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Fluvastatin-mediated down-regulation of SATB1 affects aggressive phenotypes of human non-small-cell lung cancer cell line H292

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ABSTRACT

Aims: Fluvastatin reduces tumor proliferation and increased apoptotic activity in various cancers. Special AT-rich sequence binding protein 1 (SATB1) is a genome organizer that reprogrammes the gene transcription profiles of tumors to promote growth and metastasis. The antitumor effect and molecular mechanisms of fluvastatin on lung cancer is poorly understood. This study aimed to investigate the antitumor effect of fluvastatin on lung cancer and its possible mechanics.

Main methods: Cell viability assay was used to examine the inhibition of fluvastatin on proliferation of H292 cells. In order to investigate the antitumor mechanics, SATB1 knock-down H292 cells was constructed by lentiviral transfection. RT-PCR and Western blot were performed to examine the effects of fluvastatin on expression of SATB1 and Wnt/β-catenin signaling components.

Key findings: Fluvastatin significantly inhibited proliferation and invasion of H292 cells in a time- and dose-dependent manner and promoted the apoptosis ($p < 0.05$). The expression of SATB1 was down-regulated by fluvastatin in a dose-dependent manner. The proliferation and invasion of SATB1-shRNA cells was significantly suppressed, and the apoptosis was significantly enhanced ($p < 0.05$). We also show that the common target genes were regulated by SATB1 and Wnt/β-catenin pathway simultaneously. There may be a functional link between SATB1 and Wnt/β-catenin pathway.

Significance: We presented a possible mechanism of statins that fluvastatin significantly suppressed the in vitro tumor progression of H292 cells possibly by down-regulation of SATB1 via Wnt/β-catenin pathway, which provided new therapeutic possibilities for more cancers driven by hyperexpression of SATB1 and Wnt/β-catenin pathway.

1. Introduction

Fluvastatin is a member of statin family, which targets 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of the mevalonate pathway, and it was widely used for the treatment of hyperlipidaemia [1–5]. Recently, many researches have confirmed that fluvastatin could reduce tumor proliferation and increase apoptotic activity in various cancers in vitro, such as breast cancer [6], prostate cancer [7], ovarian cancer [8], pancreatic cancer [9] and hepatocellular carcinoma [10]. However, the effectiveness of fluvastatin in vivo is generally not satisfactory and the anti-tumor mechanism of fluvastatin remains unclear. Furthermore, the anti-tumor effect and mechanisms of fluvastatin on non-small-cell lung cancer (NSCLC) has not yet been fully elucidated.

Special AT-rich sequence binding protein 1 (SATB1) is a specifically expressed matrix attachment regions (MARs)-binding protein and can act as a transcriptional repressor [11]. It was confirmed to confer malignant behavior and was associated with the tumor growth, metastasis and poor prognosis in several solid tumor, including breast cancer [12,13], liver cancer [14], esophageal cancer [15] and gastric cancer [16]. SATB1 is up-regulated in tumor and functions as a genome organizer during tumorigenesis to alter the gene expression profile of cancer cells to induce an aggressive phenotype, thereby promote the tumor growth and metastasis [12]. Recent research has shown that fluvastatin down-regulated the expression of SATB1 in a dose- and time-dependent manner in colon cancer cells [17]. In addition, Mir et al.

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Table 1
Primers used for RT-PCR analysis.

Gene	Primer sequence (Forward)	Primer sequence (Reverse)
SATB1	5′-GTGGAAGCCTTGGGAATCC-3′	5′-CTGACAGCTCTTCTTCTAGTT-3′
ACTB	5′-CTGGCACCACACCTTCTACAATG-3′	5′-CCTCGTAGATGGGCACAGTGTG-3′
C-myc	5′-AGGAGCTGGTTGTACTTGG3–3′	5′-TCTTCAGGTCTGGAAAGTATG-3′
Bcl-2	5′-GCGATGAGCTGGAGATGATCCGGC-3′	5′-CTGCAGAGAAGATGTGGCCAGCC-3′
MMP2	5′-AGCCGTGCCTTCAGCTCTAC-3′	5′-GAAAGGAGAAGAGCCTGAAGTGT-3′
MMP9	5′-GAGAAGAGAGGGCCCAGC-3′	5′-ACGTGACCTATGACATCCTGC-3′
S100A4	5′-GATGAGCAACTTGGACAGCAA-3′	5′-CTGGGCTGCTTATCTGGGAAG-3′
N-cadherin	5′-ACATTGGGGACTTCATTAATGA-3′	5′-AAGTTCACCCTGAAGTTCAGT-3′
Fibernectin	5′-CATTTGCTCCTGCA CATGCT-3′	5′-AGTTTAGATGGATCTTGGCAG-3′
IL-2	5′-TGTTCATTTAACCCTCATTGCAC-3′	5′-TACTTTGGGAGGACTTTTGGACA-3′
IL-2R	5′-TTTCTTGGTAAGAAGCCGGG-3′	5′-TTAGAAGTGGGTAGCGCTCG-3′
VEGF-B	5′-GAGATGTCCCTGGAAGAACACA-3′	5′-GAGTGGGATGGGTGATGTCAG-3′

RT-PCR, Reverse Transcription-Polymerase Chain Reaction.

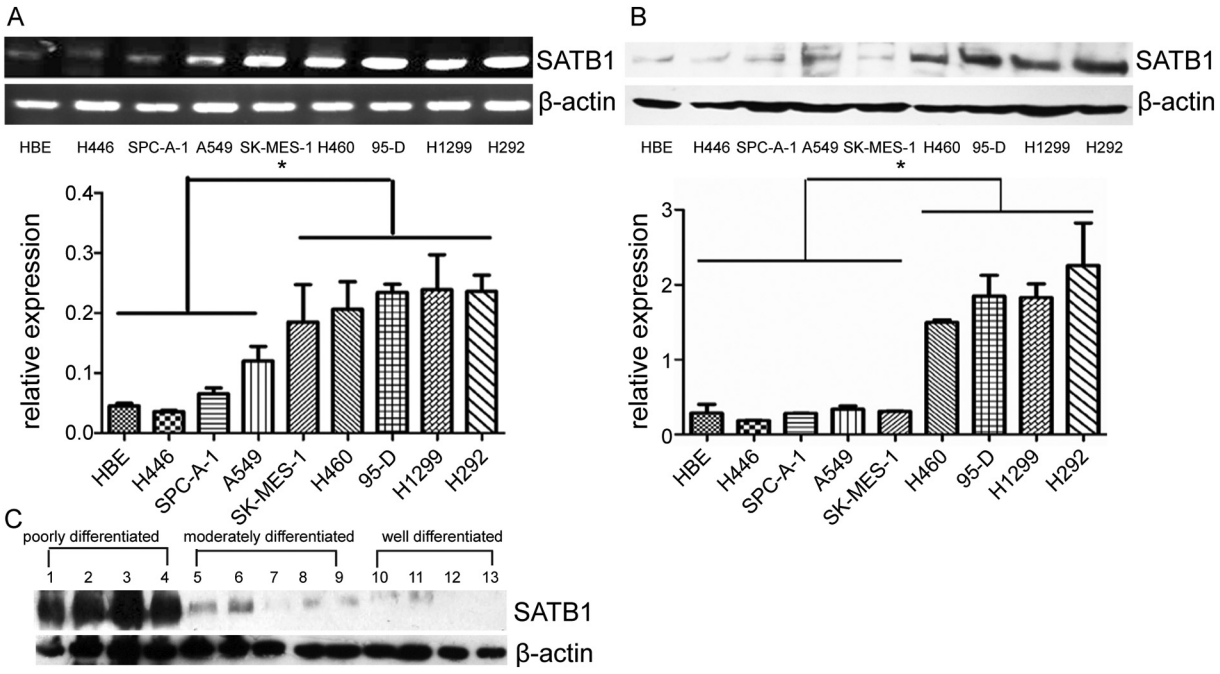

Fig. 1. SATB1 expression in nine cancer cell lines. A, SATB1 mRNA expression detected by semi-quantitative RT-PCR analysis. B, SATB1 protein expression detected by western blot analysis. C, SATB1 protein expression in different differentiated tumor tissue detected by western blot analysis. *$p < 0.05$. (n = 3 experiments).

have demonstrated that the expression of SATB1 was induced upon hyperactivation of Wnt/β-catenin pathway and functionally overlapped with Wnt/β-catenin pathway [18]. There are many same target genes such as c-myc and Bcl-2 between SATB1 and Wnt/β-catenin [19]. Therefore we supposed that there may be an important functional link between SATB1 and Wnt/β-catenin pathway in lung cancer.

The aim of this study was to investigate the antitumor effect of fluvastatin on NSCLC cell line H292 and whether the SATB1 was involved in the antitumor mechanism of fluvastatin. In addition, we explored the potential link between SATB1 and Wnt/β-catenin pathway. In NSCLC cell line H292, we demonstrated that fluvastatin significantly suppressed the proliferation, invasion and promote the apoptosis in vitro. Furthermore, we showed that SATB1 is down-regulated by fluvastatin in a time- and dose-dependent manner in H292 cells. Removal of SATB1 from H292 cells not only inhibited tumor growth and invasion but also promote apoptosis, indicating that SATB1 is a key factor in NSCLC progression. Additionally, our findings suggested potential cross-talk between SATB1 and Wnt/β-catenin pathway, providing a novel perspective to study the antitumor mechanisms of fluvastatin in NSCLC cells.

2. Materials and methods

2.1. Materials and reagents

Fluvastatin was purchased from Sigma (St. Louis, MO). Cell culture media, penicillin and streptomycin were purchased from Hyclone. Antibody to SATB1 and SATB1-shRNA lentiviral particles were from Santa Cruz Biotechnology. Cell counting Kit-8 (CCK-8) was from Dojindo Chemical Co., Japan. TUNEL assay kit was purchased from Roche. Transwells were purchased from Millipore Co., USA. Matrigel were from BD Biosciences. Lithium chloride (LiCl) and BIO were from Calbiochem-Merck, USA. H292, 95-D, H1299, A549, SPC-A-1, H460, SK-MES-1, H446, and HBE cell lines were originally obtained from National Key Laboratory of Biotherapy, Sichuan University.

2.2. Cell culture

H292, 95-D, H1299, A549, SPC-A-1, H460, SK-MES-1, H446 cells were grown in RPMI-1640; HBE cells in DMEM. Medium was supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100 U/ml penicillin and 100 μg/ml streptomycin. The medium was changed twice

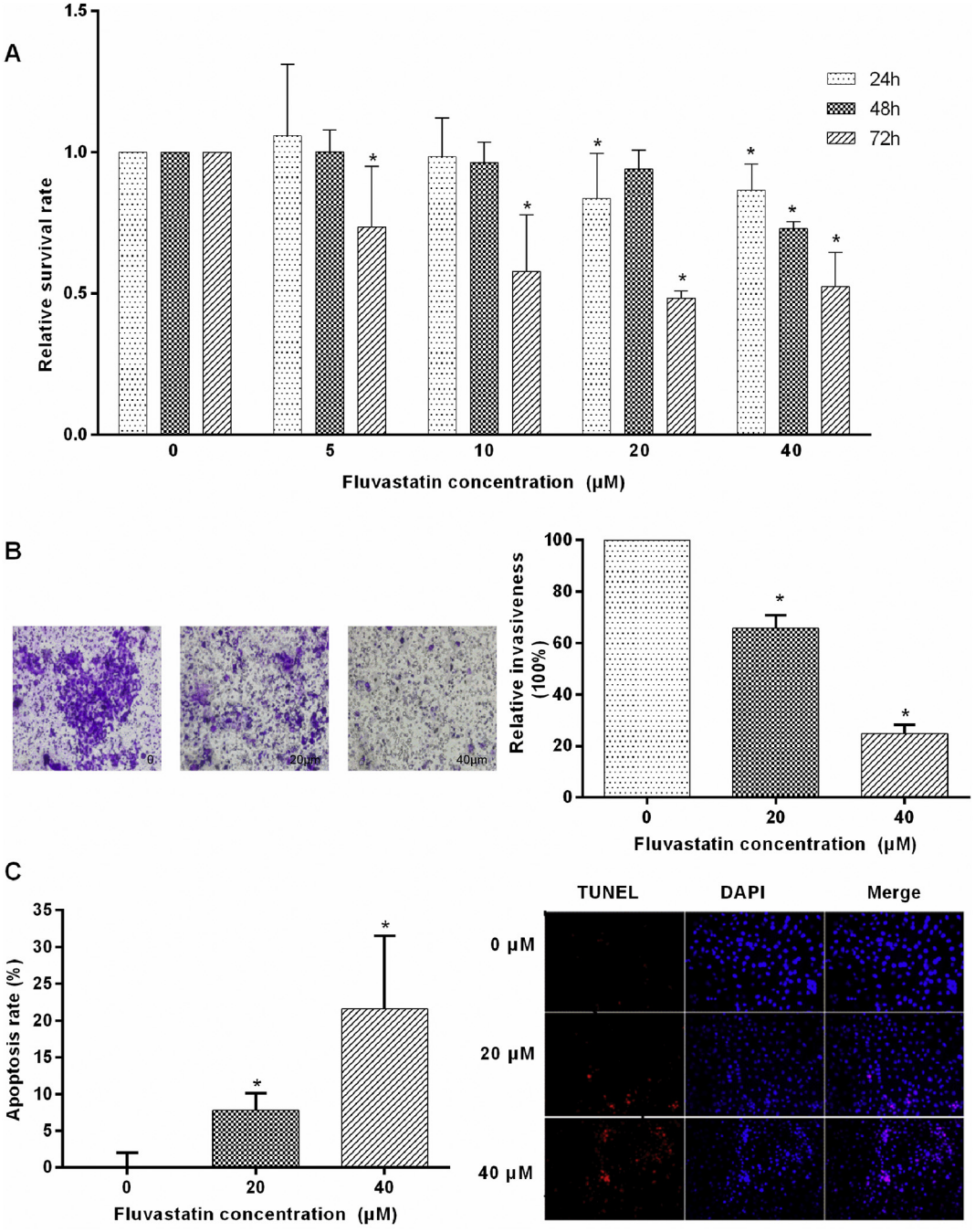

Fig. 2. Fluvastatin inhibits the activity, invasion and apoptosis of H292. A, The survival rate of H292 after exposure to fluvastatin determined by CCK-8 assay. Cells were treated with fluvastatin on varying concentrations (0, 5, 10, 20, and 40 μM) for different time periods (0, 24 h, 48 h). B, The H292 cells invasiveness detected by Transwell chamber after exposure to fluvastatin. Cells were treated with fluvastatin on varying concentrations (0, 20, and 40 μM) for 48 h. C, Cell apoptosis rate detected by TUNEL assay. Cells were treated with fluvastatin on varying concentrations (0, 20, and 40 μM) for 48 h. *$p < 0.05$ vs. control group. (n = 3 experiments).

a week and the cells were maintained in an incubator at 37 °C with a 5% CO_2 atmosphere. The cells were subcultured when confluent (> 80% confluence) using trypsin (2.5 g/l).

2.3. Treatment with fluvastatin and measure of protein expression

At ~80% confluence, cells were treated with fluvastatin at different concentrations (0, 5, 10, 20, 40 μM). After 24 h cultivation of H292 cells, cells were harvested and washed with cold PBS twice. Then cells were homogenized in 200 μl pre-cooling RIPA buffer containing 1 μl PMSF, 0.2 μl protease inhibitor cocktail and 0.2 μl dithiothreitol (DTT) for 10 min on ice. The lysate was centrifuged at 13,000 rpm for 10 min at 4 °C to collect the cytosolic protein supernatant. The remaining precipitates were lysed with lysis buffer (0.12 μl DTT, 0.1 μl protease inhibitor cocktail and 0.5 μl PMSF) for 40 min. The lysate was centrifuged at 14,000 rpm for 10 min at 4 °C to collect the nucleoprotein supernatant. Protein was determined using the bicinchoninic acid (BCA) method [20]. Expression of protein in H292 cells was measured by Western blot using antibody to SATB1. The bands intensity of western blot was analyzed using Quantity One software (Bio-Rad, USA).

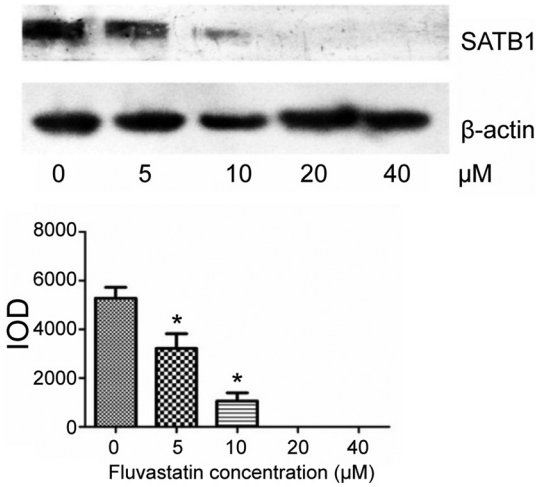

Fig. 3. Fluvastatin-mediated down-regulation of SATB1 in H292 cells. IOD, Integral Optical Density *$p < 0.05$. (n = 3 experiments).

2.4. RT-PCR

Total RNA was isolated using TRIzol reagent (invitrogen, Corp., Carlsbad, CA) and cDNA was prepared using Takara primeScript RT-PCR Kit (TaKaRa, China). PCR primer sequences were available in Table 1. ACTB was used as endogenous control. Image-Pro Plus 4.5 (IPP4.5, Media Cybernetics, Silver Spring, MD, USA) was used for the semi-quantitative analysis.

2.5. Construction of SATB1 knock-down H292 cell line

At 50% confluence, H292 cells were treated with 50 μl lentiviral plasmid copGFP-shRNA and SATB1-shRNA (1×10^5 TU/ml), respectively. Cells were maintained in an incubator at 37 °C with a 5% CO_2 atmosphere for 24 h. The medium was changed to RPMI-1640 with 3 μg/ml puromycin to select the shRNA-positive H292 cells. Cells that had a control shRNA unrelated to SATB1 sequences (copGFP-shRNA) was used as a negative control and that did not have lentiviral vector shRNA was served as blank control.

2.6. Measure of H292 cells' activity, invasion and apoptosis

Cell viability was measured by cell counting Kit-8 (Dojindo, Tokyo, Japan). CCK-8 solution (10 μl) was added into each well and the incubation continued for another 1.5 h. Absorbance was measured at 450 nm by a microplate reader (μQuant MQX200, BioTek, USA). Percentage of cell activity was calculated according to the following formula: Survival rate = (mean OD value of experimental group − mean OD value of blank control group) / (mean OD value of negative control group − mean OD value of blank control group). Cell invasion assay was assessed by using matrigel-coated Transwell chamber, according to manufacturer's instructions [21]. Pictures were taken under 400× magnification. The terminal transferase uridyl nick end labelling (TUNEL) assay was applied to investigate active cell apoptosis [22]. Percentage of cell apoptosis as calculated according to the following formula: Apoptosis rate = positive cells count / all cells count.

2.7. Statistical analysis

Statistical analysis was performed with IBM SPSS statistics 19.0 (SPSS Inc., Chicago, Illinois, USA). Results from each experiment were expressed as the mean ± standard deviation (SD) of three separate experiments. Statistical significance was determined with Student's *t*-test. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. SATB1 expression in different cells

We examined SATB1 expression in 9 lung epithelial cell lines, including normal human bronchial epithelial (HBE) cells, 5 human lung adenocarcinoma cells, 1 human lung cancer cell line, 1 squamous cell line, and 1 small cell lung cancer cell line. SATB1 mRNA and protein were only rarely detected in HBE and small cell lung cancer cell line H446. SATB1 were detected in most NSCLC cell lines (H292, 95-D, H1299, A549, SPC-A-1, H460, SK-MES-1). For mRNA expression, SATB1 expression levels were significant higher in H460, 95-D, H1299 and H292 than the other five cell lines ($p < 0.05$, Fig. 1A). For STAB1 protein expression, western blot analysis showed that STAB1 protein levels were found to be highest in H292 followed by 95-D, H1299 and H460, and relatively low levels were observed in other five cell lines (Fig. 1B). Taken together, these results suggest that higher expression of SATB1 was detected in highly metastatic cell lines (H460, 95-D, H1299 and H292). In this study, H292 cells were selected to conduct subsequent experiments since they had the highest level of STAB1 expression.

Among 13 human lung cancers with different differentiation abilities, western blot analysis showed that higher expression of SATB1 was detected in all four poorly differentiated tumor tissue. Low level SATB1 expression was found in moderately differentiated tumors, and SATB1 was hardly detected in well differentiated tumor samples (Fig. 1C).

3.2. Fluvastatin-induced cell inhibition

Cancer cell viability predicts its metastasis potential, so we assessed the effects of fluvastatin on cell viability in H292 cells using CCK-8. The cells were treated with various concentrations (0–40 μM) for different time periods (0–48 h). After 48 h of fluvastain treatment, the viability of fluvastatin-treated H292 cells (5–40 μM) was significant lower than that of the blank control ($p < 0.05$, Fig. 2A). The viability of H292 cells decreased as the increasing of fluvastain concentration. Meanwhile, the viability was also reduced with the time. Fluvastain dose- and time-dependently decreased cell viability by 50% in H292 cells, with maximum effect at 48 h and 40 μM.

Invasion is another aspect that predicts the metastatic capacity of cancer cells. Matrigel-coated Transwell invasion assay was used to examine the invasion ability of H292 cells. After 48 h, the invasiveness of H292 cells was reduced by 20% and 70% respectively, when cells were treated with 20 and 40 μM fluvastatin (Fig. 2B). The results showed that fluvastatin inhibited the invasiveness in a dose-dependent manner.

We examined the effects of fluvastatin on cell apoptosis in H292 cells using the classic method, TUNEL assay. After 48 h, the apoptosis rate of H292 cells were 0.1%, 7.8% and 21.6%, respectively, when cells were treated with 0, 20, and 40 μM fluvastatin (Fig. 2C). The results showed that fluvastatin enhanced the apoptosis rate of H292 cells dose-dependently.

3.3. Fluvastatin-mediated down-regulation of SATB1 in H292 cells

To explore the possible antitumor mechanism of fluvastatin, western blot analysis was used to examine the SATB1 protein expression in H292 cells. The cells were treated with different concentrations of fluvastatin for 48 h. After treatment for 48 h, fluvastatin significantly reduced the levels of SATB1 protein expression of H292 cells dose-dependently. When concentration was higher than 10 μM, SATB1 protein expression was not detected (Fig. 3). There was significant difference of SATB1 protein expression between fluvastatin-treated group and blank control group ($p < 0.05$).

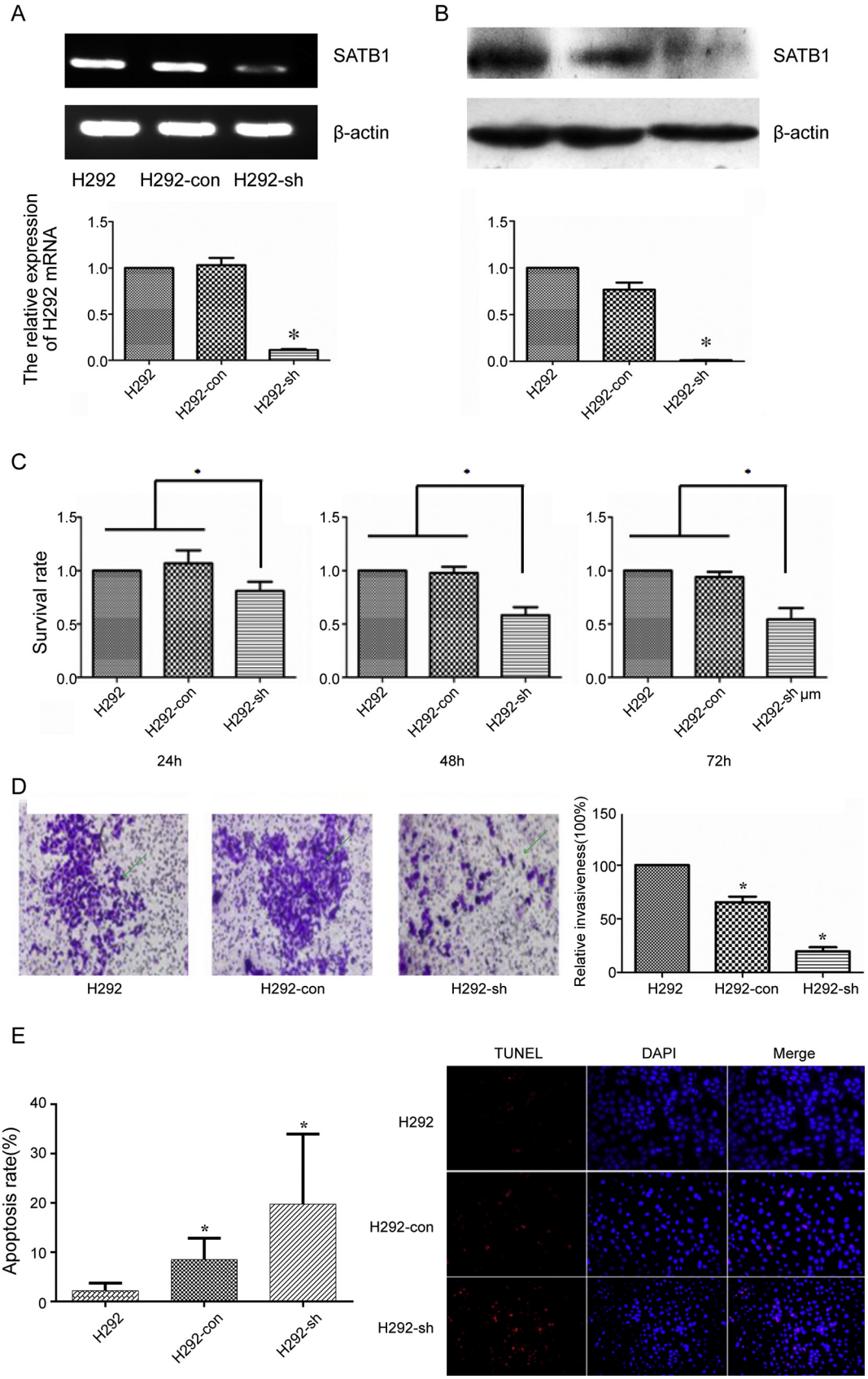

Fig. 4. SATB1 effects aggressive phenotypes of lung cancer cells in vitro. A, Knock-down SATB1 expression determined by semi-quantitative RT-PCR analysis in SATB1-shRNA cells compared with controls. B, Knock-down SATB1 expression determined by western blot analysis in SATB1-shRNA cells compared with controls. C, Cell viability detected by CCK-8 in SATB1-shRNA cells compared with controls. D, Cell invasion detected by Transwell chamber in SATB1-shRNA cells compared with controls. E, Cell apoptosis rate detected by TUNEL assay in SATB1-shRNA cells compared with controls. *$p < 0.05$. (n = 3 experiments). Note: H292, parental cell line as blank control; H292-con, control shRNA cells as negative control; H292-sh, SATB-shRNA cells.

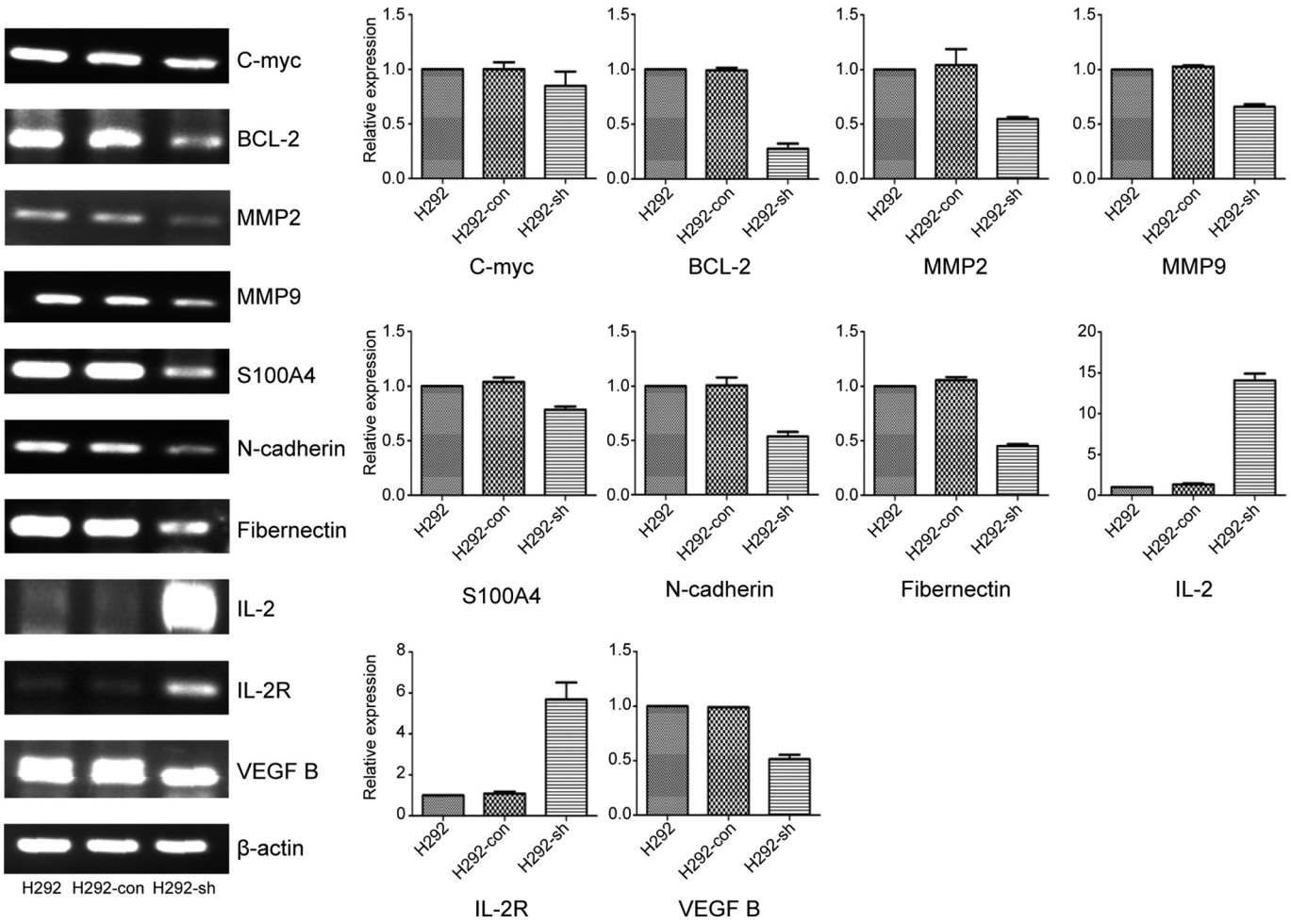

Fig. 5. SATB1 depletion affects expression levels of multiple genes confirmed with semi-quantitative RT–PCR. (n = 3 experiments).

3.4. SATB1 effects aggressive phenotypes of lung cancer cells in vitro

We investigated whether SATB1 effects the growth, invasion and apoptosis of lung cancer cells in vitro by expressing short hairpin RNAs (shRNA) to knock down SATB1 expression. We expressed shRNA in H292 cell lines. Both SATB1 mRNA and protein were hardly detected in SATB1-shRNA expressing cells. The mRNA and protein levels of SATB1 were substantially reduced by 90.3% and 97.1%, respectively (Fig. 4A, B). SATB1 expression remained unaltered in H292 cells expressing a control shRNA whose sequence did not match any known human gene.

SATB1 knock-down significantly reduced the viability of SATB-shRNA cells compared with the parental cell line and control shRNA cells ($p < 0.05$, Fig. 4C). Moreover, the invasive capacity of SATB-shRNA cells was reduced by 63% (Fig. 4D). The apoptosis rate of SATB-shRNA cells was significant higher than that of parental cell line H292 and control shRNA cells ($p < 0.05$, Fig. 4E).

3.5. Inhibition effect of fluvastatin on Wnt/β-catenin pathway in vitro

In order to clarify the possible mechanisms by which fluvastatin exerts its anticancer effect, RT–PCR was applied to assess the same target protein's expression in SATB1 and Wnt/β-catenin pathway. The expression of many genes known to play important roles in promoting proliferation and metastasis was found to be regulated by SATB1 and Wnt/β-catenin pathway. For example, proto-oncogene (c-myc) could promote the cell proliferation; matrix metalloproteases (MMPs), metastasin (S100A4) and vascular endothelial growth factor B (VEGF-B) are prometastatic genes; N-cadherin and fibernectin involved in epithelial–mesenchymal transition (EMT). Therefore, we evaluated the expression of downstream target proteins after knock-down of SATB1 in H292 cells to. Meanwhile, Wnt/β-catenin pathway activators (15 μM LiCl, 1.5 μM BIO) and inhibitor (2.5 μM DKK-1) were added into H292 cells to evaluate the expression of the same target proteins, respectively.

As shown in Fig. 5, SATB1 depletion down-regulated the expression of proliferative gene c-myc. The expression of prometastatic genes (MMP2, MMP9, S100A4 and VEGF-B) were suppressed by knocking down SATB1. Anti-apoptosis gene (Bcl-2) expression was down-regulated by SATB1 depletion. EMT related genes (N-cadherin and fibernectin) were also down-regulated in SATB-shRNA cells. In contrast, IL-2 and IL-2R which enhance the immune response was up-regulated by SATB1 depletion.

Meanwhile, Fig. 6 showed that c-myc, Bcl-2, MMP2, MMP9, S100A4, N-cadherin, fibernectin and VEGF-B were all up-regulated by Wnt/β-catenin pathway, while IL-2 and IL-2R was down-regulated by Wnt/β-catenin pathway, indicating the similar regulation effect between Wnt/β-catenin and SATB1. These findings suggested the potential cross-talk between SATB1 and Wnt/β-catenin pathway.

4. Discussion

Several recent researches have confirmed the antitumor effect of HMG-CoA reductase inhibitors (statins), including fluvastatin, simvastatin, and lovastatin, in various cancer cells [17,23]. The effectiveness of fluvastatin in vivo has also been preliminary proven [24]. For example, a population-based case–control study of patients diagnosed

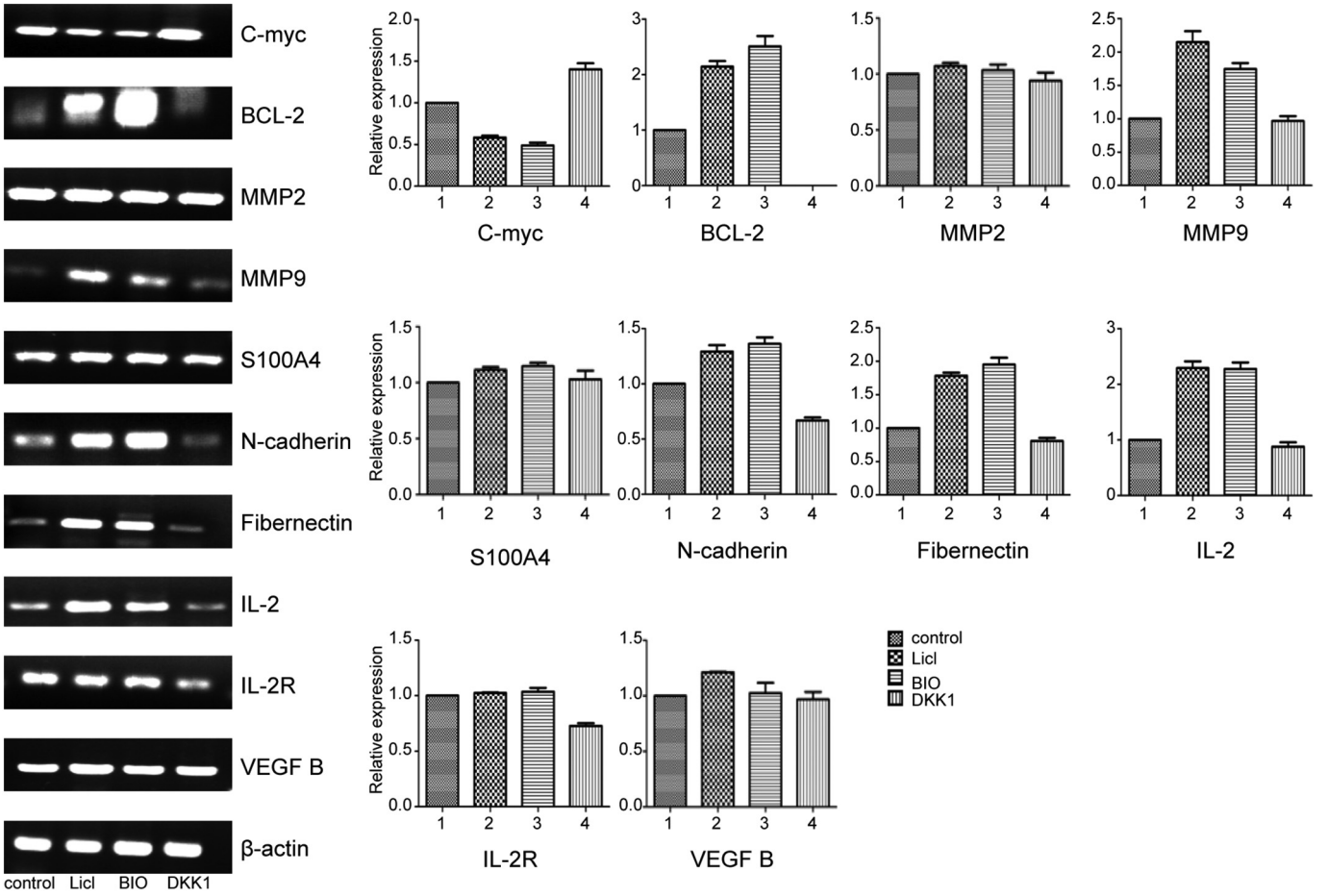

Fig. 6. Activation or inhibition of Wnt/β-catenin pathway affects expression levels of multiple genes confirmed with semi-quantitative RT–PCR. (n = 3 experiments).

with colorectal cancer has reported that statins can reduce the risk of colorectal cancer by 47% [25]. Furthermore, the antitumor mechanism of statins has been explored, which revealed that statins inhibited the metastasis and proliferation, and induced apoptosis by down-regulating the expression of SATB1 in colon cancer cells [17,26–28]. Nevertheless, there are few studies on its antitumor effects and specific mechanisms in NSCLC. Accordingly, we focused on the effect of fluvastatin in NSCLC cell lines to research its antitumor mechanism for the first time. The results demonstrated that fluvastatin down-regulated the expression of SATB1 to change the expression of downstream gene, and then induced the aggressive phenotype which could promote tumor growth and metastasis, and inhibit tumor apoptosis. Moreover, the downstream gene expression of SATB1 was also regulated by Wnt/β-catenin pathway, indicating that the two signalings had functional link. Therefore, we presented a possible model of fluvastatin-mediated gene regulation in H292, in which fluvastatin may mediate the down-regulation of SATB1 expression via Wnt/β-catenin pathway.

In this study, we detected the SATB1 expression in different cell lines. Results showed that SATB1 expression levels were significant higher in H460, 95-D, H1299 and H292 than others. It is well known that H292 is a lung adenocarcinoma with lymph node metastatic cell line; both 95-D and H1299 are recognized as highly metastatic lung adenocarcinoma cell lines and H460 is a large cell carcinoma cell line. All the four cell lines belong to highly metastatic lung cancer cell lines. Taken together, the highly metastatic cell lines had the higher expression of SATB1, which is consistent with the findings of a previous study [12]. Furthermore, we found that the highly metastatic H292 cells had the highest levels of SATB1 expression. Besides, SATB1 expression was proved to be related to the cell differentiation degree. The lower the degree of differentiation, the higher the expression of SATB1, indicating that the high levels of SATB1 expression may be associated with poor prognosis. Thus, given that the expression of SATB1 was found to be highest in H292 cells, we used these cells to study the antitumor effect of fluvastatin.

According to cell viability assay, fluvastatin was proved to inhibit cell proliferation in a dose- and time-dependent manner. Cell invasion assay using matrigel-coated Transwell chamber have been applied to evaluate the invasiveness of cancer cells in vitro. Our results suggested that fluvastatin significantly suppressed the migration and invasion capacity of H292 in vitro. In addition, TUNEL assay showed that fluvastatin enhanced the apoptosis of H292 in vitro. Overall, our studies confirmed the antitumor effect of fluvastatin in H292 cell lines in vitro. Fluvastatin could inhibit the proliferation, invasion and metastasis of cancer cells, and promote the cell apoptosis.

SATB1 as the genome organizer could alter the gene expression profile to induce an aggressive phenotype. In this study, it is worthwhile mentioning that fluvastatin significantly down-regulated SATB1 protein levels in a dose-dependent manner, which was in agreement with recent reports on colon cancer cells [17]. Then, after silencing SATB1 in H292, the cell activity and metastatic capacity were significantly inhibited, and apoptosis rate was significantly enhanced. Therefore, our studies serve as a possible mechanism that fluvastatin exerts antitumor effect by down-regulating the expression of SATB1.

Wnt/β-catenin pathway is a highly conserved signaling pathways, which is closely related to the cancer progression [29,30]. Notably, it has reported that many SATB1-targeted genes are also targeted by Wnt/β-catenin, such as c-myc, Bcl-2, IL-2, IL2-R [19]. C-myc plays roles in both cellular proliferation and apoptosis [31]. Anti-apoptosis gene (Bcl-2) is the key regulators of apoptosis [32]. Matrix metalloproteases (MMPs) degrades the basal extracellular matrix (ECM) and promotes

tumor invasion [33]. Metastasin (S100A4) is a key prometastatic proteins, which is overexpressed in various tumor and plays an important role in tumor invasion and metastasis [34,35]. N-cadherin and Fibernectin are mesenchymal markers involved in epithelial–mesenchymal transition (EMT), which is a process whereby cells acquire molecular alterations that facilitate cell motility and invasion [36]. VEGF-B, a molecule of vascular endothelial growth factor (VEGF) family, promotes cancer metastasis through the remodeling of tumor microvasculature [37]. Thus, to shed light on the potential link between fluvastatin-mediated down-regulation of SATB1 and Wnt/β-catenin pathway, the expression of downstream genes in knock-down SATB1 cells was studied. On one hand, the genes involved in promoting tumor progression were suppressed. For example, c-myc which promotes the cell proliferation was down-regulated by knocking down SATB1. Meanwhile, MMP2, MMP9, S100A4 and VEGF-B which are prometastatic genes were all down-regulated by knocking down SATB1. In addition, N-cadherin and fibernectin involved in EMT were also down-regulated by knocking down SATB1. They also were down-regulated by inhibiting Wnt/β-catenin pathway. On the other hand, the genes involved in promoting tumor apoptosis were enhanced (Bcl-2, IL-2 and IL-2R) by knocking down SATB1 or inhibiting Wnt/β-catenin pathway. On the basis of these results we speculated that fluvastatin-mediated down-regulation of SATB1 may occur through the down-regulation of Wnt/β-catenin pathway. The data presented here additionally suggest that multiple downstream genes involved in many aspects of tumor progression were regulated by SATB1, indicating the tumor growth and metastasis were induced by a large group of SATB1-targeted genes collectively via either SATB1 or Wnt/β-catenin pathway. In a word, fluvastatin significantly suppressed the in vitro tumor progression of H292 cells possibly by down-regulation of SATB1 via Wnt/β-catenin pathway.

5. Conclusion

We reported the antitumor effect of fluvastatin on non-small cell lung cancer in vitro for the first time. Fluvastatin inhibited tumor proliferation by down-regulating the expression of STAB1. Moreover, the results demonstrated that there may be a functional link between SATB1 and Wnt/β-catenin pathway in NSCLC cells. These findings indicated that fluvastatin had the potential to be used in the treatment of non-small cell lung cancer. In addition, the elucidation of the mechanisms of SATB1 and its cross-link with Wnt/β-catenin pathway indicated that SATB1 may be useful as a therapeutic target for NSCLC, and The investigations on detailed antitumor mechanism of fluvastatin remains to be further researched.

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Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent: For this type of study informed consent is not required.

References

[1] B.O. Barnstein, P.A. Paez, E.M. Kolawole, J.J. Ryan, The HMGCR inhibitor fluvastatin induces apoptosis and autophagy in primary and neoplastic mast cells, J. Immunol. 198 (1 Supplement) (2017) 67.22.
[2] T. Obata, M. Nakashima, Fluvastatin, an HMG-CoA reductase inhibitor, facilitate adenosine production in the rat hearts via activation of ecto-5′-nucleotidase, Microvasc. Res. 107 (1–5) (2016).
[3] G. Derosa, A.E. Cicero, G. Bertone, M.N. Piccinni, L. Ciccarelli, D.E. Roggeri, Comparison of fluvastatin + fenofibrate combination therapy and fluvastatin monotherapy in the treatment of combined hyperlipidemia, type 2 diabetes mellitus, and coronary heart disease: a 12-month, randomized, double-blind, controlled trial, Clin. Ther. 26 (10) (2004) 1599–1607.
[4] E. Haak, C. Abletshauser, S. Weber, C. Goedicke, N. Martin, N. Hermanns, K. Lackner, K. Kusterer, K.H. Usadel, T. Haak, Fluvastatin therapy improves microcirculation in patients with hyperlipidaemia, Atherosclerosis 155 (2) (2001) 395–401.
[5] S. Zhao, F. Wang, K. Yang, Y. Hao, G. Li, M. Yang, Z. Yang, Efficacy and safety of fluvastatin extended-release tablets in Chinese patients with hyperlipidemia: a multi-center, randomized, double-blind, double dummy, active-controlled, parallel-group study, Zhonghua Nei Ke Za Zhi 53 (6) (2014) 455–459.
[6] E.R. Garwood, A.S. Kumar, F.L. Baehner, D.H. Moore, A. Au, N. Hylton, C.I. Flowers, J. Garber, B.A. Lesnikoski, E.S. Hwang, O. Olopade, E.R. Port, M. Campbell, L.J. Esserman, Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer, Breast Cancer Res. Treat. 119 (1) (2010) 137–144.
[7] U. Sivaprasad, T. Abbas, A. Dutta, Differential efficacy of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors on the cell cycle of prostate cancer cells, Mol. Cancer Ther. 5 (9) (2006) 2310–2316.
[8] B. Taylor-Harding, S. Orsulic, B.Y. Karlan, A.J. Li, Fluvastatin and cisplatin demonstrate synergistic cytotoxicity in epithelial ovarian cancer cells, Gynecol. Oncol. 119 (3) (2010) 549–556.
[9] M. Elsayed, D. Kobayashi, T. Kubota, N. Matsunaga, R. Murata, Y. Yoshizawa, N. Watanabe, T. Matsuura, Y. Tsurudome, T. Ogino, S. Ohdo, T. Shimazoe, Synergistic antiproliferative effects of zoledronic acid and fluvastatin on human pancreatic cancer cell lines: an in vitro study, Biol. Pharm. Bull. 39 (8) (2016) 1238–1246.
[10] T.G. Simon, H. Bonilla, P. Yan, R.T. Chung, A.A. Butt, Atorvastatin and fluvastatin are associated with dose-dependent reductions in cirrhosis and hepatocellular carcinoma, among patients with hepatitis C virus: results from ERCHIVES, Hepatology 64 (1) (2016) 47–57.
[11] M. Beyer, Y. Thabet, R.U. Muller, T. Sadlon, S. Classen, K. Lahl, S. Basu, X. Zhou, S.L. Bailey-Bucktrout, W. Krebs, E.A. Schonfeld, J. Bottcher, T. Golovina, C.T. Mayer, A. Hofmann, D. Sommer, S. Debey-Pascher, E. Endl, A. Limmer, K.L. Hippen, B.R. Blazar, R. Balderas, T. Quast, A. Waha, G. Mayer, M. Famulok, P.A. Knolle, C. Wickenhauser, W. Kolanus, B. Schermer, J.A. Bluestone, S.C. Barry, T. Sparwasser, J.L. Riley, J.L. Schultze, Repression of the genome organizer SATB1 in regulatory T cells is required for suppressive function and inhibition of effector differentiation, Nat. Immunol. 12 (9) (2011) 898–907.
[12] H.J. Han, J. Russo, Y. Kohwi, T. Kohwi-Shigematsu, SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis, Nature 452 (7184) (2008) 187–193.
[13] Z. Pan, W. Jing, K. He, L. Zhang, X. Long, SATB1 is correlated with progression and metastasis of breast cancers: a meta-analysis, Cell. Physiol. Biochem. 38 (5) (2016) 1975–1983.
[14] W. Tu, M. Luo, Z. Wang, W. Yan, Y. Xia, H. Deng, J. He, P. Han, D. Tian, Upregulation of SATB1 promotes tumor growth and metastasis in liver cancer, Liver Int. 32 (7) (2012) 1064–1078.
[15] B. Huang, F. Xiong, S. Wang, X. Lang, X. Wang, H. Zhou, Effect of SATB1 silencing on the proliferation, invasion and apoptosis of TE-1 esophageal cancer cells, Oncol. Lett. 13 (5) (2017) 2915–2920.
[16] X. Lu, C. Cheng, S. Zhu, Y. Yang, L. Zheng, G. Wang, X. Shu, K. Wu, K. Liu, Q. Tong, SATB1 is an independent prognostic marker for gastric cancer in a Chinese population, Oncol. Rep. 24 (4) (2010) 981–987.
[17] C.N. Lakshminarayana Reddy, V.N. Vyjayanti, D. Notani, S. Galande, S. Kotamraju, Down-regulation of the global regulator SATB1 by statins in COLO205 colon cancer cells, Mol. Med. Rep. 3 (5) (2010) 857–861.
[18] R. Mir, S.J. Pradhan, P. Patil, R. Mulherkar, S. Galande, Wnt/beta-catenin signaling regulated SATB1 promotes colorectal cancer tumorigenesis and progression, Oncogene 35 (13) (2016) 1679–1691.
[19] D. Notani, K.P. Gottimukkala, R.S. Jayani, A.S. Limaye, M.V. Damle, S. Mehta, P.K. Purbey, J. Joseph, S. Galande, Global regulator SATB1 recruits beta-catenin and regulates T(H)2 differentiation in Wnt-dependent manner, PLoS Biol. 8 (1) (2010) e1000296.
[20] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1) (1985) 76–85.
[21] L.A. Repesh, A new in vitro assay for quantitating tumor cell invasion, Invasion Metastasis 9 (3) (1989) 192–208.
[22] Z. Darzynkiewicz, D. Galkowski, H. Zhao, Analysis of apoptosis by cytometry using TUNEL assay, Methods 44 (3) (2008) 250–254.
[23] S. Kato, S. Smalley, A. Sadarangani, K. Chen-Lin, B. Oliva, J. Branes, J. Carvajal, R. Gejman, G.I. Owen, M. Cuello, Lipophilic but not hydrophilic statins selectively induce cell death in gynaecological cancers expressing high levels of HMGCoA reductase, J. Cell. Mol. Med. 14 (5) (2010) 1180–1193.

[24] Z. Mei, M. Liang, L. Li, Y. Zhang, Q. Wang, W. Yang, Effects of statins on cancer mortality and progression: a systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals, Int. J. Cancer 140 (5) (2017) 1068–1081.
[25] J.N. Poynter, S.B. Gruber, P.D. Higgins, R. Almog, J.D. Bonner, H.S. Rennert, M. Low, J.K. Greenson, G. Rennert, Statins and the risk of colorectal cancer, N. Engl. J. Med. 352 (21) (2005) 2184–2192.
[26] B. Agarwal, S. Bhendwal, B. Halmos, S.F. Moss, W.G. Ramey, P.R. Holt, Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells, Clin. Cancer Res. 5 (8) (1999) 2223–2229.
[27] A.E. Kowalczyk, J. Godlewski, B.E. Krazinski, J. Kiewisz, A. Sliwinska-Jewsiewicka, P. Kwiatkowski, B. Pula, P. Dziegiel, J. Janiszewski, P.M. Wierzbicki, Z. Kmiec, Divergent expression patterns of SATB1 mRNA and SATB1 protein in colorectal cancer and normal tissues, Tumour Biol. 36 (6) (2015) 4441–4452.
[28] S. Ishikawa, H. Hayashi, K. Kinoshita, M. Abe, H. Kuroki, R. Tokunaga, S. Tomiyasu, H. Tanaka, H. Sugita, T. Arita, Y. Yagi, M. Watanabe, M. Hirota, H. Baba, Statins inhibit tumor progression via an enhancer of zeste homolog 2-mediated epigenetic alteration in colorectal cancer, Int. J. Cancer 135 (11) (2014) 2528–2536.
[29] R. Nusse, H. Clevers, Wnt/beta-catenin signaling, disease, and emerging therapeutic modalities, Cell 169 (6) (2017) 985–999.
[30] Y. Ma, Y. Yang, F. Wang, M.P. Moyer, Q. Wei, P. Zhang, Z. Yang, W. Liu, H. Zhang, N. Chen, H. Wang, H. Wang, H. Qin, Long non-coding RNA CCAL regulates colorectal cancer progression by activating Wnt/beta-catenin signalling pathway via suppression of activator protein 2alpha, Gut 65 (9) (2016) 1494–1504.
[31] M.A. Mansour, T. Hyodo, K.A. Akter, T. Kokuryo, K. Uehara, M. Nagino, T. Senga, SATB1 and SATB2 play opposing roles in c-Myc expression and progression of colorectal cancer, Oncotarget 7 (4) (2016) 4993–5006.
[32] J.M. Adams, S. Cory, The BCL-2 arbiters of apoptosis and their growing role as cancer targets, Cell Death Differ. 25 (1) (2018) 27–36.
[33] K. Vinnakota, Y. Zhang, B.C. Selvanesan, G. Topi, T. Salim, J. Sand-Dejmek, G. Jonsson, A. Sjolander, M2-like macrophages induce colon cancer cell invasion via matrix metalloproteinases, J. Cell. Physiol. 232 (12) (2017) 3468–3480.
[34] K. Boye, G.M. Mælandsmo, S100A4 and metastasis: a small actor playing many roles, Am. J. Pathol. 176 (2) (2010) 528–535.
[35] S.C. Garrett, K.M. Varney, D.J. Weber, A.R. Bresnick, S100A4, a mediator of metastasis, J. Biol. Chem. 281 (2) (2006) 677–680.
[36] Z. Yang, X. Zhang, H. Gang, X. Li, Z. Li, T. Wang, J. Han, T. Luo, F. Wen, X. Wu, Up-regulation of gastric cancer cell invasion by twist is accompanied by N-cadherin and fibronectin expression, Biochem. Biophys. Res. Commun. 358 (3) (2007) 925–930.
[37] X. Yang, Y. Zhang, K. Hosaka, P. Andersson, J. Wang, F. Tholander, Z. Cao, H. Morikawa, J. Tegner, Y. Yang, H. Iwamoto, S. Lim, Y. Cao, VEGF-B promotes cancer metastasis through a VEGF-A-independent mechanism and serves as a marker of poor prognosis for cancer patients, Proc. Natl. Acad. Sci. U. S. A. 112 (22) (2015) E2900–E2909.