Long Non-Coding RNA CASC2 Functions as A Tumor Suppressor in Colorectal Cancer via Modulating The miR-18a-5p/BTG3 Pathway

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Abstract

Objective: Reportedly, long non-coding RNA (lncRNA) cancer susceptibility candidate 2 (CASC2) is involved in regulating colorectal cancer (CRC) progression. However, the function and detailed downstream mechanism of CASC2 in CRC progression are not fully elucidated. The aim of the study was to investigate the potential function and molecular mechanism of CASC2 in CRC progression.

Materials and Methods: In this experimental study, quantitative real-time polymerase chain reaction (qRT-PCR) was adopted to probe CASC2, microRNA-18a-5p (miR-18a-5p) and B cell translocation gene 3 (BTG3) mRNA expression in CRC tissues and cell lines. After CASC2 was overexpressed in Colo-678 and HCT116 cell lines, methylthiazol tetrazolium (MTT) and 5-bromo-2’-deoxyuridine (BrdU) assays were employed to examine the proliferation of CRC cells. Transwell migration and invasion assays were executed to evaluate the metastatic potential of CRC cells. The targeting relationships among CASC2, miR-18a-5p and BTG3 were validated by dual luciferase reporter gene assay. Western blot assay was applied to examine the regulatory effects of CASC2 and miR-18a-5p on BTG3 protein expression.

Results: CASC2 was decreased in CRC tissues and cell lines, and its low expression in CRC tissues was associated with larger tumor size and lymph node metastasis. CASC2 overexpression restrained proliferative, migrative and invasive capabilities of CRC cells. CASC2 could function as a molecular sponge for miR-18a-5p and repress the expression of miR-18a-5p. Furthermore, the inhibitory effects of CASC2 on the malignant phenotypes of CRC cells was counteracted by miR-18a-5p mimics. Additionally, CASC2 could positively regulate BTG3 expression via suppressing miR-18a-5p.

Conclusion: CASC2 inhibits CRC development by suppressing miR-18a-5p and raising BTG3 expression.

Keywords: B Cell Translocation Gene 3, Colorectal Cancer, lncRNA CASC2, miR-18a-5p

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Introduction

Colorectal cancer (CRC) brings about a huge health burden globally (1). In 2020, there were approximately 147,950 new cases of CRC and 53,200 deaths from CRC worldwide (2). In China, the incidence of CRC is on the rise (3). Currently, colonoscopy is the most reliable screening method for CRC screening, but it has high economic and physical burdens (4). In addition, relapse after the surgery, distant metastasis and chemoresistance contribute to the adverse prognosis of CRC patients (5). It is therefore pivotal to decipher the mechanism of CRC progression to identify potential therapy targets for treating CRC.

Long non-coding RNA (IncRNA) exceeding 200nt, is vital in imprinting, epigenetic regulation, transcription and translation regulation (6). Through modulating gene expression and the function of proteins, IncRNAs participate in regulating diverse biological processes (7). In these years, some IncRNAs emerge as either oncogenes or tumor suppressors in diverse cancers (8, 9). Notably, the expression of IncRNA cancer susceptibility candidate 2 (CASC2) is reduced in pancreatic cancer and oral squamous cell carcinoma (10, 11). Importantly, it is reported that CASC2 can play a tumor suppressive role in CRC through sponging miR-18a (12). However, the mechanism of action of CASC2 in CRC still awaits more investigation.

As reported, IncRNA can serve as a competing endogenous RNA (ceRNA) to regulate targeted gene expression via sponging microRNA (miRNA) (13). Besides, miRNA dysregulation is a feature of CRC (14, 15). However, the IncRNA-miRNA network in CRC is still partly covered and requires further research. MiR-18a-5p has been confirmed to be a promoter in many cancers including CRC (16). However, the interaction mechanism between CASC2 and miR-18a-5p in CRC has not been clarified.

B cell translocation gene 3 (BTG3) belongs to B-cell translocation gene / Transducer of ERBB2 (BTG/TOB) family. BTG3 is a crucial participant in regulating cell proliferation, differentiation and apoptosis (17, 18). It has been reported that BTG3 overexpression suppresses the malignancy of CRC cells through regulating Wnt/β-
LncRNA CASC2, miR-18a-5p, BTG3 and CRC

catenin signaling (19). But the hidden role and mechanism of BTG3 relevant to the progression of CRC are inconclusive. Here we focused on the role and mechanism of CASC2 in CRC. We investigated the regulatory effects of the CASC2/miR-18a-5p/BTG3 axis on the proliferative, migrative and invasive abilities of CRC cells.

Materials and Methods

Tissue samples

In this experimental study, this work was endorsed by the Suzhou Science and Technology Town Hospital of Nanjing Medical University’s Ethics Committee (20190712-006) and all experiments were performed according to “Declaration of Helsinki”. Specifically, all subjects provided written informed consent. 65 pairs of cancerous tissues and adjacent non-cancerous tissues were available from CRC patients in the Suzhou Science and Technology Town Hospital of Nanjing Medical University. All samples were kept in liquid nitrogen immediately at -196°C after the surgery.

Cell culture and transfection

In this experimental study, the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) was the supplier of CRC cell lines HCT116, SW620, LoVo. Colo-678 cells were provided by Honsun Biological Technology Co., Ltd. (Shanghai, China). Normal human colorectal mucosal cell line FHC was acquired from the China General Microbiological Culture Collection (Beijing, China). Notably, cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS, Invitrogen, Shanghai, China), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) in 5% CO₂ at 37°C. miR-18a-5p mimics (miR-18a-5p), and the mimics negative control (miR-NC) were available from GenePharma (Shanghai, China). CASC2 sequences with full length were inserted into pcDNA3.0 plasmid to construct CASC2 overexpression plasmid. Empty pcDNA3.0 vector was adopted as negative control (NC). Lipofectamine® 3000 (Invitrogen, Carlsbad, CA, USA) was employed for transfections as protocols.

Quantitative real-time polymerase chain reaction

Total RNA of tissues and cells isolated by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) were reversely transcribed into complementary DNA (cDNA) by a PrimeScript™ RT Reagent kit (Invitrogen, Carlsbad, CA, USA). Next, qRT-PCR was conducted with SYBR® Premix-Ex Taq™ (Takara, Tokyo, Japan) on the ABI PRISM 7000 Fluorescent Quantitative PCR System. The data were analyzed with 2^(-ΔΔCt) method, with Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 as the endogenous references. The sequences of the primers are:

CASC2-
F: 5’-GGACATTGGACGTTTCC-3’
R: 5’-CCCAGTCCCTCACAGGTTCAC-3’

miR-18a-5p-
F: 5’-TCCGAGATAGACGTGATCTA-3’
R: 5’-GTGCAGGGTCCGAGGT-3’

BTG3-
F: 5’-ATGAAGAAATGAAATTGCTG-3’
R: 5’-TTAGTGAGGTGCTAACATGTG-3’

GAPDH-
F: 5’-GTCAACGGATTGGTCTATTG-3’
R: 5’-CCGTTTCTCAGCCATGTT-3’

U6-
F: 5’-CTCGCTTCGGCAGCACA-3’
R: 5’-AACGCTTTCACGAATTTCGT-3’

Subcellular fractionation analysis

Cytoplasmic and nuclear RNA were isolated and extracted from Colo-678 and HCT116 cells by the Cytoplasmic and Nuclear RNA Purification Kit (Norgen, Belmont, CA, USA). Then, the expression of CASC2 in the cytoplasm and nucleus of Colo-678 and HCT116 cells was investigated by qRT-PCR, with GAPDH as a cytoplasmic control and U6 as a nuclear control.

Methylthiazol tetrazolium (MTT) assay

Transfected Colo-678 and HCT116 cells were inoculated into 96-well plates (3×10³ cells/well). The cells were cultured for different times. At different time points, 20 μL of MTT solution (5 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) was dripped into each well, and then the cells were incubated for 4 hours at room temperature. Next, the medium was discarded, and 150 μL of DMSO (Sigma-Aldrich, St. Louis, MO, USA) was loaded into each well to dissolve the formazan. Finally, a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) was used for measuring the absorbance of the cells at 570 nm.

5-bromo-2’-deoxyuridine (BrdU) assay

The viability of transfected cells was examined by a BrdU incorporation assay kit (Wuhan AmyJet Scientific Inc. Wuhan, China). Briefly, Colo-678 and HCT116 cells in different groups were respectively plated into a 96-well plate (1×10³ cells/well). After the DNA was denatured, anti-BrdU antibody (Abcam, Cambridge, UK) was loaded into each well and was subsequently incubated at 4°C overnight. Cells were thoroughly washed with phosphate buffered solution (PBS), and cell nuclei were counterstained by Hoechst 33258 staining solution (Beyotime Institute of Biotechnology, Haimen, China) at ambient temperature for 30 minutes. Immersed in PBS again, cells were accordingly observed under a fluorescent microscope (Olympus BX51, Tokyo, Japan). The number of cells was counted using Image J. The percentage of BrdU positive
cells = the number of red fluorescent cells/the number of blue fluorescent cells × 100%.

Transwell assay

Transwell assays were accomplished with Transwell chambers (pore size: 8 μm; Corning, NY, USA). In the invasion assay, the filter was specifically covered with a layer of Matrigel (30 μg/well; BD, San Jose, CA, USA); in the migration assay, Matrigel was not used. For each well, 1 × 10⁴ cells suspended in serum-free medium was loaded into the upper chamber, with 700 μL of medium containing 20% FBS in the lower chamber as a chemoattractant. Next, cells were cultured for 36 hours, and then cells on the upper surface of the filter were wiped off, and those on the lower were subsequently fixed with ethanol and stained with 0.2% crystal violet solution. Subsequently, the cells were meticulously observed and photographed under an inverted microscope (Nikon TE2000-S, Tokyo, Japan). Specifically, the number of stained cells after migration or invasion was counted with the Image J software.

Dual-luciferase reporter gene assay

Through StarBase software, we found putative binding sites between miR-18a-5p and CASC2 or BTG2. Also, bioinformatics analysis predicted a binding site between miR-18a-5p and CASC2. Then the wild type (WT) and mutant type (MUT) predicted binding sequences of CASC2 were subsequently synthesized and cloned into pmirGLO vector (Promega, Madison, WI, USA) to construct CASC2-WT and CASC2-MUT reporter plasmids. Next, the CRC cells were co-transfected with CASC2-WT or CASC2-MUT and miR-18a-5p or miR-NC. 48 hours later, the relative luciferase activity of the cells in each group was examined by the dual-luciferase assay system (Promega). The targeting relationship between miR-18a-5p and BTG3 was validated in the same way.

RNA immunoprecipitation assay

RNA immunoprecipitation (RIP) assay was accomplished employing the Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA). Colo-678 and HCT116 cells (2 × 10⁷ cells/mL) were collected and subsequently lysed in RIPA buffer containing a protease inhibitor cocktail and RNase inhibitor. Notably, cell lysates were specifically incubated with magnetic beads containing immunoglobin G antibody (IgG, ab172730, Abcam, Cambridge, UK) or argonaute-2 antibody (Ago2, ab186733, Abcam, Cambridge, UK) at 4°C overnight. The samples were accordingly incubated with proteinase K along with shaking at 55°C. Subsequently, the immunoprecipitated RNA was isolated. Ultimately, the enrichment of CASC2 and miR-18a-5p was probed by qRT-PCR. Specifically, the group without antibody was the positive control (Input), the group with IgG antibody was the negative control (anti-IgG), and the group with Ago2 antibody was the experimental one (anti-Ago2).

Western blot

CRC cells harvested were lysed in RIPA lysis buffer (Pierce, Rockford, IL, USA) on ice for 30 minutes to extract the total protein. Notably, the total protein in each sample was respectively quantified by a BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Haimen, China). Then the protein samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore), which was blocked with 5% skimmed milk for 2 hours at 37°C and incubated with primary antibodies against BTG3 (1:1000, ab197399, Abcam, Cambridge, UK) or GAPDH (1:1000, ab181602, Abcam) overnight at 4°C. Next, the membranes were rinsed three times with Tris-buffered saline containing Tween-20 (TBST) and subsequently incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour at ambient temperature. At last, the enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Little Chalfont, UK) was adopted for observing the protein band. GAPDH was utilized as the endogenous reference.

Statistical analysis

The analysis was fulfilled by SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Each experiment was independently replicated 3 times, with data expressed as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used to test the for normality and equal variance of the data. For data that were skewed distributed, comparisons between two groups were performed by the Mann-Whitney test. For normally distributed data, comparisons between two groups were accomplished by t test and one-way analysis of variance. Overall survival analysis was operated with Kaplan-Meier plots and log rank tests. Pearson’s correlation analysis was wielded to delve into the relationships among CASC2, miR-18a-5p and BTG3 mRNA. Chi-square (χ²) test was executed to analyze the relations between CASC2 expressions and the clinicopathological features of CRC patients. P<0.05 denoted meaningful difference.

Results

CASC2 expression characteristics in CRC and its clinical significance

In the beginning, we evaluated CASC2 expression characteristics in cancer tissues and tissues adjacent to cancer from 65 patients with CRC by qRT-PCR. As against normal tissues, CASC2 expression in CRC tissues was remarkably down-regulated (Fig.1A). qRT-PCR was also employed to detect CASC2 expressions in human normal colon epithelial cell line (FHC cells) and 4 human CRC cell lines (Colo-678, HCT116, SW620, and LoVo cells). It was found that compared with FHC cells, CASC2 expression in all 4 CRC cell lines was down-regulated (Fig.1B). Subsequently, we analyzed the subcellular
distribution of \( CASC2 \) and observed that \( CASC2 \) was mainly located in the cytoplasm of CRC cells (Fig.1C). Chi-square test was used to analyze the relations between \( CASC2 \) expressions and the clinicopathological indicators of CRC patients, and the results highlighted that \( CASC2 \) low expression was in close association with the larger tumor size and lymph node metastasis of the patients (Table 1). Besides, Kaplan-Meier analysis indicated that low expression of \( CASC2 \) was associated with poor overall survival of CRC patients (Fig.1D). These findings implied that \( CASC2 \) could probably participate in repressing tumor progression in CRC.

**Table 1: Correlations between \( CASC2 \) expression and multiple clinicopathological characteristics in colorectal cancer patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (n=65)</th>
<th>( CASC2 ) expression</th>
<th>( \chi^2 )</th>
<th>P value</th>
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<tr>
<td></td>
<td>Low (n=33)</td>
<td>High (n=32)</td>
<td></td>
<td></td>
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<td>Age (Y)</td>
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<td></td>
<td>0.7753</td>
<td>0.3785</td>
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<td>&lt;60</td>
<td>35</td>
<td>16</td>
<td>19</td>
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<tr>
<td>≥60</td>
<td>30</td>
<td>17</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.1641</td>
<td>0.6854</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
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<td>12</td>
<td></td>
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<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td>0.1269</td>
<td>0.7216</td>
</tr>
<tr>
<td>I–II</td>
<td>27</td>
<td>13</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>III–IV</td>
<td>38</td>
<td>20</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td>6.9350</td>
<td>0.0085**</td>
</tr>
<tr>
<td>&lt;5</td>
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<td>8</td>
<td>18</td>
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<td>≥5</td>
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<td>Smoking history</td>
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<td>17</td>
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<tr>
<td>No</td>
<td>27</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td>4.5241</td>
<td>0.0334*</td>
</tr>
<tr>
<td>Present</td>
<td>39</td>
<td>24</td>
<td>15</td>
<td></td>
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<tr>
<td>Absent</td>
<td>26</td>
<td>9</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
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<td>3.4529</td>
<td>0.0631</td>
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<tr>
<td>Well/moderate</td>
<td>29</td>
<td>11</td>
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<td></td>
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<tr>
<td>Poor</td>
<td>36</td>
<td>22</td>
<td>14</td>
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*; \( P<0.05 \) and **; \( P<0.01 \).
**Fig.1:** CASC2 was down-regulated in CRC tissues and cells. A. qRT-PCR was used to detect the expression of CASC2 in CRC tissues and adjacent normal tissues (n=65). B. qRT-PCR was adopted to detect the expression of CASC2 in normal colorectal mucosal cell line (FHC) and CRC cell lines (Colo-678, HCT116, SW620, LoVo cells). C. Subcellular fractionation assay was used to measure the expression of CASC2, GAPDH and U6 in the nuclei and cytoplasm of Colo-678 and HCT116 cells. D. Kaplan-Meier analysis showed a correlation between CASC2 expression and overall survival of CRC patients. Data are represented as the mean ± SD (n=3). **; P<0.01, ***; P<0.001, CRC; Colorectal cancer, and qRT-PCR; Quantitative real-time polymerase chain reaction.

**CASC2 overexpression impeded the malignant progression of CRC cells**

To work out the biological function of CASC2 in CRC cells, we transfected CASC2 overexpression plasmids into Colo-678 and HCT-116 cells to construct cell models with CASC2 overexpression (Fig.2A). MTT, BrdU and Transwell assays revealed that in comparison with the control group, CASC2 overexpression greatly inhibited the proliferative, migrative and invasive abilities of Colo-678 and HCT-116 cells (Fig.2B-E).

**CASC2 directly targeted miR-18a-5p in CRC cells**

To expound the downstream mechanism of CASC2, bioinformatics analysis was performed, and a binding site between CASC2 and miR-18a-5p was predicted (Fig.3A). Besides, miR-18a-5p expressions in 65 pairs of CRC tissues and tissues adjacent to cancer were then evaluated by qRT-PCR. As against normal tissues adjacent to cancer, miR-18a-5p was observed to be highly expressed in CRC tissue (Fig.3B). Additionally, miR-18a-5p expression was inhibited in Colo-678 and HCT116 cells with CASC2 overexpression (Fig.3C). Besides, we observed a negative correlation between CASC2 and miR-185-5p expression in CRC tissue (Fig.3D). In addition, dual-luciferase reporter gene assay revealed that miR-18a-5p mimics could demonstrably restrain the luciferase activity of wild-type CASC2 reporter, but make no difference on that of mutant CASC2 reporter (Fig.3E). RIP assay highlighted that CASC2 and miR-18a-5p were significantly enriched in anti-Ago2 group of Colo-678 and HCT116 cells (Fig.3F). Collectively, CASC2 could target miR-18a-5p and regulate its expression negatively.

**Fig.2:** CASC2 overexpression inhibited CRC cell proliferation, migration and invasion. A. qRT-PCR was used to detect the expression of CASC2 in Colo-678 and HCT116 cells transfected with the CASC2 overexpression plasmid. B, C. MTT and BrdU assays were employed to detect the proliferation of CRC cells with CASC2 overexpression (scale bar: 100 μm). D, E. Transwell assay was used to detect the migration and invasion of CRC cells with CASC2 overexpression (scale bar: 250 μm). The data were analyzed by independent samples t test or one-way ANOVA. Data are represented as the mean ± SD (n=3). *; P<0.05, **; P<0.01, ***; P<0.001, NC; Negative control, CRC; Colorectal cancer, qRT-PCR; Quantitative real-time polymerase chain reaction, MTT; Methylthiazol tetrazolium, and BrdU; 5-bromo-2’-deoxyuridine.

**MiR-18a-5p counteracted the biological functions of CASC2 in CRC cells**

To expound the role of CASC2/miR-18a-5p axis in CRC, miR-18a-5p mimics was transfected into Colo-678 and HCT116 cells with CASC2 overexpression.
qRT-PCR showed that the transfection of miR-18a-5p mimics reversed the declination of miR-18a-5p expression caused by CASC2 overexpression (Fig.4A). Next, MTT assay, wound healing assay and transwell assay revealed that miR-18a-5p restoration counteracted the inhibiting impacts of CASC2 overexpression on the proliferative, migrative and invasive abilities of Colo-678 and HCT116 cells (Fig.4B-E). These results implied that CASC2 inhibited the progression of CRC cells via targeting miR-18a-5p.

**BTG3 was the target of miR-18a-5p**

Next, we searched the targets of miR-18a-5p using the StarBase database, and the results showed that miR-18a-5p could probably bind to BTG3 3'-UTR (Fig.5A). Dual-luciferase reporter gene assay indicated that the transfection of miR-18a-5p mimics dramatically repressed the luciferase activity of wild-type BTG3 reporter but could not repress that of the mutant type BTG3 reporter (Fig.5B). qRT-PCR revealed that BTG3 mRNA expression was markedly reduced in CRC tissues and cells (Fig.5C, D). Additionally, western blot assay showed that in comparison with CRC cells, BTG3 protein expression was declined in FHC cells. Transfection of miR-18a-5p mimics reduced BTG3 mRNA and protein expressions in CRC cells; CASC2 overexpression induced the up-regulation of BTG3 in CRC cells, and this effect could be diminished by miR-18a-5p overexpression (Fig.5F, G). Notably, BTG3 mRNA expression was in negative correlation with miR-18a-5p in CRC tissue (Fig.5H), but in positive correlation with CASC2 expression (Fig.5I). The aforementioned results confirmed that BTG3 was a downstream target of miR-18a-5p, and its expression was negatively modulated by miR-18a-5p and positively modulated by CASC2.
Discussion

In recent years, multiple IncRNAs have been identified as potential biomarkers and therapy targets for CRC prognosis and treatment (20). As reported, CASC2 is frequently dysregulated in diverse cancers and modulates a wide range of biological processes (21-23). For instance, CASC2 up-regulation inhibits the viability and metastasis of breast cancer cells by inhibiting the TGF-β signaling pathway (21); CASC2 inhibits the malignant biological processes of hepatocellular carcinoma cells through the MAPK signaling pathway (23). Here, we validated that CASC2 expression was reduced in CRC tissues and cell lines and demonstrated that CASC2 low expression was associated with the unfavorable clinicopathological indexes; functionally, we also demonstrated that CASC2 overexpression can suppress the malignant phenotypes of CRC cells. Our findings are consistent with the previous reports (12).

Reportedly, lncRNA can interact with miRNA as a molecular sponge of miRNA, thereby regulating the expression of the target genes of miRNA (24, 25). Besides, some studies have revealed several target miRNAs of CASC2 in cancer. For example, in hepatocellular carcinoma, CASC2 inhibits cell viability and induces apoptosis via modulating miR-24-3p (26). In addition, CASC2 inhibits the tumorigenesis of hepatocellular carcinoma, melanoma, nasopharyngeal carcinoma and other malignant tumors through sponging miR-18a-5p (27-29). However, there are few reports about the target miRNA of CASC2 in CRC. Here, we confirmed that miR-18a-5p was a direct target of CASC2 in CRC cells.

As reported, miR-18a-5p is pivotal in the pathogenesis of many human diseases including cancers. For example, miR-18a-5p accelerates osteosarcoma cell migration and invasion via directly targeting IRF2 (30); in renal cell carcinoma, miR-18a-5p up-regulation can promote cancer cells proliferation, migration, invasion and inhibit apoptosis (31). The present work proved that miR-18a-5p expression level was elevated in CRC tissues, which is consistent with previous reports (16). Additionally, we focused on the interactions between CASC2 and miR-18a-5p in CRC. Our research proved that miR-18a-5p was a direct target of CASC2, and CASC2 could negatively modulate miR-18a-5p expression. Moreover, up-regulation of miR-18a-5p could counteract the inhibitory effect of CASC2 overexpression on the proliferative, migrative and invasive abilities of CRC cells.

To further understand the downstream mechanism of CASC2, we focused on studying the downstream target of miR-18a-5p. Interestingly, a hidden binding site between miR-18a-5p and BTG3 was revealed by searching the StarBase database. Reportedly, BTG3 is an important participant in regulating cell growth, differentiation, migration, and DNA damage repair (32, 33). In gastric cancer, BTG3 down-regulation facilitates the proliferative, migrative and invasive abilities of gastric cancer cells (18). BTG3 overexpression represses the multiplication and invasion of epithelial ovarian cancer cells via modulating AKT/GSK3β/β-Catenin signaling (34). In this work, we validated that BTG3 was weakly expressed in CRC tissues and cells. Besides, we confirmed for the first time that there was a targeting relation between miR-18a-5p and BTG3 in CRC cells. Moreover, we reported that CASC2 could positively regulate BTG3 expression, probably via suppressing miR-18a-5p. Collectively, CASC2, miR-18a-5p and BTG3 could form a ceRNA network to regulate the CRC development.

Conclusion

According to our findings, CASC2 expression is reduced in CRC tissues and cell lines. In addition, CASC2 can
repress the proliferative, migrative and invasive abilities of CRC cells via targeting miR-18a-5p and increasing BTG3 expression. This work provides a novel mechanism to explain the progression of CRC cells, and restoration of CASC2 may be a novel therapy strategy for CRC treatment.

Acknowledgments

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Authors’ Contributions

X.H.S.; Conceive and experiments design. L.M.K., J.S., J.L.; Experiments performing. F.X., Q.L.Z.; Data analysis. L.M.K., X.H.S.; Manuscript writing. All authors read and approved the final manuscript.

References


