MicroRNA-192 acts as a tumor suppressor in colon cancer and simvastatin activates miR-192 to inhibit cancer cell growth

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Abstract. Colon cancer is one of the most common malignant tumors worldwide. Understanding the underlying molecular mechanisms is crucial for the development of therapeutic strategies for the treatment of patients with colon cancer. In the present study, a novel tumor suppressive microRNA, miR-192, was demonstrated to be markedly downregulated in colon cancer cells compared with normal colon cells. By overexpressing miR-192 in colon cancer HCT-116 cells, the results of the present study revealed that miR-192 inhibits cell proliferation, migration and invasion. Bioinformatics were used to determine the target gene of miR-192 and Ras-related protein Rab-2A (RAB2A) was identified as a downstream target of miR-192. Following the determination of the role of the miR-192-RAB2A pathway in colon cancer, small molecules that may regulate miR-192 were screened and the results demonstrated that simvastatin is an activator of miR-192. Furthermore, simvastatin upregulated miR-192 and inhibited the expression of downstream targets of miR-192, which subsequently led to suppressed proliferation, migration and invasion of colon cancer cells. In conclusion, the present study identified a novel colon cancer cell suppressor, as well as a small-molecule activator of the tumor suppressor miR-192, which may represent a therapeutic strategy for the treatment of patients with colon cancer.

Introduction

Colon cancer is one of the leading causes of cancer-associated mortality worldwide and its incidence has increased in recent years. In China, colon cancer is the fifth most common type of cancer and has the fifth highest mortality rate (1). It has been estimated that >370,000 cases of colon cancer and 190,000 colon cancer-associated fatalities occur annually in China. Despite advances in conventional cancer therapies, including surgery, radiotherapy and chemotherapy, the 5-year relative survival rate of colon cancer remains poor, with lymphatic and distant metastasis being the main causes of colon cancer-associated mortality (2). Therefore, there is an urgent need to determine the molecular basis of colon cancer and to discover novel therapeutic targets for its treatment.

MicroRNAs (miRNAs/miRs) are endogenous non-coding RNAs with a length of ~22 nucleotides that can regulate the expression of target genes at the post-transcriptional level (3). It has been well established that miRNAs are involved in numerous physiological processes by regulating the expression of different target genes. In addition, abnormal expression and function of miRNAs are associated with a number of human diseases, including cancer (4). miR-192 has been reported to be abnormally expressed in numerous malignant tumors, including lung, gastric and bladder cancer (5-7). However, the role of miR-192 in colon cancer and the underlying molecular mechanisms remain unclear.

Elucidating the roles of specific miRNAs in cancer may enable the development of novel treatments via the regulation of these miRNAs. Small molecules may regulate endogenous miRNAs, thereby affecting therapeutic outcomes (8,9). For example, enoxacin has been demonstrated to be a universal miRNA activator and to inhibit cancer growth (10,11). Polylysine and trypaflavine have been found to be universal inhibitors of miRNAs that may reverse the occurrence of tumors via modulation of endogenous miRNA expression (12). Therefore, the aim of the present study was to investigate the association between miR-192 and colon cancer, and identify a potential small molecule that regulates miR-192 expression and subsequently inhibits cancer growth.

Materials and methods

Cells and reagents. The HCT-116, HT-29, SW480 and RKO human colon cancer cell lines, as well as the FHC normal colon epithelial cell line and the 293T cell line, were acquired from the Shanghai Institute for Biological Sciences (Shanghai, China). The cells were cultured in McCoy's 5A (modified)

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medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and incubated at 37˚C in 5% CO_2. Cells in exponential growth phase (~80% confluence) were used for subsequent experiments. Statins (pravastatin, simvastatin, fluvastatin, compactin, lovastatin, rosuvastatin, atorvastatin and pitavastatin) were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany) and dissolved in dimethyl sulfoxide (DMSO) to establish 10 mM stock solutions.

Bioinformatics analysis. TargetScan was used to predict target mRNAs for miR-192 (13).

Cell proliferation assay. Post-digestion, HCT-116 cells in the logarithmic growth phase were counted and plated in 96-well plates ($5x10^3$ cells/well per 100 μl medium). Following incubation overnight, HCT-116 cells were treated with various statins concentrations (0, 1, 2, 4, 8, 16, 32, 64, 128 and 256 μM; 5 wells per dosage group) for 48 h. A total of 100 μl MTT solution (1 mg/ml; Sigma-Aldrich; Merck KGaA) was then added and the resultant solution was incubated for a further 4 h at 37˚C. Following removal of the culture medium, 100 μl DMSO (Sigma-Aldrich; Merck KGaA) was added to each well. The absorbance values were then measured at a wavelength of 560 nm using a Multiskan Spectrum (Molecular Devices, LLC, Sunnyvale, CA, USA). Half-maximal inhibitory concentration (IC_{50}) values were determined using Graphpad Prism 5.01 software (GraphPad Software, Inc., La Jolla, CA. USA).

To determine the effect of miR-192 on cell activity, HCT-116 cells (100 μl) were seeded into 96-well plates at a density of $5x10^4$/ml per well. Following incubation overnight, the cells were transfected with either miR-192 mimics (sense strand, 5'-cugaccuaugaauugacagcc-3'; passenger strand, 5'-cugccaauuccauaggucacag-3') or miR-negative control mimics (sense strand, 5'-uucuccgaacgugucacguuu-3'; passenger strand, 5'-aaacgugacacguucggagaa-3') (Synthgene Biotech, Nanjing, China) using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. 10 μl MTT (5 mg/ml) was added to the medium for 0, 12, 24 and 48 h post-transfection, in accordance with the manufacturer's protocol. After 4 h of incubation, medium was removed and 100 μl DMSO was added to each well for 30 min at 37˚C. The absorption of each well at 490 nm was collected on using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) to assess cell viability.

Wound-healing assay. Wound-healing assays were performed to evaluate cancer cell migration as previously described by Liu *et al* (14). Briefly, HCT-116 cells were plated in a 12-well plate at $2x10^4$ cells/well. When the cells reached confluence, a horizontal scratch was created using a 10-μl pipette tip. At 0 and 48 h post-wound infliction, the cell migration status was determined using a light microscope (scale bar, 500 μM).

Transwell assay. Cell migration and invasion abilities were investigated using specialized Transwell chambers (8-μm pore size; BD Biosciences, Franklin Lakes, NJ, USA). For migration assays, HCT-116 cells ($2x10^5$/ml) and 4 μM simvastatin suspension were added to the upper chamber and 450 μl culture medium supplemented with 15% FBS was added to the lower chamber. Following incubation for 48 h at 37˚C in 5% CO_2, any non-migrating or non-invading cells on the upper surface were removed. The cells in the lower chamber were then fixed with methanol and stained with hematoxylin for 30 min at 37˚C. The number of invading cells was counted under a light microscope (magnification, x200; three visual fields/well).

In order to perform invasion assays, Matrigel (BD Biosciences) kept in a -20˚C refrigerator was defrosted on ice and the pipette tips, Eppendorf tubes and medium were precooled at 4˚C. Subsequently, Matrigel and medium were mixed at a ratio of 1:8. A total of 40 μl mixed medium was then added to the upper chamber and the chamber was incubated at 37˚C for 4 h. The cell suspension (150 ml; $3x10^5$ cells/ml) and the drug suspension were added to the upper chamber, and 600 μl complete medium containing 15% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added to the lower chamber. A total of three replicates were performed per group. Following 48 h of incubation, Matrigel and non-invading cells were removed using cotton swabs, fixed with methanol at 37˚C for 30 min and then stained with crystal violet solution for 30 min at room temperature. The number of invading cells was counted under a light microscope.

RNA preparation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. The analysis was conducted with the $2^{-\Delta\Delta Cq}$ quantification method as described by Livak and Schmittgen (15). Total RNA was isolated from HCT-116 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to a previously published protocol by Beekman *et al* (16). To quantify miRNAs, TaqMan probes (Thermo Fisher Scientific, Inc.) were used in accordance with the manufacturer's protocol. Briefly, 1 μg total RNA was reverse-transcribed to cDNA using AMV reverse transcriptase (Takara Biotechnology Co., Ltd., Dalian, China) and an RT primer. The reaction conditions were 16˚C for 30 min, 42˚C for 30 min and 85˚C for 5 min. qPCR was performed using TaqMan QPCR Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The reactions were performed in a 96-well plate at 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 1 min. U6 was used as an internal control. The primers for U6 were 5'-CGCTTCGGCAGCACA TATACTA-3' (forward) and 5'-CGCTTCACGAATTTGCGT GTCA-3' (reverse).

The expression levels of Ras-related protein Rab-2A (RAB2A), epithelial (E)-cadherin, β-catenin and twist mRNA were detected using a SYBR QPCR kit (Synthgene Biotech) according to the manufacturer's protocol. The reactions were performed in a 96-well plate at 95˚C for 5 min, followed by 40 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec. GAPDH was used as an internal control. The primers used in this experiment were as follows: RAB2A 5'-GGCGAC ACAGGTGTAGAGTT-3' (forward) and 5'-TGATTGCCTG CATGTGTTGC-3' (reverse); E-cadherin 5'-TGCCCAGAA AATGAAAAAGG-3' (forward) and 5'-GTGTATGTGGCA ATGCGTTC-3' (reverse); β-catenin 5'-GAAACGGCTTTC AGTTGAGC-3' (forward) and 5'-CTGGCCATATCCACC AGAGT-3' (reverse); twist 5'-GGAGTCCGCAGTCTTACG AG-3' (forward) and 5'-TCTGGAGGACCTGGTAGAGG-3'

(reverse); and GAPDH 5'-TGTTGCCATCAATGACCCCTT-3' (forward) and 5'-CTCCACGACGTACTCAGCG-3' (reverse).

Luciferase reporter assay. The entire 3'-untranslated region (UTR) of RAB2A was inserted into a luciferase reporter plasmid named pMIR-REPORT™ Luciferase (Synthgene Biotech). To investigate the binding specificity, sequences that interacted with miR-192 were mutated and mutant RAB2A 3'-UTRs were then inserted into an equivalent luciferase reporter plasmid. In order to perform the luciferase reporter assay, 293T cells ($2x10^4$ cells/ml) were plated in 24-well plates and 0.3 μg luciferase reporter plasmid and 0.2 μg β-galactosidase plasmid (internal control) were added to each well. After 4 h, 15 pmol of miR-192 mimics and negative control (NC) mimics were transfected into 293T cells using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following 48 h of incubation, firefly and *Renilla* luciferase activities were measured using a Dual-Luciferase® Reporter Assay System in accordance with the manufacturer's protocol (Promega Corporation, Madison, WI, USA).

Western blotting. HCT-116 cells were treated with either 4, 8 or 16 μmol/l, respectively. The HCT-116 cells were washed twice with ice-cold PBS and centrifuged at 12,000 x g for 10 min at 4˚C, lysed using radioimmunoprecipitation assay buffer (Beijing Solarbio Science & Technology, Co., Ltd., Beijing, China) and incubated on ice for 20 min. The protein concentration of the supernatant was determined with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). Extracted proteins of 20 μg/lane were diluted in 1X SDS loading buffer, pre-denatured and then resolved via 10% SDS-PAGE. Subsequently, proteins were transferred to a polyvinylidene difluoride membrane, blocked using 5% non-fat milk with Tris-buffered saline containing Tween-20 [TBST; 20 mM Tris-HCl, 150 mM NaCl and 0.1% (v/v) Tween-20; pH 7.4] at room temperature for 1 h , and then incubated with the following primary antibodies at 4˚C overnight: Anti-E-cadherin (1:1,000; cat. no. ab1416), anti-β-catenin (1:1,000; cat. no. ab16051), anti-twist (1:1,000; cat. no. ab50581), anti-RAB2A (1:1,000; cat. no. ab154729), anti-phosphatidylinositol 3-kinase (PI3K; 1:1,000; cat. no. ab32089), anti-extracellular signal-regulated kinase (ERK; 1:1,000; cat. no. ab166847) and anti-GAPDH (1:2,000; cat. no. ab8245; all Abcam, Cambridge, MA, USA). After washing, the membrane was further incubated with HRP-conjugated secondary antibodies (1:1,000; cat. no. A0208; Beyotime Institute of Biotechnology) for 1 h at room temperature and proteins were then visualized using electrochemiluminescence reagents (Bio-Rad Laboratories, Inc.) by ImageJ Software version 1.6 (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. All experiments were performed in triplicate and the data are expressed as the mean ± standard error of the mean. Unless stated otherwise, statistical analysis was performed using GraphPad Prism 5.01 software (GraphPad Software, Inc.). The statistical significance of the differences between groups was assessed using one-way analysis of variance followed by Tukey's post-hoc test for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

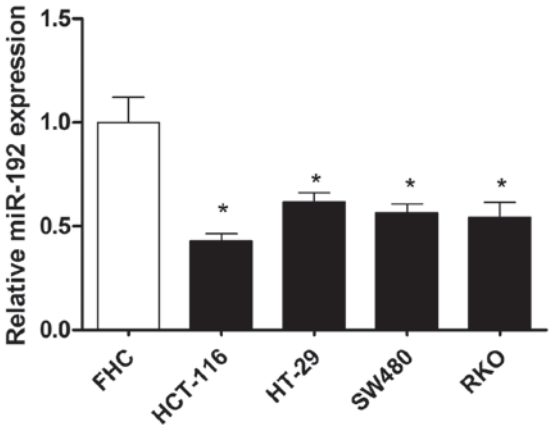

Figure 1. miR-192 is downregulated in colon cancer cells. *P<0.05 vs. FHC. miR, microRNA.

Results

miR-192 is downregulated in colon cancer cells. RT-qPCR was performed to investigate the expression of miR-192 in colon cancer cells. The results revealed that the expression of miR-192 in colon cancer cells was significantly decreased compared with normal colon cells and this effect was most prominent in HCT-116 cells ($P<0.05$; Fig. 1). Therefore, it may be suggested that downregulation of miR-192 is associated with the occurrence and progression of colon cancer.

miR-192 inhibits the proliferation, migration and invasion of HCT-116 cells. To determine the association between miR-192 expression and the occurrence and metastasis of colon cancer, the effects induced by transfection with miR-192 mimics and miR-192 inhibitors on the proliferation, migration and invasion of HCT-116 cells were observed. As presented in Fig. 2A, the relative expression of miR-192 significantly increased following transfection with the miR-192 mimic ($P<0.05$), while the relative expression of miR-192 significantly decreased following transfection with the miR-192 inhibitor ($P<0.05$). As determined by MTT assays, upregulation of miR-192 expression significantly inhibited the proliferation of HCT-116 cells, while downregulation of miR-192 expression significantly enhanced the proliferation of HCT-116 cells ($P<0.05$; Fig. 2B). Subsequently, the migration and invasion of HCT-116 cells were investigated, and the results revealed that expression of miR-192 mimics significantly decreased cell migration and invasion ($P<0.05$), whereas the miR-192 inhibitors exerted the opposite effect compared with the control (Fig. 2C and D).

RAB2A is a direct target of miR-192 in HCT-116 cells. To identify genes regulated by miR-192, TargetScan (13) was used to predict potential targets. RAB2A, a member of the RAS family and a previously determined oncogene, was demonstrated to be a potential target gene of miR-192. It has been reported that an abnormal increase in RAB2A expression is associated with carcinogenesis and the expression of RAB2A in cancer cells is enhanced compared with that in normal cells (17). Following prediction by TargetScan, the results demonstrated that miR-192-5p may bind with the 3'-UTR of RAB2A mRNA and that the predicted binding site was close to the 218-225 bp region.

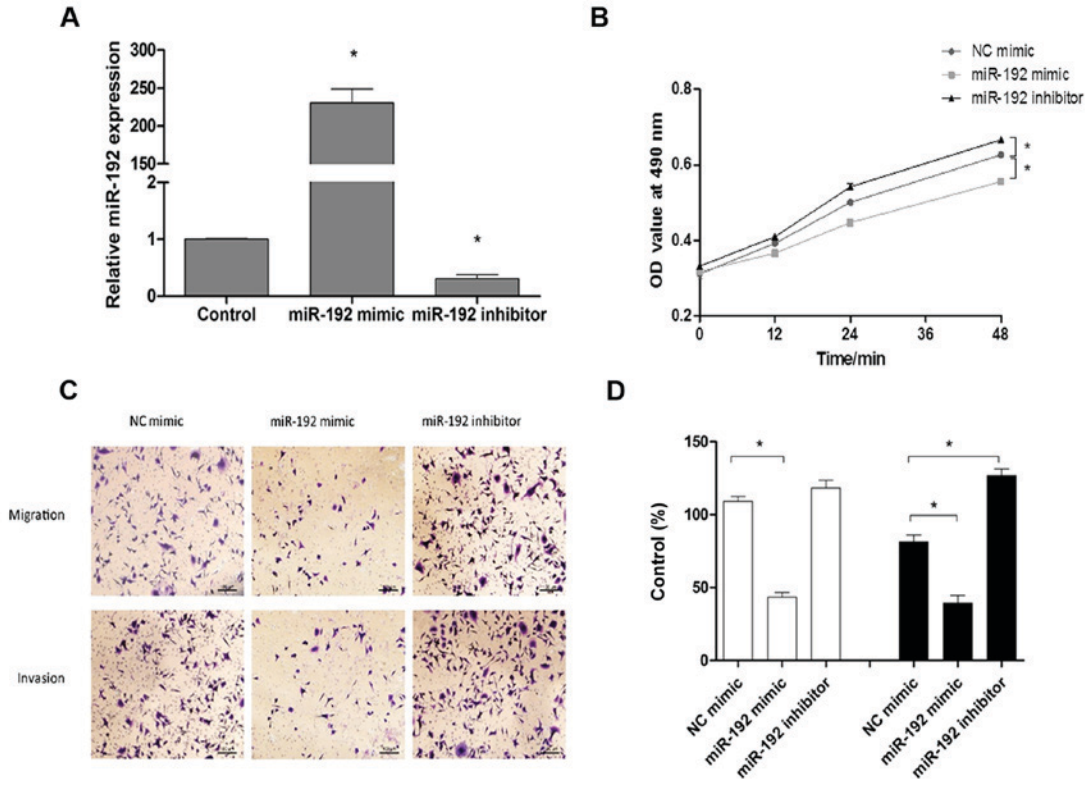

Figure 2. miR-192 inhibits the proliferation, migration and invasion of HCT-116 cells. (A) The relative miR-192 expression transfection with miR mimics/inhibitors. (B) MTT analysis of cell proliferation. P<0.05 vs. the control. Effects of miR-192 on the migration and invasion abilities of HCT-116 cells are presented as a (C) representative image (scale bar, 100 μM) and (D) quantitative analysis. White, migration; black, invasion. *P<0.05 vs. the NC mimic. miR, microRNA; NC, negative control; OD, optical density.

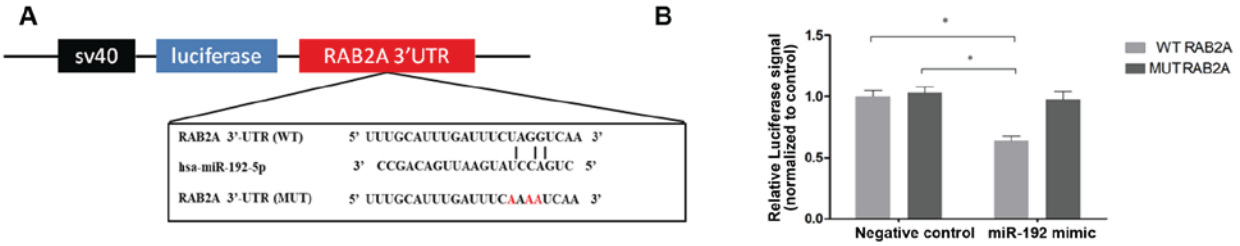

Figure 3. Identification of RAB2A as a direct target of miR-192 in HCT-116 cells. (A) Binding sites of miR-192-5p in the 3'-UTR of RAB2A were predicted using TargetScan software. (B) At 24 h post-transfection of cells with miR-NC or miR-192 mimics containing WT or MUT 3'-UTR RAB2A, dual-luciferase reporter assays were performed. *P<0.05. UTR, untranslated region; miR, microRNA, WT, wild-type; MUT, mutant; RAB2A, Ras-related protein Rab-2A.

To determine whether the regulatory effect of miR-192 on RAB2A expression is due to the binding of miR-192-5p to the predicted sites in the 3'-UTR of RAB2A (Fig. 3A), full-length RAB2A 3'-UTRs were inserted into the firefly luciferase gene. The resultant plasmid was then co-transfected with miR-192 mimics into 293T cells and luciferase signals were then detected to determine whether miR-192-5p can bind to the 3'-UTR of RAB2A. As presented in Fig. 3B, luciferase signals were significantly decreased in 293T cells transfected with miR-192 mimics, but not in those transfected with NC mimics (P<0.05). Furthermore, to confirm that miR-192-5p can bind to the predicted sites, the predicted miR-192-5p binding sites were mutated in the 3'-UTR of RAB2A (Fig. 3A) and subsequently inserted into the luciferase gene. The results demonstrated that luciferase signals were not markedly decreased in these cells; therefore, RAB2A appears to be a target gene of miR-192.

Screening of small molecules that activate miR-192 in HCT-116 cells. Statins are associated with the prevention and treatment of colon cancer. It has been previously demonstrated that statins may serve a protective role against the development of adenomatous polyps and are used during the early treatment stages of colonic polypoid tumors (18). It has also been reported that the regulation of miR-92a expression may represent a novel clinical target of statins when used to treat endothelial cell dysfunction in patients with coronary heart disease (19). In the present study, eight variants of small molecules were screened using the RAB2A 3'-UTR luciferase system. Following treatment with statins for 48 h, the relative luciferase signals exhibited by cells were determined (Fig. 4). The results revealed that the relative luciferase signals in cells treated with simvastatin were significantly decreased compared with the other small molecules investigated

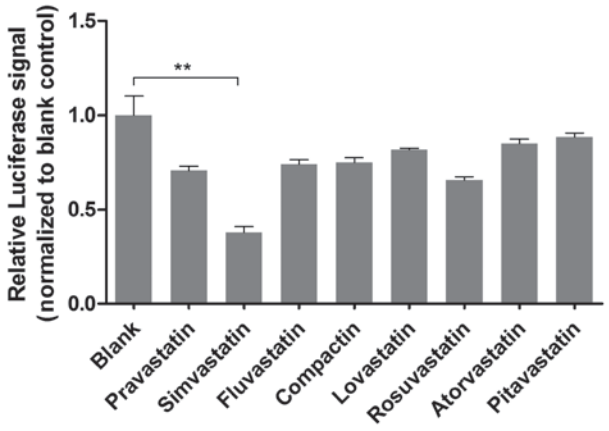

Figure 4. Screening of small molecules that activate miR-192 in HCT-116 cells. **P<0.01. miR, microRNA.

Table I. HCT-116 colon cancer cells were treated with different statins.

Compound	IC_{50} (μM)
Pravastatin	81
Simvastatin	16
Fluvastatin	93
Campactin	82
Lovavastatin	109
Rosuvastatin	80
Atorvastatin	100
Pitavastatin	120

IC_{50}, half-maximal inhibitory concentration.

(P<0.01), indicating that simvastatin markedly upregulated miR-192 expression.

Simvastatin affects the expression of miR-192 and associated downstream pathway proteins in HCT-116 colon cancer cells. Epithelial-to-mesenchymal transition (EMT) is required for the migration and invasion of epithelium-derived malignant tumor cells and is considered to represent an important regulatory mechanism of tumor growth, invasion and metastasis. During the process of EMT, the expression of the epithelial marker E-cadherin decreased and then the contact between the tumor cells and the surrounding cells decreased. The decrease or loss of intercellular adhesion leads to the increase of movement and invasion ability of tumor cells, therefore infiltrating and transferring to the surrounding tissues. As a marker of interstitial cells, the elevated expression level of β-catenin indicates the transformation of epithelial cells into mesenchymal cells. In contrast, the reduced level of β-catenin expression is a sign that the EMT process is suppressed (20-22). In the present study, the effect of simvastatin on the mRNA levels of downstream pathway proteins was investigated. Cells were treated with 4, 8 and 16 μM of simvastatin according to the IC_{50} value previously determined by MTT assays. The results of RT-qPCR revealed that following treatment with simvastatin the level of E-cadherin mRNA significantly increased and the levels of β-catenin and twist mRNA decreased in a dose-dependent manner, indicating that the EMT process was inhibited (P<0.05; Fig. 5A-C). Subsequently, the protein expression levels of E-cadherin, β-catenin, twist, RAB2A, PI3K and ERK were determined via western blot analysis. As presented in Fig. 5D and E, the protein expression levels of β-catenin, twist, RAB2A, PI3K and ERK were significantly decreased (P<0.05), and the protein expression of E-cadherin was markedly increased in a dose-dependent manner following treatment with simvastatin. These results suggested that simvastatin inhibited the EMT process by regulating the expression of EMT-associated proteins in the downstream pathway of miR-192.

Effect of statins on the proliferation of HCT-116 cells. Various concentrations of statins were established (0, 1, 2, 4, 8, 16, 32, 64, 128 and 256 μM) and added to HCT-116 cells for 48 h. Subsequently, the effect of statins on cell activity was investigated using MTT assays and the results revealed that cell activity was markedly decreased following treatment with statins (Table I). Using the growth inhibition curve of compound concentration as well as cell viability, the IC_{50} values of numerous drugs were determined (Table I). The results demonstrated that simvastatin inhibited the growth of HCT-116 cells to a greater extent compared with the other statins, which is in agreement with the small molecule screening results presented in Fig. 4.

Simvastatin inhibits the migration and invasion of HCT-116 cells by upregulating miR-192-5p expression. The role of simvastatin on miR-192-5p expression in HCT-116 colon cancer cells was investigated. As presented in Fig. 6, the expression of miR-192-5p in HCT-116 cells was significantly increased following treatment with simvastatin (P<0.05). To determine the effects of simvastatin on the migration and invasion of HCT-116 cells, the migration and invasion rates of the untreated group, the simvastatin group, and the simvastatin + miR-192 inhibitor group were investigated, and the results demonstrated that inhibition of miR-192-5p attenuated the effects of simvastatin on the migration and invasion of HCT-116 cells (Fig. 7). These results indicated that simvastatin may inhibit the migration and invasion of colon cancer via upregulation of miR-192-5p.

The expression levels of the RAB2A protein in HCT-116 cells were then evaluated by western blotting. As presented in Fig. 8, simvastatin inhibited RAB2A protein expression via regulation of miR-192. Therefore, miR-192 appears to regulate RAB2A expression in colon cancer cells by directly binding to its 3'-UTR.

Discussion

Colon cancer is a common malignancy of the digestive tract and one of the leading causes of cancer-associated mortality. Elucidation of the molecular mechanism underlying colon cancer development is urgently required, as is the discovery of novel therapeutic targets for the treatment of colon cancer patients. miRNAs regulate protein expression and serve an important role in tumor progression by mediating the occurrence and development of tumors via regulation of

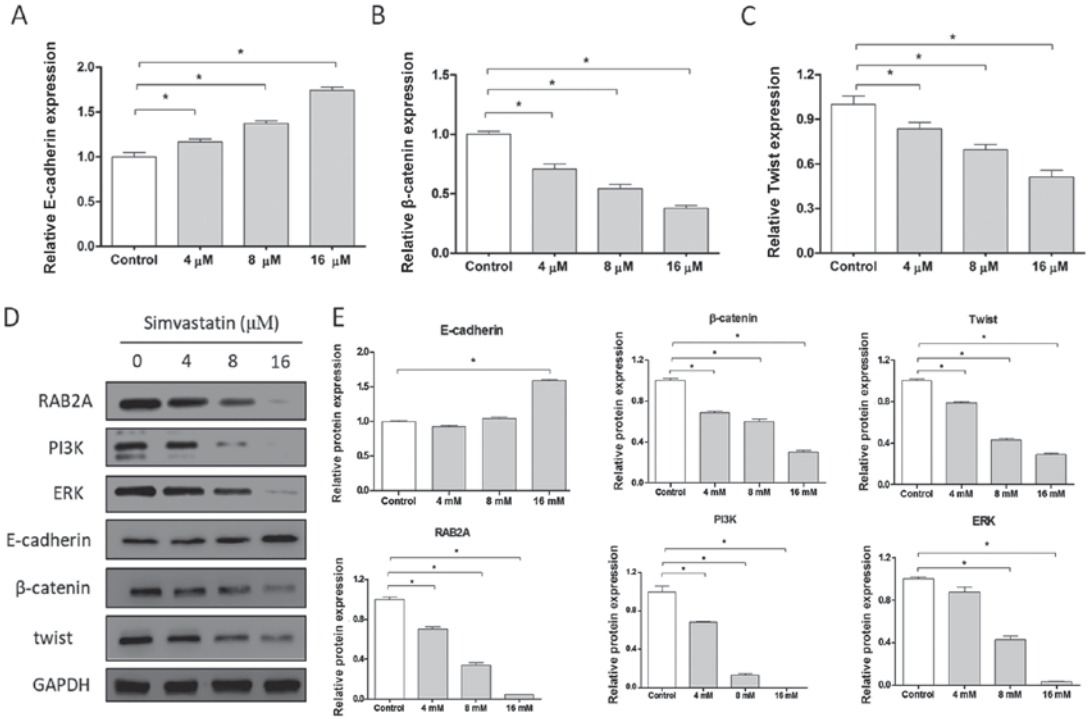

Figure 5. Simvastatin inhibits EMT and the PI3K/Akt pathways in HCT-116 cells. mRNA expression levels of EMT-associated proteins (A) E-cadherin, (B) β-catenin and (C) twist following treatment with various concentrations of simvastatin. Protein expression levels of E-cadherin, β-catenin, twist, RAB2A, PI3K and ERK in HCT-116 cells, presented as a (D) representative image and (E) quantitative analysis. *P<0.05. RAB2A, Ras-related protein Rab-2A; PI3K, phosphatidylinositol 3-kinase; EMT, epithelial-to-mesenchymal transition; Akt, protein kinase B; ERK, extracellular signal-regulated kinase; E, epithelial.

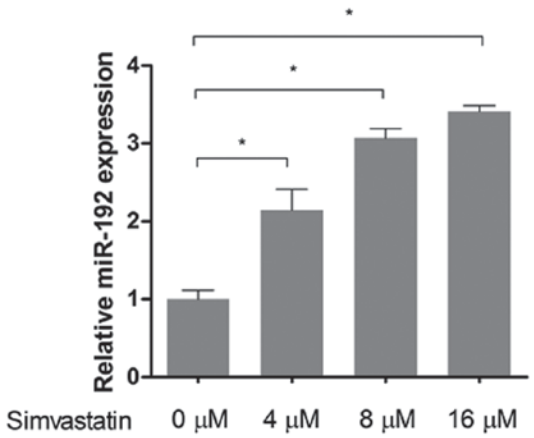

Figure 6. Simvastatin enhances miR-192-5p expression in HCT-116 cells. *P<0.05. miR, microRNA.

downstream target genes. miR-192 has been demonstrated to be abnormally expressed in various tumors. For example, miR-192 was demonstrated to be downregulated in lung cancer tissues compared with normal lung tissues. Previous *in vitro* analyses have demonstrated that overexpression of miR-192 may inhibit the proliferation and apoptosis of lung cancer cells via regulation of the RB transcriptional corepressor 1 gene (5). In addition, overexpression of miR-192 may inhibit the migration and invasion of renal cell carcinoma cells (23). In the present study, the expression of miR-192 in colon cancer cells was demonstrated to be markedly decreased compared with that in normal colon cells and this result was most prominent in HCT-116 cells. Furthermore, overexpression of miR-192 was demonstrated to inhibit the proliferation, migration and invasion of HCT-116 cells. In addition, the present study identified RAB2A as a novel oncogene in colon cancer, which is regulated by miR-192 via direct binding with its 3'-UTR. These results suggested that miR-192 acts as a tumor suppressor gene in colon cancer cells.

As important endogenous biomolecules, the regulation of miRNAs is becoming increasingly important. The regulation of miRNAs by small molecules has been extensively investigated. Statins are lipid-modifying drugs that inhibit the synthesis of cholesterol by selectively and competitively inhibiting hydroxymethylpentacyl-coenzyme A reductase expression, and are predominantly used as lipid-lowering drugs for the prevention of cerebrovascular and cardiovascular diseases. Recent studies have demonstrated that statins are highly effective in the treatment of colon, lung, pancreatic and breast cancer, as well as other solid tumors (24,25). It has also been reported that different doses of statins may reduce the risk of colon cancer by 94% (26). In the present study, small molecules that regulate miR-192 were screened and simvastatin was found to represent a novel activator of miR-192. Furthermore, it was demonstrated that simvastatin upregulated miR-192 and inhibited the expression of the downstream targets of miR-192, which subsequently led to suppressed proliferation, migration and invasion of colon cancer cells. In addition, it was observed that simvastatin inhibited the growth of colon cancer cells *in vitro* and exerted the most potent inhibitory effect among all the small molecules investigated. Furthermore, the results of the present study indicated that simvastatin may inhibit the migration and invasion of colon

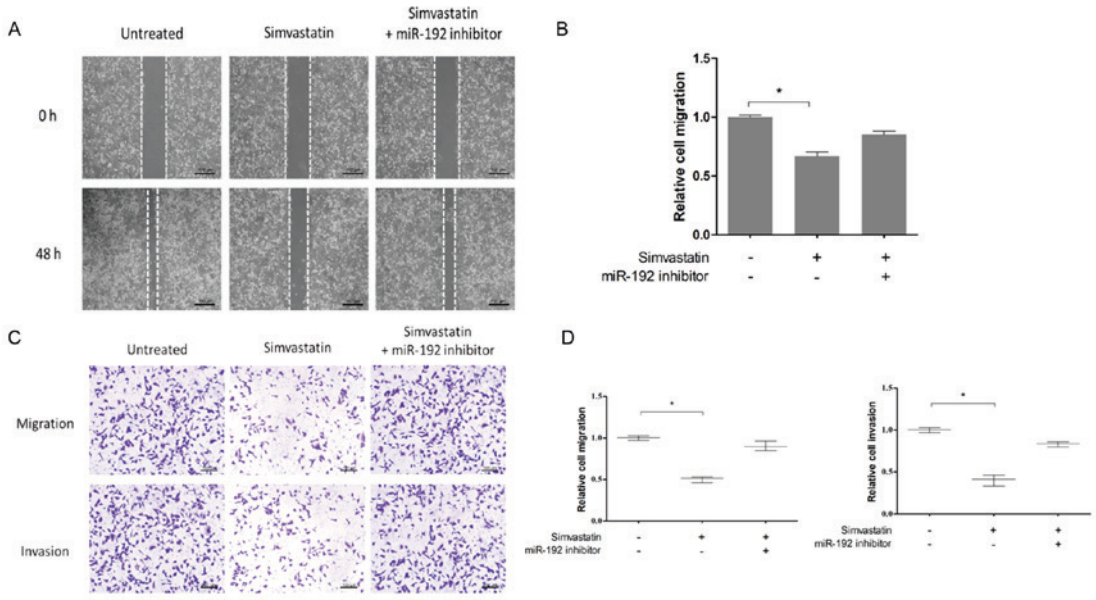

Figure 7. Inhibition of miR-192-5p suppresses the effect of simvastatin on the migration and invasion of HCT-116 cells. Effects of simvastatin and miR-192-5p on the wound healing of HCT-116 colon cancer cells are presented as a (A) representative image and (B) quantitative analysis. The effect of simvastatin and miR-192-5p on the migration and invasion of HCT-116 colon cancer cells are presented as a (C) representative image (scale bar, 100 μM) and (D) quantitative analysis of migration and invasion rates. *P<0.05. miR, microRNA.

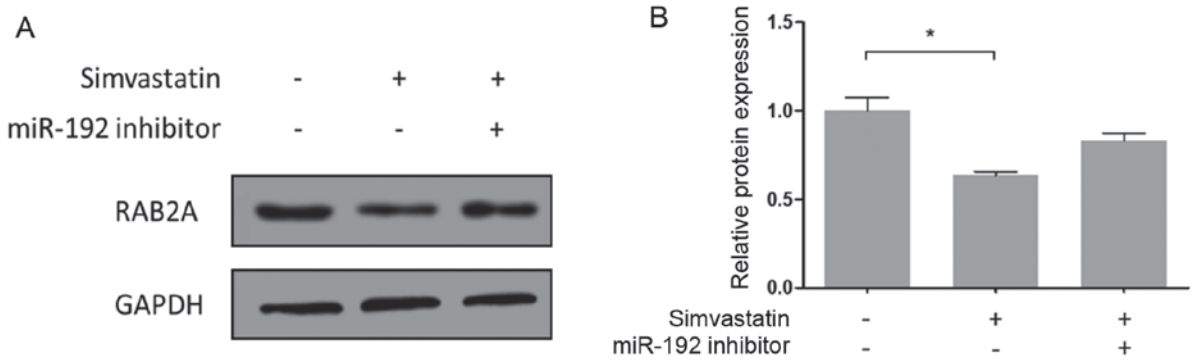

Figure 8. Simvastatin inhibits the expression of the RAB2A protein in HCT-116 cells via regulation of miR-192, the results of which are presented as a (A) representative image and (B) quantitative analysis. *P<0.05. miR, microRNA; RAB2A, Ras-related protein Rab-2A. miR, microRNA.

cancer cells. Therefore, simvastatin appears to upregulate miR-192, thereby inhibiting cancer growth.

In conclusion, miR-192 was identified as a tumor suppressor in colon cancer and simvastatin was found to be an activator of miR-192, which may represent a novel therapeutic approach to the treatment of patients with colon cancer.

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Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XZ and KL designed the experiments. XZ, XW, RZ and XL performed the experiments. XZ, XW, RZ and XL analyzed the data. XZ and KL wrote the manuscript. XZ and KL revised the manuscript. All authors reviewed the revised manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Van CE, Sagaert X, Topal B, Haustermans K and Prenen H: Gastric cancer. Lancet 388: 2654-2664, 2016.
2. Elias D, Faron M, Iuga BS, Honoré C, Dumont F, Bourgain JL, Dartigues P, Ducreux M and Goéré D: Prognostic similarities and differences in optimally resected liver metastases and peritoneal metastases from colorectal cancers. Ann Surg 261: 157-163, 2015.
3. Lin H and Hannon GJ: Micro RNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 5: 522-531, 2004.
4. Uchino K, Takeshita F, Takahashi RU, Kosaka N, Fujiwara K, Naruoka H, Sonoke S, Yano J, Sasaki H, Nozawa S, *et al*: Therapeutic effects of MicroRNA-582-5p and -3p on the inhibition of bladder cancer progression. Mol Ther 21: 610-619, 2013.
5. Feng S, Cong S, Zhang X, Bao X, Wang W, Li H, Wang Z, Wang G, Xu J, Du B, *et al*: MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells. Nucleic Acids Res 39: 6669-6678, 2011.
6. Jin Z, Selaru FM, Cheng Y, Kan T, Agarwal R, Mori Y, Olaru AV, Yang J, David S, Hamilton JP, *et al*: MicroRNA-192 and -215 are upregulated in human gastric cancer in vivo and suppress ALCAM expression in vitro. Oncogene 30: 1577-1585, 2011.
7. Jin Y, Lu J, Wen J, Shen Y and Wen X: Regulation of growth of human bladder cancer by miR-192. Tumour Biol 36: 3791-3797, 2015.
8. Li J, Tan S, Kooger R, Zhang C and Zhang Y: MicroRNAs as novel biological targets for detection and regulation. Chem Soc Rev 43: 506-517, 2014.
9. Li J, Zhang W, Zhou M, Kooger R and Zhang Y: Small molecules modulating biogenesis or processing of microRNAs with therapeutic potentials. Curr Med Chem 20: 3604-3612, 2013.
10. Shan G, Li Y, Zhang J, Li W, Szulwach KE, Duan R, Faghihi MA, Khalil AM, Lu L, Paroo Z, *et al*: A small molecule enhances RNA interference and promotes microRNA processing. Nat Biotechnol 26: 933-940, 2008.
11. Melo S, Villanueva A, Moutinho C, Davalos V, Spizzo R, Ivan C, Rossi S, Setien F, Casanovas O, Simo-Riudalbas L, *et al*: Small molecule enoxacin is a cancer-specific growth inhibitor that acts by enhancing TAR RNA-binding protein 2-mediated microRNA processing. Proc Natl Acad Sci USA 108: 4394-4399, 2011.
12. Watashi K, Yeung ML, Starost MF, Hosmane RS and Jeang KT: Identification of small molecules that suppress microRNA function and reverse tumorigenesis. J Biol Chem 285: 24707-24716, 2010.
13. TargetScan: http://www.targetscan.org/vert_71/.
14. Liu JJ, Liu JY, Chen J, Wu YX, Yan P, Ji CD, Wang YX, Xiang DF, Zhang X, Zhang P, *et al*: Scinderin promotes the invasion and metastasis of gastric cancer cells and predicts the outcome of patients. Cancer Lett 376: 110-117, 2016.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. Beekman JM, Reischl J, Henderson D, Bauer D, Ternes R, Peña C, Lathia C and Heubach JF: Recovery of microarray-quality RNA from frozen EDTA blood samples. J Pharmacol Toxicol Methods 59: 44-49, 2009.
17. Kajiho H, Kajiho Y, Frittoli E, Confalonieri S, Bertalot G, Viale G, Di Fiore PP, Oldani A, Garre M, Beznoussenko GV, *et al*: RAB2A controls MT1-MMP endocytic and E-cadherin polarized Golgi trafficking to promote invasive breast cancer programs. EMBO Rep 17: 1061-1080, 2016.
18. Broughton T, Sington J and Beales IL: Statin use is associated with a reduced incidence of colorectal adenomatous polyps. Int J Colorectal Dis 28: 469-476, 2013.
19. Wang H, Lu HM, Yang WH, Luo C, Lu SH, Zhou Y and Lin YZ: The influence of statin therapy on circulating microRNA-92a expression in patients with coronary heart disease. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 24: 215-218, 2012 (In Chinese).
20. Rodriguez JA, Huerta-Yepez S, Law IK, Baay-Guzman GJ, Tirado-Rodriguez B, Hoffman JM, Iliopoulos D, Hommes DW, Verspaget HW, Chang L, *et al*: Diminished expression of CRHR2 in human colon cancer promotes tumor growth and EMT via persistent IL-6/Stat3 signaling. Cell Mol Gastroenterol Hepatol 1: 610-630, 2015.
21. Uwafuji S, Goi T, Naruse T, Kurebayashi H, Nakazawa T, Hirono Y and Yamaguchi A: Protein-bound polysaccharide K reduced the invasive ability of colon cancer cell lines. Anticancer Res 33: 4841-4845, 2013.
22. Bao H, Zhang Q, Zhu Z, Xu H, Ding F, Wang M, Du S, Du Y and Yan Z: BHX, a novel pyrazoline derivative, inhibits breast cancer cell invasion by reversing the epithelial-mesenchymal transition and down-regulating Wnt/β-catenin signalling. Sci Rep 7: 9153, 2017.
23. Khella HW, Bakhet M, Allo G, Jewett MA, Girgis AH, Latif A, Girgis H, Von Both I, Bjarnason GA and Yousef GM: miR-192, miR-194 and miR-215: A convergent microRNA network suppressing tumor progression in renal cell carcinoma. Carcinogenesis 34: 2231-2239, 2013.
24. Li L and Zhuang Z: Anticancer effects of statins and application in prevention and treatment of esophageal cancer. Chin J Gastroenterol 18: 493-496, 2013 (In Chinese).
25. Yu X, Pan Y, Ma H and Li W: Simvastatin inhibits proliferation and induces apoptosis in human lung cancer cells. Oncol Res 20: 351-357, 2013.
26. Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, Low M, Greenson JK and Rennert G: Statins and the risk of colorectal cancer. N Engl J Med 352: 2184-2192, 2005.