

Overexpression of CCDC34 in colorectal cancer and its involvement in tumor growth, apoptosis and invasion

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Abstract. It has been previously reported that increased expression of coiled-coil domain containing 34 (CCDC34), a member of the CCDCs family, may promote the proliferation and invasion of bladder cancer cells. However, its role in colorectal cancer (CRC) remains unclear. The present study investigated CCDC34 expression in CRC tissues and determined the association between CCDC34 expression and biological characteristics in patients with CRC. Additionally, the variation of cell activity, apoptosis, invasion and associated mechanisms were evaluated following CCDC34 inhibition in SW620 cells with small interfering RNA (siRNA). The role of CCDC34 in CRC growth, apoptosis and invasion was investigated. In the current study, immunohistochemistry revealed an overexpression of CCDC34 in CRC tissues compared with paracancerous tissue ($\chi^2=29.810$; $P<0.001$). Furthermore, CCDC34 expression was revealed to be associated with tumor invasion depth and lymphatic metastasis ($\chi^2=4.343$, $P=0.037$; $\chi^2=7.915$, $P=0.005$). Additionally, the inhibition of CCDC34 expression in SW620 cells led to reduced tumor cell activity, increased apoptosis rate and reduced invasion ability, and expression of apoptosis and invasion-associated genes varied simultaneously which demonstrated that B cell leukemia/lymphoma 2, survivin, N-cadherin, and MMP-9 were decreased, whereas E-cadherin increased significantly in cells of CCDC34-siRNA group compared with the control group ($P<0.05$). Therefore, CCDC34 may contribute to CRC development by inhibiting apoptosis of cancer cells and promoting invasion.

Introduction

Colorectal cancer (CRC) is one of the most common malignant cancers worldwide (1), with considerable medical cost to society and suffering of patients with CRC (2,3). As CRC is

characterized by rapid progression, majority of patients are diagnosed with advanced stage at the first hospital visit and cannot undergo operation to remove tumor (4). Patients that have had the tumor removed surgically, still have high risk of recurrence and metastasis postoperatively (5,6) due to rapid growth (7), resistance to apoptosis (8) and invasion of CRC tumor cells (9). Thus, it is important to identify the genes relevant to regulation of apoptosis and invasion of CRC cells to improve the diagnosis and comprehensive treatments for CRC.

Coiled-coil domain containing (CCDC) proteins, one family of protein with coiled-coil structures, possess a wide range of physiological functions (10), and the potential associations between some members of CCDC family and cancers have been previously reported. Previous studies have confirmed that CCDC67, CCDC6 and CCDC134 were associated with apoptosis and invasion of thyroid, lung and stomach cancer cells (11-14). CCDC34 is also a member of CCDC family, and has been reported to contribute to apoptosis and invasion of bladder cancer cells (15); however, it is currently uncertain whether CCDC34 can exacerbate this process in CRC cells. In the current study, the expression of CCDC34 protein in paraffin-embedded tissue samples was detected and clinical pathological data of patients with CRC was also collected. The association between CCDC34 expression and the biological characteristics of patients with CRC was analyzed. Additionally, endogenous CCDC34 expression in SW620 cells was suppressed with small interfering RNA (siRNA) and the changes of cell metabolic activity, apoptosis and invasion ability were detected following the siRNA transfection. Subsequently, reverse-transcription quantitative polymerase chain reaction (RT-qPCR) and western blotting was used to detect the expression levels of apoptosis and invasion-associated genes, including B cell leukemia/lymphoma 2 (Bcl-2), survivin, E-cadherin, N-cadherin and matrix metalloproteinase-9 (MMP-9) after endogenous CCDC34 in CRC cells was inhibited. The current findings suggested that the detection of CCDC34 may be valuable for the evaluation of patients with CRC and provided evidence for the investigation of its role in invasion and metastasis of CRC.

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Materials and methods

Patients and tissue specimens. In the current study, a total of 85 paraffin specimens of tumor tissues were obtained from

patients with CRC diagnosed and received surgical treatment at Hebei General Hospital (Shijiazhuang, China) between January 2015 and June 2016. In addition, 60 paraffin specimens of paracancerous tissues were selected as controls, which were >3 cm from edge of cancer and no cancer cells were observed with microscopic examination. In total, 59 male patients and 26 female patients were recruited, and the patients aged from 41-76 (mean age, 57.29±7.49). All enrolled patients did not suffer from other cancers and were pathologically confirmed as adenocarcinoma with no preoperative treatments such as radiotherapy, chemotherapy or targeted therapy. The current study has been approved by the Medical Ethics Committee of Hebei General Hospital and informed consent was obtained from all participants.

Cell lines and reagents. HCT8, HCT116, SW620, SW480, LS-174T, HT29 human colon cancer cell lines were purchased from the Cell Resource Centre of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China), and passaged and preserved in Hebei General Hospital. Cells after 5-8 passages were used for the study. Rabbit anti-human polyclonal antibodies against CCDC34 (SAB1303750) were from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany), and antibodies against Bcl-2 (sc-783), survivin (sc-10811), E-cadherin (sc-7870), N-cadherin (sc-7939), MMP-9 (sc-10737) and β -actin (sc-8432) were all from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). The immunohistochemistry (IHC) kit was purchased from Fuzhou Maixin Biotechnology Co., Ltd. (Fuzhou, China). Culture medium Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and trypsin were purchased from Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Primers for qPCR and siRNA for CCDC34 were synthesized by Shanghai Sangon Biotech, Co., Ltd. (Shanghai, China). Lipofectamine 2000 reagent was obtained from Invitrogen; Thermo Fisher Scientific, Inc. Apoptosis detection kits containing Annexin V-FITC and PI were purchased from Wuhan Boster Biological Technology, Ltd., (Wuhan, China). Caspase-3 and -8 Activity Assay kits were obtained from EMD Millipore (Billerica, MA, USA).

IHC analysis. Sections were fixed with 4% neutral buffered formalin for 24 h, and cut from each paraffin tissue block of CRC with 4 μ m thickness, and antigen retrieval was performed according to following steps: Sections were immersed in citrate buffer (pH=6.0), and the pressure cooker antigen retrieval method was utilized for 4 min. Sections were removed, the buffer was allowed to come to room temperature and the sections were rinsed 3 times (5 min each time). They were subjected to the IHC procedure following the manufacturer's protocol and the results were examined by a professional pathologist. The number and the percentage of positive cells were calculated in 100 cells from randomly chosen 5 fields in each section using a TE2000-U microscope (x400; Nikon Corporation, Tokyo, Japan). The dilutions of antibodies were as following: CCDC34 (1:200), Bcl-2 (1:400), survivin (1:400), E-cadherin (1:400), N-cadherin (1:250), MMP-9 (1:400). The duration of each incubation step was performed at 37°C for 3 h according to the relevant manufacturer's instructions. The cells with yellow or brown

particles in the cytoplasm were considered to be positive for CCDC34, Bcl-2, survivin, E-cadherin, N-cadherin and MMP-9. The scoring was performed with the percentage of positive cells (0, 0-25%; 1, 25-50%; 2, 51-75%; 3, 76-100%) and intensity of staining (0, no staining; 1, pale yellow; 2, yellow; 3, brown). The percentage of positive cells and intensity of staining were added up to produce the result for each case: 0, Negative staining (-), 1-2 as mild staining (+), 3-4, moderate staining (++) , 5-6, intense staining (+++). A result of (-) was recorded as negative and results of (+), (++) , and (+++) were recorded as positive.

Cell culture. HCT8, HCT116, SW620, SW480, LS-174T, HT29 human colon cancer cell lines which were cultured in DMEM containing 10 % FBS and incubated at 37°C supplemented with 5% CO₂ was passaged every 2-3 days. The cells at logarithmic growth phase were used for the subsequent experiments. This procedure was repeated three times.

CCDC34-siRNA transfection and groups. The synthesized CCDC34-siRNA sequence was 5'-GCCUGAGAGGAAUGG AGUUTT-3' and negative control (NS) siRNA sequence 5'-UUCUCCGAACGUGUCACGUTT-3'. SW620 cells were transfected with various concentrations of CCDC34-siRNA or NS-siRNA using Lipofectamine 2000 and used for subsequent experiments after 48 h. The cells were assigned into CCDC34-siRNA group, NS-siRNA group and blank control group (treated only with Lipofectamine 2000 reagent).

MTT assay. Tumor single-cell suspensions were prepared and seeded in 96-well plate (10⁵ cells/well). MTT (20 μ l) solution (5 mg/ml) was added to each well 4 h prior the end of the experiment and then following a 4 h incubation at room temperature, the culture medium was removed and 150 μ l DMSO was added to each well. The absorbance values were determined following shaking at room temperature for 15 min at 490 nm using a microplate reader.

Cell apoptosis assay. The cells of all groups transfected for 48 h were collected by trypsinization after rinsed with PBS and the cell density was adjusted to 5x10⁵ cells/ml. Then, 500 μ l binding buffer was added to suspend cells and 10 μ l Annexin V-FITC or PI was added and mixed respectively. The cells were counted by FACS flow cytometry after incubation in dark for 15 min and subjected to calculate percentage of apoptotic cells.

Cell invasion ability assay. SW620 cells were seeded in 24-well plates with 10⁵ cells/well and treated as the different groups, which were transfected with CCDC34-siRNA or NS-siRNA. Transwell chambers were coated with 100 μ l Matrigel was treated with ultraviolet radiation. SW620 cells from each group were seeded in 200 μ l in the upper chamber with serum free DMEM, and DMEM medium containing 20% FBS was added to the lower chamber. After 24 h, Matrigel glue and extra SW620 cells in the upper chamber were wiped with cotton swabs. Methanol was utilized to fix the membranes for 10 min. The cells penetrating to the lower membrane were counted following crystal violet staining. Each experiment was repeated 3 times.

Table I. Primer sequences for reverse transcription-quantitative polymerase chain reaction.

Gene	Forward (5'-3')	Reverse (5'-3')
CCDC34	ACAGAAACAGGTGCGCTTACC	CAGCCGGTCACGTTCTTCTTT
Bcl-2	TGTGTGGAGAGCGTCAACC	TGGATCCAGGTGTGCAGGT
Survivin	GCCAGATTTGAATCGCGGGA	GCAGTGGATGAAGCCAGCCT
E-cadherin	CACTCGTCGCTGGATCTGTCA	CACAGCCTACTGCATGGCTCA
N-cadherin	GAGATCCTACTGGACGGTTCCG	TCTTGGCGAATGATCTTAGGA
MMP-9	AGAACCAATCTCACCGACAGG	CGACTCTCCACGCATCTCT
GAPDH	GACCCCTTCATTGACCTCAAC	CGTCTCTGGAAGATGGTGAT

CCDC34, coiled-coil domain containing 34; Bcl2, B cell leukemia/lymphoma 2; MMP-9, matrix metalloproteinase-9.

RT-qPCR. The cells were collected 48 h after transfection and total RNA was isolated using the TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RNA (2 μ g) was reverse transcribed to synthesize first-strand cDNA according to the instruction of Reverse Transcription System kit (Promega Corporation, Madison, WI, USA) according to following steps: 25°C for 5 min, 42°C for 60 min, 70°C for 15 min. cDNA templates (2 μ g) were amplified by qPCR using SYBR Green PCR kit (Promega Corporation) to establish a 25 μ l reaction system on an RT-qPCR machine. PCR reaction started with 1 cycle of 95°C for 5 min, followed by 45 cycles of 2 steps as 95°C for 30 sec, 72°C for 30 sec and 72°C for 5 min. The quantification cycle (Cq) (16) was obtained from the reaction curve and the expression of targeted genes was normalized to the housekeeping gene GAPDH. The primers used are listed in Table I.

Western blotting. Cell protein was extracted with cell lysis buffer (Sigma-Aldrich; Merck KGaA) 48 h after transfection and protein concentrations were determined by Bradford assay. Protein (40 μ g) was loaded and separated on a 12% SDS-PAGE gel and transferred to a polyvinylidene fluoride membrane. Membranes were blocked with 5% fetal bovine serum (Thermo Fisher Scientific, Inc.) in Tris-buffered saline with 0.05% Tween-20 (TBST) for 1 h at room temperature and incubated with primary antibodies of interest overnight at 4°C. The dilutions of antibodies were as following: CCDC34 (1:800), Bcl-2 (1:1,000), survivin (1:400), E-cadherin (1:800), N-cadherin (1:1,000), MMP-9 (1:400). After being washed with TBST 3 times, membranes were incubated with IgG secondary antibody (1:1,000, sc-2007; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature and subjected to color development. The absorbance of target bands was detected with an Odyssey Infrared Imaging System (9120; Li-COR Biosciences, Lincoln, NE, USA) to determine the relative expression intensity of the proteins.

Caspase-3 and -8 activity assays. The activity of caspase-3 and -8 was detected by spectrophotometry. Cells were collected, lysed for 20 min using the cold lysis buffer, and centrifuged for 10 min at 12,500 \times g. The supernatant was transferred to test the protein concentration. The analysis was performed according to the manufacturer's protocol of the activity detection kits. The absorbance values at the

wavelength of 405 nm were obtained from a microplate reader. The caspase activity was presented as caspase enzyme units in per unit cell protein.

Statistical analysis. Statistical analyses were performed with SPSS software version 19.0 (IBM Corp., Armonk, NY, USA). Chi-squared test, Mann-Whitney rank sum test, Spearman correlation, one-way analysis of variance and Dunnett's test were used for data analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

CCDC34 protein expression in CRC tissues and adjacent normal mucosa. The IHC results illustrated the CCDC34-positive rate in the 85 CRC tissues was 74.12% (63/85) and 28.33% (17/60) in paracancerous tissues. As presented in Fig. 1, the expression of CCDC34 was increased in CRC tissues compared with adjacent tissues ($\chi^2 = 29.810$, $P < 0.001$).

Association between CCDC34 protein expression and CRC clinical pathology parameters. Findings are presented in Table II and revealed that the protein expression of CCDC34 was related to invasion depth of the tumor, differentiation and metastasis in the lymph node. CCDC34-positive CRC tissues were characterized with deeper infiltrating of the tumor and higher positive rate of lymphatic metastasis ($P < 0.05$). No significant association was identified between CCDC34 protein expression and the remaining biological characteristics ($P > 0.05$).

Alteration of CCDC34 protein expression in SW620 cells post CCDC34-siRNA treatment. The protein expression levels of CCDC34 in SW620 cells transfected with CCDC34-siRNA for 48 h was detected with western blotting. As demonstrated in Fig. 2, CCDC34 protein expression in the CCDC34-siRNA transfected group was significantly lower than the negative control and blank control groups ($P < 0.05$), whereas there was no obvious difference between the NS-siRNA group and the blank control groups ($P > 0.05$).

Metabolic activity of SW620 cells following CCDC34-siRNA treatment. In Fig. 3, metabolic activity of SW620 cells transfected

Table II. Relationship between coiled-coil domain containing 34 protein expression and clinicopathological characteristics of patients with colorectal cancer.

Clinicopathological parameters	Positive (63)	Negative (22)	Total	χ^2	P
Sex					
Male	45	14	59	0.466	0.495
Female	18	8	26		
Age (years)					
≥ 60	18	9	27	1.145	0.285
< 60	45	13	58		
Tumor differentiation					
Well-differentiated	45	17	62	0.282	0.595
Poorly differentiated	18	5	23		
Depth of invasion					
Serosal infiltration	49	12	61	4.343	0.037
No serosal infiltration	14	10	24		
Lymphatic metastasis					
Positive	51	11	62	7.915	0.005
Negative	12	11	23		
TNM stages					
I	5	4	9	2.012	0.570
II	10	4	14		
III	43	12	55		
IV	5	2	7		
Distant metastasis					
Positive	5	2	7	0.029	0.865
Negative	58	20	78		

TNM, tumor-node-metastasis.

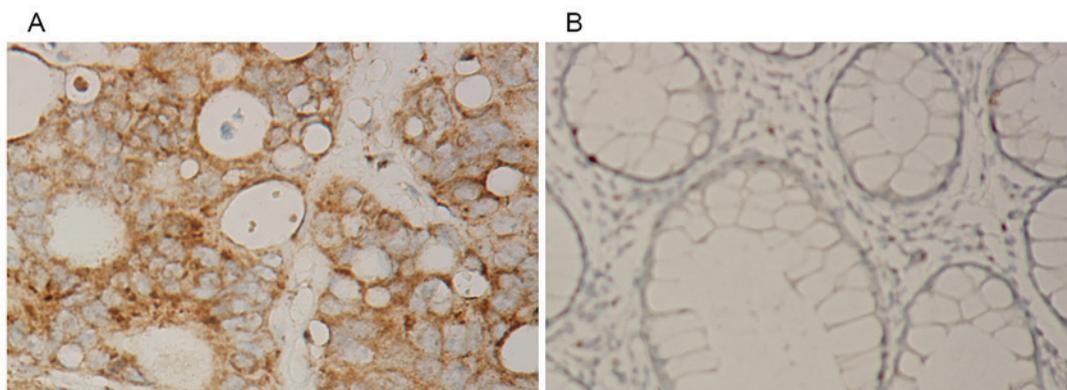


Figure 1. Expression of CCDC34 protein in CRC and adjacent normal mucosa tissues. A total of 85 paraffin tissues from patients with CRC were collected to determine CCDC34 protein expression using immunohistochemistry. Positive staining for CCDC34 protein was located in cell membrane and/or cytoplasm. Expression level of CCDC34 protein in (A) CRC tissues and (B) adjacent normal mucosa tissues. CCDC34, coiled-coil domain containing 34; CRC, colorectal cancer.

with CCDC34-siRNA for 48 h was 0.232 ± 0.031 , and was significantly lower compared with the NS-siRNA group (0.283 ± 0.029) and the blank group (0.306 ± 0.041 ; $P < 0.05$), whereas there was no difference observed between con-siRNA group and the blank control group ($P > 0.05$).

Apoptosis rate of SW620 cells post CCDC34-siRNA treatment. In Fig. 4, the apoptotic rate of SW620 cells transfected with CCDC34-siRNA was $28.62 \pm 4.57\%$ compared with $12.65 \pm 3.05\%$ in the NS-siRNA group and $11.26 \pm 2.26\%$ in the blank group. This indicated that the

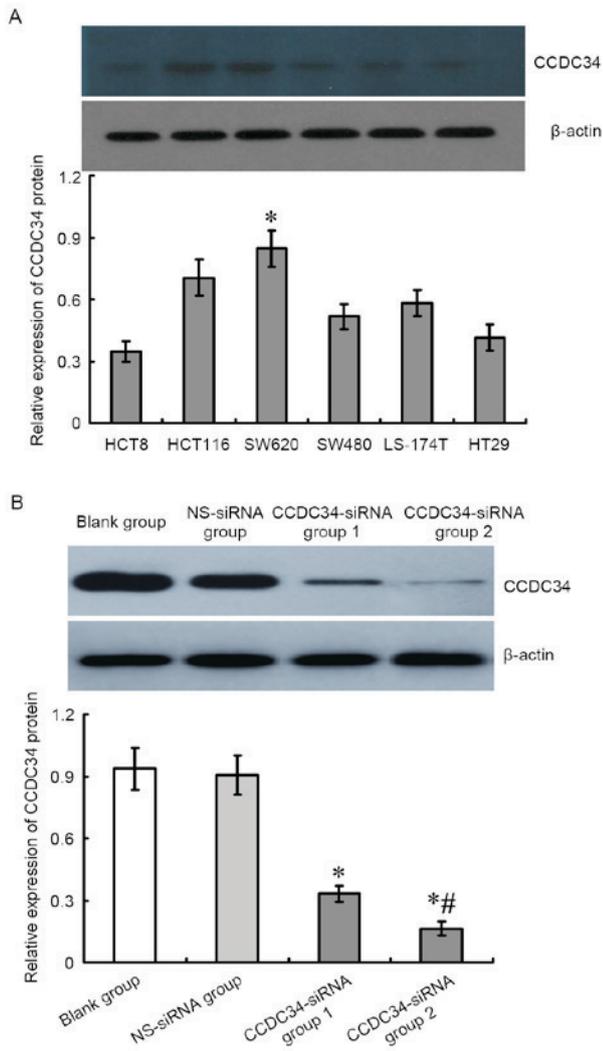


Figure 2. Different expression levels of CCDC34 protein in CRC cell lines and inhibitory effect of CCDC34-siRNA on CCDC34 expression in SW620 cells. (A) From the 6 CRC cell lines, the highest CCDC34 protein expression was detected in the SW620 cells; therefore, the SW620 cells were used for subsequent experiments. * $P < 0.05$ vs. HCT8, HCT116, SW480, LS-174T, HT29 cells. (B) CCDC34-siRNA was transfected into SW620 cells, CCDC34 expression level was significantly downregulated following CCDC34-siRNA transfection. Group 1, 20 $\mu\text{mol/l}$; Group 2, 80 $\mu\text{mol/l}$; * $P < 0.05$ vs. blank or NS-siRNA groups, # $P < 0.05$ vs. CCDC34-siRNA group 1. CCDC34, coiled-coil domain containing 34; NS, negative control; siRNA, small interfering RNA; CRC, colorectal cancer.

apoptotic rate of the CCDC34-siRNA group was evidently elevated when compared with the two other groups ($P < 0.01$).

Cell invasion ability of SW620 cells following CCDC34-siRNA treatment. As presented in Fig. 5, Transwell assay results highlighted the lower number of SW620 cells crossing the Transwell chamber membrane in the CCDC34-siRNA group (50.17 ± 6.15) compared with the NS-siRNA group (83.50 ± 6.47) and the blank group (85.67 ± 5.05 ; $P < 0.05$), whereas there was no obvious difference between the NS-siRNA and blank groups ($P > 0.05$).

Alteration of the expression of apoptosis-associated Bcl-2 and caspase-3, -8 activity in SW620 cells following CCDC34-siRNA treatment. In Fig. 6A-C, mRNA and protein

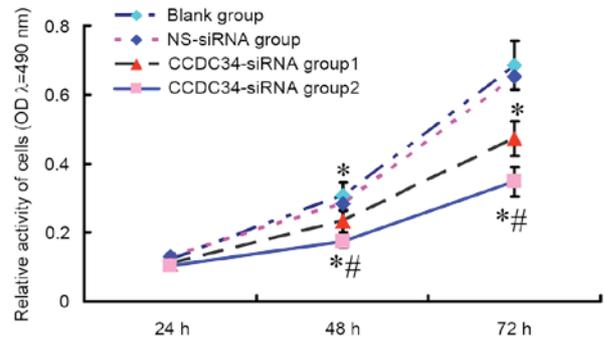


Figure 3. Effect of CCDC34-siRNA on the metabolic cell activity of SW620 cells. The metabolic activity of SW620 cells varied with different concentrations and time following CCDC34-siRNA transfection. Compared with NS-siRNA group and blank group, activity of SW620 cells in CCDC34-siRNA group decreased significantly in the time-dependent manner. Group 1, 20 $\mu\text{mol/l}$; Group 2, 80 $\mu\text{mol/l}$; * $P < 0.05$ vs. blank or NS-siRNA groups, # $P < 0.05$ vs. CCDC34-siRNA group 1. CCDC34, coiled-coil domain containing 34; NS, negative control; siRNA, small interfering RNA; OD, optical density.

expression levels of Bcl-2 and survivin were significantly decreased in cells of CCDC34-siRNA group when compared with the NS-siRNA and blank groups ($P < 0.05$). No obvious difference was identified between the NS-siRNA group and the blank group ($P > 0.05$). Additionally, the activity of caspase-3 and -8 in cells of CCDC34-siRNA group was higher compared with the control groups ($P < 0.05$), whereas there was no obvious difference between the NS-siRNA and blank groups ($P > 0.05$; Fig. 6D).

Alteration of the expression of invasion-associated genes in SW620 cells following CCDC34-siRNA treatment. As presented in Fig. 7, western blotting and RT-qPCR revealed mRNA and protein expression of E-cadherin was significantly increased in cells of the CCDC34-siRNA group compared with the control groups ($P < 0.05$), whereas that of N-cadherin and MMP-9 were significantly reduced ($P < 0.05$). No obvious difference between the NS-siRNA group and the blank group was identified ($P > 0.05$).

Discussion

CRC is one of the most common cancers globally (17). Although the treatments have improved in terms of surgical technique (18), radiotherapy (19), chemotherapeutics and targeted drugs for patients CRC (20,21), the overall therapeutic efficacy is unsatisfactory with a high mortality rate, which leads to suffering patients and also a considerable burden to society (22). Therefore, investigating the pathogenesis and developing novel therapies have been hotspots in CRC research. Recurrence and metastasis are primary causes of death for CRC patients; therefore, methods blocking this process may potentially improve CRC treatment. Previous studies have reported that CRC cells which have high anti-apoptotic and invasion abilities are prone to rapid progression and metastasis (23,24). The anti-apoptosis and invasion of CRC cells may be due to the combined effects of multiple genes, and considering that, it is important to identify the key genes relevant to this process. Some genes have been previously identified associated with

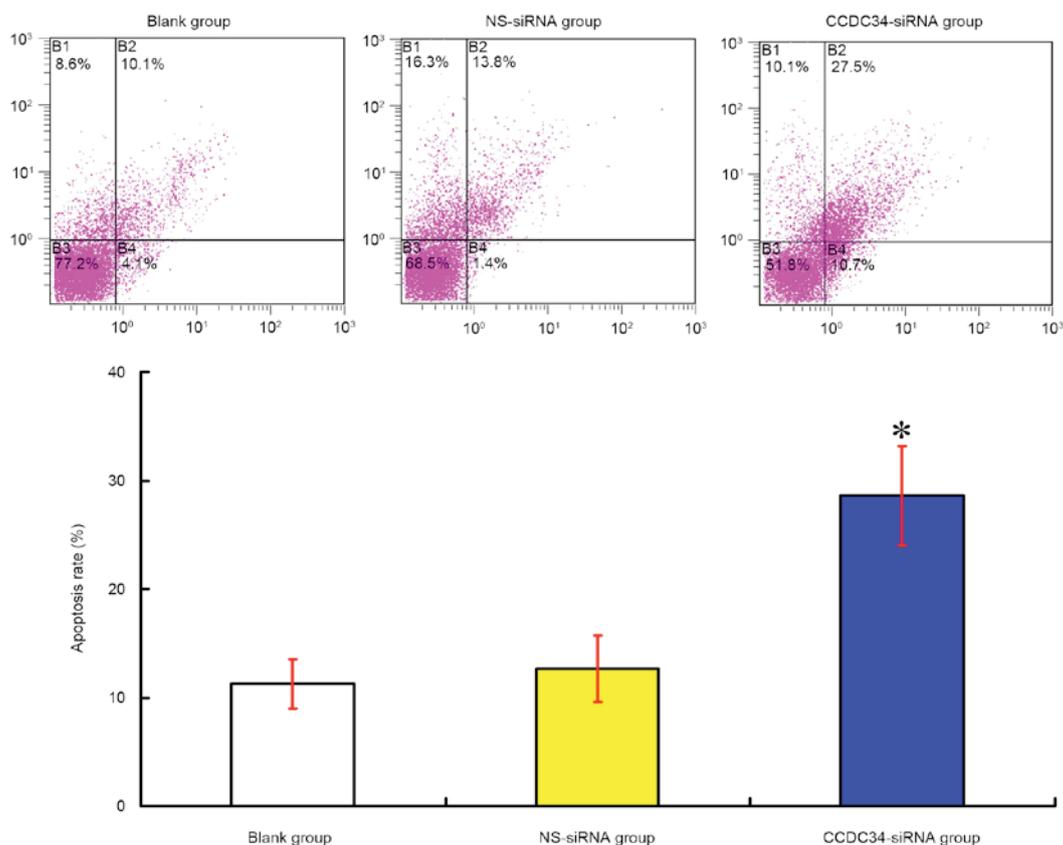


Figure 4. Effects of CCDC34-siRNA on apoptosis of SW620 cells. SW620 cells were transfected with CCDC34-siRNA and were then subjected to flow cytometry to detect the apoptotic rate and the apoptotic rate of SW620 cells was increased after CCDC34-siRNA transfection. * $P < 0.05$ vs. blank or NS-siRNA groups. CCDC34, coiled-coil domain containing 34; NS, negative control; siRNA, small interfering RNA.

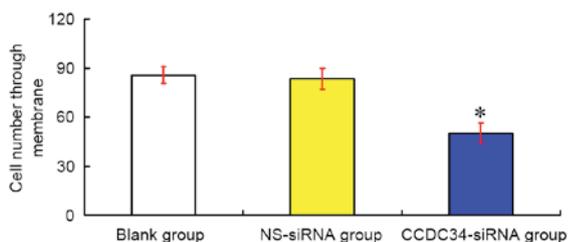


Figure 5. Effects of CCDC34-siRNA on invasion of SW620 cells. SW620 cells were transfected with CCDC34-siRNA and then were subjected to Transwell assay to detect cell invasion activity. Invasion of SW620 cells was decreased after CCDC34-siRNA transfection. * $P < 0.05$ vs. blank or NS-siRNA groups. CCDC34, coiled-coil domain containing 34; NS, negative control; siRNA, small interfering RNA.

anti-apoptosis and invasion of CRC cells; however, specific mechanisms remain to be elucidated. CCDC, a protein with a coiled-coil structure, has the functions of metabolism regulation, cell membrane channel and molecular chaperone (25,26). The association between some members of the CCDC family and cancers has been investigated previously. Gong *et al* (15) revealed that abnormal high expression of CCDC34, a member of CCDC family, was detected in bladder cancer cells, and suppression of CCDC34 contributed to the reduced proliferation and invasion and increased apoptosis of cancer cells. The present study observed high expression of CCDC34 in CRC tissues, particularly in tissues with deep tumor invasion and lymphatic metastasis. This infers that CCDC34 contributed to

CRC progression and metastasis, and detection of CCDC34 in CRC tissues may provide information to evaluate patients' condition. Anti-apoptosis, invasion and metastasis of cancer cells have important roles in progression of CRC. As CRC cells have anti-apoptotic ability, previous studies regarding CRC treatment are focused on how to suppress anti-apoptotic ability of CRC cells (27,28). In addition, considering CRC cells have strong invasive and metastatic ability, suppression of these abilities may control tumor development (29,30). In the *in vitro* experiments in the present study, reduced cell metabolic activity, increased apoptotic rate and decreased invasion were observed following the suppression of the expression of CCDC34 in the SW620 cell line, which may indicate that CCDC34 may have the ability to regulate cell apoptosis and invasion. In order to determine the role of CCDC34 in apoptosis and invasion of CRC cells, the expression levels of apoptosis and invasion-associated genes in CRC cells were detected following the suppression of CCDC34 expression and the role of CCDC34 in CRC was investigated.

Bcl-2 is an important gene that regulates apoptosis through the mitochondrial pathway and it is able to suppress cell apoptosis in various ways (31,32). Survivin, a member of inhibitor of apoptosis proteins family, is able to suppress apoptosis by suppressing caspase-3 and -8 activity which are apoptosis promoting molecules (33,34). In the current study, reduced expression of Bcl-2 and survivin was detected in the SW620 cell line following CCDC34 inhibition, whereas the activity of caspase-3 and -8 was increased. This suggested that CCDC34

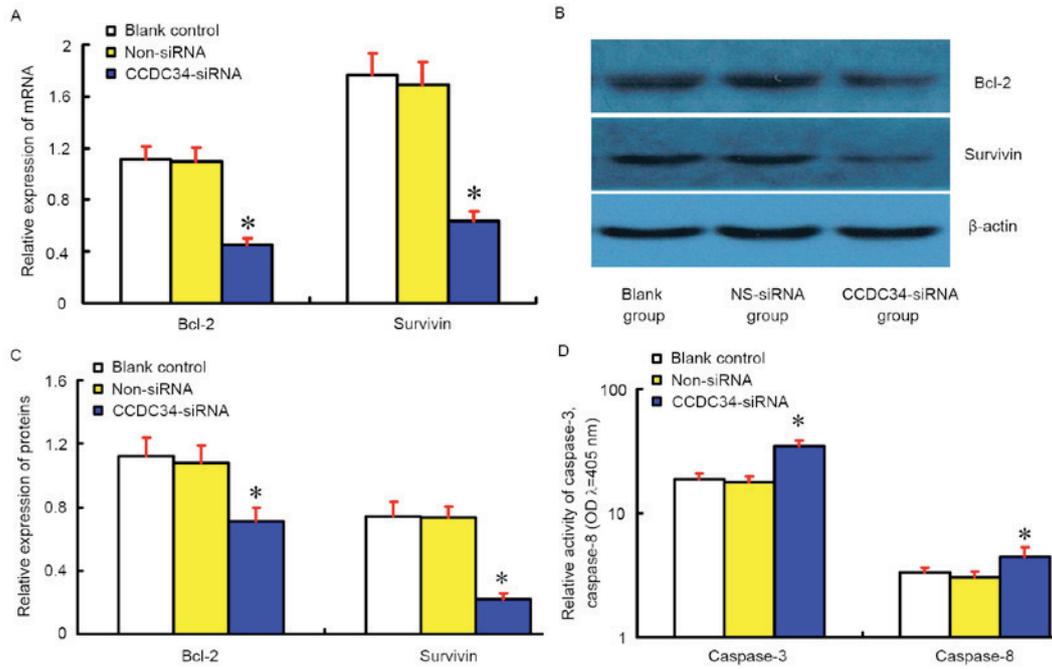


Figure 6. Effect of CCDC34-siRNA on the mRNA expression levels of Bcl-2, survivin, and caspase-3 and 8 activity in SW620 cells. SW620 cells were transfected with CCDC34-siRNA and were then subjected to (A) reverse transcription-quantitative polymerase chain reaction and (B and C) western blotting, to detect the mRNA and protein expression levels of Bcl-2 and survivin, respectively. (D) The activity of caspase-3 and -8 increased significantly after CCDC34-siRNA transfection. * $P < 0.05$ vs. blank or NS-siRNA groups. CCDC34, coiled-coil domain containing 34; NS, negative control; siRNA, small interfering RNA; Bcl-2, B cell leukemia/lymphoma 2.

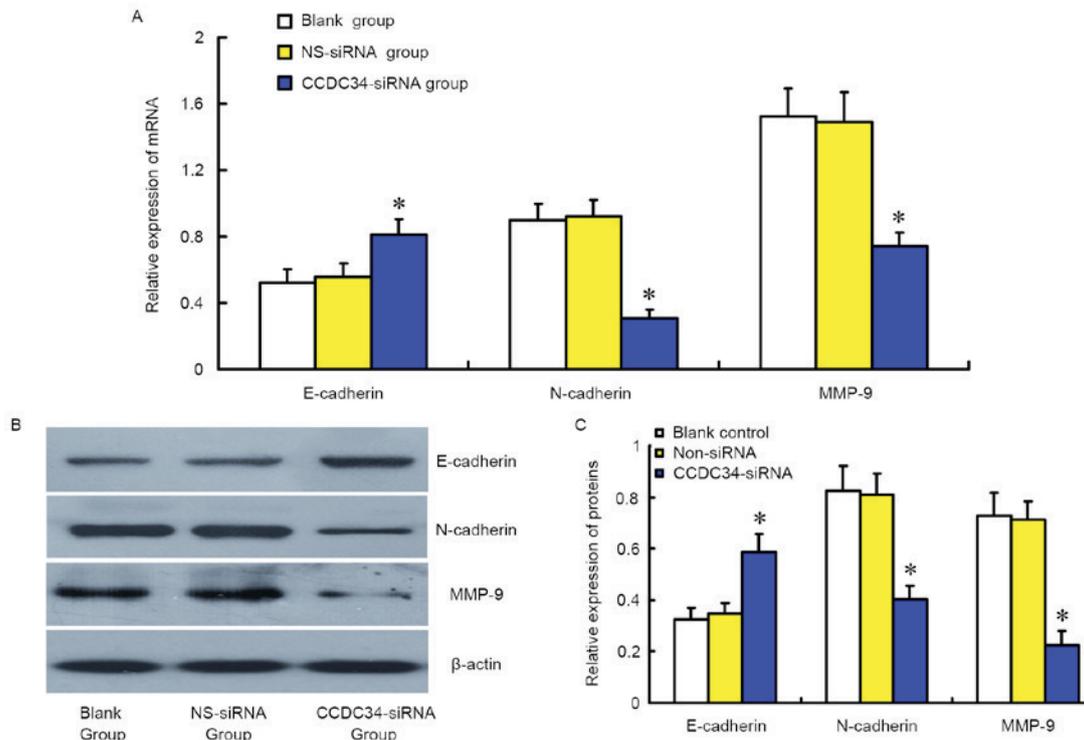


Figure 7. Effect of CCDC34-siRNA on the expression levels of E-cadherin, N-cadherin and MMP-9 in SW620 cells. SW620 cells were transfected with CCDC34-siRNA and were then subjected to (A) reverse transcription-quantitative polymerase chain reaction and (B and C) western blotting. E-cadherin expression was increased, whereas N-cadherin and MMP-9 decreased in SW620 cells. * $P < 0.05$ vs. blank or NS-siRNA groups. CCDC34, coiled-coil domain containing 34; NS, negative control; siRNA, small interfering RNA; MMP-9, matrix metalloproteinase-9.

increased apoptosis resistance by activating Bcl-2 and survivin and suppressing caspase-3 and caspase-8.

Epithelial-mesenchymal transition (EMT) has been identified to participate in cancer invasion and metastasis (35).

In the current study, the changes of EMT-associated genes in SW620 cell line following suppression of CCDC34 was also detected. E-cadherin, a transmembrane glycoprotein in epithelial cells, is essential for cell junction and integrity of structure (36,37). Previous studies have revealed that the downregulation of E-cadherin expression may trigger the invasion and expansion of basement membrane, which may lead to tumor invasion and metastasis (38,39). N-cadherin is one of the important mesenchymal markers and its upregulated expression is the hallmark of EMT, as well as an indicator of tumor invasion and metastasis (40,41). MMP-9, one of the important members of the MMPs family is involved in the degradation of extracellular matrix and contribution to metastasis in tumors (7,8,42,43) and regulated by E-cadherin (44). The present study determined that E-cadherin expression was significantly increased following the inhibition of the endogenous CCDC34 expression by RNA interference, whereas expression of N-cadherin and MMP-9 was decreased. This indicates that CCDC34 is involved in CRC EMT, which may lead to cancer invasion and metastasis by suppressing E-cadherin and promoting N-cadherin and MMP-9. However, the corresponding molecular mechanisms should be further clarified by future studies.

In conclusion, the present study demonstrated increased expression of CCDC34 protein in CRC tissues was associated with reduced apoptosis and increased metastasis in CRC cell line. CCDC34 may promote anti-apoptosis and invasion by regulating Bcl-2, survivin, E-cadherin, N-cadherin and MMP-9. However, the sample size in the present study was limited and the *in vitro* experiments are insufficient. Despite the limitations, it may be concluded that CCDC34 had an important role in CRC invasion and metastasis. Further investigation of the functions of CCDC34 may be beneficial to CRC evaluation and CCDC34 may also be regarded as the target gene for controlling CRC progression and metastasis.

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