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Simvastatin induces G_1 arrest by up-regulating GSK3β and down-regulating CDK4/cyclin D1 and CDK2/cyclin E1 in human primary colorectal cancer cells

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Simvastatin (SIM), a widely used cholesterol-lowering drug, also exhibits tumor-suppressive potentials in several types of malignancy. Colorectal cancer (CRC), the third most common malignant neoplasm, accounts for the second most leading cause of cancer-related deaths worldwide. In the present study, we investigated the anticancer effects of SIM on CRC using primary cancer cells lines (CPs: CP1 to CP5) isolated from five Taiwanese colorectal cancer patients as a model for colorectal cancer. We treated all five CPs with SIM for 24–72 hr and observed the respective cell viability by an MTT assay. SIM increased DNA content of the G_1 phase, but did not induce apoptosis/necrosis in CPs as shown by flow cytometry with propidium iodide (PI)/annexin V double staining and PI staining. The expression of G_1 phase-related proteins was analyzed by RT-PCR and Western blotting. SIM suppressed cell growth and induced cell cycle G_1-arrest by suppressing the expression of CDK4/cyclin D1 and CDK2/cyclin E1, but elevating the expression of glycogen synthase kinase 3β in CPs. Our findings indicate that SIM may have antitumor activity in established colorectal cancer.

KEYWORDS
colorectal cancer, cyclin D1, G_1 arrest, glycogen synthase kinase 3β, simvastatin

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant neoplasm, accounting for the second most leading cause of cancer-related deaths worldwide (Patel, 2014). The development of CRC proceeds through multiple stages, and the determination of the colorectal malignant potential relies on polyp histology. Colorectal adenomatous polyps are considered as precursor lesions of malignancy, where most sporadic CRC cases appear to advance through the transition of adenomatous polyps to cancer (Cappell, 2008). Once in the advanced stage, clinical intervention of CRC, including surgery, chemotherapy, radiation therapy, and immunotherapy, often leads to unfavorable consequences.

Hypercholesterolemia is a growing health issue worldwide. Statins, widely used for reducing plasma cholesterol concentration, inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thereby suppressing the conversion of HMG-CoA to mevalonate, the rate-limiting step of cholesterol biosynthesis (Reiner, 2015). In

addition to the role in lipid-lowering, mounting evidence indicates that statins also exhibit potential anti-tumor benefits, including reducing tumor cell growth and inducing apoptosis as well as impairing angiogenetic and metastatic processes (Demierre, Higgins, Gruber, Hawk, & Lippman, 2005; Zhong et al., 2015).

Simvastatin (SIM), one type of statins used in treating hypercholesterolemia, exhibits tumor-suppressive potentials by mediating cell proliferation, angiogenesis, and/or metastasis in several types of malignancy, including lung cancer (Liang et al., 2013), colorectal cancer (Cho et al., 2008), breast cancer (Park, Jung, Ahn, & Im, 2013), leukemia (Chen & Chang, 2014), and hepatocellular carcinoma (Relja et al., 2010). SIM exhibits a potentially protective effect against the development of adenomatous polyps in a significant time- and dose-response manner (Broughton, Sington, & Beales, 2013). The potential prophylactic effect of SIM may prevent susceptible individuals from the subsequent development of colorectal malignancy.

In this report, we sought to investigate the effect of SIM on primary cancer cells (CP1 to CP5) isolated directly from colorectal cancer patients. Our data indicated that SIM suppressed cell growth and induced G_1 arrest by upregulating p27 and glycogen synthase kinase 3β (GSK3β), inhibitors of cyclin-dependent kinases (CDKs) and cyclin D1, respectively, and downregulating CDKs, CDK4 and CDK2, and cyclins, cyclin D1 and cyclin E1. The non-cardiovascular benefits of statins could potentially lead to a translational application to reduce the side effects of the traditional measures and assist the intervention of colorectal cancer.

2 | MATERIALS AND METHODS

2.1 | Materials

All chemicals (analytical grade) and cell culture supplies were obtained from Sigma, Inc. (St. Louis, MO) and Invitrogen (Carlsbad, CA), respectively, unless indicated otherwise. Antibodies were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX) except that β-actin antibodies (AC-15) were from Sigma. Polyvinylidene difluoride (PVDF) membranes were from EMD Millipore Corp. (Billerica, MA), and molecular weight markers were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA).

2.2 | Cell culture

CP1 to CP5, five primary colorectal cancer cell lines, were obtained from MedicoGenomic Research Center, Kaohsiung Medical University as described before (Hsu, Weng, Lin, & Chien, 2007). Cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah) in a humidified atmosphere with 5% CO_2 at 37°C.

2.3 | Cell proliferation assay

Cells were seeded into 96-well culture plates at a density of 5,000 cells per well. After the incubation of 0–50 μM SIM for 24–72 hr, the cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) for 4 hr. The reaction was stopped by the addition of dimethyl sulfoxide, and the optical density was measured at 540 nm by a multi-well plate reader. Results were expressed as percentage of vehicle control, which was considered as 100%.

2.4 | Immunohistochemistry

Tissue specimens were fixed in 10% phosphate-buffered formaldehyde, and the dehydrated tissues were embedded in paraffin wax and then cut at 5 μm thickness. Serial sections were stained for p63 (SC-8431) and visualized using ABC kit and DAB substrate (Santa Cruz Biotechnology, Inc.). The sections were counterstained with hematoxylin. The detection of p63 positive cells was achieved by TissueFAXS Plus and HistoQuest Analysing Software (TissueGnostics USA Ltd., Tarzana, CA).

2.5 | Western blotting

Fifty micrograms of total cell lysates were separated by SDS–polyacrylamide gel electrophoresis and transferred onto PVDF membranes in a tank blotter containing 25 mM Tris (pH 8.3), 192 mM glycine, and 20% methanol. The membranes were blocked with a freshly made 5% non-fat milk solution in 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.05% Tween-20, and then incubated with antibodies against β-actin, p63 (SC-8431), CDK4 (SC-601), Cyclin D1 (SC-753), CDK2 (SC-748), or GSK3β (SC-9166) for 1.5–2 hr. The blots were further incubated with respective IRDye® Secondary Antibodies (LI-COR Biotechnology, Lincoln, NE). The detection was then visualized and analyzed by Odyssey® CLx Imaging System (LI-COR Biotechnology) or Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare Bio-Sciences, Marlborough, MA) followed by autoradiography.

2.6 | Evaluation of apoptosis/necrosis

CRC cells were treated with 0–50 μM SIM for 6 hr; the apoptotic cells were detected by ApopNexin Annexin V FITC Apoptosis Kit (EMD Millipore Corp.) and flow cytometry (BD FACSCalibur™ System, BD Biosciences, San Jose, CA).

2.7 | Cell cycle analysis

Propidium iodide (PI), a fluorescent nucleic acid dye, was used to identify the proportion of cells each interphase stage of the cell cycle. Cells were treated with 0–50 μM SIM or serum starvation for 24 hr, and then harvested and fixed in 1 ml cold 70% ethanol for 8 hr at −20°C. DNA was stained in a PI/RNaseA solution, and the distribution of the cell cycle was detected by flow cytometry (Facscalibur, BD). Data were analyzed by WinMDI 2.9 free software (BD).

2.8 | RT-PCR

A reverse transcriptase system (Promega, Southampon, UK) was used to synthesize cDNA from 1 μg of total RNA, where 2–4 μl of

cDNA were used for PCR analysis. PCR reactions were performed using 100 ng of each primer and 1 unit of Dynazyme II (Flowgen, Lichfield, UK) in a total volume of 50 µl. Thermal cycling was conducted for 35 cycles at the following temperature/durations: 98°C for 10 s, 66°C for 30 s, and 72°C for 1 min using a Progene thermal cycler (Cambridge, UK). A final extension of 72°C was performed for 10 min at the end of the 35 cycles. The primers used for amplification of the target genes were checked against the sequences of other genes for specificity. PCR reactions were

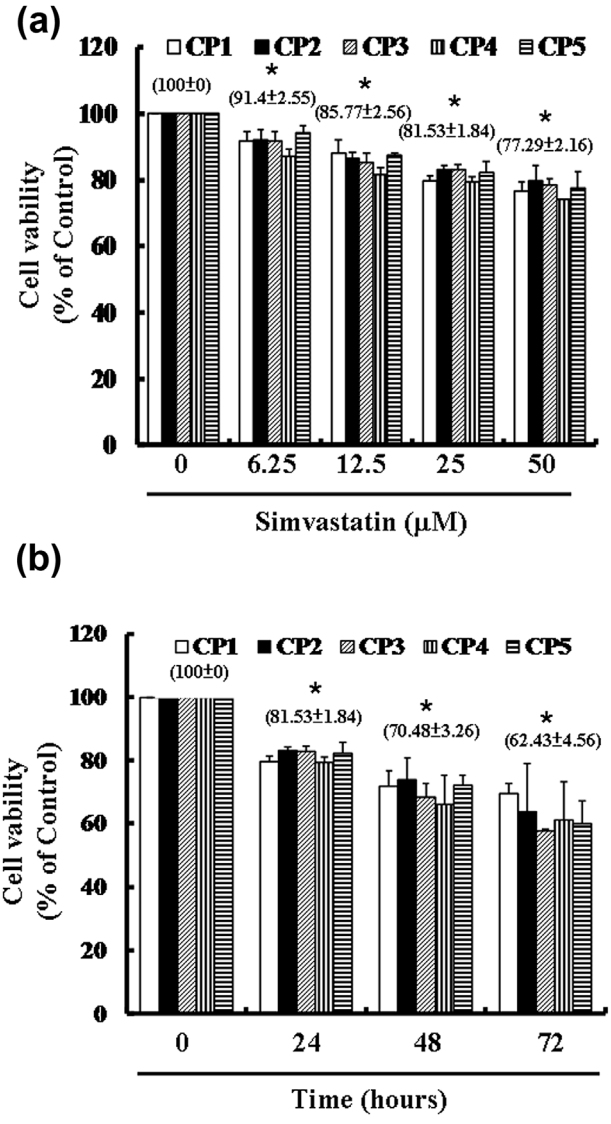

FIGURE 1 Simvastatin (SIM) suppressed cell growth in five human primary colorectal cancer (CRC) cell lines (CP1–CP5, $n = 6$ per group). (a) CP1–CP5 were exposed to various doses of SIM (0–50 µM) for 24 hr, and their cell viability was measured by an MTT assay. (b) The CRC cell lines were each first treated with SIM 25 µM for 24 hr, and then incubated in fresh media for 24–48 hr to study their growth recovery by an MTT assay. All data were reported as means ± SEM from at least three separate experiments and expressed as a percentage of control, which was considered as 100%. *$p < 0.05$ as compared with Control, 0 µM (a) or 0 hr (b)

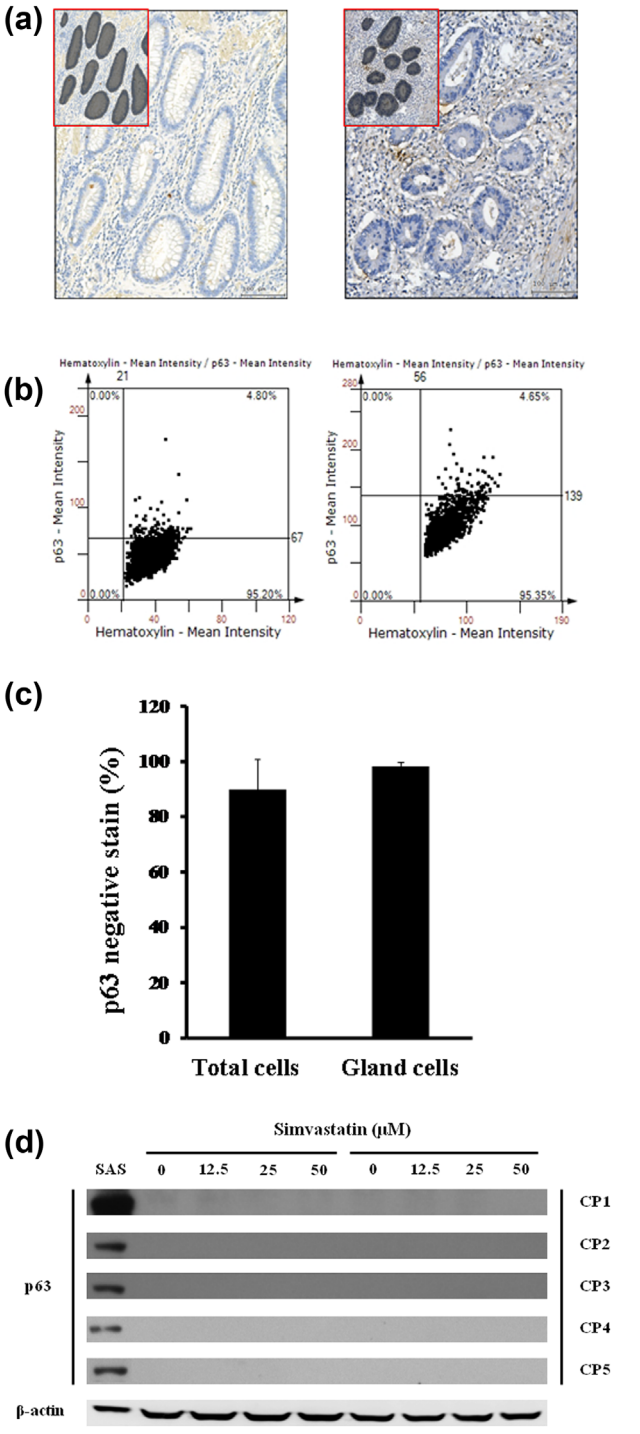

FIGURE 2 Simvastatin (SIM) did not alter the expression of p63 in colorectal cancer tissue. (a) Immunohistochemical staining of a representative CRC tissue (20×). Brown-colored DAB deposits, if present, would be positive indicative of p63. The insert, a miniature of TissueFAXS Plus scan view, indicates the area where the machine scanned, instead of showing brown-colored DAB deposits. (b) Immunohsitochemical data analyzed by HistoQuest software. (c) Results were expressed as a percentage of p63(−) cells, which was considered as 100%. All data were reported as means ± SEM from at least three separate experiments. (d) CP1–CP5 were treated with SIM (0–50 µM) for 24 hr, and then analyzed for the expression of p63 by Western blotting where β-actin was the loading control. SAS cells served as a positive control of p63 expression

analyzed on 1.5% agarose/TAE minigels and stained by 0.5 μg/ml ethidium bromide. Gels were visualized using an Apligene UV CCD camera system.

2.9 | Quantitative real-time polymerase chain reaction (qRT-PCR)

Real-time PCR was conducted using SYBR Green PCR MasterMix according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed using approximately 200 ng of SYBR Green PCR MasterMix and primers in an ABI 7300 system (Applied Biosystems, Foster City, CA). PCR conditions were 95°C for 120 s, 60°C for 30 s, and 72°C for 30 s for 40 cycles. Sample cells from three plates were run in duplicate, using the threshold suggested by the software for the instrument to calculate Ct. To normalize readings, we used Ct values from 18S as the internal control for each run, obtaining a delta Ct value for each gene.

2.10 | Statistical analysis

All data were reported as means ± standard errors of the mean (SEM) from at least three separate experiments. Statistical significance was determined by Student's *t*-test at the level of $p < 0.05$. Where appropriate, a regression line, $y = ax + b$, illustrated the relationship between the independent (x) and dependent (y) variables in the graph where a correlation coefficient, R^2 (R-squared), to express how fit the data were to the line. In the equation "$y = ax + b$," $a > 0$ indicated an increasing tendency and $a < 0$ indicated a decreasing tendency.

3 | RESULTS

3.1 | Effect of SIM on cell viability in five primary CRC cell lines

To explore the anti-tumor effect of SIM on colorectal cancer, we exposed five primary CRC cell lines with various doses of SIM (0–50 μM) for 24 hr, and measured cell viability by an MTT assay. As shown in Figure 1a, cell viability upon SIM treatment for 24 hr decreased in a dose-dependent manner in each primary CRC cell line (CP1–CP5), but not in normal cells, including Hs-68 normal human skin fibroblasts and MRC-5 human fetal lung fibroblasts (data not shown). Moreover, following SIM exposure (12.5 μM) for 6–24 hr, these primary colon cancer cells underwent significant morphological changes; in some cases, primary CRC cells detached from the dish and suspended in the medium (data not shown). To further determine if the growth-inhibitory effect of SIM was reversible, we collected the SIM-treated primary colon cancer cells, re-cultivated them in fresh culture media for additional 24–48 hr, and then assessed their recovery of cell proliferation. The bar graphs in Figure 1b indicated

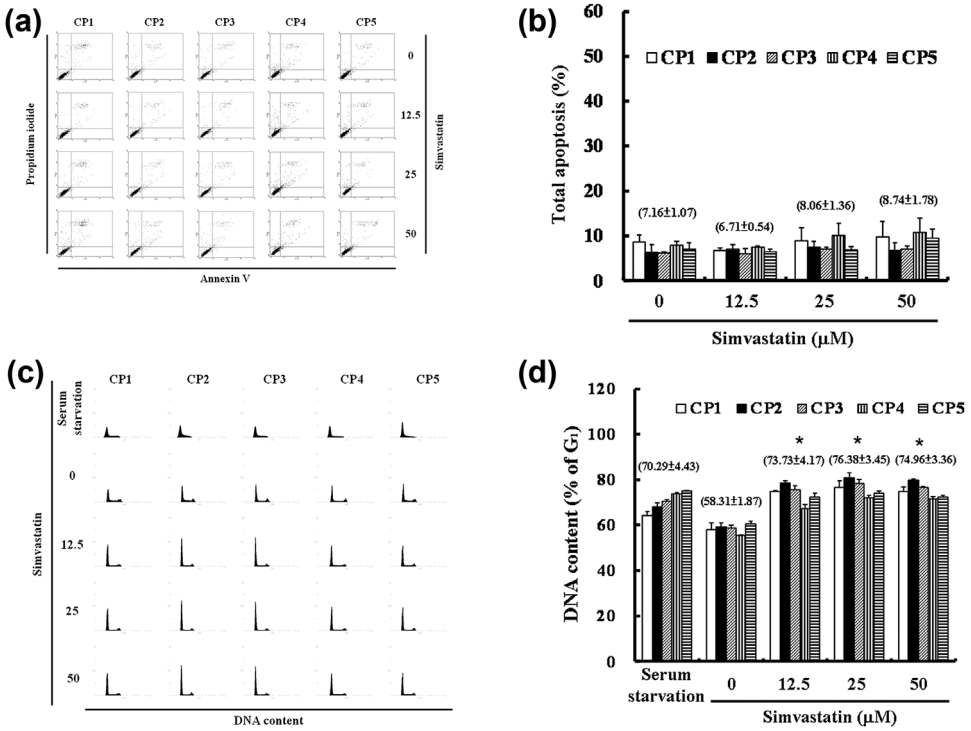

FIGURE 3 Simvastatin (SIM) did not induce apoptosis or necrosis, but caused G_1 cell cycle arrest in primary colorectal cancer (CRC) cell lines. (a,b) CP1–CP5 were incubated with SIM (0–50 μM) for 6 hr and analyzed for the apoptotic/necrotic effect by annexin V/propidium iodide staining and flow cytometry. Results were expressed as a percentage of total number of the cells undergoing early or late apoptosis from at least three independent experiments. (c,d) Primary CRC cell lines were incubated with SIM (0–50 μM) for 24 hr and analyzed for cell cycle progression/distribution by flow cytometry. SIM induced a significant increase in the G_1 phase. $^*p < 0.05$ as compared with 0 μM

that the growth-inhibitory effect of SIM still remained even after a washout period of 24–48 hr. These data suggested that SIM imposed a dominant growth-suppressive effect on the primary colorectal cancer cells.

3.2 | Effect of SIM on p63 protein expression in CPs

To explore the expression of p63 in CRC, immunohistochemistry was performed using an antibody specific to p63. A representative specimen from a CRC patient was analyzed for the expression of p63, where brown-colored DAB deposits, if present, would be positive indicative of p63 by immunohistochemical staining. As shown in Figure 2a, no significant expression of p63 protein was detected in the tissue; the dot-plot of the expression of p63 versus hematoxylin further indicated a non-significant increase in the percentage of p63-expressing cells (Figures 2b and 2c). Next, we explored the expression of p63 in five primary CRC cell lines (CP1–CP5) upon SIM exposure. As shown in Figure 2d, p63 protein expressed in SAS cells (oral squamous cell carcinoma cells), but not in any CP cell line, regardless of SIM treatment. These data indicated that SIM had no effect on the expression of p63.

3.3 | Effect of SIM on apoptosis/necrosis in primary CRC cells

Loss of the mitochondrial membrane potential, a classical hallmark of apoptosis, is an early event that coincides with caspase activation. In order to investigate if SIM induced apoptosis in primary CRC cells, we employed a membrane-permeant JC-1 dye with flow cytometry to determine the mitochondrial membrane potential in various doses of the SIM-treated groups, and we found that SIM-exposed CRC cell lines did not exhibit a significant reduction in the mitochondrial membrane potential; neither did we observe caspase 3 activation in any case (data not shown). Next, we employed Annexin V-FITC and propidium iodide (PI) staining and assessed the percentage of the apoptotic cells by flow cytometric analysis to reconfirm the result. The dot-plot analysis of the fluorescence (Annexin V-FITC versus PI) indicated a non-significant increase in the apoptotic cells treated with SIM in five CPs when compared with untreated cells (Figure 3a). There was no indicative of necrosis or apoptosis (Figure 3b) ($y = 0.0376x + 6.8453$, $R^2 = 0.7823$) in the five CRC cell lines examined. Therefore, SIM did not appear to induce apoptosis in CPs.

3.4 | SIM-induced accumulation of G_1 phase in primary CRC cells

The data of Figures 1, 3a, and 3b indicated that SIM inhibited the proliferation of these primary CRC cells by means other than apoptosis. Therefore, we analyzed the cell-cycle distribution by flow cytometry in primary CRC cells after their 24 hr exposure of SIM. As shown in Figure 3c, exposure to SIM increased the number of the G_1 phase cells while simultaneously reduced the number of cells in the G_2/M phase ($y = 0.2781x + 64.762$, $R^2 = 0.4961$; Figure 3d), indicating that the CRC cells underwent cell cycle arrest (*$p < 0.05$ vs. SIM 0 μM).

3.5 | Genes involved in G_1 arrest following SIM exposure in primary CRC cell lines

The observation of SIM-induced cell cycle arrest prompted us to further identify the relevant genes involved in G_1 accumulation. As shown in Figures 4a (RT-PCR) and 4b (qPCR), SIM treatment reduced the expression of *CCND1* (cyclin D1), *CDK4*, *CCNE1* (cyclin E1), and *CDK2* genes, but elevated the levels of *GSK3B* (GSK3β) and *CDKN1B* ($p27^{KIP1}$) genes in primary CRC cell lines. Specifically, SIM-treated CRC cell lines exhibited substantial downregulation of cyclin D1 ($y = -22.404x + 109.7$, $R^2 = 0.8232$), CDK4 ($y = -16.236x + 109.36$, $R^2 = 0.8932$), cyclin E1 ($y = -9.875x + 110.78$, $R^2 = 0.9922$),

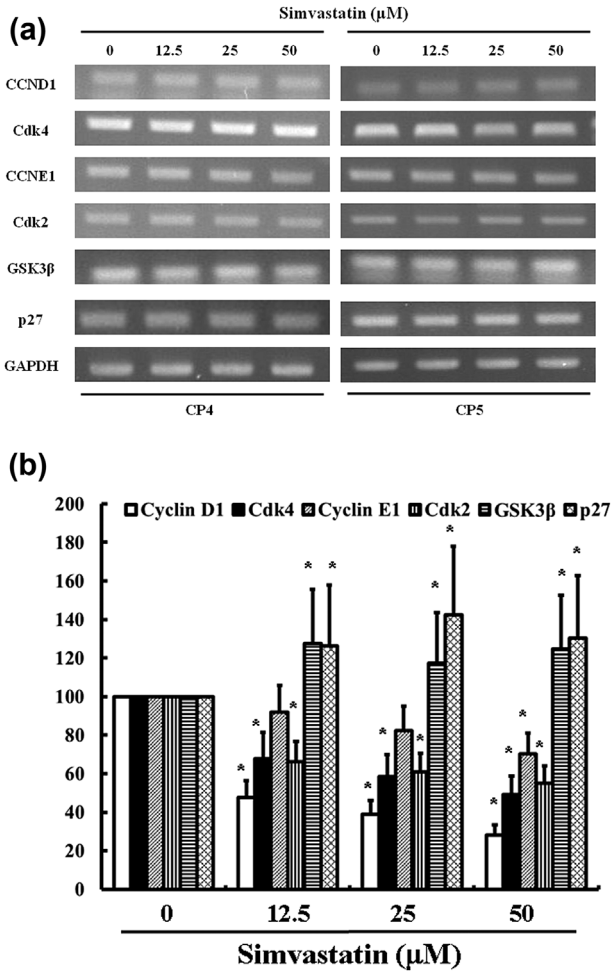

FIGURE 4 SIM suppressed the mRNA expression of *CCND1* (cyclin D1), *CDK4*, *CCNE1* (cyclin E1), and *CDK2*, but increased the mRNA expression of *GSK3B* (GSK3β) and *CDKN1B* ($p27^{KIP1}$) in primary CRC cell lines. Cells were treated with SIM (0–50 μM) for 24 hr, and the mRNA expression levels of the G_1 relevant genes were subsequently detected by RT-PCR (a) and quantitative RT-PCR (b). Data were reported as means ± SEM from at least three separate experiments. *$p < 0.05$ as compared with 0 μM

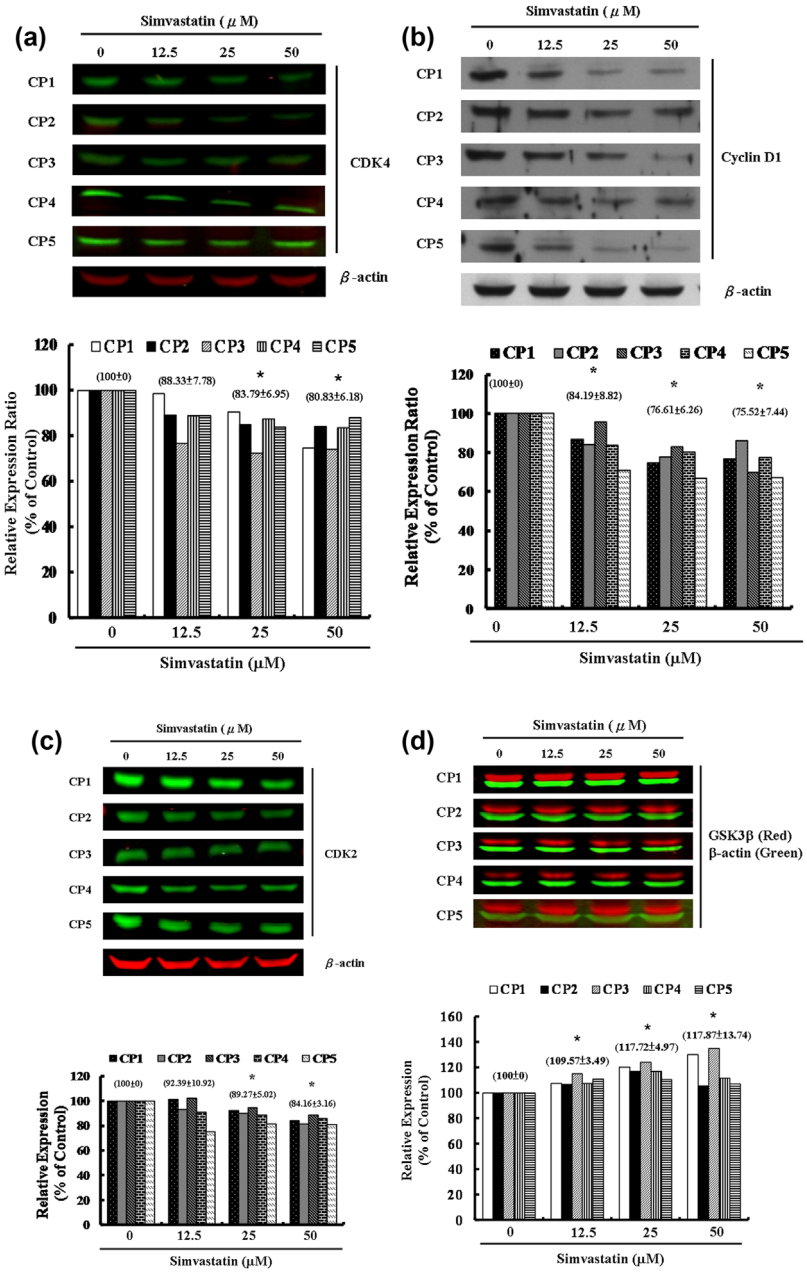

FIGURE 5 SIM downregulated CDK4, cyclin D1, and CDK2, but upregulated GSK3β in primary colorectal cancer cell lines. CP1–CP5 were treated with SIM (0–50 μM) for 24 hr, and the expression levels of the relevant cell cycle proteins were subsequently detected by Western blotting. Representative images (upper panels) and quantification (lower panels) of the Western blot data with antibodies against CDK4 (a), cyclin D1 (b), CDK2 (c), and GSK3β (d). All data were reported as mean ± SEM from at least three separate experiments. $^*p < 0.05$ as compared with 0 μM

and CDK2 ($y = -13.986x + 105.55$, $R^2 = 0.8046$), but notable upregulation of GSK3β ($y = 6.4371x + 101.3$, $R^2 = 0.4532$) and p27 ($y = 10.703x + 97.993$, $R^2 = 0.5998$) (Figure 4b). We next determined the protein expression of these genes by immunoblotting and then quantified by measuring relative intensities. Following incubation with SIM, the protein levels of CDK4 ($y = -0.3513x + 95.924$, $R^2 = 0.7924$), cyclin D1 ($y = -0.4485x + 93.893$, $R^2 = 0.7192$), and CDK2 ($y = -0.3416x + 97.184$, $R^2 = 0.889$) were significantly lower (Figures 5a, 5b, and 5c), whereas GSK3β exhibited a notable upregulation ($y = 0.3425x + 103.8$, $R^2 = 0.7457$) (Figure 5d). These findings indicate that the SIM-induced accumulation of the G_1 phase in primary CRC cells mainly resulting from the downregulation of CDK4, cyclin D1, CDK2 and the upregulation of the GSK3β.

4 | DISCUSSION

When studying the role of SIM in cell growth in primary CRC cells, we identified the cytostatic effect of SIM such that SIM suppressed cell growth of CPs by upregulating cyclin-dependent kinase (CDK)

inhibitors, p27 and GSK3β, as well as downregulating cyclin-dependent kinases (CDKs) and cyclins to induce G_1 arrest. Statins, such as SIM, are used for the intervention of cardiovascular disease to prevent further damage from hypercholesterolemia. In addition to this traditional role, the non-cardiovascular benefits of SIM could potentially apply to colorectal cancer treatment.

The progression of the cell cycles in mammalian cells is governed by the periodic activation and inactivation of CDKs and their inhibitors such as $p21^{Waf1}$ and $p27^{Kip1}$. CDK4 and CDK6 are responsible for the progression of the early to mid-G_1 phase, and CDK2 is mainly for the progression from the late G_1 to the S phase. G_1 cyclins, cyclin D and cyclin E, bind to CDK4/6 and CDK2, respectively, and ultimately initiate the G_1–S transition. Cyclin D1/CDK4 and cyclin D1/CDK6 sequester $p21^{Waf1}$ and $p27^{Kip1}$ from cyclin E/CDK2, activate cyclin E/CDK2, and induce the expression of genes responsible for the S phase entry (Sherr, 1995).

Cyclin D1 plays a pivotal role during the G1-S progression in cell cycle; therefore, the expression of proper cyclin D1 levels is critical in maintaining normal cell cycle progression. Overexpression of cyclin D1 have been found in several types of cancer, where cyclin D1 is involved in tumorigenesis. Glycogen synthase kinase 3β (GSK3β) is one important regulatory protein to control the expression of cyclin D1. GSK3β, a serine/threonine protein kinase, can phosphorylate cyclin D1 and thus export cyclin D1 from the nucleus (Takahashi-Yanaga & Sasaguri, 2008). Quintayo et al. reported an inverse relationship between GSK3β and cyclin D1 expression in early breast cancer; the authors suggested that GSK3β negatively regulate the gene expression of cyclin D1, repress the activation of cyclin D1 and CDK4/6, and suppress the entry into the G_1/S phase (Quintayo et al., 2012). This rationale appears to fit into our current work such that SIM-induced G_1 cell cycle arrest was accompanied by a marked reduction in cyclin D1 and CDK4 and an up-regulation of GSK3β.

Other statins have been investigated for their chemopreventive effect in cancer cells. Rao et al. reported that, independent of the status of p53, lovastatin, another statin, mediates G1 arrest by increasing the binding of both p21 and p27 to CDK2, thereby decreasing the activity of cyclin/CDK2 in breast cancer cells (Rao, Lowe, Herliczek, & Keyomarsi, 1998). In the primary CRC cells examined in the current work, we found that SIM induced an accumulation of the G_1 phase with a down-regulation of CDK2 and an upregulation of p27 (Figures 3–5). Although we did not investigate the binding activity between p21 or p27 with CDK2, the data of increased p27 and decreased CDK2 were likely also responsible for the G_1 arrest, again, supporting an anti-carcinogenic and protective role of SIM in CPs.

TP63, one of the p53 family members, encodes tumor protein p63. p63 and p53 share common functions, and both can induce cell cycle arrest and apoptosis (Napoli & Flores, 2017). However, in contrast to the tumor suppressor function of p53, the expression of p63 may result from at least two different promoters with opposite roles, such as the transcriptionally active TAp63 or the truncated dominant-negative ΔNp63 (Napoli & Flores, 2017). In addition, p63 may exhibit its standalone effect on cancer cells, regardless the expression of p53. Endogenous TAp63α may be induced by some chemotherapeutic agents, and suppression of TAp63α may lead to chemoresistance (Gressner et al., 2005). Employing a TAp63-conditional mouse model, Guo et al. found that TAp63 induces senescence and suppresses tumorigenesis in vivo, irrespective of p53 status (Guo et al., 2009). In the current study, the expression of p63 was negligible in the CRC tissue examined; neither did we detect p63 in primary CRC cell lines upon SIM exposure. Therefore, SIM-induced growth inhibition was unlikely through p63-mediated senescence or apoptosis in primary CRC cell lines.

While others found the SIM-induced suppressive effect of cell growth mainly by apoptosis, our findings suggested that cell cycle G_1 arrests occurred primarily at three SIM doses in the five CRC cell lines (Figure 3). Also as reported previously, our group also found that SIM induced cell cycle arrest, but not apoptosis, in NCI-H460 non-small cell lung cancer cells (Liang et al., 2013). Hoques et al. found that simvastatin reduced cell viability by inducing apoptosis and G_1 phase arrest in PC3, DU145, and LnCap prostate cancer cell lines (Hoque, Chen, & Xu, 2008). Cho et al. reported that SIM induced apoptosis, but not cell cycle arrest, in COLO205 and HCT116 human colon cancer cells (Cho et al., 2008). Therefore, the inhibition of proliferation through the induction of apoptosis or cell cycle arrest is likely dependent upon the types of cancer and the doses of SIM. Agarwal et al. found that lovastatin induced apoptosis in four colon cancer cell lines, SW480, HCT116, HT29, and LoVo, but each with various sensitivity, and they suggested that the effect of lovastatin also depends on the specific oncogenic mutation of the CRC cells (Agarwal et al., 1999). Although we did not examine the mutation status of the primary CRC cell lines employed in our study, the fact that CRC cells tend to carry a variety of oncogenic mutations may explain the disparity of our findings from others'.

5 | CONCLUSION

In conclusion, we have demonstrated for the first time that SIM inhibits tumor growth by arresting the cell cycle in the G_1 phase via repressing the gene expression of CDKs/cyclins in primary colorectal cancer cells. Because the progression of CRC is often related to genetic mutations and environmental risk factors (Peters, Bien, & Zubair, 2015), chemoprevention is of great significance in the individuals prone to hereditary and/or environmental predisposition to the development of colorectal cancer (Bardou, Barkun, & Martel, 2010). Our findings suggest a potential translational application of SIM as an adjuvant of chemotherapy. The combination use of statins with other drugs may be applicable to both the prevention and treatment of colorectal cancer.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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