Abstract. Circular RNAs (circRNAs/circs) have gained attention as a class of potential biomarkers for the early detection of multiple cancers. However, the functions and mechanisms of circRNAs in the oncogenesis of human colorectal cancer (CRC) remain to be elucidated. The present study aimed to investigate the roles of hsa_circ_0000523 and its parental gene methyltransferase-like 3 (METTL3) in regulating cell proliferation, apoptosis and invasion in the HCT116 human CRC cell line. To uncover the regulated function of hsa_circ_0000523 in HCT116 cells, a dual-luciferase reporter assay, flow cytometry, reverse transcription-quantitative PCR, Cell Counting Kit-8 assay, cell invasion and western blot assay were used. In HCT116 cells, hsa_circ_0000523 indirectly regulated METTL3 expression by suppressing the transcription of microRNA (miR)-let-7b. The expression of METTL3 promoted cell proliferation and suppressed apoptosis. In the present study, it was found that miR-let-7b promoted cell viability and inhibited apoptosis and invasion, while circ_0000523 exerted the opposite effects. Higher levels of METTL3 expression were associated with more aggressive tumor invasion. The present results suggest that circRNAs and METTL3 may be applied for highly sensitive diagnosis of CRC and for predicting prognosis in patients who have undergone therapy.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide (1,2). More than 1 million new cases of CRC were diagnosed worldwide in 2012, which accounts for ~10% of the global cancer burden (3). Currently, CRC is the fourth most common cause of cancer-related mortality (4,5). Although conventional treatments such as surgery, chemotherapy, radiotherapy and immunotherapy have improved the survival of patients with CRC, the mortality and relapse rates remain high (5,6). Therefore, more specific mechanism-based treatments are greatly needed.

Circular RNAs (circRNAs/circs) have gained attention as a class of potential biomarkers for the early detection of CRC (7). circRNA is a newly discovered type of non-coding RNA, which is distinct from the traditional linear RNA that has 5'- and 3'-ends (8). circRNAs are formed by exon skipping or back-splicing events, during which the 5'-end of an upstream exon is spliced together with the 3'-end of the downstream exon to form a circular molecule of RNA (8,9). circRNAs have been demonstrated to serve an important role in post-transcriptional regulation by acting as microRNA (miRNA/miR) sponges to competitively inhibit RNA/miRNA transcriptional regulation (10,11).

The abnormal expression of circRNA is closely associated with various diseases, including CRC (12-14). Jiang et al (15)
identified a large set of differentially expressed circRNAs in a primary CRC cell line (SW480) and a metastatic CRC cell line (SW620), relative to a normal colon cell line (NCM460). 

hsa_circ_000984 has been reported to promote colon cancer growth and metastasis by sponging miR-106b (16). Furthermore, Bachmayr-Heyda et al (17) demonstrated a global reduction in circRNA abundance in CRC cell lines and clinical CRC specimens compared with normal tissues. The authors described five specific circRNAs (circ0817, circ3203, circ6229, circ7374 and circ7780) and proposed a potentially negative association between global circRNA abundance and cell proliferation.

Among the five circRNAs identified, the present study focused on circ6229 (also known as circ_0000523) and its corresponding gene/linear mRNA, methyltransferase-like 3 (METTL3). As a major RNA N6-adenosine methyltransferase, METTL3 is widely implicated in mRNA biogenesis, decay and translation control (18). METTL3 has previously been demonstrated to promote the growth, survival and invasion of numerous human cancers (19), such as lung cancer (20), hepatocellular carcinoma (21), breast cancer (22) and pancreatic cancer (23). However, the precise role of METTL3 in the tumorigenesis and pathogenesis of CRC remains largely unknown.

HCT116 cells are a population of malignant cells that were isolated from the primary cell culture of a single human colonic carcinoma (24). In present study, the regulatory role of hsa_circ_0000523 in HCT116 cells was explored in detail. Dual-luciferase reporter assay, reverse transcription-quantitative PCR (RT-qPCR) and some rescue experiments were applied to confirm the existence of the hsa_circ_0000523/miR-let-7b/METTL3 axis. FCM was used to count the apoptotic cells. Cell Counting Kit-8 (CCK-8) and invasion assays were performed to monitor the activities of the HCT116 cells. The present study suggests a potential role for the hsa_circ_0000523/miR-let-7b/METTL3 axis in the tumorigenesis and pathogenesis of human CRC.

Materials and Methods

Cell culture. The HCT116 human CRC cell line (cat. no. CCL-247) was obtained from American Type Culture Collection. Cells were maintained in DMEM (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.), 100 units/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, Inc.). Cells were grown in a humidified atmosphere with 5% CO₂ at 37°C. Cells were cultured in 75-cm² flasks or 10-cm plates (Corning, Inc.), with the confluence maintained below 80%. After digestion with 0.25% (w/v) trypsin and 0.05 mM EDTA (Thermo Fisher Scientific, Inc.), cells were passaged every 2-3 days with 0.25% (w/v) trypsin and 0.5 mM EDTA (Thermo Fisher Scientific, Inc.).

Plasmid constructs and small interfering RNA (siRNA) oligos. Total RNA was extracted from the HCT116 cells using TRizol® reagent (Thermo Fisher Scientific, Inc.) and further purified with two phenol-chloroform treatments, and then treated with RQ1 DNase (Promega Corporation) to digest DNA. The quality and quantity of the purified RNAs were measured by a Nano Photometer spectrometer with the absorbance at 260/280 nm. The mixture was verified by 1.2% agarose gel electrophoresis. The cDNA was synthesized with random primers with the High-capacity cDNA Reverse-Transcription Kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. The full-length cDNA for human METTL3 (GenBank accession no. NM_019852.5) was obtained from the cDNA library of HCT116 cells by PCR, using the following protocol: 98°C for 10 min, followed by 98°C for 10 sec, 55°C for 5 sec and 72°C for 2 min for 35 cycles, and then 72°C for 10 min. The primers are listed in Table 1. The vector used to generate full-length wild-type (WT) METTL3 and hsa_circ_0000523 was mammalian expression vector pcDNA3.1 (Thermo Fisher Scientific, Inc.). The METTL3 coding sequence or circ_0000523 fragment was subcloned into the pcDNA3.1 vector via KpnI and BamHI double-enzymatic sites. Positive clones were selected, and the plasmid expressing METTL3 or hsa_circ_0000523 was verified by sequencing. The siRNA oligos used in the present study were designed and synthesized by Wuhan GeneCreate Biological Engineering Co., Ltd. The sequences for these siRNA oligos were as follows: Negative control siRNA oligo (METTL3-con) sense, 5'-GCUACUCAGAGGAGAUAUT dTdT-3'; METTL3-specific siRNA oligo (METTL3-si) sense, 5'-GCUGACUCAGAGGAGAUAUTdTdT-3'; negative control oligo for hsa_circ_0000523 (circ-con) sense, 5'-CAACAG AGCAAGAGAGAGAGAGAUC UAddTdT-3'; and circ_0000523-specific siRNA oligo (circ-si) sense, 5'-CAACAGAGAGAGAGAGAUC UAddTdT-3'.

Transfection of plasmids and siRNA oligos. HCT116 cells were transfected with the empty pcDNA3.1 vector (METTL3-EV), METTL3-expressing pcDNA3.1 vector (METTL3-OE), METTL3-con, METTL3-si, circ-EV, circ-OE, circ-con, circ-si, mimics, mimics NC, inhibitor and inhibitor NC using Lipofectamine® 2000 reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. miR-let-7b mimic (5'-UGAGGUAGUGUGUGUGGUU-3'), its negative control (5'-UCAACACCCUCCUAAAGAGAGUGA-3'), inhibitor (5'-AACCAACACCAACCUACACCUA-3') and inhibitor NC (5'-UCACUCUUUCGUAGGUGUGUG-3') were synthesized by Wuhan GeneCreate Biological Engineering Company. The RNA concentrations of mimics and inhibitor were 0.32 and 0.26 µg/µl, respectively. HCT116 cells (1x10⁶) were seeded in 6-well plates and maintained at a confluence of 70-80% in complete culture medium. Then, 2.5 µg plasmid or 100 pmol siRNA oligo was added to 250 µl Opti-MEM (Thermo Fisher Scientific, Inc.), and 5 µl Lipofectamine 2000 reagent was added to 250 µl Opti-MEM. The diluted plasmid or siRNA oligo was mixed with diluted Lipofectamine 2000 reagent and kept at room temperature for 20 min. After 500 µl serum-free medium was added to each well, the Opti-MEM mixture was added. After transfected cells were incubated at 37°C for 4 h, the culture medium was changed to regular DMEM supplemented with 10% FBS. After 48 h, the subsequent experiments were performed.

RNA isolation and reverse transcription. Total RNA was extracted from cells using TRizol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. Briefly, after treatment, cell pellets were resuspended in...
PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer’s protocols and Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher) protein was extracted from the treated cells using NE‑PER. Various treatments were washed twice using PBS buffer. Total RNA extraction was performed by adding 1 ml TRIzol and mixed well. Then, 0.2 ml chloroform was added to the cell suspension, which was shaken vigorously for 15 sec and then incubated for 3 min at room temperature. After centrifugation of the suspension at 12,000 x g for 10 min at 4˚C, supernatant was collected and subjected to RNA precipitation by adding 0.5 ml isopropanol at 40˚C. The RNA pellet was obtained by centrifugation at 12,000 x g for 10 min at 4˚C, and then washed with 75% ethanol. The appropriate amount of RNase‑free distilled water was used to dissolve extracted RNA. The concentration of RNA was measured, and the gray level was assessed using ImageJ 1.8.0 software (National Institutes of Health). The primary antibodies were: Anti‑METTL3 antibody (EPR18810; 1:2,000 dilution; cat. no. ab195352; Abcam) and GAPDH monoclonal Antibody (catalog no. SA00001‑2; ProteinTech Group, Inc.). All antibodies used in the WB assay were diluted in 5% skimmed milk for 45 min at 37˚C, and then probed with primary antibodies for 12 h at 4˚C. The membrane was washed three times and probed with the secondary antibodies for 1 h at 25˚C. The signal was detected using an ECL system (MilliporeSigma) and the gray level was assessed using ImageJ 1.8.0 software (National Institutes of Health). The primary antibodies were: Anti‑METTL3 antibody (EPR18810; 1:2,000 dilution; cat. no. ab195352; Abcam) and GAPDH monoclonal Antibody (catalog no. 60004‑1‑lg; ProteinTech Group, Inc.). The secondary antibody was HRP‑conjugated Affinipure Goat Anti‑Rabbit IgG(H+L) (1:10,000 dilution; catalog no. A00001‑2; ProteinTech Group, Inc.). All antibodies used in the WB assay were diluted in 5% skimmed milk powder.

Dual‑luciferase reporter assay. The binding sites among miR‑let‑7b, circ_0000523 and METTL3 were predicted by Bielefeld Bioinformatics Service online software (https://bibiserv.cebitec.uni‑bielefeld.de/prevres.jsf). The WT miR‑let‑7b along with the 3′‑UTR of METTL3 that contained the predicted miR‑let‑7b binding site or a sequence with mutations (MUT) were amplified and inserted into the pmirGLO vector (Promega Corporation), resulting in the WT luciferase reporter construct. The vectors (miR‑let‑7b‑WT, miR‑let‑7b‑MUT, METTL3‑WT or METTL3‑MUT, miR‑let‑7b mimics and negative control mimics, which were synthesized by Wuhan GeneCreate Biological Engineering Company, were transfected into CRC cells using Lipofectamine 3000 (Thermo Fisher Scientific, Inc.). After 48 h, the luciferase activity was measured using a luciferase reporter system (Promega Corporation), with Renilla luciferase activity as an internal control. The sequences of mimics miR‑let‑7b and mimics NC were as follows: miR‑let‑7b mimic sense, 5′‑UGA GGU AGU AGG UUG UGU GGU U‑3'; NC sense, 5′‑UCA CAA CCU CCU AGA AAG AGU AGA‑3'.

CCK‑8 assay to measure cell proliferation. After transfection of empty pcDNA3.1 vector, METTL3‑expressing pcDNA3.1 vector, METTL3‑con oligo or METTL3‑si oligo, HCT116 cells were seeded into flat‑bottomed 96‑well plates at a density of 3x10^3‑6x10^3 cells per well in 100 µl culture medium. Each treatment condition was replicated in nine wells. Cells were incubated for 24, 48 or 72 h in an incubator with 5% CO₂ at 37˚C. Cell proliferation was analyzed with a CCK‑8 assay (Beyotime Institute of Biotechnology) according to the manufacturer’s protocol. Briefly, 20 µl CCK‑8 reagent (5 mg/ml)
was added to the culture medium, and cells were cultured for an additional 4 h. The optical density values of all wells were measured with a plate reader (Thermo Fisher Scientific, Inc.) at 450 nm.

**Cell apoptosis.** HCT116 cells seeded in 6-well plates were transfected with empty pcDNA3.1 vector, METTL3-expressing pcDNA3.1 vector, METTL3-con oligo or METTL3-si oligo, as aforementioned. At 48 h after transfection, cells were detached by incubation with 0.25% trypsin-EDTA solution (Thermo Fisher Scientific, Inc.) and were harvested. Flow cytometry analysis was performed to detect apoptosis using the Annexin V-FITC/PI apoptosis kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Briefly, after one wash in PBS and one wash in binding buffer, cells were stained with Annexin V-FITC/PI for 20 min at room temperature in the dark. After another wash in binding buffer, labelled cells were detected immediately using a flow cytometer (CytoFLEX S; Beckman Coulter, Inc.). Data were analyzed with CytExpert 2.0 Software (Beckman Coulter, Inc.).

**Cell invasion.** The cellular potential for invasion was determined using a 24-well Transwell plate with 8.0-µm Pore Polyester Membrane Inserts (Corning, Inc.). Matrigel (Corning, Inc.) was melted overnight at 4°C and diluted to a final concentration of 1 mg/ml in pre-cooled serum-free medium. Then, 100 µl diluted Matrigel was added to the bottom of the upper chamber. The plate was then incubated at 37°C for 4-5 h to dry the Matrigel. At 24 h after transfection, HCT116 cells in the logarithmic growth phase were seeded in triplicate at a density of 1x10^6 cells/chamber. Cells were seeded on top of the Transwell plate in 100 µl DMEM supplemented with 0.1% bovine serum albumin (Thermo Fisher Scientific, Inc.). Then, 0.8 ml DMEM supplemented with 10% FBS (Thermo Fisher Scientific, Inc.) was added to the lower chamber as a chemoattractant. After 24 h of incubation at 37°C, cells on the top surface of the insert were removed with a cotton swab. Cells that had invaded the lower surface of the membrane were fixed with 4% paraformaldehyde at 37°C for 15 min and stained with 800 µl Giemsa solution at 37°C for 20 min (Beyotime Institute of Biotechnology). Cells were visualized using a light microscope (CKX-41; Olympus Corporation). Cell counts were obtained from three randomly selected optical fields.

**Statistical analysis.** All data were analyzed using SPSS Version 21.0 software (IBM Corp.). All values are presented as the mean ± SD. Three independent experiments were performed. Quantitative data were compared using the χ^2 test. For only two comparisons, statistically significant differences between means were determined using an unpaired Student's t-test. For multiple comparisons, the significance was identified by simple one-way ANOVA followed by a Tukey's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

**Results**

*hsa_circ_0000523* regulates METTL3 expression by modulating levels of miR-let-7b in HCT116 cells. To explore the role of *hsa_circ_0000523* circRNA in the tumorigenesis and pathogenesis of CRC, an *in vitro* model was established by manipulating the expression of *hsa_circ_0000523* in the HCT116 human CRC cell line. As shown in Fig. 1A, the mRNA expression levels of *circ_0000523* were successfully increased more than 20-fold in the circ overexpression (circ-OE) group compared with cells transfected with empty vector (circ-EV). Next, this circRNA was knocked down in HCT116 cells. Efficient knockdown of *hsa_circ_0000523* was observed in HCT116 cells transfected with *hsa_circ_0000523*-specific siRNA oligo (circ-si), in which *hsa_circ_0000523* expression was 80% less than that in HCT116 cells transfected with control siRNA oligo (circ-con) (Fig. 1A). Next, the effect of *hsa_circ_0000523* expression on METTL3 and miR-let-7b, a microRNA that may be a target of *hsa_circ_0000523*, was evaluated in HCT116 cells. A negative association between the expression of *hsa_circ_0000523* and miR-let-7b was observed (Fig. 1B). Among the various treatment conditions, HCT116 cells transfected with *hsa_circ_0000523*-expressing plasmid (circ-OE) displayed the highest level of *hsa_circ_0000523* expression and the lowest level of miR-let-7b expression.

**Positive association between METTL3 expression and proliferation in HCT116 cells.** As METTL3 was the endpoint effector molecule for the *hsa_circ_0000523/miR-let-7b/METTL3* axis, it was important to elucidate the effects of METTL3 expression on proliferation, apoptosis and invasion of HCT116 cells in the present study. Similarly, HCT116 cells with overexpression or knockdown of METTL3 were generated by transfection of a METTL3-expressing pcDNA3.1 plasmid or a METTL3-specific siRNA oligo, respectively. The levels of METTL3 mRNA were quantified using RT-qPCR and WB analysis. The results demonstrated that METTL3 overexpression resulted in a >200-fold increase in METTL3 transcript levels. The WB results were consistent with the RT-qPCR findings. Compared with the METTL3-con group, the METTL3-si group exhibited a >70% decrease in METTL3 transcript levels (Fig. 2A).
After transfection with plasmids or siRNA oligos, HCT116 cells were further cultured for 24, 48 or 72 h. Cell proliferation was measured at these time points using the CCK-8 assay. There were no significant differences in cell proliferation among the untreated WT, empty vector-transfected and negative control siRNA oligo-transfected HCT116 cells (Fig. 2B). Compared with the empty vector-transfected HCT116 cells (METTL3-EV group), HCT116 cells with ectopic expression of METTL3 (METTL3-OE group) exhibited a significantly higher rate of proliferation at each time point. Cell proliferation was decreased in HCT116 cells with knockdown of METTL3 compared with cells transfected with negative control siRNA oligos (Fig. 2B). Furthermore, the effects of miR-let-7b and circ_0000523 on HCT116 cell proliferation were evaluated. Compared with the mimic NC groups, proliferation was obviously inhibited in the mimic groups. By contrast, cell viability was significantly promoted in inhibitor groups (Fig. 2C). As shown in Fig. 2D, overexpression of circ_0000523 promoted HCT116 cell proliferation at all time points. Furthermore, miR-let-7b reversed the effects of circ_0000523 overexpression on human HCT116 cell characteristics. Taken together, these results indicated that has_circ_0000523 may regulate proliferation of CRC cells via miR-let-7b.

METTL3 regulates apoptosis in HCT116 cells. An important question was whether and how the expression of METTL3...
impacts apoptosis in HCT116 cells; therefore, this was evaluated using Annexin V and PI staining. There were no significant differences in apoptotic rates among the untreated WT, empty vector-transfected and negative control siRNA oligo-transfected HCT116 cells, all of which exhibited an apoptotic rate of ~15% (Fig. 3A and B). The rate of apoptosis was significantly decreased in HCT116 cells with ectopic expression of METTL3 compared with HCT116 cells transfected with empty vector (12% vs. 15%, respectively). The rate of apoptosis was higher in HCT116 cells with knockdown of METTL3 than in cells transfected with negative control siRNA oligos (20% vs. 15%, respectively; Fig. 3A and B). Therefore, a negative association between METTL3 expression and the apoptotic rate was observed, which suggests that METTL3 inhibits apoptosis in HCT116 cells. In addition, the effect of circ_0000523 and miR-let-7b on HCT116 cell apoptosis was investigated by flow cytometry. Overexpression of circ_0000523 significantly reduced apoptosis of CRC cells (Figs. 3C and S1A). Compared with that in the mimics NC group, the number of apoptotic cells was increased in the mimics group. By contrast, adding an inhibitor suppressed HCT116 cell apoptosis (Figs. 3D and S1B).
Figs. 3E and S1C, circ_0000523 reversed the antitumor effects of miR-let-7b on human CRC cell line characteristics.

Higher METTL3 expression is associated with more aggressive tumor invasion in HCT116 cells. To explore the role of METTL3 in regulating CRC metastasis, Transwell assays were performed to examine the invasion of HCT116 cells with various levels of METTL3 expression. After plasmid (overexpression) or siRNA oligo (knockdown) transfection, the number of cells that had migrated to the surface of the lower chamber (filled with DMEM containing 10% FBS) was used as an index for the metastatic ability of HCT116 cells. As shown in Fig. 4A, a higher number of Giemsa-positive stained cells were observed in the group with METTL3 overexpression compared with the group with METTL3 knockdown.

Statistical analysis confirmed the positive association between METTL3 expression and the invasion of HCT116 cells. There were no significant differences in invasion among the untreated WT, empty vector-transfected and negative control siRNA oligo-transfected HCT116 cells (Fig. 4A). Compared with empty vector-transfected HCT116 cells (METTL3-EV group), HCT116 cells with ectopic expression of METTL3 (METTL3-OE group) exhibited significantly higher invasion. Consistently, invasion was decreased in HCT116 cells with METTL3 knockdown compared with cells transfected with negative control siRNA oligos (Fig. 4A). These results suggest that higher expression of METTL3 is associated with more aggressive tumor invasion in HCT116 cells. Next, it was examined whether circ_0000523 and miR-let-7p mediate CRC cell invasion. Compared with those in the circ-EV group, cells in the lower chamber were increased by 43.2% in the circ-OE
suggesting significant levels of invasion in CRC cells (Fig. 4B). The cell invasion of miR-let-7b-transfected cells (mimics group) obviously decreased compared with the control group (mimics NC group), while inhibitor treatment resulted in an obvious increase in CRC cell invasion ability (Fig. 4C). Furthermore, transfection of miR-let-7b mimics reduced the invasion of CRC cells; however, this was restored by circ_0000523 overexpression (Fig. 4D).
Discussion

circRNAs modulate the expression of parental genes by regulating alternative splicing or transcription and acting as competitive sponges for endogenous RNA or miRNA (26). circRNAs may provide more comprehensive information on the key genes involved in oncogenesis and the development of numerous cancers (27,28). circRNAs are easily accessible and noninvasive biological markers for the early detection of CRC (29,30). However, few studies have reported on the specific functions of circRNAs in the tumorigenesis and pathogenesis of human CRC. In the present study, the expression patterns of hsa_circ_0000523 and its parental gene METTL3 were explored in the HCT116 human CRC cell line. The present results identified a potential hsa_circ_0000523/miR‑let‑7b/METTL3 axis. The results of gain-of-function and loss-of-function assays demonstrated that expression of METTL3 promoted cell proliferation and invasion and inhibited apoptosis in HCT116 cells. Rescue experiments were performed to confirm the regulatory effects of hsa_circ_0000523 on miR‑let‑7b. It was found that circ_0000523 reversed the antitumor effects of miR‑let‑7b on human CRC cell line characteristics.

Accumulating evidence has demonstrated the abnormal expression and important biological functions of circRNAs in CRC (12‑14). circRNA plays various roles in CRC, including in proliferation, metastasis and apoptosis (6). Notably, most circRNAs previously identified by bioinformatic approaches have been found to be downregulated in CRC cell lines and clinical CRC tissues (17). Huang et al (31), demonstrated that the expression of circRNA itchy E3 ubiquitin ligase (circ_ITCH) was lower in CRC tissues compared with adjacent noncancerous tissues. circ_ITCH was found to sponge tumorigenic miR‑7 and miR‑20a, which contribute to the malignancy of CRC (31). A positive association between transcription of circ_ITCH and the parental gene ITCH has been identified (31). Notably, expression of circ_ITCH was found to suppress proliferation in CRC cells (31). Similarly, Zhu et al (32) reported that circ‑BANP was expressed in 35 CRC tissues, and knockdown of circ‑BANP decreased the proliferation of CRC cells.

Indeed, there have been some studies reporting the regulatory role of circRNAs in tumourigenesis and progression of human CRC in previous years (33,34). Jin et al (35) found a deficiency in hsa_circ_0000523 could activate the Wnt/β‑catenin signaling pathway, which induced the development of CRC. To the best of our knowledge, the present study found for the first time that hsa_circ_0000523 successfully promotes CRC via the miR‑let‑7b/METTL3 axis.

In the potential target miR‑let‑7b, and its parental gene METTL3 were evaluated in HCT116 cells. Similar to the expression patterns of circ_ITCH and ITCH, the transcript levels of hsa_circ_0000523 were positively associated with mRNA levels of linear METTL3 in HCT116 cells. Although the effects of hsa_circ_0000523 expression on cell proliferation were not measured, the ability of METTL3 expression to increase HCT116 cell proliferation suggests a similar proliferative role for hsa_circ_0000523. However, a previous report by Jin et al (35) demonstrated that hsa_circ_0000523 exerted anti-proliferative effects and promoted apoptosis in two other human CRC cell lines (SW480 and SW620). Therefore, although hsa_circ_0000523 can modulate METTL3 expression by acting on miR‑let‑7b, additional studies will be necessary to determine whether the expression of METTL3 can impact the transcription of hsa_circ_0000523 in HCT116 cells.

circRNAs may regulate the proliferation of CRC by sequestering multiple miRNAs. For instance, circ‑homeodomain interacting protein kinase 3 (circ‑HIPK3) has been demonstrated to be upregulated in CRC tissue compared with normal tissue and regulates cell proliferation by sponging nine miRNAs with 18 potential binding sites (36). Specifically, circ‑HIPK3 has been reported to bind to and inhibit the activity of miR‑124, a tumor suppressor that is typically down-regulated in CRC (37). Therefore, circRNAs may modulate the tumorigenetic proliferation of CRC cells by diminishing the antitumor effects of certain tumor-suppressive miRNAs.

miR‑let‑7b, shown here to be a potential target of hsa_circ_0000523, is also a tumor suppressor in multiple cancers (38). Human miR‑let‑7b has been demonstrated to be downregulated in various cancers, and the induction of tumorigenesis was revealed to be inhibited in normal cells by ectopic expression of miR‑let‑7b (39,40). Human miR‑let‑7b has been reported to inhibit cancer growth by targeting various oncogenes and inhibiting key regulators of several mitogenic pathways in cancer (38). These results point to a therapeutic potential for human miR‑let‑7 in cancer treatment (38). Therefore, high levels of hsa_circ_0000523 and low levels of miR‑let‑7 may contribute to tumorigenesis in CRC.

On the other hand, circRNAs may regulate the proliferation of CRC by indirectly targeting oncogenes. METTL3, a target gene of miR‑let‑7b, was shown to promote growth, survival and invasion in human lung cancer (20). Furthermore, the oncogenic roles of METTL3 have been demonstrated in numerous solid tumors and hematopoietic malignancies (41,42). For instance, it was found METTL3 depletion inhibited tumorigenicity and sensitized lung cancer cells to bromodomain-containing protein 4 inhibition (42). To the best of our knowledge, the present study was the first report to describe the role of METTL3 in CRC cells. METTL3 expression in HCT116 cells was found to be positively associated with proliferation and cancer invasion and to be negatively associated with apoptosis. These findings suggest that METTL3 functions as an oncogene in CRC, potentially by promoting the translation of other oncogenes. By contrast, METTL3 has been found to be a tumor suppressor in renal cell carcinoma (43). METTL3 has been demonstrated to promote cell proliferation, invasion and invasion in two human renal cell carcinoma cell lines (CAKI‑1 and CAKI‑2). These effects are mediated by modulation of the epithelial-to-mesenchymal transition and the PI3K‑Akt‑mTOR signaling pathway (43). The molecular mechanisms underlying the diverse roles of METTL3 in renal cell carcinoma and CRC remain to be elucidated.

Through gain-of-function and siRNA oligo mediated loss-of-function experiments, a potential hsa_circ_0000523/miR‑let‑7b/METTL3 axis, which contributed to tumorigenesis and pathogenesis in the HCT116 human CRC cell line, was identified. METTL3 was demonstrated to promote proliferation and invasion and to inhibit apoptosis in HCT116 cells. The present study on the hsa_circ_0000523/miR‑let‑7b/METTL3 axis may facilitate highly
sensitive diagnosis of CRC and improved prognosis prediction in patients following therapy.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants from the Scientific Research Fund Project of Yunnan Education Department (grant no. 2019J1306). The funders had no role in study design, data collection, data analysis, decision to publish or preparation of the manuscript.

Availability of data and materials

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

Authors’ contributions

HL and YT designed and supervised the study. YW, BZ and LL were responsible for analyzing the data, writing the manuscript and revising it. YuZ, YoZ, LL and TS contributed to the statistical analysis of the data and text correction. All authors have read and approved the final manuscript. YW and BZ confirm the authenticity of all the raw data.

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. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-E386, 2015.
2. Marley AR and Nan H: Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet 7: 105-114, 2016.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
4. Zhang Y, Chen Z and Li J: The current status of treatment for colorectal cancer in China: A systematic review. Medicine (Baltimore) 96: e2842, 2017.
5. Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi RE and Corcione F: Worldwide burden of colorectal cancer: A review. Updates Surg 68: 7-11, 2016.
6. Fakhig MG: Metastatic colorectal cancer: Current state and future directions. J Clin Oncol 33: 1809-1824, 2015.
7. Taborda MI, Ramirez S and Bernal G: Circular RNAs in colorectal cancer: Possible roles in regulation of cancer cells. World J Gastrointest Oncol 9: 62-69, 2017.
8. Salzman J: Circular RNA expression: Its potential regulation and function. Trends Genet 32: 309-316, 2016.
9. Meng X, Li X, Zhang P, Wang J, Zhou Y and Chen M: Circular RNA: An emerging key player in RNA world. Brief Bioinform 18: 547-557, 2017.
10. Memczak S, Jens M, Elefantes A, Torti F, Krueger A, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, et al: Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495: 333-338, 2013.
11. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK and Kjems J: Natural RNA circles function as efficient microRNA sponges. Nature 495: 384-388, 2013.
12. Wang P and He X: Current research on circular RNAs associated with colorectal cancer. Scand J Gastroenterol 52: 1203-1210, 2017.
13. Zhang P, Zuo Z, Shang W, Wu A, Bi R, Wu J, Li S, Sun X and Jiang L: Identification of differentially expressed circular RNAs in human colorectal cancer. Tumor Biol. Mar 28, 2017 (Epub ahead of print).
14. Kristensen LS, Hansen TB, Vento MT and Kjems J: Circular RNAs in cancer: Opportunities and challenges in the field. Oncogene 37: 555-565, 2018.
15. Jiang W, Zhang X, Chu Q, Lu S, Zhou L, Lu X, Liu C, Mao L, Ye C, Timko MP, et al: The circular RNA profiles of colorectal tumor metastatic cells. Front Genet 9: 34, 2018.
16. Xu XW, Zheng BA, Hu ZM, Qian ZY, Huang CJ, Liu XQ and Wu WD: Circular RNA hsa_circ_000984 promotes colon cancer growth and metastasis by sponging miR-106b. Oncotarget 8: 91674-91683, 2017.
17. Bachmayr-Heyda A, Reiner AT, Auer K, Sukhbaatar N, Amt S, Bachleiter-Hofmann T, Mesteri I, Grunt TW, Zeilinger R and Pils D: Correlation of circular RNA abundance with proliferation-exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues. Sci Rep 5: 8057, 2015.
18. Maita A and Das B: N6-methyladenosine modification in mRNAs: Machinery, function and implications for health and diseases. FEBS J 283: 1607-1630, 2016.
19. Deng X, Su R, Weng H, Huang H, Li Z and Chen J: RNA N6-methyladenosine modification in cancers: Current status and perspectives. Cell Res 28: 507-517, 2018.
20. Lin S, Choe J, Du P, Triboulet R and Gregory RI: The m(6)A methyltransferase METTL3 promotes translation in human cancer cells. Mol Cell 62: 335-345, 2016.
21. Chen M, Wei L, Law CT, Tsang FH, Shen J, Cheng CL, Tsang LH, Ho DW, Chiu DK, Lee JM, et al: RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2. Hepatology 67: 2254-2270, 2018.
22. Cai X, Wang X, Cao C, Gao Y, Zhang S, Yang Z, Liu Y, Zhang X, Zheng W and Ye L: HBXIP-elevated methyltransferase METTL3 promotes the progression of breast cancer via inhibiting tumor suppressor let-7g. Cancer Letters 415: 11-19, 2018.
23. Taketo K, Konno M, Asai A, Koseki J, Toratani M, Satoh T, Doki Y, Mori M, Ishi H and Ogawa K: The epitranscriptome m6A writer METTL3 promotes chemo- and radioresistance in pancreatic cancer cells. Int J Oncol 52: 621-629, 2018.
24. Brattain MG, Fine WD, Khaled FM, Thompson J and Brattain DE: Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res 41: 1751-1756, 1981.
25. 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.
26. Shao T, Pan YH and Xiong XD: Circular RNA: An important player with multiple facets to regulate its parental gene expression. Mol Ther Nucleic Acids 23: 369-376, 2020.
27. Huang X, Zhang W and Shao Z: Prognostic and diagnostic significance of circRNAs as expression in lung cancer. J Cell Physiol 234: 18459-18465, 2019.
28. Ma Y, Zheng L, Gao Y, Zhang W and Xu Y: A comprehensive overview of circRNAs: Emerging biomarkers and potential therapeutics in gynecological cancers. Front Cell Dev Biol 9: 709512, 2021.
29. Tian J, Xi X, Yu J, Huang Q, Ma R, Zhang X, Li H and Wang L: CircRNA hsa_circ_0004585 as a potential biomarker for colorectal cancer. Cancer Manag Res 11: 5413-5423, 2019.
30. Li RD, Guan M, Zhou Z, Dong SX and Liu Q: The role of circRNAs in the diagnosis of colorectal cancer: A meta-analysis. Front Med (Lausanne) 8: 766208, 2021.
31. Huang G, Zhu H, Shi Y, Wu W, Cai H and Chen X: cirITCH plays an inhibitory role in colorectal cancer by regulating the Wnt/β-catenin pathway. PLoS One 10: e0131225, 2015.
32. Zhang M, Xu Y, Chen X and Yan F: Circular RNA hsa_circ_0172468, an upregulated circular RNA that modulates cell proliferation in colorectal cancer. Biomed Pharmacother 88: 138-144, 2017.
33. Peng K, Jiang P, Du Y, Zeng D, Zhao J, Li M, Xia C, Xie Z and Wu J: Oxidized low-density lipoprotein accelerates the injury of endothelial cells via circ-USP36/miR-98-5p/VCAM1 axis. IUBMB Life 73: 177-187, 2021.

34. Wu D, Jia H, Zhang Z and Li S: Circ-PRMT5 promotes breast cancer by the miR-509-3p/TCF7L2 axis activating the PI3K/AKT pathway. J Gene Med 23: e3300, 2021.

35. Jin Y, Yu LL, Zhang B, Liu CF and Chen Y: Circular RNA hsa_circ_0000523 regulates the proliferation and apoptosis of colorectal cancer cells as miRNA sponge. Braz J Med Biol Res 51: e7811, 2018.

36. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, et al: Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nat Commun 7: 11215, 2016.

37. Liu K, Yao H, Lei S, Xiong L, Qi H, Qian K, Liu J, Wang P and Zhao H: The miR-124-p63 feedback loop modulates colorectal cancer growth. Oncotarget 8: 29101-29115, 2017.

38. Barh D, Malhotra R, Ravi B and Sindurani P: MicroRNA let-7: An emerging next-generation cancer therapeutic. Curr Oncol 17: 70-80, 2010.

39. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, et al: The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res 67: 7713-7722, 2007.

40. Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA and Jacks T: Suppression of non-small cell lung tumor development by the let-7 microRNA family. Proc Natl Acad Sci USA 105: 3903-3908, 2008.

41. Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, Chou T, Chow A, Saletore Y, MacKay M, et al: The N6-methyladenosine (m6A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat Med 23: 1369, 2017.

42. Choe J, Lin S, Zhang W, Liu Q, Wang L, Ramirez-Moya J, Du P, Kim W, Tang S, Sliz P, et al: mRNA circularization by METTL3-eIF3h enhances translation and promotes oncogenesis. Nature 561: 556-560, 2018.

43. Li X, Tang J, Huang W, Wang F, Li P, Qin C, Qin Z, Zou Q, Wei J, Hua L, et al: The M6A methyltransferase METTL3: Acting as a tumor suppressor in renal cell carcinoma. Oncotarget 8: 96103-96116, 2017.