantong, China).

Cell lines and culture and drugs. NSCLC cell lines, A549 and GLC-82, provided by the American Type Culture Collection (ATCC; Manassas, VA, USA), were cultured in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA) containing 5% CO₂ at 37°C. The two cell lines, treated with different concentrations of lovastatin (0, 2.5, 5, 10 and 20 μ M), were assessed using the colorimetric method of MTT [3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (5 mg/ml; Sigma Chemical Co.) assay. The cells were incubated at a density of 1×10^6 cells/well in plates of 24-wells, and allowed to adhere overnight. Then lovastatin treatment was carried out for 24, 48, 72 and 96 h, respectively. MTT solution (400 μ g/ml) was added and incubated for 1 h. The formazan crystals were dissolved using dimethylsulfoxide (DMSO). Quantification was provided by an absorbance reading at a wavelength of 540 nm. Each test was performed at least 3 times.

RNA interference (RNAi). A549 and GLC-82 cells were transfected with siRNA against MCM2 or control siRNA using Lipofectamine 2000TM (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. We designed the MCM2 siRNA-targeting sequence, 5'-GACCUGAGGAAAGAAUCUATT, and a random siRNA sequence, 5'-UAGAUUCUUUCCUCAGGUCTT, as the control-targeted sequence. For follow-up tests, RNAi oligonucleotides were performed 48 h later using Lipofectamine 2000. Cells were harvested and used for real-time PCR and western blotting 48 h after transfection. A549 and GLC-82 cells were labeled with propidium iodide (PI) for the cell cycle assay, or stained with Annexin V for the cell apoptosis assay.

Western blot analysis. Total protein extracts were separated by electrophoresis on 10% SDS-PAGE amide gels, and then transferred to PVDF membranes. The PVDF membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) and then incubated with primary antibodies overnight at 4°C, washed with TBST 3 times for 10 min, then exposed to peroxidase-conjugated secondary antibodies for 2 h at room temperature and washed with TBST 3 times for 10 min. Proteins were detected using

enhanced chemiluminescence (ECL; Pierce, Rockford, IL, USA).

RNA extraction and real-time PCR. Total RNA was extracted from lovastatin-treated and untreated cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA levels were measured with the SYBR Premix ExTaq Quantitative PCR kit (Takara, Otsu, Japan). Primers for MCM2 and GAPDH genes were designed as follows: MCM2 forward, AGAATCTATGGCGACAG and reverse, ACCTGCTCTGCCACTAACTG; GAPDH forward, GGAAAGCCTGCCGGTGAC and reverse, ACCTGCTCTGCCACTAACTG. MCM2 gene expression was analyzed using the Applied Biosystems 7500 Real Time PCR machine. The expression of the target gene relative to GAPDH was determined using the formula $2^{-\Delta\Delta C_t}$. The experiment was repeated 3 times for each group and the mean values were calculated.

Apoptosis detection. The apoptosis of lovastatin-treated or untreated cells was detected with the Annexin V fluorescein isothiocyanate (FITC) apoptosis kit (Beyotime Biotechnology) according to the manufacturer's instructions. Differently treated cells were trypsinized and washed 3 times with cold PBS, and then 5 μ M Annexin V-FITC was added to the differently treated cells for 10 min at room temperature and analyzed on a FACSsort (Becton-Dickinson, San Jose, CA, USA).

Flow cytometric analysis. Our cell cycle analysis was performed on a flow cytometer and analyzed using the ModFit LT2.0 software (Coulter). A549 and GLC-82 cells were synchronized overnight in serum-free medium which was replaced by complete medium. The two cell lines were treated with lovastatin for 24, 48, 72 and 96 h, respectively prior to being harvested. The treated and control cells (5×10^5) were washed with ice-cold PBS 3 times for 10 min and fixed in cold 70% ethanol solution, then washed with PBS, treated with RNase (1 μ g/ml), and stained with PI (50 μ g/ml) (both from Sigma Chemical Co.) for 30 min at 37°C. DNA distributions were analyzed on a FACSsort with Cell Quest software (version 313).

Statistical analysis. Statistical analysis was performed using SPSS software, version 12.0 (SPSS, Chicago, IL, USA). Each experiment was repeated at least 3 times, and the results are expressed as means \pm SD. We considered $P < 0.05$ to indicate a statistically significant result.

Results

Lovastatin induces anti-proliferation, G1/S phase arrest, and apoptosis in NSCLC cells. To confirm the anti-proliferative effects of lovastatin, we cultured two NSCLC cell lines, A549 and GLC-82, and treated them with lovastatin at various concentrations (0, 2.5, 5, 10 and 20 μ mol/l) for 1 to 4 days, respectively (Fig. 1A). The MTT assay showed that lovastatin treatment produced time- and dose-dependent growth inhibition in the two cell lines, which was apparent 48 h after treatment and this time period was used for further experiments.

To investigate the exact mechanism involved in the anti-proliferative effects of lovastatin, cell cycle progression in the NSCLC cells was determined. We examined the effect of

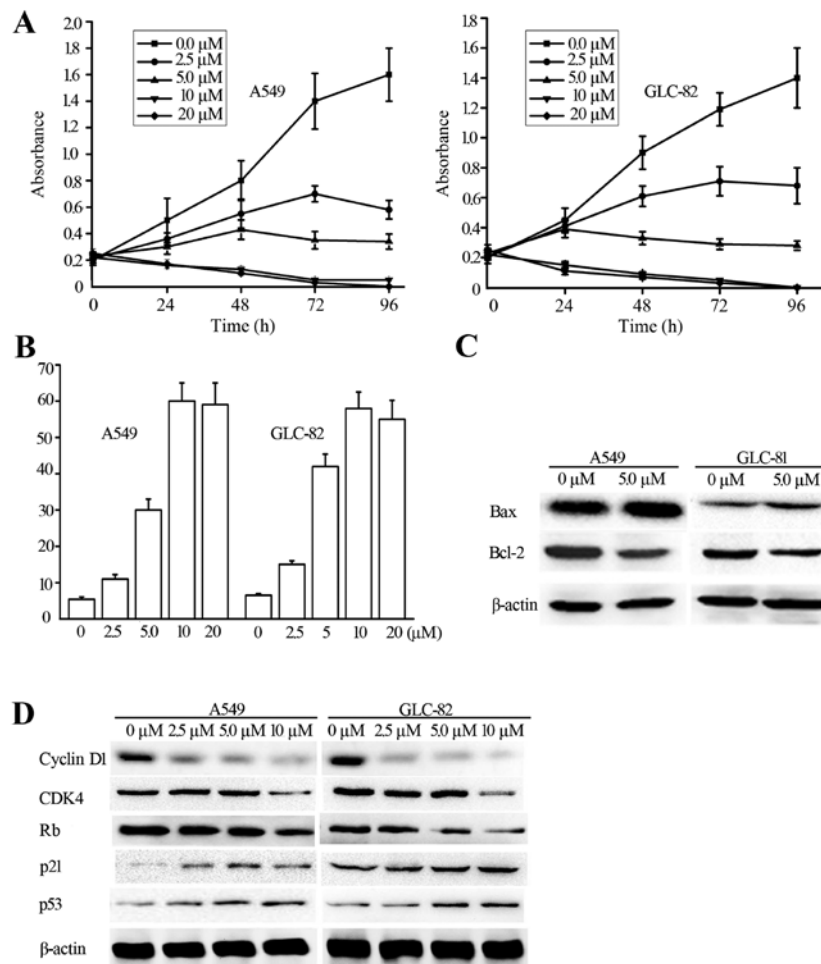


Figure 1. Effects of lovastatin on cell growth, cell cycle progression and apoptosis in NSCLC cells. (A) Human NSCLC A549 and GLC-82 cells were treated with different concentrations (0, 2.5, 5, 10 and 20 μ M) of lovastatin for 1, 2, 3 and 4 days, and cell viability was measured by MTT assay. Data represent means \pm SD of triplicate experiments; * P <0.05, significantly different compared with the alcohol-treated control. (B) Cell apoptosis was analyzed in A549 and GLC-82 cells following treatment with lovastatin at serial concentrations (0, 2.5, 5, 10 and 20 μ M), as measured by Annexin V staining. (C) Protein levels of Bcl-2 and Bax were determined by western blotting in the A549 and GLC-82 cells, and β -actin was used as the sample loading control. (D) A549 and GLC-82 cells were treated with lovastatin at a series of concentrations of 0, 2.5, 5 and 10 μ M for 48 h. Protein levels of CDK4, cyclin D1, Rb, p53 and p21 were determined by western blot analysis, and β -actin was used as the sample loading control. All experiments were performed in triplicates. NSCLCs, human non-small cell lung carcinomas.

Table I. Lovastatin-induced cell cycle arrest at the G1 phase.

| Cells | Treatment | Cells in each phase of the cell cycle (%) | | |
|--------|-------------------------|---|----------------|----------------|
| | | G0/G1 | S | G2/M |
| A562 | 0 | 50.1 \pm 1.5 | 32.8 \pm 0.5 | 14.1 \pm 0.2 |
| | 2.5 μ M lovastatin | 56.8\pm1.3 | 28.5 \pm 1.6 | 14.7 \pm 0.5 |
| | 5.0 μ M lovastatin | 64.7\pm0.8 | 23.9 \pm 2.3 | 11.4 \pm 0.5 |
| | 10.0 μ M lovastatin | 73.4\pm1.1 | 17.7 \pm 1.1 | 8.9 \pm 0.6 |
| GLC-82 | 0 | 59.5 \pm 2.1 | 29.5 \pm 2.1 | 11.0 \pm 0.4 |
| | 2.5 μ M lovastatin | 64.2\pm1.4 | 24.5 \pm 1.1 | 11.3 \pm 0.5 |
| | 5.0 μ M lovastatin | 67.2\pm1.6 | 22.9 \pm 1.8 | 9.9 \pm 0.2 |
| | 10.0 μ M lovastatin | 73.5\pm1.2 | 17.7 \pm 0.9 | 8.8 \pm 0.3 |

lovastatin on cell cycle distribution with PI staining and flow cytometry. As shown in Table I, for the A549 cells, the cell population in the G1 phase was 50.1, 56.8, 64.7 and 73.4% at

concentrations of lovastatin 0, 2.5, 5.0 and 10 μ M, respectively, and, for GLC-82 cells, 59.5, 64.2, 67.2 and 73.5% at concentrations of 0, 2.5, 5.0 and 10 μ M, respectively. Lovastatin

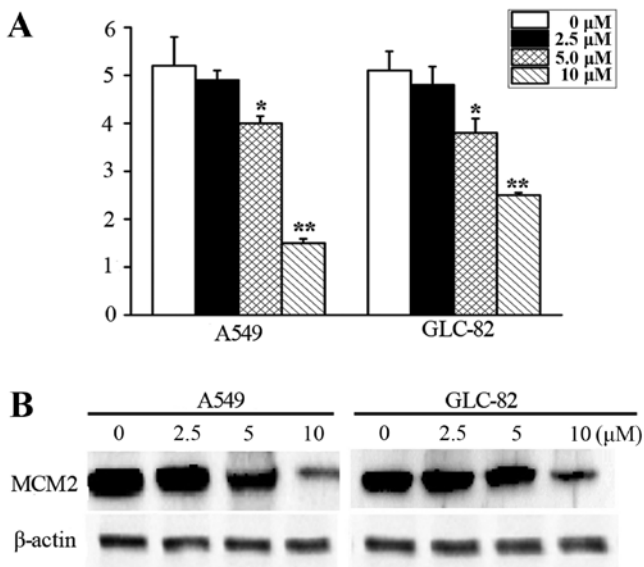


Figure 2. Lovastatin treatment inhibits MCM2 expression at the mRNA and protein levels in A549 and GLC-82 cells. (A) A549 and GLC-82 cells were treated with lovastatin at a series of concentrations of 0, 2.5, 5 and 10 μ M for 48 h. The MCM2 mRNA level was determined by real-time PCR, and GAPDH was used as the internal control. Results are expressed as the mean \pm SD of triplicate experiments; * P <0.05, ** P <0.01, Student's t-test. (B) Protein levels of MCM2 were determined by western blot analysis, and β -actin was used as the sample loading control. All experiments were performed in triplicates. MCM, minichromosome maintenance.

treatment markedly increased the G1 phase population, causing G1/S arrest.

We also aimed to ascertain whether lovastatin treatment is involved in apoptosis. Annexin V was determined in the A549 cells after lovastatin treatment, and the percentages of apoptotic cells were increased after 48 h of lovastatin treatment (Fig. 1B). To validate the results further, western blot analysis of several apoptosis-related markers, such as Bcl-2 and Bax was carried out. As shown in Fig. 1C, Bax was found to increase quickly and Bcl-2 was downregulated. The results showed that apoptosis could be responsible for lovastatin-mediated growth inhibition.

Lovastatin treatment affects G1/S transition-related regulators. The G1/S transition of the cell cycle is regulated by the CDK/cyclin complex, CKI, and Rb. To further clarify the mechanism of G1/S arrest following lovastatin treatment, we examined the expression of G1/S transition-related regulators. First, we used a western blot analysis to test the cell cycle-related genes CDK4, cyclin D1, Rb, p53, and p21 following lovastatin treatment. As summarized in Fig. 1D, among these genes, cyclin D, CDK4, and Rb were greatly inhibited, whereas p21 and p53 were profoundly upregulated.

Lovastatin treatment inhibits MCM2 expression. MCM2 plays an important role in the origination of eukaryotic genome replication. However, the exact mechanisms of MCM members related to lovastatin treatment remain unknown. We investigated MCM2 mRNA following lovastatin treatment for 48 h by RT-PCR in the A549 and GLC-82 cells. As shown in Fig. 2A, we observed that lovastatin treatment induced a significant decrease in MCM2. We then confirmed the inhibi-

tion of MCM2 following lovastatin treatment by measuring the protein levels. Our western blot analysis data showed that MCM2 expression was substantially downregulated after lovastatin treatment in the A549 and GLC-82 cells (Fig. 2B).

MCM2 knockdown decreases proliferation and induces G1/S transition arrest in NSCLC cells. Lovastatin treatment induces anti-proliferation and cell cycle arrest, as well as the downregulation of MCM2 expression. Thus, we aimed to ascertain whether siMCM2 could induce similar effects. First, we transfected siMCM2 oligonucleotide duplexes into A549 and GLC-82 cells. As a result, we determined the efficacy of anti-proliferation and cell cycle arrest following siMCM2. As shown in Fig. 3A, MCM2 expression was suppressed after MCM2 knockdown at the mRNA and protein levels in the A549 and GLC-82 cells. Next, we evaluated the apoptosis of cancer cells after MCM2 knockdown. siMCM2 was found to increase the apoptosis of cancer cells compared with the control (Fig. 3B). Moreover, our assessment of PI staining and flow cytometry showed that G1/S arrest occurred following MCM2 knockdown at 48 h (Fig. 3C and D). Thus, MCM2 may be associated with G1/S arrest following lovastatin treatment.

Changes in G1/S transition-related regulators and apoptosis factors after silencing MCM2 in NSCLC cells. Rb inactivation is a key event in G1 to S transition, mediated by CDK4/cyclin D1, where the expression of Rb, CDK4, and cyclin D1 were detected. We found that Rb was markedly inhibited by MCM2 knockdown in a concentration-dependent manner after 48 h (Fig. 4A). In addition, cyclin D1 and CDK4 were significantly downregulated in both cell lines (Fig. 4A). p21 and p53 proteins, meanwhile, were significantly upregulated in the MCM2-knockdown cell lines when compared to levels in the cells with siCon. In conclusion, MCM2 knockdown affected Rb expression, cyclin D1 and CDK4. In contrast, p21 and p53 increased the rates of G1/S arrest.

To investigate MCM2 knockdown-induced apoptosis-related proteins, the activity of caspase-3 was examined after lovastatin treatment for 48 h. As shown in Fig. 4B, caspase-3 was significantly increased. We also examined the expression of Bax and Bcl-2 (Fig. 4B). The results showed that Bax was upregulated and Bcl-2 was downregulated.

JNK pathway activation is involved in the downregulation of MCM2 by lovastatin treatment. It has been confirmed in previous studies that JNK pathway activation is involved in statin treatment in many types of cancers. To further clarify the mechanism involved in the proliferation of the inhibitory effects of MCM2, we investigated whether the JNK pathway is involved in the lovastatin-induced downregulation of MCM2 in NSCLC cells. We investigated the expression of p-JNK under the effects of lovastatin treatment. From the observations (Fig. 5A), p-JNK was inhibited in a dose- and time-dependent manner. We then used a specific inhibitor of JNK, SP600125, to treat the lung cancer cells. As expected, the protein MCM2 was downregulated by lovastatin treatment alone, while MCM2 was not changed when treated with the inhibitor SP600125 alone (Fig. 5B). When we combined these two factors, downregulation of MCM2 by lovastatin was restored (Fig. 5B). These results indicated that the JNK

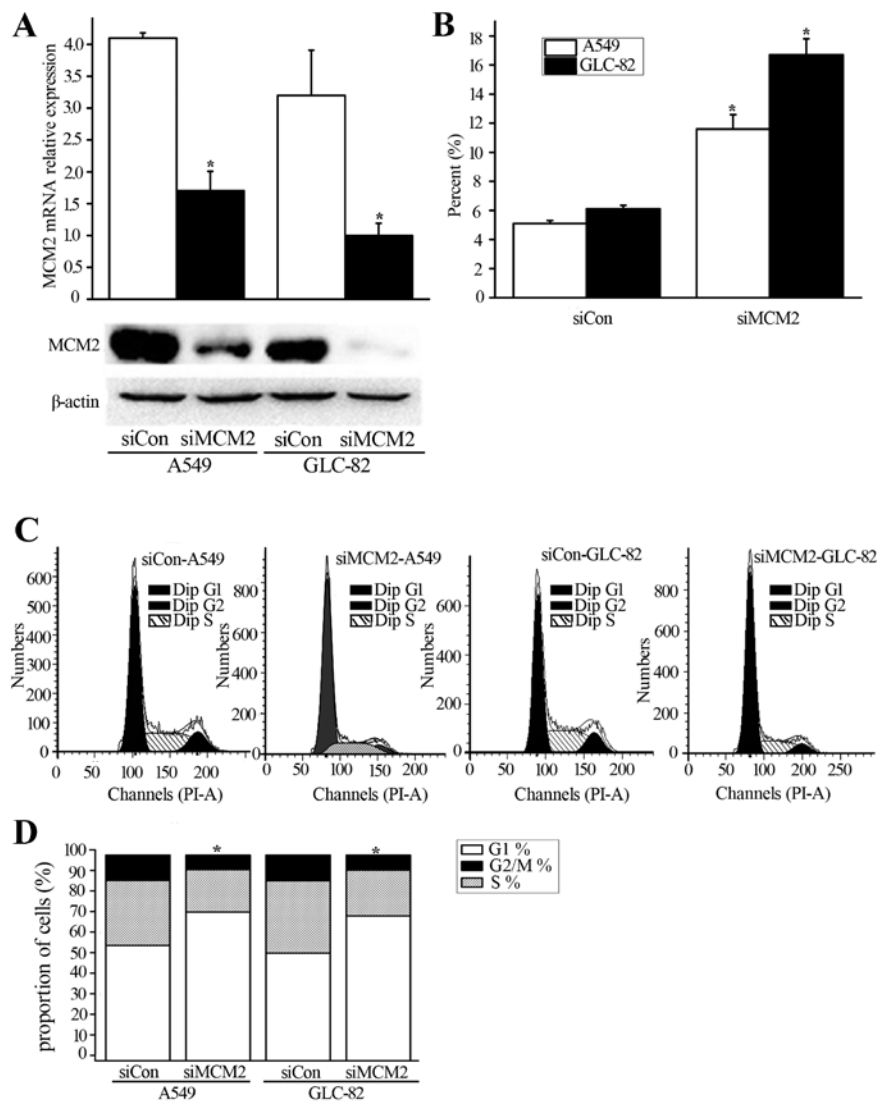


Figure 3. Effects of siMCM2 on cell growth and cell cycle progression in NSCLC cells. A549 and GLC-82 cells were cultured for 24 h, synchronized by serum-free medium for 24 h, and then transfected with MCM2 for 48 h. (A) Upper panel: MCM2 mRNA levels were determined by real-time PCR, and GAPDH was used as the internal control. Results are expressed as mean \pm SD of triplicate experiments. Lower panel: MCM2 protein level was assessed by western blot analysis. β -actin was used as the internal control. The results are representative of 3 experiments. * $P < 0.05$, Student's t-test. (B) Apoptosis was determined after MCM2 knockdown by Annexin V staining using flow cytometry. (C) PI staining and flow cytometry were used to analyze the cell cycle progression. The percentages of cells in the G1, S and G2/M phases are indicated. Data represent means \pm SD of triplicate experiments; (D) Histogram showing the results of C. * $P < 0.05$, was significantly different compared with siCon. NSCLCs, human non-small cell lung carcinomas; MCM, minichromosome maintenance.

pathway is associated with lovastatin treatment in NSCLC cells.

Discussion

Many studies have confirmed that statins, including lovastatin, inhibit cell growth and induce cell cycle arrest and apoptosis in many cancer types, such as human hepatocellular carcinoma (13,14), Barrett's esophageal adenocarcinoma (15), melanoma (15,16), ARH77 multiple myeloma (17), colon cancer (18), murine melanoma (19), prostate (20) and breast cancer (21), and NSCLCs *in vitro* and *in vivo* (3,22-25). Recent data have demonstrated that atorvastatin combined with carboplatin or lovastatin combined with ionizing radiation may offer an effective strategy against NSCLCs (22). Pelaia *et al* showed that simvastatin induced pro-apoptotic and anti-proliferative activity in NSCLC cells by inhibition of the Ras/Raf/MEK/

ERK signaling cascade (25). Furthermore, Falcone *et al* used lung cancer tissues to explore the inhibitory effects of simvastatin and rosuvastatin on MMP-2, MMP-9 and NF- κ B expression (23). Although statins have been shown to inhibit NSCLC cells growth *in vitro* and *in vivo* via different pathways, the particular mechanism of anti-proliferation involving lovastatin remains to be elucidated comprehensively.

We, first, confirmed the effects of lovastatin on the anti-proliferation of NSCLCs. The results demonstrated that lovastatin indeed triggered cell proliferation inhibition, cell cycle arrest and apoptosis (Fig. 1). Previous studies reported that CDK4 and cyclin D activation of the G1 phase and Rb protein depend on the full expression of MCMs and fully forming pre-RC (26). Cyclin D1 and CDK4 particularly are linked to replication licensing. After lovastatin treatment, Rb, cyclin D1 and CDK4 were inhibited in NSCLC cells. Therefore, we inferred that lovastatin treatment deactivated the

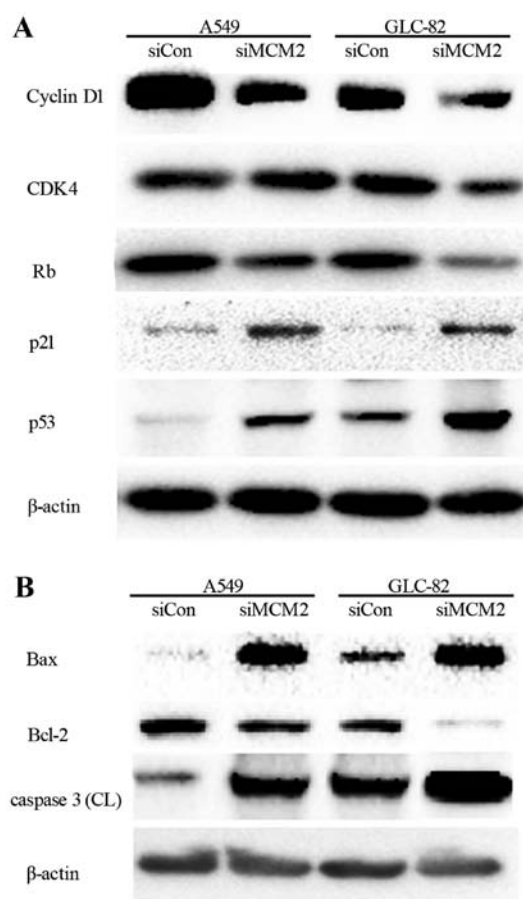


Figure 4. Cell cycle regulators and apoptosis factors following MCM2 knockdown in NSCLC cells. (A) Following knockdown of MCM2 for 48 h, the proteins in the siMCM2 cells and siCon cells were extracted, and western blotting was carried out to assess the expression of CDK4, cyclin D1, Rb p53 and p21 in the A549 and GLC-82 cells. β-actin was used as the internal control. The results are representative of 3 experiments. (B) Protein levels of Bcl-2, Bax and caspase-3 (cleaved) were determined by western blotting in the A549 and GLC82 cells, and β-actin was used as the sample loading control. All experiments were performed in triplicates. NSCLCs, human non-small cell lung carcinomas; MCM, minichromosome maintenance.

cyclin D1/CDK4 complex, which repressed Rb protein expression p53 can lead to the stabilization of this tumor suppressor, and it triggers the arrest of G1/S or G2/M through the induction of cyclin-dependent kinase inhibitor (CDKI) p21cip14, which can bind to cyclin D/CDK4 to repress their activity, leading to cell cycle arrest. In the present study, p21 and p53 were upregulated in siMCM2 cells, which contributed to G1/S arrest, but not in the siCon-transfected NSCLC cells (Fig. 4A). The induction of these kinase inhibitors and inhibition of cell cycle-related kinases by lovastatin could thus contribute to the observed cell cycle arrest.

MCMs, a highly conserved helicase and key regulatory component of eukaryotic DNA replication, are overexpressed in many types of human malignancies and have been important targets for cancer chemotherapy. Thus inhibiting eukaryotic replicative helicase activity of MCM2, would be of significant research utility. Some small molecular inhibitors of specific replication factors, such as ciprofloxacin and Trichostatin A, have been reported as potential therapeutic targets of MCMs (12,27). In the present study, we explored whether lovastatin can be a therapeutic target of MCM2. We

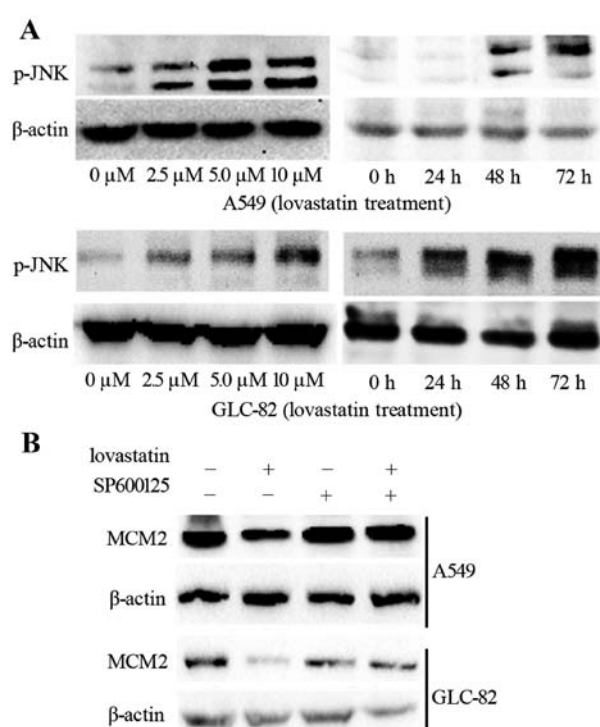


Figure 5. JNK pathway activation is involved in the downregulation of MCM2 by lovastatin treatment. (A) Phosphorylated JNK was detected in the A549 and GLC82 cells following lovastatin treatment, in a dose- and time-dependent manner. (B) We treated A549 and GLC-82 cells with 5 μM lovastatin or combined with SP600125. Total proteins were extracted and used for testing MCM2 expression. β-actin was used as the sample loading control. All results were repeated at least 3 times. MCM, minichromosome maintenance.

surprisingly found that MCM2 expression decreased at the mRNA and protein levels (Fig. 2A and B) after lovastatin treatment in two NSCLC cell lines. We suggest that lovastatin treatment-mediated cell cycle arrest and cell apoptosis may be bound up with the inhibition of MCM2, which in turn regulates the sets of genes responsible for DNA replication.

The relationship between MCM2 expression and apoptosis is controversial. One study found that MCM2 overexpression induced apoptosis in HL60 cells (28). Another showed that there is no relationship between MCM2 expression and apoptosis in malignant fibrous histiocytomas (29). Liu *et al*, however, demonstrated that the silencing of MCM2 expression by siRNA induced apoptosis in HCT116 cells (12). Caspases are essential for the apoptosis process, and apoptosis signals can activate caspase-9, which in turn activates effector caspases, such as caspase-3. These caspases can mediate cellular destruction. The conflicting results in regards to apoptosis and MCM2 expression may be due to the cell type examined (12). Our data showed that MCM2 knockdown activated caspase-3, upregulated Bax expression and downregulated Bcl-2 expression, which led to NSCLC cell apoptosis.

JNK, one subgroup of MAPKs, is activated in response to a variety of extracellular signals. It has been demonstrated that JNKs may act as tumor suppressors (30). Previous studies revealed that statin treatment could increase p-JNK in many cancers, including ovarian (31) and human breast cancer (32). Ogunwobi *et al* confirmed that blocking the p-JNK pathway induces apoptosis (15). Our data demonstrated that p-JNK

was increased after lovastatin treatment alone for 48 h, but that the pretreatment of NSCLC cells with SP600125 (a JNK inhibitor) significantly reduced the lovastatin-induced expression of MCM2, confirming the role of p-JNK in the induction of MCM2 in NSCLC cells.

In conclusion, the present study describes a novel anti-proliferation mechanism of MCM2 under the conditions of lovastatin treatment for NSCLCs. We found that lovastatin treatment significantly altered expression of cell cycle-related regulators, and MCM2 knockdown resulted in i) G1/S cell cycle arrest and ii) activation of apoptosis. In addition, we found that lovastatin activated the JNK pathway involved in the downregulation of MCM2. We suggest that MCM2 may be a potential target of lovastatin treatment in NSCLCs.

Acknowledgements

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