European Review for Medical and Pharmacological Sciences 2018; 22: 118-126

Silencing of hsa_circ_0007534 suppresses proliferation and induces apoptosis in colorectal cancer cells

R. ZHANG[2], J. XU[2], J. ZHAO[2], X. WANG[1]

[1]Department of Ophthalmology, the Fourth Affiliated Hospital, China Medical University, China
[2]Department of Colorectal Surgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital and Insititute, Dadong District, Shenyang, Liaoning, China

Abstract. – OBJECTIVE: Although some circRNAs have been found to regulate the progression of colorectal cancer (CRC). However, their functions have not been completely clarified. In the present study, a novel circRNA hsa_circ_0007534 was investigated in CRC tumor tissues and cell lines.

PATIENTS AND METHODS: The expression profile of circRNAs in CRC tumor tissues was performed by human circRNA microarray. The CCK-8 and apoptosis assay were measured in CRC cell lines after transfected with si-circRNA and si-NC.

RESULTS: We analyzed a cohort of 33 patients with CRC and demonstrated that the expression of hsa_circ_0007534 was significantly up-regulated in CRC tumor tissues as compared to adjacent non-tumorous tissues. Moreover, hsa_circ_0007534 expression was correlated with tumor stage and lymph node metastasis. Furthermore, the silence of hsa_circ_0007534 by siRNA significantly inhibited proliferation and induced apoptosis of CRC cells.

CONCLUSIONS: Hsa_circ_0007534 plays a crucial role in the initiation and progression of CRC and may be a potential therapeutic target of CRC.

Key Words:
hsa_circ_0007534, Non-coding RNA, Colorectal cancer, Cell apoptosis.

Introduction

Colorectal cancer (CRC) is the fourth most common malignancy and accounts for approximately 9%, and the incidence of CRC is more than 376,000 and corresponding to about 191,000 death incidents in China by 2015[1]. Although there are mounting progresses in clinical treatment for CRC, the overall survival time has not markedly improved in recent years[2]. The important reason is that the molecular and genetic basis of CRC carcinogenesis has not been clearly elucidated. Therefore, it is necessary to explore the pathogenesis of CRC to improve the diagnosis and treatment of CRC.

Although circular RNAs (circRNAs) were first reported as endogenous RNA splicing products, which were considered byproducts of splicing errors, in 1979, they received little attention until recent years[3,4]. With the development of microarray and high-throughput RNA sequencing (RNA-Seq), more than 20,000 circRNAs have been identified in eukaryotes[5]. They are classified into four major categories: exons, introns, exon-intron or intergenic splicing[6]. CircRNAs perform a wide variety of biological functions in eukaryotic cells by acting competing endogenous RNAs (ceRNAs) or miRNA sponges, interacting with RNA binding proteins, modulating the stability of mRNAs, regulating gene transcription and translating proteins[7-11]. Recent studies[12,13] have shown that circRNAs play a crucial role in the initiation and progression of malignancies. Notably, Bachmayr-Heyda et al[14] found that differentially expressed circRNAs have been confirmed in CRC tissues. Subsequently, Xie et al[15] suggested that hsa_circ_001569 is significantly up-regulated in human CRC tissues and promotes cell proliferation and invasion in CRC. In addition, Zhang et al [16] also showed that hsa_circ_0020397 promotes cell viability and invasion of CRC cells and inhibits their apoptosis. These findings suggest that circRNAs play a major role in tumorigenesis.

In the present work, we examined the expression profile of circRNAs in CRC tissues with microarray and found that hsa_circ_0007534 was significantly overexpressed. Hsa_circ_0007534 is located in chr17: 61869771-61877977, the spliced sequence length is 400 nt, and its associated-gene

Corresponding Author: Xinling Wang, MD; e-mail: wxinling@126.com

symbol is DDX42 (DEAD-box helicase 42; circBase database, http://www.circbase.org/). However, the relationship between the expression level of hsa_circ_0007534 and CRC is unclear. Therefore, we down-regulated hsa_circ_0007534 levels in CRC cell lines and performed cell proliferation and apoptosis experiments to elaborate its function *in vitro*.

Patients and Methods

Patients and Specimens

Thirty-three pairs of CRC tumor tissues and matched adjacent non-tumorous tissues were collected from patients who had undergone surgery at the Department of Colorectal Surgery, Cancer Hospital of China Medical University (Shenyang, China) between Jan 2014 and June 2016. All of the patients were not subjected to preoperative radiotherapy or chemotherapy and diagnosed with CRC based on the histopathological evaluation. All collected tissue samples were immediately stored in liquid nitrogen. Human samples were obtained with written informed consent from all patients. The study was approved by the Ethics Committee of the Department of colorectal surgery, Cancer Hospital of China Medical University (Shenyang, China).

Cell Culture

FHC, a normal human colon mucosal epithelial cell line (American Type Culture Collection, ATCC, Manassas, USA), and five CRC cell lines (SW620, HCT116, LoVo, SW480, and HT29) were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin at 37°C in 5% CO_2.

Human circRNA Microarray Analysis

Total RNA was extracted from three pairs of CRC tumor tissues and matched adjacent non-tumorous tissues. CircRNA was enriched with RNase R to digest linear RNA (Epicentre, Madison, WI, USA). RNA was labeled with Arraystar Human circRNA Array (8×15 K, Arraystar, Rockville, MD, USA) and was scanned using an Agilent Scanner G2505C (Jamul, CA, USA).

CCK-8 Proliferation Assay

The CCK-8 proliferation assay of si-RNA transfected CRC cells was determined as previously described[17]. Cell (1×10^4) proliferation was measured using CCK-8 Cell Proliferation/Viability Assay Kit (Dojindo Kumamoto, Japan).

Cell Apoptosis and Caspase-3 Activity Assay

The cell apoptosis and caspase-3 activity assay were determined as previously described[17,18]. Annexin V-FITC apoptosis detection kit and caspase-3 activity assay kit were purchased from Invitrogen (Carlsbad, CA, USA) and Beyotime (Beyotime Institute of Biotechnology, Haimen, China), respectively.

Cell Transfection

The small interfering RNA (si-RNA) was utilized for cell transfection and was synthesized by GenePharma Co., Ltd. (Shanghai, China). The targeted sequence of the functional si-hsa_circ_0007534 was 5'-GATCATTCAGAGCTATTTTGA-3'. SW620 and LoVo cells were transfected with si-hsa_circ_0007534 for 48 h at 37°C using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR)

RNA was extracted using TRIzol (Invitrogen; Thermo Fisher Scientifc, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Moloney Murine Leukemia Virus reverse transcriptase (Promega Corporation, Madison, WI, USA) was used to synthesize cDNA. Divergent primers were designed to amplify the head-to-tail splicing of circRNA using ABI7300 System (Applied Biosystems, Foster City, CA, USA) with SYBR Select Master Mix (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utilized to normalize the expression of the circRNAs. The PCR primers were used in this study as follows: hsa_circ_0007534, forward 5'-GTGACGGAAATCCAATTGCACC-3' and reverse 5'-ATGGAATTGCTGGCGAGTTG-3'; GAPDH, forward 5'-GCACCGTCAAGCTGAGAAC-3' and reverse 5'-TGGTGAAGACGCCAGTGGA-3'. The relative expression levels of circRNAs and RFC3 were calculated using the $2^{-\Delta\Delta Cq}$ method[19].

Western Blotting

Protein was extracted using RIPA Lysis Buffer (Beyotime Institute of Biotechnology, Hai-

men, China). The concentration was determined using the Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich, St Louis, Mo, USA); Merck KGaA, Darmstadt, Germany). Samples containing 50 μg of protein were separated by 10% SDS-PAGE gel and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Primary antibodies Bcl-2 (cat.no: sc-56015, dilution, 1:1,000) and BAX (cat.no: sc-6236, dilution, 1:1,000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (cat.no: sc-516102; dilution: 1:10,000; Santa Cruz Biotechnology), following visualized using chemiluminescence (Thermo Fisher Scientific, Inc.). α-tubulin (cat.no: sc-134237; dilution: 1:2,000; Santa Cruz Biotechnology) was used to as the control antibody.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Student's *t*-test was used to analyze two-group differences. Inter-group differences were analyzed by one-way analysis of variance, followed by a post-hoc Tukey test for multiple comparisons. Fisher χ^2-test was used to evaluate for categorical variables. $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Differential Expression of circRNAs in CRC Tissues

Three pairs of CRC tissues and adjacent non-tumorous tissues were collected and performed the circRNA expression profiles using human circRNA microarray. Differentially expressed circRNAs were selected by $p < 0.01$ and fold change ≥ 2 or fold change ≤ -2. The results revealed twenty-four significantly differentially expressed circRNAs, eight of them up-regulated and sixteen down-regulated in tumor tissues (Table I). Moreover, the gene annotation of these circRNAs was summarized, including gene symbols, spliced length and position according to the circBase database (http://www.circbase.org). Based on the fold change of circRNAs in tumor tissues, hsa_circ_0007534 was selected to focus on our study. To further validate the expression levels of hsa_circ_0007534, RT-qPCR was performed to measure hsa_circ_0007534 expression in thirty-three pairs of CRC tissues and adjacent non-tumorous tissues. The divergent primer was designed to detect the expression of hsa_circ_0007534, and the result indicated that hsa_circ_0007534 was significantly overexpressed in CRC tissues compared with non-tumorous tissues, and the average upregulation fold was 2.77 (Figure 1A).

We next evaluated the association between hsa_circ_0007534 and clinical-pathological parameters. As shown in Table II, tumor stage and lymph node metastasis were significantly associated with the expression level of hsa_circ_0007534. We found that patients with tumor stage III-IV showed higher hsa_circ_0007534 levels than patients with tumor stage I-II (stage III-IV *vs.* stage I-II: 3.08 *vs.* 1.94; $p = 0.002 < 0.01$; Figure 1B). In addition, patients with lymph node metastasis had a significant increase in hsa_circ_0007534 as compared to patients with no lymph node meta-

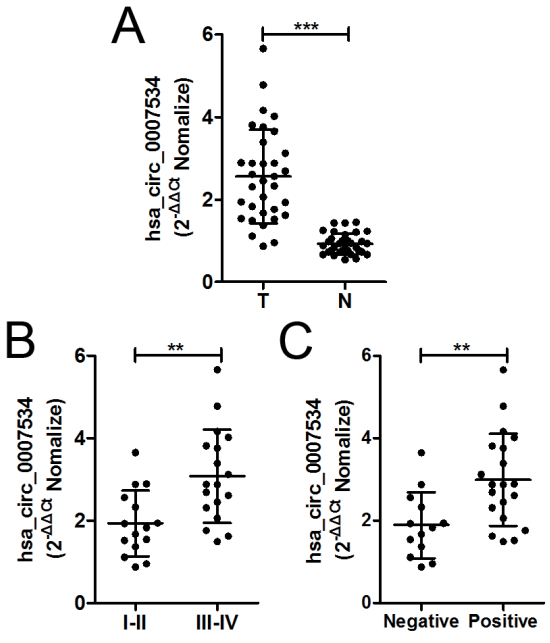

Figure 1. Correlation between hsa_circ_0007534 expression and clinical parameters. Hsa_circ_0007534 expression in the thirty-three pairs of CRC tumor tissues and matched adjacent non-tumorous tissues was measured by RT-qPCR ***(A),*** Correlation between hsa_circ_0007534 expression and clinical stage in thirty-three patients with CRC ***(B),*** Correlation between hsa_circ_0007534 expression and lymph node metastasis in thirty-three patients with CRC ***(C),*** $^{**}p < 0.01$; $^{***}p < 0.001$.

Table I. Differentially expressed circRNAs and gene annotation.

circRNA	p-value	Log2FC	Regulation	Gene symbol	Spliced length	Position
hsa_circ_0007534	3.45E-05	4.34	up	DDX42	400	chr17:61869771-61877977
hsa_circ_0103973	1.20E-06	3.56	up	ZNF280D	663	chr15:56970861-56981668
hsa_circ_0100731	4.25E-03	3.07	up	NEK3	486	chr13:52722549-52728308
hsa_circ_0100748	5.72E-06	2.85	up	None	147	chr13:54937267-54937414
hsa_circ_0001569	6.91E-04	2.49	up	IRF4	271	chr6:393097-393368
hsa_circ_0001959	8.34E-04	2.31	up	ATG7	3792	chr3:11396894-11468400
hsa_circ_0002086	1.08E-04	2.27	up	CLEC16A	692	chr16:11114049-11145498
hsa_circ_0100499	3.87E-07	2.08	up	DGKH	2455	chr13:42742575-42795530
hsa_circ_0102478	6.34E-08	-5.62	down	RP11-1012A1.4	221	chr14:68129193-68129907
hsa_circ_0002111	5.78E-07	-5.13	down	PSD3	538	chr8:18622958-18662408
hsa_circ_0104220	2.89E-05	-4.66	down	TRIP4	405	chr15:64710739-64717830
hsa_circ_0020397	5.17E-06	-4.47	down	DOCK1	2738	chr10:128768965-128926028
hsa_circ_0104851	6.12E-04	-4.23	down	TCONS_00023192	284	chr15:89931047-89932713
hsa_circ_0102442	2.65E-03	-4.03	down	TCONS_00022532	120	chr14:66584537-66584657
hsa_circ_0100236	1.87E-04	-3.75	down	FRY	500	chr13:32731409-32745407
hsa_circ_0001072	3.65E-05	-3.41	down	GTDC1	267	chr2:144966169-144969146
hsa_circ_0104215	6.17E-07	-3.29	down	CSNK1G1	7996	chr15:64568727-64576723
hsa_circ_0102810	7.91E-06	-3.17	down	EML5	1735	chr14:89171825-89221015
hsa_circ_0103890	3.82E-04	-3.12	down	ARPP19	595	chr15:52850476-52851071
hsa_circ_0000996	5.27E-05	-2.95	down	CAMKMT	3042	chr2:44729827-44732869
hsa_circ_0001498	4.17E-06	-2.76	down	WDR41	181	chr5:76758919-76760634
hsa_circ_0102001	6.37E-05	-2.58	down	SOS2	981	chr14:50641171-50671127
hsa_circ_0104683	3.29E-04	-2.34	down	TCONS_00023483	10068	chr15:81701455-81711523
hsa_circ_0103984	2.17E-07	-2.13	down	ZNF280D	661	chr15:57119721-57209892

stasis (positive *vs.* negative: 2.99: 1.89; $p = 0.010$; Figure 1C). Furthermore, the expression of hsa_circ_0007534 was not correlated with other clinical factors such as age, gender, tumor size and location (Table II). Overall, these findings validated that hsa_circ_0007534 expression was upregulated in CRC tumor tissues and was correlated with tumor stage and lymph node metastasis.

Expression of hsa_circ_0007534 in CRC Cell Lines

To investigate its function *in vitro*, we first examined the expression of hsa_circ_0007534 in CRC cell lines by RT-qPCR. The expression of hsa_circ_0007534 was significantly up-regulated in all of the CRC cell lines when normalized to FHC (Figure 2A). We found hsa_circ_0007534 was most upregulated in SW620 and LoVo cells. Therefore, SW620 and LoVo cells were selected as our experimental cell lines. Next, we designed siRNA to inhibit hsa_circ_0007534 expression. RT-qPCR results showed that si-circ_0007534 had remarkable inhibition efficacy of hsa_circ_0007534 expression in SW620 and LoVo cells (Figure 2B).

Silencing of hsa_circ_0007534 Suppresses Proliferation and Induces Apoptosis in CRC Cell Lines

To assess the effect of hsa_circ_0007534 on the proliferation of CRC cells, cell lines SW620 and LoVo were transfected with si-circ_0007534

Table II. The association between hsa_circ_0007534 expression levels and clinical physiological and pathological parameters.

Variables		hsa_circ_0007534		*p*-value
		Low	High	
Age	> 60	6	11	0.728
	≤ 60	7	9	
Gender	Male	8	12	0.714
	Female	5	8	
Tumor size (cm)	> 5	4	10	0.310
	≤ 5	9	10	
Location	Colon	5	11	0.481
	Rectum	8	9	
Tumor stage	I-II	10	5	0.005
	III-IV	3	15	
Lymph node metastasis	Negative	9	4	0.010
	Positive	4	16	

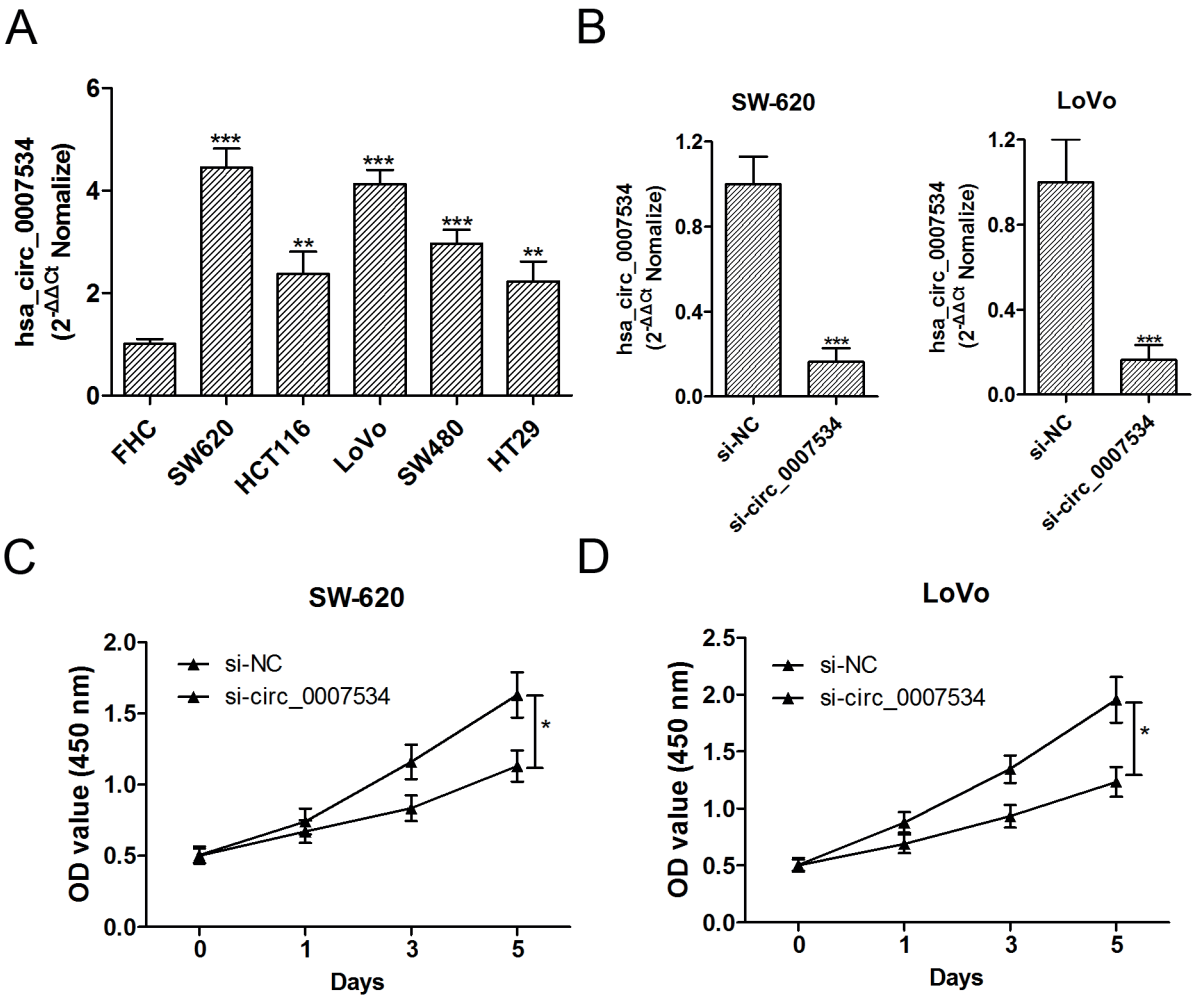

Figure 2. Silencing of hsa_circ_0007534 suppressed CRC cells proliferation. The levels of hsa_circ_0007534 expression were carried out using RT-qPCR among FHC, a normal human colon mucosal epithelial cell line, and five CRC cell lines (SW620, HCT116, LoVo, SW480 and HT29) ***(A)***. After transfected with si-circ_0007534, hsa_circ_0007534 expression was performed by RT-qPCR in SW620 and LoVo cell lines ***(B)***. After transfected with si-circ_0007534, SW620 ***(C)*** and LoVo ***(D)*** cells proliferation were monitored by CCK-8 assay at day 1, day 3 and day 5. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. n= 3 in each group.

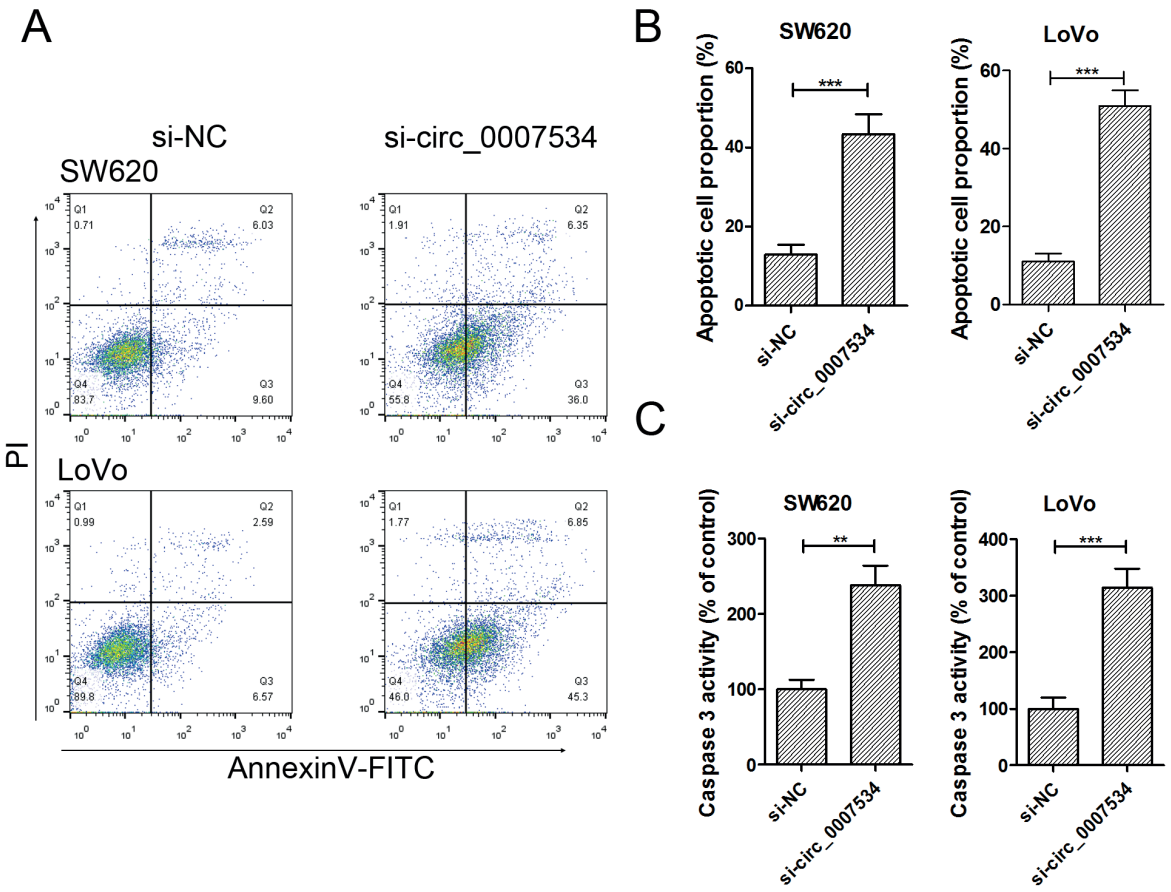

Figure 3. Silencing of hsa_circ_0007534 induced CRC cells apoptosis. Apoptosis analysis was detected by flow cytometry in SW620 and LoVo cells transfected with si-circ_0007534 or si-NC ***(A*** and ***B)***. Transfected with si-circ_0007534 significantly increased caspase-3 levels in SW620 and LoVo cells ***(C)***. $^{**}p < 0.01$; $^{***}p < 0.001$. n= 3 in each group.

or negative control (si-NC). Results of CCK-8 assay showed that knockdown of hsa_circ_0007534 significantly inhibited the proliferation of both SW620 and LoVo cell lines compared with the control group (Figure 2C and 2D). We then performed flow cytometry analysis to further evaluate whether hsa_circ_0007534 regulates CRC cells proliferation by altering apoptosis. The rate of apoptosis was significantly increased when SW620 and LoVo cells were transfected with si-circ_0007534 (Figure 3A and 3B). Moreover, the caspase-3 activity assay was performed following transfection of the SW620 and LoVo cells with si-circ_0007534 or si-NC for 48 h. The results indicated that the activity of caspase-3 in the si-circ_0007534 group was significantly higher, compared with that of the si-NC group (Figure 3C). Furthermore, apoptosis-associated proteins were measured using western blot analysis in SW620 and LoVo cells transfected with si-circ_0007534 or si-NC for 48 h. Bcl-2 is an anti-apoptotic gene, and Bax is a proapoptotic gene, their ratio is a profound indicator of cell survival, which can be altered either by increased Bcl-2 expression or decreased BAX expression[20]. In si-circ_0007534 transfected SW620 and LoVo cells, the Bcl-2/Bax ratio was significantly decreased as compared to control group (Figure 4A and 4B). Based on above findings, we demonstrate that hsa_circ_0007534 inhibits CRC cells proliferation, at least partly, by inducing apoptosis.

Discussion

The cumulative evidence indicates that circRNAs play an important role in the initiation and development of gastric cancer[21], breast cancer[17], hepatocellular carcinoma[22] and lung adenocarcinoma[23]. As a novel gene regulator, circRNAs play a regulatory function primarily through harboring miRNAs (known as miRNA spon-

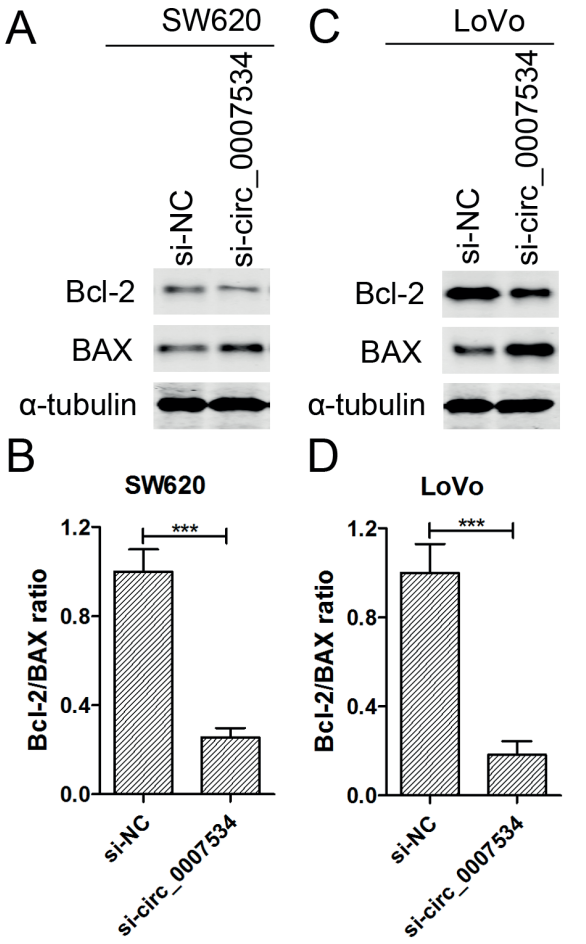

Figure 4. Silencing of hsa_circ_0007534 regulated apoptosis-related protein expression. After transfected with si-circ_0007534, the protein expression of Bcl-2 and BAX was measured by western blotting in SW620 ***(A)*** and LoVo ***(C)*** cells, and Bcl-2/BAX ratio in was calculated in SW620 (B) and LoVo (D) cells. $^{***}p < 0.001$. n= 3 in each group.

ges)[3]. Attributed to their conservative property and stability, miRNAs are also considered as ideal biomarkers in the diagnosis of cancers[3,6,15]. Currently, approximately 10 studies have examined the correlation between circRNA expression and CRC cells proliferation, migration, invasion, apoptosis, and chemoradiation resistance[24,25].

In our study, we identified a new circRNA hsa_circ_0007534 and hypothesized that it is related to CRC cells proliferation, apoptosis and the development of CRC. In fact, we found that hsa_circ_0007534 was significantly up-regulated in CRC tumor tissues and cell lines. We also showed that the overexpression of hsa_circ_0007534 was related to tumor stage and lymph node metastasis. Therefore, we assume that hsa_circ_0007534 has a high potential for assessing the risk of CRC and can serve as a biomarker of CRC detection. Although there is no correlative literature to elaborate the clinical diagnosis value of circRNAs in CRC, hsa_circ_0013958, hsa_circ_0004277, and hsa_circ_0005075 are used as a new biomarker for lung adenocarcinoma, acute myeloid leukemia, and hepatocellular carcinoma, respectively[23,26,27].

We observed that silencing of hsa_circ_0007534 suppressed proliferation and induced apoptosis of CRC cell lines *in vitro*. Therefore, we suggest that hsa_circ_0007534 inhibits the proliferation of CRC cells by accelerating cell apoptosis. Recent studies[16,24] indicate that circRNAs are associated with CRC cells proliferation and apoptosis. For example, hsa_circ_0020397 regulates colorectal cancer cell viability and apoptosis by promoting the expression of miR-138 target genes[16]. The up-regulation of hsa_circ_0000069 promotes cell proliferation and migration in CRC HT-29 cells[24]. These results indicate that circRNAs play an important regulatory role of tumorigenesis in CRC.

Accumulating evidence has demonstrated that the main function of circRNA is regulating miRNAs expression acting as ceRNAs[22,28,29]. For example, circRNA MYLK can bind competitively with miRNA-29a-3p increasing target gene DNMT3B, VEGFA and ITGB1 expressions in bladder carcinoma[30]. Hsa_circ_100338 functions as an endogenous sponge for miR-141-3p in HCC[22]. These researches excavate a new gene regulatory network, circRNA-miRNA-mRNA co-expression network[22,30]. In the present study, we had discovered just a key circRNA hsa_circ_0007534, regulating CRC cells proliferation and apoptosis. However, hsa_circ_0007534 related co-expression network had not predicted by bioinformatics methods, and these mechanisms need to be further investigated *in vivo* and *in vitro* experiments.

Conclusions

Taken together, circRNA expression profile by microarray assay showed 24 circRNAs changing remarkably in CRC tumor tissues, and preliminarily determined hsa_circ_0007534 is a key regulator of CRC. *In vitro* experiments manifested that down-regulation of hsa_circ_0007534 could inhibit CRC cells proliferation and induce apoptosis.

Acknowledgement
This research was supported by the National Natural Science Foundation of China (grant no. 81672427), "Liaoning

BaiQianWan Talents Program" (grant no. [2017] No.45) and Liaoning Clinical Research Center for Colorectal Cancer (grant no. 2015225005).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
2) Bopanna S, Ananthakrishnan AN, Kedia S, Yajnik V, Ahuja V. Risk of colorectal cancer in Asian patients with ulcerative colitis: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2017; 2: 269-276.
3) Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, Wu M. CircRNA: functions and properties of a novel potential biomarker for cancer. Mol Cancer 2017; 16: 94.
4) Hsu MT, Coca-Prados M. Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. Nature 1979; 280: 339-340.
5) Glazar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. Rna 2014; 20: 1666-1670.
6) Wang F, Nazarali AJ, Ji S. Circular RNAs as potential biomarkers for cancer diagnosis and therapy. Am J Cancer Res 2016; 6: 1167-1176.
7) Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature 2013; 495: 384-388.
8) Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res 2016; 44: 2846-2858.
9) Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol 2015; 22: 256-264.
10) Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ, Kjems J. miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. EMBO J 2011; 30: 4414-4422.
11) Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, Fatica A, Santini T, Andronache A, Wade M, Laneve P, Rajewsky N, Bozzoni I. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. Mol Cell 2017; 66: 22-37.e29.
12) Li Y, Dong Y, Huang Z, Kuang Q, Wu Y, Li Y, Li M. Computational identifying and characterizing circular RNAs and their associated genes in hepatocellular carcinoma. PLoS One 2017; 12: e0174436.
13) Guarnerio J, Bezzi M, Jeong JC, Paffenholz SV, Berry K, Naldini MM, Lo-Coco F, Tay Y, Beck AH, Pandolfi PP. Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. Cell 2016; 165: 289-302.
14) Bachmayr-Heyda A, Reiner AT, Auer K, Sukhbaatar N, Aust S, Bachleitner-Hofmann T, Mesteri I, Grunt TW, Zeillinger R, Pils D. Correlation of circular RNA abundance with proliferation--exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues. Sci Rep 2015; 5: 8057.
15) Xie H, Ren X, Xin S, Lan X, Lu G, Lin Y, Yang S, Zeng Z, Liao W, Ding YQ, Liang L. Emerging roles of circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer. Oncotarget 2016; 7: 26680-26691.
16) Zhang XL, Xu LL, Wang F. Hsa_circ_0020397 regulates colorectal cancer cell viability, apoptosis and invasion by promoting the expression of the miR-138 targets TERT and PD-L1. Cell Biol Int 2017; 41: 1056-1064.
17) Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL. Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. Am J Cancer Res 2017; 7: 1566-1576.
18) Gong J, Wang ZX, Liu ZY. miRNA1271 inhibits cell proliferation in neuroglioma by targeting fibronectin 1. Mol Med Rep 2017; 16: 143-150.
19) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-408.
20) Khanna A, Muthusamy S, Liang R, Sarojini H, Wang E. Gain of survival signaling by down-regulation of three key miRNAs in brain of calorie-restricted mice. Aging (Albany NY) 2011; 3: 223-236.
21) Dang Y, Ouyang X, Zhang F, Wang K, Lin Y, Sun B, Wang Y, Wang L, Huang Q. Circular RNAs expression profiles in human gastric cancer. Sci Rep 2017; 7: 9060.
22) Huang XY, Huang ZL, Xu YH, Zheng Q, Chen Z, Song W, Zhou J, Tang ZY, Huang XY. Comprehensive circular RNA profiling reveals the regulatory role of the circRNA-100338/miR-141-3p pathway in hepatitis B-related hepatocellular carcinoma. Sci Rep 2017; 7: 5428.
23) Zhu X, Wang X, Wei S, Chen Y, Chen Y, Fan X, Han S, Wu G. hsa_circ_0013958: a circular RNA and potential novel biomarker for lung adenocarcinoma. FEBS J 2017; 284: 2170-2182.
24) Guo JN, Li J, Zhu CL, Feng WT, Shao JX, Wan L, Huang MD, He JD. Comprehensive profile of differentially expressed circular RNAs reveals that hsa_circ_0000069 is upregulated and promotes cell proliferation, migration, and invasion in colorectal cancer. Onco Targets Ther 2016; 9: 7451-7458.
25) Hsiao KY, Lin YC, Gupta SK, Chang N, Yen L, Sun HS, Tsai SJ. Noncoding effects of circular RNA CCDC66 promote colon cancer growth and metastasis. Cancer Res 2017; 77: 2339-2350.

26) Li W, Zhong C, Jiao J, Li P, Cui B, Ji C, Ma D. Characterization of hsa_circ_0004277 as a new biomarker for acute myeloid leukemia via circular RNA profile and bioinformatics analysis. Int J Mol Sci 2017; 18. pii: E597.

27) Shang X, Li G, Liu H, Li T, Liu J, Zhao Q, Wang C. Comprehensive circular RNA profiling reveals that hsa_circ_0005075, a new circular RNA biomarker, is involved in hepatocellular crcinoma development. Medicine (Baltimore) 2016; 95: e3811.

28) Yang ZG, Awan FM, Du WW, Zeng Y, Lyu J, Wu, Gupta S, Yang W, Yang BB. The circular RNA interacts with STAT3, increasing its nuclear translocation and wound repair by modulating Dnmt3a and miR-17 function. Mol Ther 2017; 25: 2062-2074.

29) Han D, Li J, Wang H, Su X, Hou J, Gu Y, Qian C, Lin Y, Liu X, Huang M, Li N, Zhou W, Yu Y, Cao X. Circular RNA MTO1 acts as the sponge of miR-9 to suppress hepatocellular carcinoma progression. Hepatology 2017; 66: 1151-1164.

30) Huang M, Zhong Z, Lv M, Shu J, Tian Q, Chen J. Comprehensive analysis of differentially expressed profiles of lncRNAs and circRNAs with associated co-expression and ceRNA networks in bladder carcinoma. Oncotarget 2016; 7: 47186-47200.