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Ginsenoside Rg3 inhibits cell growth, migration and invasion in Caco-2 cells by downregulation of lncRNA CCAT1

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ABSTRACT

Background: Colorectal cancer (CRC) is a troublesome disease with high morbidity and mortality. Ginsenoside Rg3 possesses anti-cancer properties. Colon Cancer Associated Transcript 1 (CCAT1) participates in the genesis, development, invasion and metastasis of colorectal cancer. In our study, we explored the effects of Rg3 on CRC cell line Caco-2 by regulating CCAT1.

Methods: CRC tissue was obtained from hospital and Caco-2 cells were purchased. Caco-2 cells were treated with Rg3 and/or transfected with pc- CCAT1 or pcDNA3.1. The group without Rg3 treatment was treated as control. Cell viability, cell apoptosis, cell migration and invasion were detected by Cell Counting Kit-8 assay, flow cytometry and Transwell chamber migration/invasion assay, respectively. The expression of CyclinD1, apoptosis related proteins (p53, Bcl-2, Bax, pro-/Cleaved-Caspase-3), migration and invasion related proteins (MMP-9 and vimentin), and phosphatidylinositol 3′-kinase (PI3K)/protein kinase B (AKT) related proteins (p/t-PI3K, p/t-AKT) were examined by western blot. The expression of CCAT1 was measured by quantitative real time RCR (qRT-PCR).

Results: Rg3 significantly decreased cell viability, migration and invasion, and promoted apoptosis. Meanwhile, the expression of Cyclin D1, matrix metalloproteinase (MMP)-9 and vimentin was downregulated. The expression of apoptosis-related proteins p53, Bax, and Cleaved-Caspase-3 were upregulated while Bcl-2 was downregulated by the treatment of Rg3 compared with control. Furthermore, CCAT1 was upregulated in CRC tissue and Rg3 negatively regulated CCAT1 expression. Transfection with pc-CCAT1 led to the opposite results as compared with transfection with pcDNA3.1 in Rg3 treated cells. In addition, Rg3 decreased the phosphorylation of PI3K and AKT.

Conclusion: Ginsenoside Rg3 inhibits migration and invasion, and promotes apoptosis of Caco-2 cells by suppression expression of LncRNA CCAT1.

1. Introduction

Colorectal cancer (CRC) is a troublesome grisly disease with high morbidity and mortality (Guinney et al., 2015). Recent study showed that CRC already became the third common cancer and a major cancer-related death in the United States (Imperiale et al., 2014). A report showed that the most common occurrence age for CRC in United States is a range from 65 to 74 years old, and moreover, the mean age for CRC-related death is 73 years old (Bibbins-Domingo et al., 2016). Accordingly, the mortality of CRC increased into 50%, which means that half of patients died from CRC if they were confirmed as this disease. On the other hand, only limited 10% has possible to survive more than five years after their diagnosis (Wang and Yuan, 2008). This depressing situation required novel medicine or methods to make a breakthrough in the treatment of CRC.

Ginsenoside Rg3, is the main active compound purified from ginseng (Kim et al., 2003). Increasing evidences have proved that Rg3 has anti-tumor properties. For example, Rg3 was reported to affect cell growth and angiogenesis in ovarian cancer (Xu et al., 2007); Rg3 revealed inhibition of vasculogenic mimicry in pancreatic cancer (Guo et al., 2014); Rg3 combined with cyclophosphamide in the lower concentration revealed antiangiogenic effect in Lewis lung cancer (Zhang et al., 2006); Rg3 possessed suppressing effects in on ovarian cancer cell growth and metastasis (Xu et al., 2008). Importantly, as an anti-cancer agent, Rg3 modulated the expression of genes in CRC cells (Luo et al., 2008). Moreover, Rg3 has been proved to suppress CRC cell growth via inactivation of Wnt/β-catenin signal pathway (He et al., 2011). Therefore, we aimed to investigate whether Rg3 affected CRC cell

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growth or metastasis.

Long non-coding RNAs (lncRNAs) referred to a group of RNAs without protein-coding in length > 200 nucleotides (Xin et al., 2016). Recent studies elucidated that lncRNAs participate in a wide range of biological activities and various kinds of diseases, including cancer (Huarte, 2015). The aberrant expression of lncRNAs often related with cancers and several lncRNAs play vital roles in regulation of cell proliferation, cell apoptosis, cell migration and invasion, such as Highly upregulated in liver cancer (HULC) (Zhao et al., 2014), H19 (Yang et al., 2012), and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (Wang et al., 2015). Among these identified lncRNAs, Colon Cancer Associated Transcript 1 (CCAT1) was reported to be often upregulated in various cancers, such as colon cancer (He et al., 2014), gastric carcinoma (Yang et al., 2013) and gallbladder cancer (Ma et al., 2015). Especially, CCAT1 receives attention due to its close association with CRC. For instance, upregulation of CCAT1 contributed tumorigenesis by enhancing colon cancer cell proliferation and invasion (He et al., 2014). This valuable information motivated us to consider whether CCAT1 was also joined in the effects of Rg3 on CRC.

In our study, we used human colon carcinoma cell line Caco-2 to mimic CRC in vitro and investigated the effects of Rg3 on Caco-2 cells and also explored the underlying mechanisms. Our study might provide a novel insight into the research of CRC in the future.

2. Material and methods

2.1. Clinical specimen collection

Between June 2016 to May 2017, clinical human colon cancer tissues (n = 23) and the corresponding non-colon cancer tissues (n = 23) were attained from patients undergoing colectomy with each patient's informed consent from Jining No.1 People's Hospital (Jining, China). These patients information was as follows: colon cancer (twelve males and eleven females, average age 55.3 ± 3.5 years old; the non-colon cancer samplings without other diseases (twelve males and eleven females, average age 52.2 ± 5.6 years old). None of the patients received any therapies preoperative radiotherapy or chemotherapy prior to surgery. Informed consents from every patient were obtained, and the present study was approved by the Medical Ethics Committee of Jining No.1 People's Hospital.

2.2. Cell culture and treatment

Human colon carcinoma cell line Caco-2 was provided by Procell life science and technology Co., Ltd. (Cat. No.: CL-0050, Wuhan, China). The culture medium for Caco-2 was MEM (Procell, PM150410) and 20% FBS (Procell, 164210-500) according to the Caco-2 cell culture instructions. The cells were cultured in the environment with 95% air and 5% CO_2, and keep the temperature at 37 °C. The medium renewed 1–2 times per week.

Ginsenoside Rg3 (≥ 98% (HPLC), Cat. No. SML0184) was purchased from Sigma-Aldrich (St Louis, MO, USA), dissolved in dimethylsulfoxide (DMSO) and then diluted into concentrations in a range of 0–100 μg/ml. Cells were treated in ginsenoside Rg3 for 24 h.

2.3. Cell viability assay

Cell viability was performed using Cell Counting Kit-8 (CCK-8, Yeasen, Shanghai, China) after Caco-2 cells treated with different concentrations of Rg3. In brief, Caco-2 cells were collected and diluted into the density of 2×10^5 cells/well and then were seeded in 96-well plate. Rg3 at different concentrations were added into the culture medium and then cells were cultured at 37 °C in humidified air with 5% CO_2 for 24 h. Afterward, added 10 μl CCK-8 solution into plates and cells were incubated for 1 h. After incubation, absorption values were read at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

2.4. Cell apoptosis assay

Propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining (Yeasen, Shanghai, China) was used for determining cell apoptosis. In brief, the cells at the density of 100,000 cells/well were seeded in 6 well-plate. Treated cells were washed twice with precooling phosphate buffer saline (PBS) and centrifuged to resuspend in binding buffer. Then added 5 μl Annexin V-FITC, mixed gently and put in the dark and incubated for 15 min. In addition, added 5 μl PI to the samples. The apoptotic cells rate was measured with flow cytometer (Beckman Coulter, USA).

2.5. Migration and invasion assay

Cell migration was evaluated by using a modified two-chamber migration assay with a pore size of 8 μm. Cell suspension 100 μl (around 2×10^5 cells/ml) without serum was added to upper Transwell. Then 600 μl culture medium with 10% FBS was added in the lower compartment of 24-well Transwell. Caco-2 cells were maintained for 24 h at 37 °C with humidified air with 5% CO_2. After incubation, cells at the upper surface of the filter were removed by a cotton swab, and the filter was fixed with methanol for 5 min. Caco-2 cells at the lower surface of the filter were stained by Giemsa for 15 min. Cells were counted by 100 × microscope. Cell invasion was conducted in the similar process as cell migration, but the inserts were coated with 50 μg of Matrigel (BD Biosciences, Bedford, MA, USA).

2.6. Cell transfection

To clarify the function of CCAT1, pc-CCAT1 and its corresponding negative control (NC) pcDNA3.1 (GenePharma Co., Shanghai, China) were transfected into Caco-2 cells. Pre-treated cells at the density of 2×10^5 cells/well were seeded and incubated until the cells arrived at 70–80% confluence, they were transfected with pc-CCAT1 or NC using Lipofectamine 2000 reagent (Invitrogen).

2.7. qRT-PCR

The total RNA obtained from clinical tissue was process as below: 100 mg CRC tissue was treated with 1 ml of Trizol (Invitrogen, Carlsbad, CA, USA), and mashed using homogenizer (APV Company Ltd., Crawley, Surrey, England). In addition, tissue was isolated with chloroform, precipitated with isopropanol, washed with 75% ethanol and dissolved in DECP water. The whole procedure was done on ice to keep the tissue in a lower temperature. RNA concentration was measured with ultraviolet spectrophotometry after extraction. Qualified samples were preserved in −80 °C fridge.

On the other hand, total RNA from Caco-2 cells was obtained using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) was used for Real-Time PCR analysis to determine the expression level of CCAT1. GAPDH was the internal control for CCAT1.

2.8. Western blot

Western blot was used in our study to detect all the protein expression. Protein was obtained from Caco-2 cells using RIPA lysis buffer (Cat. No:R0010, Solarbio, Beijing, China) supplemented with protease inhibitors (Thermo Fisher Scientific). The BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA) was used for determining proteins concentration. The western blot system was established using a Bio-Rad Bis-Tris Gel system following the manufacturer's instructions. Primary antibodies included: anti-Cyclin D1 antibody (ab134175), anti-p53 antibody (ab31333), anti-Bcl-2 antibody (ab32124), anti-Bax antibody (ab32503), anti-pro Caspase 3 antibody (ab32150), anti-cleaved Caspase-3 antibody (ab32042), anti-matrix metalloproteinase (MMP)-9

antibody (ab73734), anti-vimentin antibody (ab137321), anti-phosphatidylinositol 3′-kinase (PI3K) antibody (ab191606), anti-phospho-PI3K antibody (ab182651), anti-protein kinase B (AKT) antibody (ab8805), anti-phospho-AKT antibody (ab8933), anti-β-actin antibody (ab8227), all from Abcam (Cambridge, UK). Primary antibodies were prepared in 5% blocking buffer and diluted according to the product instruction. These primary antibodies were incubated in membrane and maintained at 4 °C overnight at recommended concentration. Then for second antibody incubation, incubate with horseradish peroxidase (HRP) conjugated second antibody. Detection was performed by capturing the signals and analyzing the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

2.9. Statistical analysis

All data are presented as mean ± standard deviation (SD) which based on at least three times experiments. Statistical analyses were performed using Graphpad 6.0 statistical software (GraphPad, San Diego, CA, USA). The *P*-values were calculated using a one-way analysis of variance (ANOVA) and student's *t*-test. * ($P < .05$), ** ($P < .01$), and *** ($P < .001$) all considered as statistically difference.

3. Results

3.1. Rg3 inhibited cell viability and induced cell apoptosis

Caco-2 cells were exposed into different concentrations of Rg3 (25, 50 and 100 μM) for 24 h, and cell viability was determined by CCK-8 assay. Results showed that cell viability was significantly decreased with the increasing concentration of Rg3 (25 μM, $P < .05$; 50 and 100 μM, $P < .01$). Cyclin D1 is an important cell cycle related proteins. Result showed that the expression of Cyclin D1 was downregulated by Rg3 ($P < .01$, Fig. 1B), which indicated that Rg3 (50 μM) could affect cell proliferation. Based on this, Rg3 at the concentration of 50 μM and pre-treatment for 24 h was applied for the subsequent experiments. Afterward, we detected the effects of Rg3 on cell apoptosis. Obviously, the apoptotic cell rate was significantly increased compared with control ($P < .001$, Fig. 1C). Simultaneously, the expression of pro-apoptotic proteins p53 ($P < .01$) and Bax ($P < .001$) was upregulated, and the ratio of cleaved-Caspase-3/pro-Caspase-3 ($P < .001$) was significantly increased (Fig. 1D–E). On the other hand, the expression of anti-apoptotic protein Bcl-2 was downregulated ($P < .05$, Fig. 1D–E). Taken together, Rg3 inhibited Caco-2 cell growth.

3.2. Rg3 alleviated cell migration and invasion

Strong migration and aggressive abilities are key factors for cancer progression (Yamaguchi and Condeelis, 2007), therefore we investigated the effects of Rg3 on Caco-2 cell migration and invasion. As shown in Fig. 2A and C, Rg3 statistically decreased cell migration and invasion ($P < .05$ or $P < .01$). Meanwhile, it is well known that MMP-9 and vimentin are migration and invasion related proteins (Wu et al., 2012; Wu et al., 2009). In our study, we found that the production of MMP-9 and vimentin was decreased ($P < .05$ or $P < .01$, Fig. 2B and D).

3.3. CCAT1 was upregulated in tumor tissue while Rg3 downregulated CCAT1 expression

CCAT1, as the name itself suggested, CCAT1 was closely related with colon cancer (Alaiyan et al., 2013). Interestingly, we found that the expression of CCAT1 was upregulated (Fig. 3A) while in the following study, Rg3 pre-treatment at different concentrations (25, 50 and 100 μM)) for 24 h, was observed to have potential to downregulate CCAT1 expression as compared to the control ($P < .05$ or $P < .01$, Fig. 3B). Taken together, CCAT1 might be involved in the protective effects of Rg3 on Caco-2 cells.

3.4. Rg3 induced cell apoptosis, and inhibited cell migration and invasion by downregulation of CCAT1

To clarify the functions of CCAT1, pc-CCAT1 and its NC pcDNA3.1 were transfected into Caco-2 cells. Overexpression of CCAT1 by transfection with pc-CCAT1 indicated the high transfection efficiency ($P < .001$, Fig. 4A). We thereafter detected the effects of overexpression of CCAT1 on cell apoptosis, cell migration and invasion. Interestingly, co-treatment with Rg3 and transfection with pc-CCAT1 significantly decreased cell apoptosis ($P < .01$, Fig. 4B). The apoptotic proteins Bcl-2 ($P < .01$) was upregulated while p53 ($P < .01$), Bax ($P < .01$) and cleaved-Caspase-3 ($P < .01$) were all inhibited by co-treatment with Rg3 with transfection with pc-CCAT1 (Fig. 4C–D). Similarly, we determined the influence of CCAT1 overexpression on cell migration and invasion. We found that co-treatment with Rg3 and transfection with pc-CCAT1 increased migration ($P < .01$, Fig. 4E) get along with upregulation of MMP-9 ($P < .01$, Fig. 4F). In addition, we also observed that co-treatment with Rg3 and transfection with pc-CCAT1promoted invasion ($P < .01$, Fig. 4G) and upregulated the expression of vimentin ($P < .01$, Fig. 4H). In conclusion, Rg3 could inhibit cell apoptosis, and enhance cell migration and invasion through downregulation of CCAT1.

3.5. Rg3 inactivated PI3K/AKT signal pathway through downregulation of CCAT1

PI3K/AKT was involved in CRC progression (Ke et al., 2015), hence, we explored the effects of Rg3 on PI3K/AKT pathway. In our study, we found that Rg3 decreased the phosphorylation of PI3K ($P < .01$)and AKT ($P < .05$) compared with control (Fig. 5A–B). On the other side, overexpression of CCAT1 led to upregulation of PI3K and AKT (both $P < .01$) as compared with Rg3 and NC treatment group (Fig. 5A–B). Above all, Rg3 inactivated PI3K/AKT signal pathway through downregulation of CCAT1.

4. Discussion

CRC is a kind of catastrophic disease with low outcome in patients (Wang and Yuan, 2008). Cetuximab and chemotherapy are often used for the initial treatment for CRC (Van Cutsem et al., 2009), which increased the survival ratio of CRC patients in a certain extent. However, the cure rate for CRC is still very low, which made the requirement for new medicine and effective treatment methods becoming urgent. In our study, we investigated the effects of ginsenoside Rg3 on Caco-2 cells. Results showed that Rg3 inhibited cell growth, migration and invasion. In addition, the clinical tissue results revealed that CCAT1 was upregulated while Rg3 downregulated the expression of CCAT1. Further experiments showed that Rg3 inhibited cell growth, migration and invasion was through downregulation of CCAT1. In addition, Rg3 inactivated PI3K and AKT through downregulation of CCAT1.

With the increasing attention been turn to the traditional medicine, we also started to consider whether we could use traditional medicine in the treatment of CRC. In our study, Rg3 was used to investigate its effects on CRC cell line due to its anti-cancer effects (Park et al., 2014). Previous studies have demonstrated that Rg3 had effects in suppressing cell growth and inducing cell death in different kinds of cancers (Shan et al., 2015; Tian et al., 2016; Zhang et al., 2015). For example, Rg3 revealed inhibitive effects on CRC cell growth in three different CRC cell lines (HCT116, HT29, SW480) (Yang et al., 2017). In our study, we used another CRC cell line Caco-2 and similarly, Rg3 administration demonstrated anti-cancer effects by decreasing cell viability and increasing cell apoptosis in Caco-2 cells.

CyclinD1 is a crucial gene which links in the cell cycle progression. In particular, abnormal expression of Cyclin D1 is frequently occurred

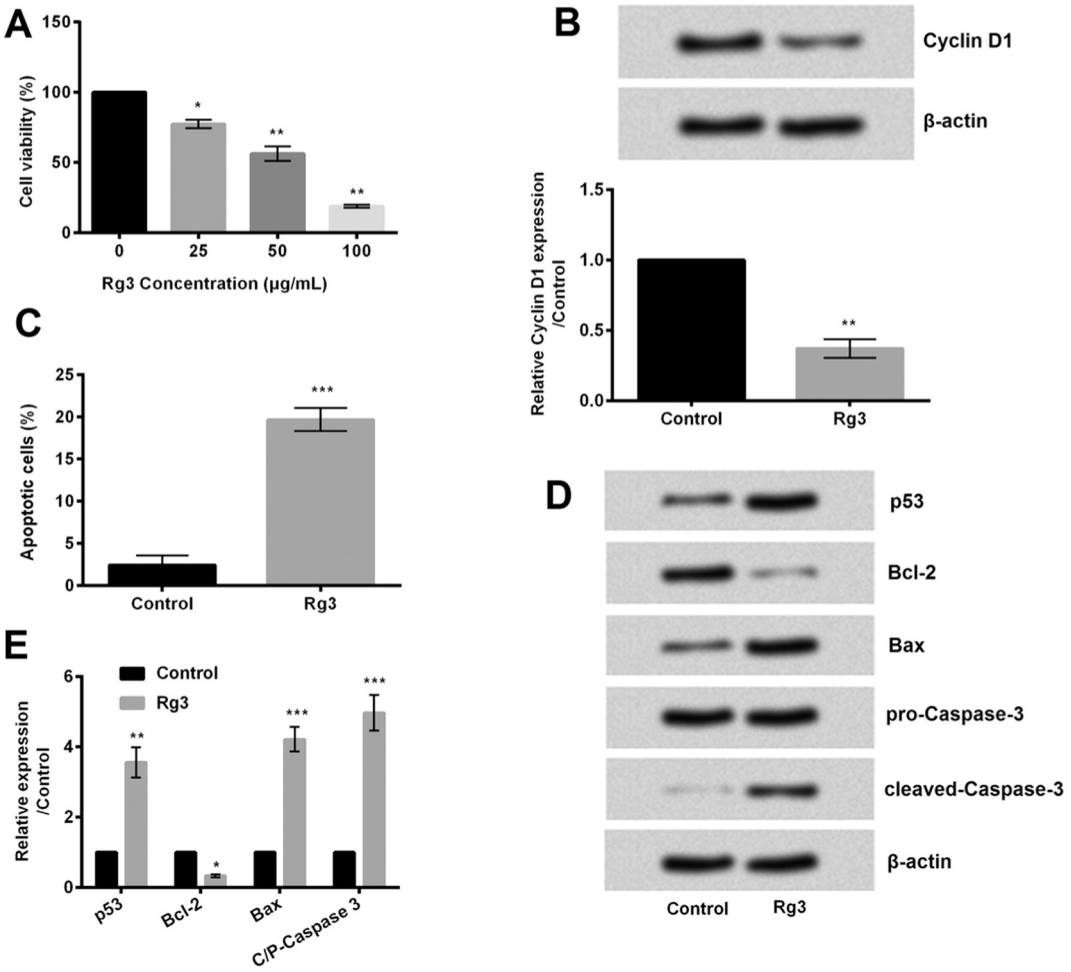

Fig. 1. Ginsenoside Rg3 decreased cell viability and increased cell apoptosis. (A) Cell viability was detected by Cell Counting Kit-8. (B) The expression of Cyclin D1 was analyzed by western blot. (C) Cell apoptosis by Rg3 was detected by flow cytometry. (D-E) The expression of cell apoptosis-related proteins was examined by western blot. All data represented as mean ± standard deviation (SD). Asterisk * ($P < .05$), ** ($P < .01$) and *** ($P < .001$) were all considered as significant results.

in most human cancers (Hydbring et al., 2016). In our study, we found that Cyclin D1 was downregulated by Rg3, which validated the finding that Rg3 decreased cell proliferation. Accordingly, this finding was also observed in the research from Duo et al. (Liu and Duo, 2018). Hence, it provided evidence that Rg3 inhibited cell proliferation.

In addition, the expression of apoptosis-related protein (p53, Bcl-2, Bax, Caspase-3) was also examined by western blot. p53 is an interesting factor both lead to cell cycle arrest or cell apoptosis (Paek et al., 2016). The decreased cell proliferation and increased cell apoptosis, indicating that p53 might achieve a threshold in leading to cell apoptosis induced by Rg3. Anti-apoptotic protein Bcl-2, and pro-apoptotic proteins Bax and cleaved Caspase-3 play vital roles in cell apoptosis (Ashkenazi et al., 2017; Ralph et al., 2006). The downregulation of Bcl-2 and upregulation of Bax and cleaved Caspase-3 suggested the effects of Rg3 on inducing cell apoptosis. This finding revealed the same properties of Rg3 from Yuan et al. that Rg3 increased Bax and cleaved-Caspase-3 expression and inhibited Bcl-2 expression in breast cancer (Yuan et al., 2017). The expression of proteins as evidenced that Rg3 could increase apoptosis in Caco-2 cells.

It is well-known that cell migration and invasion are two key steps for tumor progression and becoming malignant tumor (Appert-Collin et al., 2015). Previous study proved that Rg3 demonstrated effects of suppressing cell migration and invasion in lung cancer cells (Kim et al., 2014). Hence, we investigated to determine whether Rg3 influence migration and invasion in CRC cells. As expected, Rg3 revealed significant effects in inhibition of migration and invasion in CRC cells. Similarly, we also found evidence about Rg3 in inhibiting cell migration and invasion in lung cancer from former literature (Tian et al., 2016). MMP-9 and vimentin are closely related with cell migration and invasion (Dai et al., 2016). Simultaneously, we also found that MMP-9 and vimentin are both downregulated by treatment with Rg3, which verified the effects of Rg3 on migration and invasion.

Increasing evidences have proved that CCAT1 was closely involved in the progression of multiple cancers. For example, one study pointed out that CCAT1 enhanced gallbladder cancer development via negatively regulating miR-218-5p (Ma et al., 2015). Another study illuminated effects of CCAT1 in gastric cancer through activating c-Myc (Yang et al., 2013). From these data above, we inferred that CCAT1 might play important roles in promoting cancer cell development. In clinical tissue result, CCAT1 was upregulated in CRC tumor tissue as compared to normal control, which consistent with previous study that CCAT1 was observed to be elevated expression in CRC and treated as a biomarker for CRC (Ozawa et al., 2017). Clinical data provided more persuade words on the study of CCAT1 in cancers. Previous study proved that CCAT1 played an important role in the genesis, development, invasion and metastasis in colorectal cancer and its clinical data also revealed that CCAT1 was upregulated from the statistics of 37 colorectal cancer tissue (Ye et al., 2015), which stood in the same line with our results.

Based on what have obtained, we therefore detected the expression of CCAT1 by the treatment of Rg3 in Caco-2 cells to evaluate how Rg3

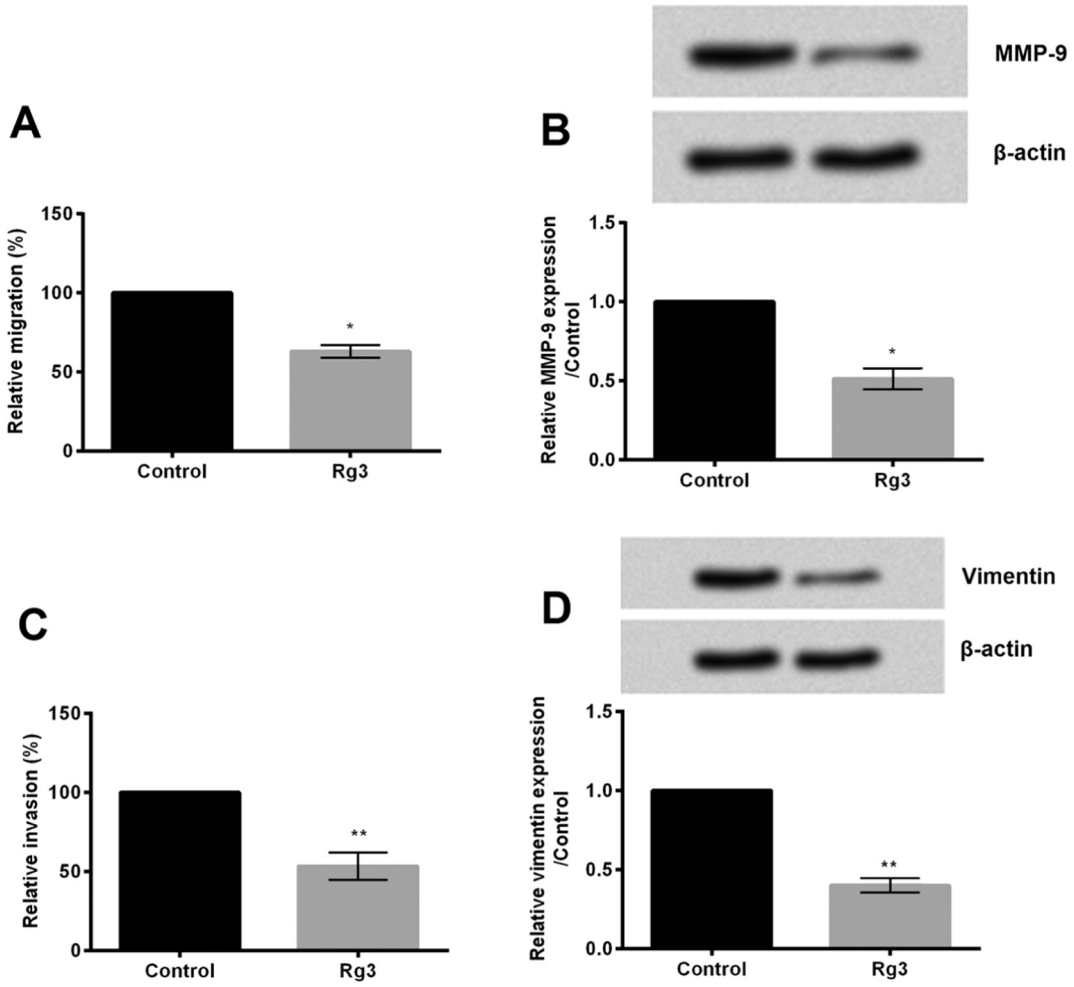

Fig. 2. Rg3 suppressed cell migration and cell invasion in Caco-2 cells. (A and C) Cell migration and cell invasion were detected by Transwell chamber assay. (B and D) The expression of matrix metalloproteinase-9 (MMP-9) and vimentin was examined by western blot. All data represented as mean ± standard deviation (SD). Asterisk * ($P < .05$) and ** ($P < .01$) were both considered as significant results.

could affect the product of CCAT1. Interestingly, we found that Rg3 negatively regulated the expression of CCAT1. Previous study reported that CCAT1 has the potential to be treated as effective approach in screening for CRC (Zhao et al., 2015). Based on this, we explored the functions of CCAT1 in Caco-2 cells combined with Rg3.

Overwhelming evidence proved that CCAT1 was involved in regulation of cell proliferation, apoptosis, cell migration and cell invasion. For example, CCAT1 reduced cell apoptosis in renal cancer cell (Chen et al., 2017). CCAT1 silence repressed cell proliferation and migration in esophageal squamous tumor cells (Zhang et al., 2017). Importantly, CCAT1 was observed to promote colon cancer cell growth and invasion (He et al., 2014). However, it is the first time that CCAT1 was investigated to be involved in the anti-tumor effects of Rg3. In our study, overexpression of CCAT1 promoted Caco-2 cells growth and migration

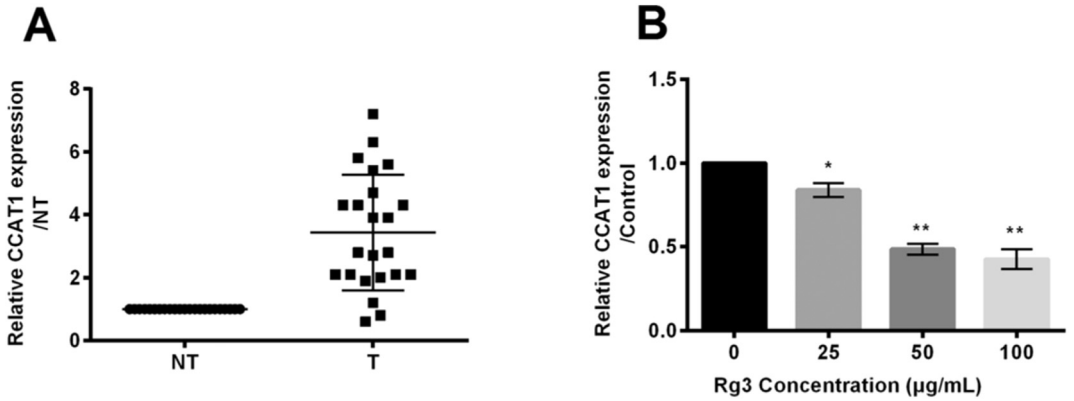

Fig. 3. Colon cancer associated transcript1 (CCAT1) was upregulated in CRC tissue and Rg3 downregulated the expression of CCAT1 in Caco-2 cells. (A and B) The expression of CCAT1 was detected by qRT-PCR. All data represented as mean ± standard deviation (SD). Asterisk * ($P < .05$) and ** ($P < .01$) were both considered as significant results.

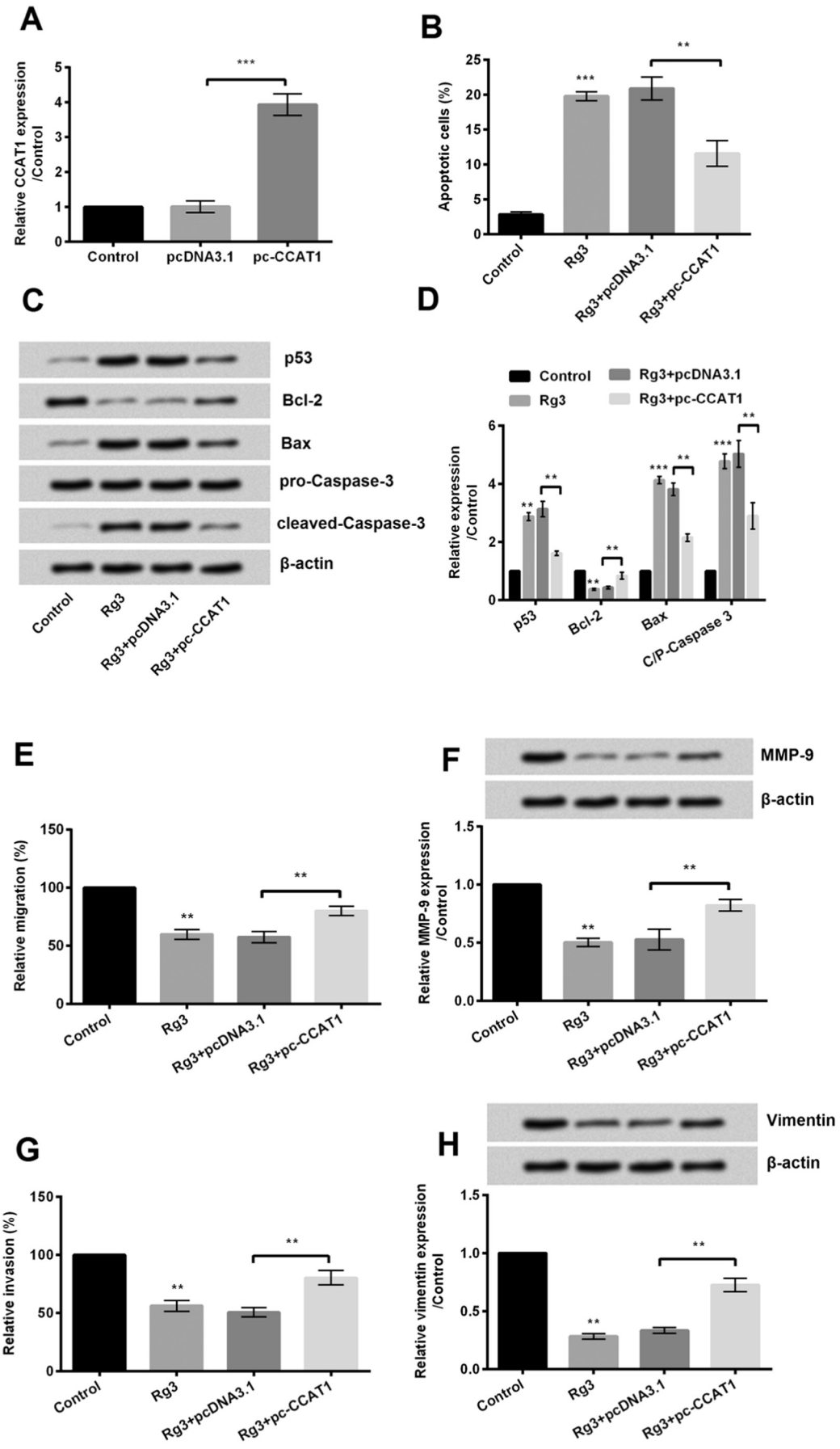

Fig. 4. Rg3 decreased cell growth and metastasis via downregulation of colon cancer associated transcript1 (CCAT1). (A) pc-CCAT1 was transfected into Caco-2 cells and the expression of CCAT1 was detected by qRT-PCR. (B) Cell apoptosis was detected by flow cytometry. (C-D) The expression of cell apoptosis-related proteins was examined by western blot. (E and G) Cell migration and cell invasion were detected by Transwell chamber assay. (F and H) The expression of matrix metalloproteinase-9 (MMP-9) and vimentin was examined by western blot. All data represented as mean ± standard deviation (SD). Asterisk * ($P < .05$), ** ($P < .01$) and *** ($P < .001$) were all considered as significant results.

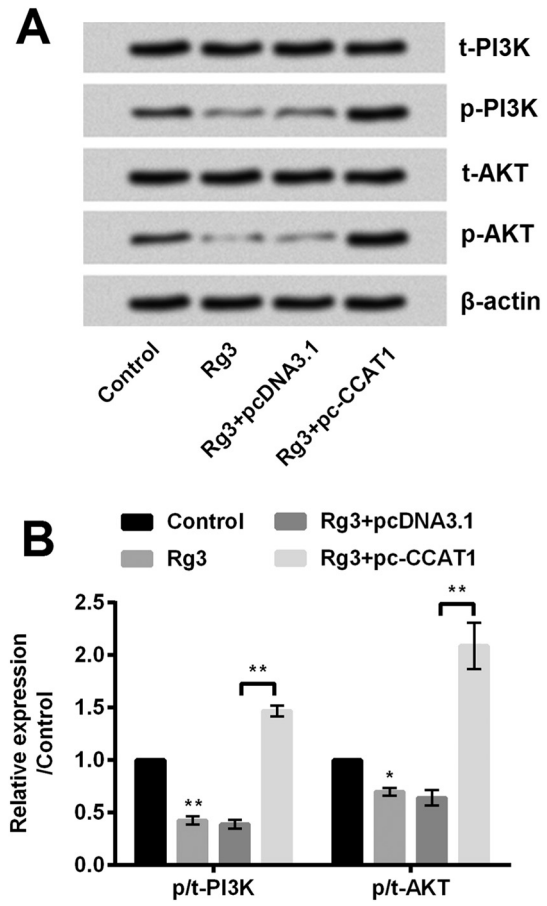

Fig. 5. Rg3 inactivated phosphatidylinositol 3′-kinase (PI3K)/protein kinase B (AKT) signal pathway. (A-B) The phosphorylation of PI3K and AKT was detected by western blot. All data represented as mean ± standard deviation (SD). Asterisk * ($P < .05$) and ** ($P < .01$) were both considered as significant results.

and invasion, which indicated that the anti-tumor effects of Rg3 was via downregulation of CCAT1.

PI3K/AKT was reported to be participated in CRC progression (Ke et al., 2015). In our study, we found that Rg3 inactivated PI3K/AKT pathway while overexpression of CCAT1 activated this pathway. This mechanism was same as what Xie et al. found in lung cancer that inactivation of PI3K/AKT pathway was triggered by anti-tumor effects of Rg3 (Xie et al., 2017). Thus, we inferred that the anti-tumor effect of Rg3 might be through inactivation of PI3K/AKT pathway.

5. Conclusions

In conclusion, our experiments were designed to investigate the effects of Rg3 on Caco-2 cells and the underlying mechanisms. We found that Rg3 revealed the anti-tumor effects in CRC Caco-2 cells presented by decreasing cell viability, migration and invasion, and increasing cell apoptosis. Further study validated that this process was through downregulation of CCAT1 and along with inactivation of PI3K/AKT pathway.

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Conflict of interests

Authors declare that there is no conflict of interests.

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