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ORIGINAL RESEARCH ARTICLE

Circular RNA hsa_circ_0053277 promotes the development of colorectal cancer by upregulating matrix metallopeptidase 14 via miR-2467-3p sequestration

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Abstract

Colorectal cancer (CRC), a kind of human gastrointestinal cancer, has been reported to be one of the most common malignant tumors worldwide. Increasing evidence has indicated that circular RNAs exert significant effects on the development of multiple cancers. Nevertheless, whether hsa_circ_0053277 regulates the progression of CRC remains to be explored. In this study, our results showed that the expression of hsa_circ_0053277 was markedly upregulated in CRC tissues and cells. Knockdown of hsa_circ_0053277 inhibited cell proliferation, migration, and epithelial-mesenchymal transition (EMT) process in CRC. miR-2467-3p had a binding site for hsa_circ_0053277. Molecular mechanism assays confirmed that hsa_circ_0053277 could bind with miR-2467-3p. In addition, hsa_circ_0053277 accelerated cell proliferation rate by acting as a sponge for miR-2467-3p in CRC. Matrix metalloproteinase 14 (MMP14) expression was notably upregulated in CRC cells and MMP14 was a downstream target gene of miR-2467-3p. Besides, hsa_circ_0053277 positively regulated MMP14 expression while miR-2467-3p negatively regulated MMP14 expression. Rescue assays verified that MMP14 knockdown countervailed the function of miR-2467-3p inhibitor on cell proliferation, migration, and EMT process in CRC. To sum up, hsa_circ_0053277 facilitated the development of CRC by sponging miR-2467-3p to upregulate MMP14 expression.

KEYWORDS

CRC, hsa_circ_0053277, miR-2467-3p, MMP14

1 | INTRODUCTION

Colorectal cancer (CRC), regarded as one of the most common malignant cancers worldwide among both men and women, has been threatening human's health and life (Torre et al., 2015). CRC progression is a multistep process involving multiple factors such as genetic mutation (Shen et al., 2017). The tumorigenesis and metastasis in CRC lead to many deaths (Brody, 2015; Sasaki et al., 2008). In addition, the incidence of CRC is increasing while the 5-year overall survival rate is still low (Torre et al., 2015). Although many traditional therapeutic strategies have been adopted to treat patients with CRC, including surgery, radiotherapy, and chemotherapy, the outcome remains disappointing (Cunningham et al., 2010). Therefore, it is urgent to understand the molecular regulatory mechanisms in CRC development, which is crucial for researchers to figure out an efficient therapeutic target for CRC patients.

Circular RNAs (circRNAs), a member of noncoding RNAs, have attracted a great of attention owing to their crucial biological roles in cancers (Du et al., 2017). CircRNAs are characterized by a covalently closed loop without either a 5′ cap or 3′ polyadenylate tail and regarded as promising candidates for cancer diagnosis and prognosis (Wang & Li, 2018; Yang et al., 2018). A growing number of

investigations have suggested that circRNAs participate in the tumorigenesis and development of CRC. For instance, circ_0026344 as a prognostic biomarker inhibits the progression of CRC via miR-21 and miR-31 (Yuan, Liu, Zhang, Zhang, & Sun, 2018). The hsa_circ_0020397 modulates cell viability, apoptosis, and invasion in CRC by sponging miR-138 to upregulate the expression of telomerase reverse transcriptase (TERT) and PD-L1 (Zhang, Xu, & Wang, 2017). The hsa_circ_0000523 regulates cell proliferation and apoptosis in CRC by acting as a sponge for microRNA (miRNA; Jin, Yu, Zhang, Liu, & Chen, 2018). The hsa_circRNA_103809 regulated the progression of CRC by targeting miR-532-3p/FOXO4 axis (Bian et al., 2018). However, hsa_circ_0053277 is a novel circRNA that has not been studied in any cancers yet. The specific role of hsa_circ_0053277 in CRC remains to be explored.

In this study, we intended to probe the biological role and molecular regulatory mechanism of hsa_circ_0053277 in CRC. The results of our study showed that hsa_circ_0053277 contributed to the progression of CRC by targeting miR-2467-3p/matrix metalloproteinase (MMP14) axis, which provided a new and potential therapeutic target for CRC patients.

2 | MATERIALS AND METHODS

2.1 | Tissue samples

Three pairs of CRC tissues and normal tissues were collected from patients who were diagnosed with CRC in the Fourth Affiliated Hospital, Harbin Medical University. All patients have signed informed consent. This study has been approved by the ethics committee of the Fourth Affiliated Hospital, Harbin Medical University. Tissues were collected and maintained for microarray analysis.

2.2 | Microarray analysis

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) and then the NanoDrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE) was used to quantify RNA. As for circRNA microarray analysis, the circRNAs chip (Arraystar Human circRNAs chip; ArrayStar, Rockville, MD) was specifically used for human circRNAs splicing sites. After hybridization and washing, CRC tumor tissues and corresponding nontumor tissues were analyzed by using the circRNAs chips. As for miRNA microarray analysis, the target miRNAs were fluorescently labeled by a MiRCURY™ Array Labeling Kit (Exiqon, Vedbaek, Denmark) and hybridized on a miRNA microarray chip (Exiqon). A GenePix 4000B laser scanner (Axon Instruments, Foster City, CA) was used to collect the hybridization data and the GenePix 4.0 software (Axon Instruments) was applied to analyze the images.

2.3 | Cell culture and transfection

Human CRC cell lines (HCT116, SW480, SW620, HT29, RKO, and LOVO) and normal human colonic epithelial cell line (HCoEpiC) were obtained from the American Type Culture Collection. Cells were then cultured in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco) added with 10% fetal bovine serum (FBS) (Gibco) and 1% antibiotics at 37°C in humid air with 5% CO_2.

Sh-hsa_circ_0053277, sh-MMP14, miR-2467-3p mimics, miR-2467-3p inhibitor, and negative controls were bought from Invitrogen, and then transfected into CRC cells by using Lipofectamine 2000 (Invitrogen) based on the manufacturer's instructions.

2.4 | RNase R treatment and RT-qPCR

Total RNA was cultured at 37°C for 15 min, with or without 3 U/mg RNase R (Epicentre Technologies, Madison, WI), and was used for RNase R treatment. TRIzol reagent (Thermo Fisher Scientific, Waltham, MA) was used to isolate total RNA from CRC tissues or cells in accordance with the manufacturer's instructions. The Transcriptor First Strand Complementary DNA (cDNA) Synthesis Kit (Roche, Germany) or TaqMan™ Advanced miRNA cDNA Synthesis Kit (Waltham, MA) was used to reverse transcribe RNA into cDNA. The SYBR Premix Ex Taq (Takara) for circRNA and messenger RNA (mRNA) analysis and the Taqman Universal Master Mix II for miRNA detection were used to conduct real-time quantitative polymerase chain reaction (RT-qPCR) on an ABI 7300 system (ABI). The $2^{-\Delta\Delta Ct}$ method was applied to examine gene expression. U6 and GAPDH were used as internal controls.

2.5 | Nucleic acid electrophoresis

Two percent agarose gel electrophoresis was used to investigate the cDNA and genomic DNA (gDNA) PCR products in Tris base-acetic acid-Ethylenediaminetetraacetic acid mixture (TAE) running buffer. DNA was isolated by electrophoresis at 120 V. After 30 min electrophoresis, Super DNA Marker (cat. CW2583MCWBIO; Beijing) was used to mark DNA. Afterward, ultraviolet irradiation was utilized to examine the bands.

2.6 | Cell Counting Kit-8 assay

Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) assay was applied to measure cell proliferation. Transfected cells (1×10^3) were seeded in a 96-well plate. After incubation for 0, 24, 48, 72, and 96 hr, CCK-8 solution was added to each well and then cultured at 37°C for 2 hr. The absorbance at 450 nm was measured by a Multiskan Go spectrophotometer (Thermo Fisher Scientific, Inc.)

2.7 | Colony formation assay

Cells (1×10^3) were seeded into six-well plate. After being transfected with different plasmids, cells were cultured in DMEM medium (containing 10% FBS) for 2 weeks. The cells were fixed in methanol and then stained with crystal violet at room temperature for half an hour. The images were caught by a microscope (XSP-11CC; Caikon, Jiading, Shanghai, China) and cell colonies larger than 0.1 mm in diameter were counted.

2.8 | Transwell assay

Transwell chamber (Costar, Washington, DC) without Matrigel (BD Biosciences, Bedford, MA) was utilized to perform migration assay. Transfected cells (1×10^3) were added to the top chamber with 200 ml serum-free medium. Whereas, 600 ml medium containing 10% FBS was planted into the bottom chamber. After 24 hr, migrated cells were fixed by paraformaldehyde and then stained by crystal violet solution (Beyotime, Beijing, China). Images were caught by a light microscope (Olympus Corporation, Tokyo, Japan) and then stained cells were counted.

2.9 | Western blot assay

Cells were lysed in RIPA buffer, and then Protein Extraction Kit (KGP9100; Key Gene) was used to extract total proteins. The expression of total proteins was examined by Bicinchoninic Acid Assay Kit (Biodragon Biotech, Haidian, Beijing, China). Afterward, total proteins were isolated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica). After being blocked with 5% bovine serum albumin (BSA), membranes were incubated at 4°C overnight with primary antibody. Next, membranes were washed with phosphate buffer saline (PBS)-Tween-20 buffer for three times and then were cultured with secondary antibody at room temperature for about 1 hr. A chemiluminescence detection system (Thermo Fisher Scientific) was used to detect visualized protein bands. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

2.10 | Immunofluorescence analysis

The CRC cell lines were seeded in cell culture dishes (NEST Biotech, Wuxi, China) at 37°C with 5% CO_2 in a humid atmosphere overnight. After being washed with PBS two times, cells were fixed with formaldehyde and permeabilized with Triton X-100. Afterward, cells were blocked with 1% BSA for 30 min and were cultured with specific primary antibody at 4°C overnight. Successively, corresponding secondary antibody and 4',6-diamidino-2-phenylindole (DAPI) used for nuclear staining were put in a specially designed dish. At last, a confocal microscope (Nikon, Japan) was used to observe the cells.

2.11 | Subcellular fractionation assay

PARIS™ Kit (Invitrogen) was used to separate cytoplasmic and nuclear RNAs from CRC cells according to the manufacture's suggestions. The cellular localization of hsa_circ_0053277 was detected by RT-qPCR. U6 was served as the nuclear control while GAPDH was used as cytoplasmic control.

2.12 | Luciferase reporter assay

To demonstrate the interaction between hsa_circ_0053277 and miR-2467-3p, hsa_circ_0053277 containing the binding sequence with miR-2467-3p (hsa_circ_0053277-WT) or not (hsa_circ_0053277-Mut) was subcloned into pmirGLO vectors (Promega, Madison). Similarly, the whole sequence of MMP14 3′-untranslated region containing binding sites with miR-2467-3p (MMP14-WT) or not (MMP14-MUT) was subcloned into pmirGLO vectors (Promega). RKO and LOVO cells were incubated in the 24-well plate overnight. Vectors, as mentioned above, were then cotransfected into RKO and LOVO cells along with miR-2467-3p mimics or negative control (NC) mimics by using Lipofectamine 2000 in accordance with the manufacturer's instructions. 24 hr later, the luciferase activity was measured by Dual-Luciferase Reporter Gene Assay Kit (Promega) and was normalized to Renilla luciferase activity (Promega).

2.13 | RIP assay

EZ-Magna RNA Immunoprecipitation (RIP) Kit (Millipore) was used to perform RIP assay. In brief, cells were lysed in the RIP lysis buffer, and then cultured with RIP buffer supplemented with magnetic beads coated with human Ago2 antibody. Proteinase K was used for digestion of the protein. Immunoprecipitated RNAs were then isolated and purified. RT-qPCR was carried out to detect the relative expression of these RNAs. Immunoglobulin G (IgG) was treated as a negative control.

2.14 | Statistical analysis

SPSS software version 20.0 (SPSS, Inc., Chicago) was used to analyze the results. Data were displayed as the mean ± standard deviation. Each experiment was operated in triplicate. One-way analysis of variance or *t* test was adopted to determine *p* value. The $p < .05$ was considered as statistical significance.

3 | RESULTS

3.1 | The expression of hsa_circ_0053277 is upregulated in CRC tissues and cells, and hsa_circ_0053277 knockdown suppresses cell proliferation

Studies have indicated that circRNAs elicit crucial effects on the progression of numerous cancers (Ma, Yao, Yu, Chen, & Li, 2018; Shen et al., 2019; Zong et al., 2018). To probe the biological role of circRNAs in CRC, the following experiments were performed. First, through heatmap, we found circRNAs with significant expression differences in CRC tissue samples and noncancer tissue samples, including 482 circRNAs with upregulated expression (Figure 1a). The expression of hsa_circ_0053277 was found to be observably upregulated in CRC tissues from the heatmap. Then hsa_circ_0053277 was chosen to conduct RT-qPCR assay. The results delineated that the expression of hsa_circ_0053277 was observably higher in CRC cell lines (HCT116, SW480, SW620, HT29, RKO, and LOVO) than that in normal human colonic epithelial cell line (HCoEpiC; Figure 1b). According to the results of PCR analysis, divergent primers could produce the circular

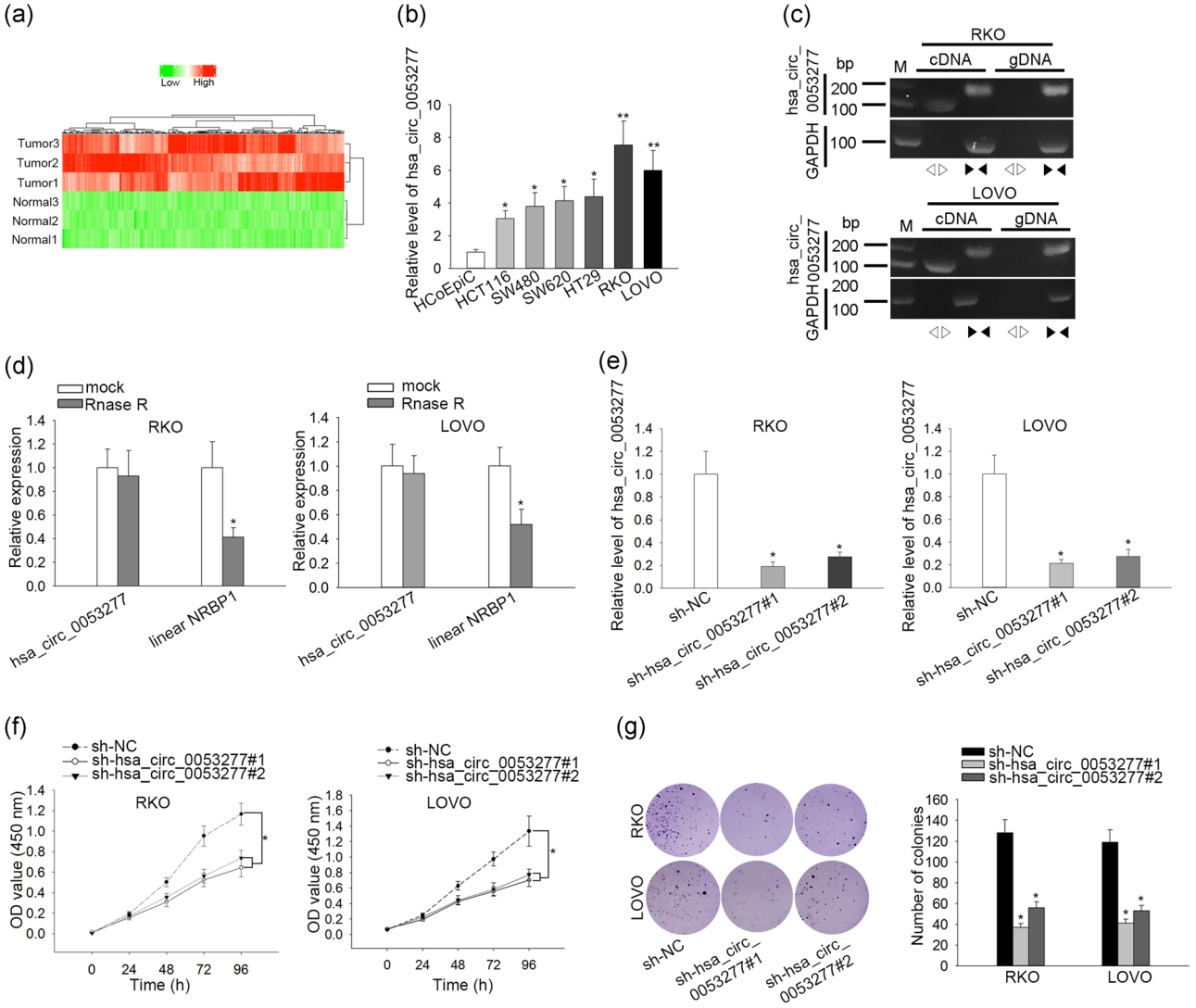

FIGURE 1 The expression of hsa_circ_0053277 is upregulated in CRC tissues and cells, and hsa_circ_0053277 knockdown suppresses cell proliferation. (a) 482 circRNAs, with upregulated expression in CRC tissue samples and noncancer tissue samples, were displayed by heatmap. (b) The RT-qPCR assay was used to detect the expression of hsa_circ_0053277 in CRC cell lines (HCT116, SW480, SW620, HT29, RKO, and LOVO) and normal human colonic epithelial cell line (HCoEpiC). (c) The existence of hsa_circ_0053277 was validated in RKO and LOVO cell lines by RT-PCR. Divergent primers amplified hsa_circ_0053277 in cDNA but not in gDNA. GAPDH was regarded as a negative control. (d) The expression of hsa_circ_0053277 and linear NRBP1 mRNA in both RKO and LOVO cells treated with or without RNase R was detected by RT-PCR. Mock-treated cells served as normal control. (e) The expression level of hsa_circ_0053277 was detected by RT-qPCR in RKO and LOVO cells transfected with sh-hsa_circ_0053277#1/2 or sh-NC. (f–g) CCK-8 and colony formation assays were conducted to examine the proliferation ability of transfected cells. *$p < .05$. cDNA, complementary DNA; CRC, colorectal cancer; gDNA, genomic DNA [Color figure can be viewed at wileyonlinelibrary.com]

isoform of hsa_circ_0053277 with cDNA but not with gDNA, whereas convergent primers could amplify the linear isoform of hsa_circ_0053277 from both cDNA and gDNA in RKO and LOVO cells (Figure 1c). In addition, the fragment of linear NRBP1 was digested by RNase R, but hsa_circ_0053277 remained after RNase R treatment in RKO and LOVO cells (Figure 1d). To explore the function of hsa_circ_0053277 in CRC, sh-hsa_circ_0053277#1/2 with sh-NC as scramble control was utilized to silence hsa_circ_0053277. RT-qPCR assay displayed that the expression of hsa_circ_0053277 was notably decreased by hsa_circ_0053277 silencing in RKO and LOVO cells (Figure 1e). Moreover, CCK-8 and colony formation assays suggested that knockdown of hsa_circ_0053277 inhibited the proliferation of RKO and LOVO cells (Figure 1f–g). Overall, hsa_circ_0053277 is highly expressed in CRC tissues and cells, and hsa_circ_0053277 depletion inhibits cell proliferation.

3.2 | Knockdown of hsa_circ_0053277 inhibits cell migration and epithelial-mesenchymal transition process in CRC

Afterward, experiments were performed to further study the biological role of hsa_circ_0053277 in CRC. Transwell assay was

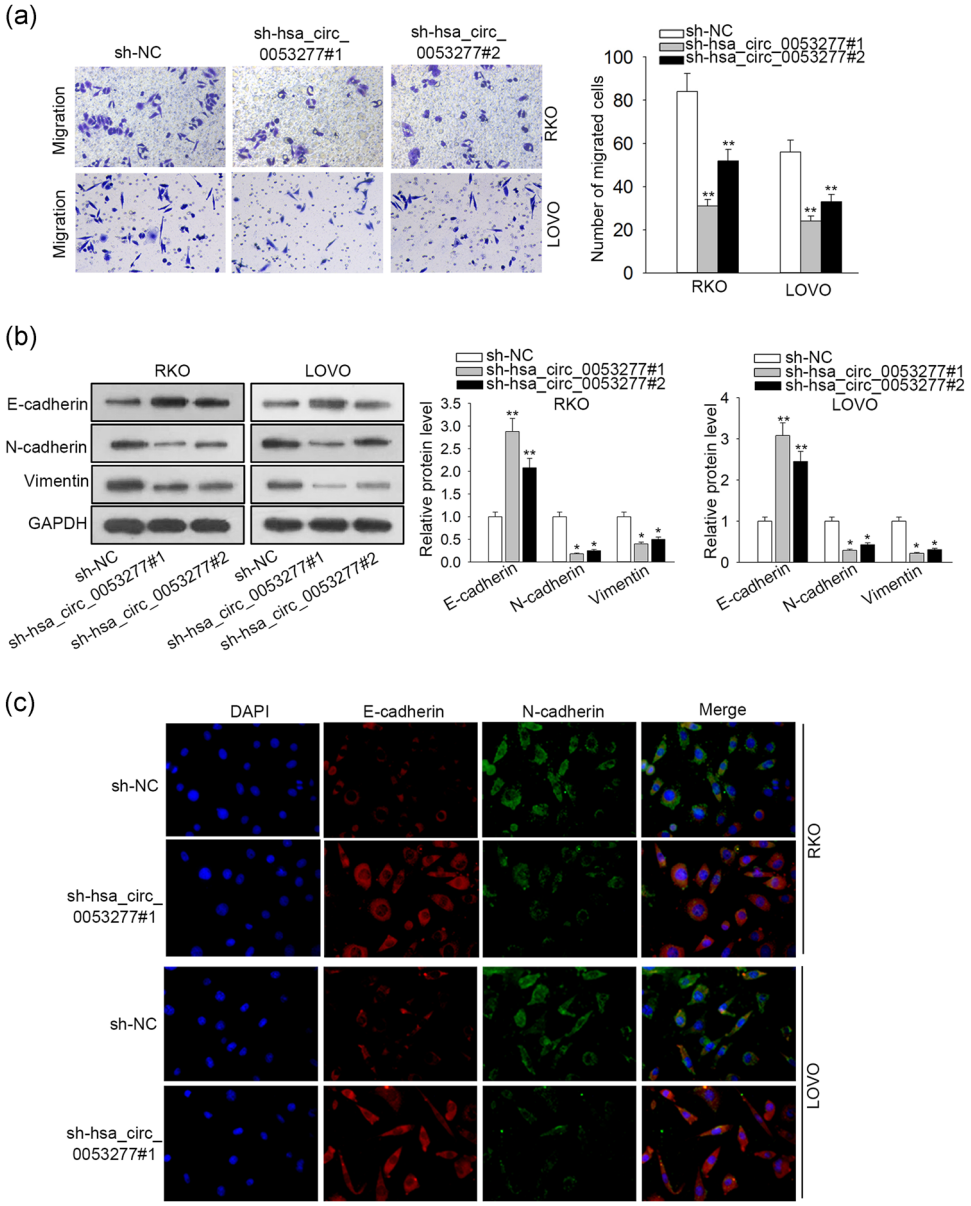

FIGURE 2 Knockdown of hsa_circ_0053277 inhibits cell migration and EMT process in CRC. (a) The migration ability of transfected cells was analyzed by transwell assay. (b) The protein expression of E-cadherin, N-cadherin, and Vimentin was evaluated by western blot assay. GAPGH was used as an internal control. (c) Immunofluorescence assay was applied to assess the expression level of E-cadherin and N-cadherin in transfected cells. $^{*}p < .05$. CRC, colorectal cancer; EMT, epithelial-mesenchymal transition [Color figure can be viewed at wileyonlinelibrary.com]

applied to detect cell migration ability. The number of migrated cells was remarkably reduced in RKO and LOVO cells transfected with sh-hsa_circ_0053277#1/2, indicating that hsa_circ_0053277 downregulation restrained cell migration (Figure 2a). Western blot assay showed that knockdown of hsa_circ_0053277 significantly increased the protein expression of E-cadherin whereas decreased the protein expression of N-cadherin and Vimentin in RKO and LOVO cells, which suggested that hsa_circ_0053277 knockdown inhibited epithelial-mesenchymal transition (EMT) process (Figure 2b). Besides, immunofluorescence assay demonstrated that knockdown of hsa_circ_0053277 successfully enhanced the expression level of E-cadherin but suppressed the expression level of N-cadherin, confirming that hsa_circ_0053277 silencing could inhibit EMT process (Figure 2c). Taken together, hsa_circ_0053277 knockdown suppresses the migration and EMT process of CRC cells.

3.3 | Hsa_circ_0053277 acts as a sponge for miR-2467-3p in CRC

To investigate the molecular mechanism of hsa_circ_0053277 in CRC, we first detected the distribution of hsa_circ_0053277 in CRC cells. The results of subcellular fractionation assay verified that hsa_circ_0053277 was mainly enriched in the cytoplasm (Figure 3a). Hundred miRNAs that could possibly bind with hsa_circ_0053277 were found from Heatmap. In

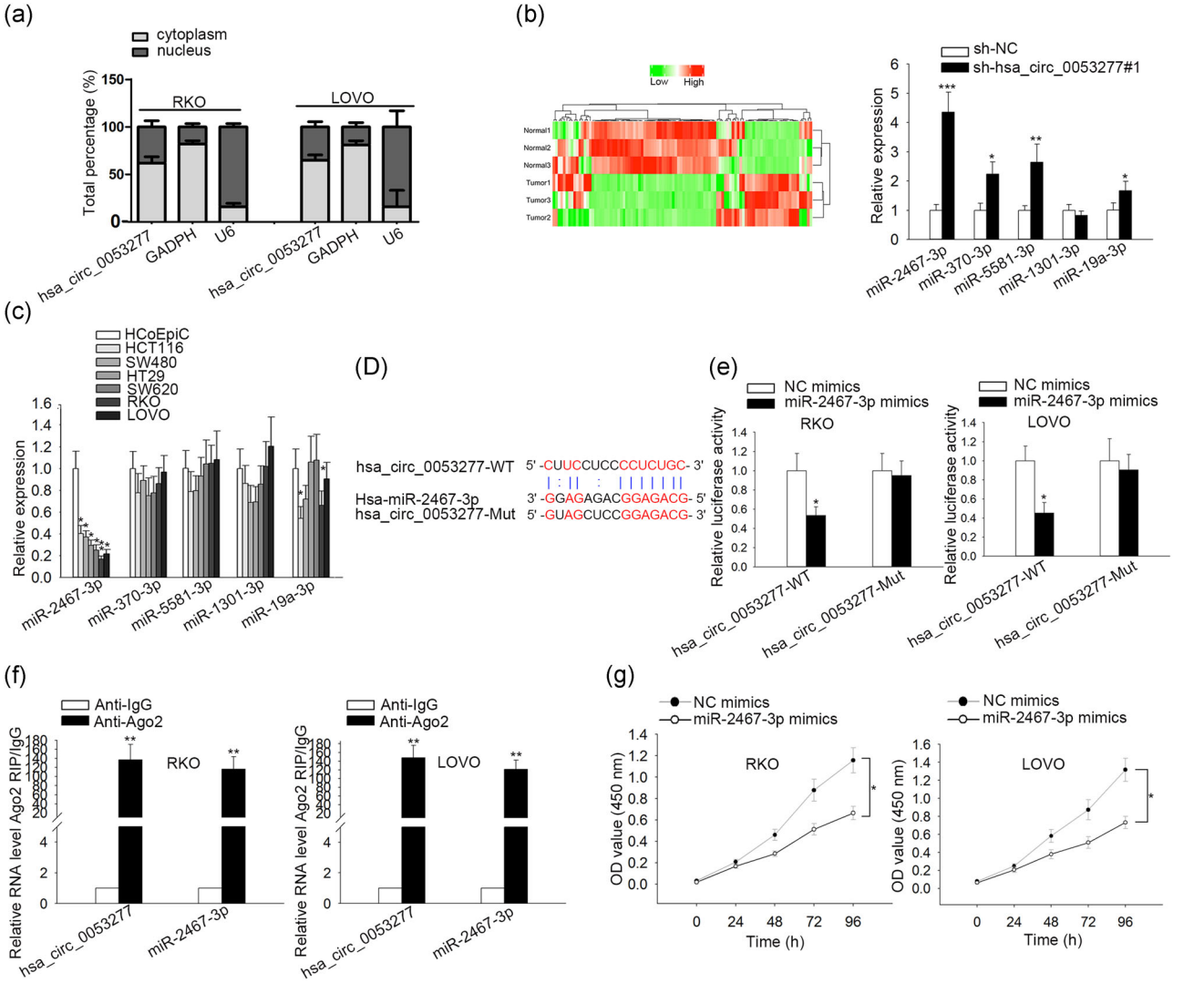

FIGURE 3 Hsa_circ_0053277 acts as a sponge for miR-2467-3p in CRC. (a) Subcellular fractionation assay was performed to probe the distribution of hsa_circ_0053277. (b) miRNAs that could possibly bind with hsa_circ_0053277 were found from the heatmap. RT-qPCR assay was used to detect the expression of five miRNAs (miR-2467-3p, miR-370-3p, miR-5581-3p, miR-1301-3p, and miR-19a-3p) in sh-hsa_circ_0053277#1-transfected cells. (c) The expression of five miRNAs was examined by RT-qPCR in CRC cell lines and normal human colonic epithelial cell line. (d) miR-2467-3p had a binding site for hsa_circ_0053277. (e, f) The interaction between hsa_circ_0053277 and miR-2467-3p was confirmed by luciferase reporter and RIP assays. (g) Cell proliferation capability in different groups was measured by CCK-8 assay. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. CRC, colorectal cancer; miRNA, microRNA [Color figure can be viewed at wileyonlinelibrary.com]

addition, five miRNAs (miR-2467-3p, miR-370-3p, miR-5581-3p, miR-1301-3p, and miR-19a-3p) were chosen to perform RT-qPCR assay. An obvious upregulation of miR-2467-3p was observed in sh-hsa_circ_0053277#1-transfected cells (Figure 3b). Furthermore, miR-2467-3p expression was downregulated in CRC cell lines compared with the other four miRNAs (Figure 3c). Through searching starBase, miR-2467-3p was found to have a binding site for hsa_circ_0053277 (Figure 3d). In addition, the luciferase activity of pGL3-hsa_circ_0053277-WT (wild type) was reduced by miR-2467-3p mimics while the luciferase activity of pGL3-hsa_circ_0053277-Mut (mutant type) displayed no evident change between NC mimics and miR-2467-3p mimics group (Figure 3e). Furtherly, hsa_circ_0053277 and miR-2467-3p were distributed in the anti-Ago2 group but not in the anti-IgG group (Figure 3f). CCK-8 assay testified that miR-2467-3p overexpression suppressed the proliferation of RKO and LOVO cells (Figure 3g). Collectively, hsa_circ_0053277 sponges miR-2467-3p in CRC.

3.4 | MMP14 functions as a downstream target gene of miR-2467-3p in CRC

Previous studies have suggested that miRNAs modulate cancer progression by targeting mRNAs (Feliciano et al., 2017; Yu et al., 2019). To figure out the underlying target gene of miR-2467-3p in CRC, we searched microT, miRmap, and RNA22 databases and the results showed that there were 33 potential target genes of miR-2467-3p (Figure 4a). Owing to the fact that MMP14 has been

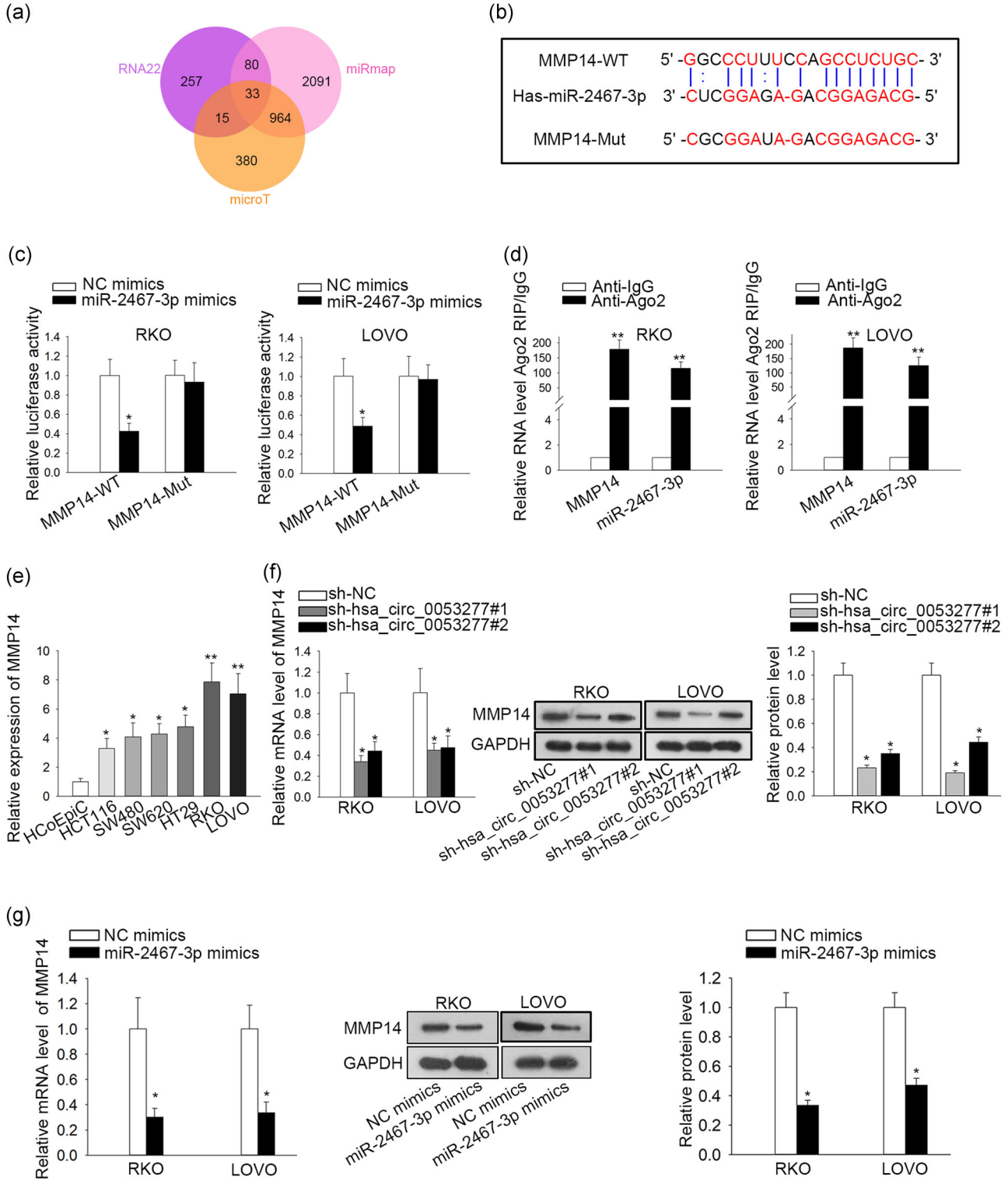

FIGURE 4 MMP14 functions as a downstream target gene of miR-2467-3p in CRC. (a) Schematic illustration displayed the overlap of the target genes of miR-2467-3p predicted by microT, miRmap, and RNA22. (b) MMP14 had a binding site for miR-2467-3p. (c,d) The interaction between miR-2467-3p and MMP14 was confirmed by luciferase reporter and RIP assays. (e) The expression of MMP14 was examined by RT-qPCR assay in CRC cell lines and normal human colonic epithelial cell line. (f,g) RT-qPCR and western blot assays were respectively utilized to measure the mRNA and protein expression of MMP14 in transfected cells. GAPGH was an internal control. $^{*}p < .05$, $^{**}p < .01$. CRC, colorectal cancer; MMP, matrix metalloproteinase [Color figure can be viewed at wileyonlinelibrary.com]

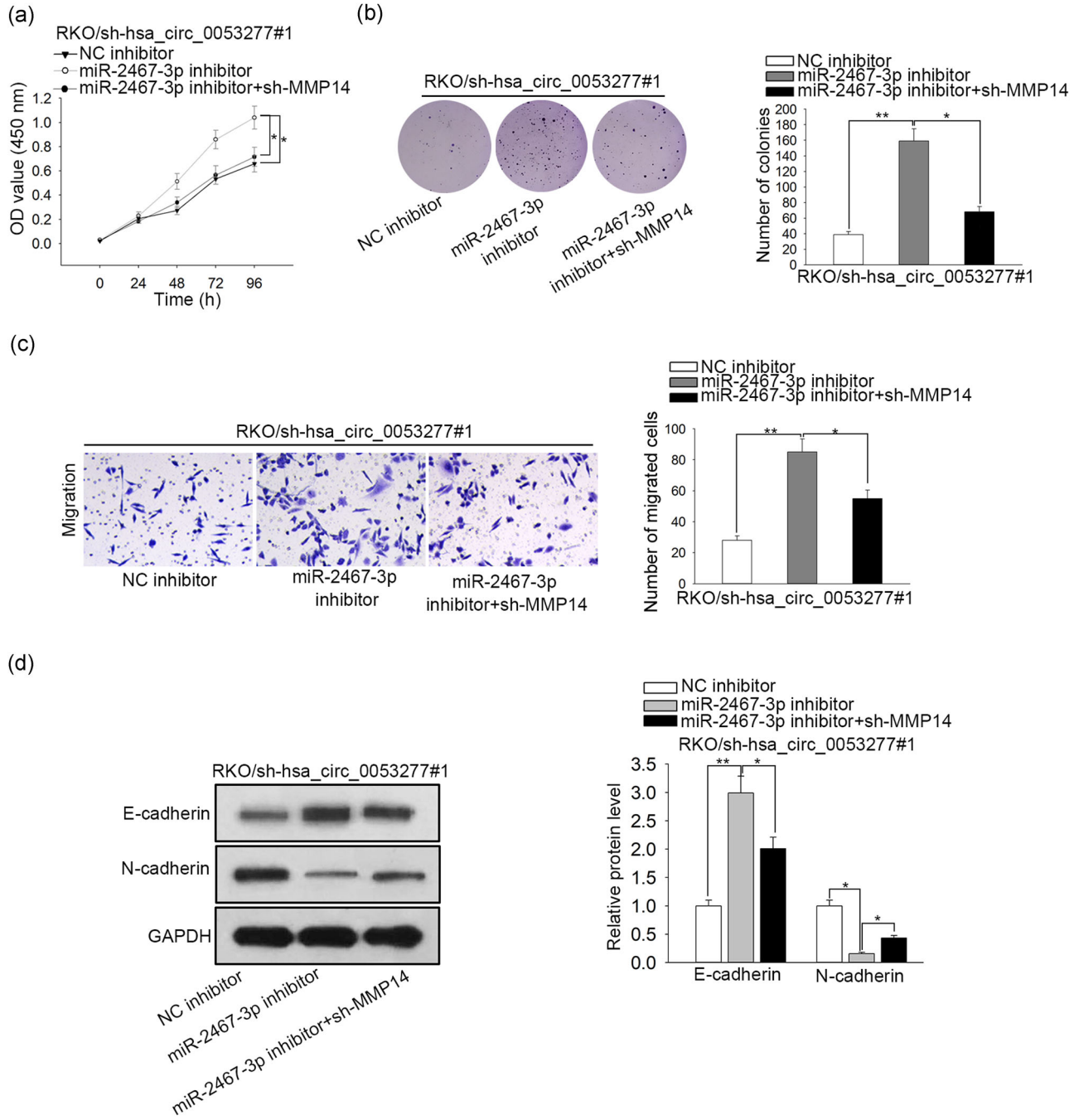

FIGURE 5 The hsa_circ_0053277 facilitates CRC progression via miR-2467-3p/MMP14 axis. (a, b) The proliferation ability of transfected cells was estimated by CCK-8 and colony formation assay. (c) The migration ability of transfected cells was evaluated by transwell assay. (d) The EMT process was analyzed by western blot assay. GAPDH was treated as an internal control. $^{*}p < .05$, $^{**}p < .01$. EMT, epithelial-mesenchymal transition [Color figure can be viewed at wileyonlinelibrary.com]

reported to be an oncogenic gene (Chen et al., 2016; Zuo et al., 2015), MMP14 was chosen to do the follow-up experiments. MMP14 was discovered to have a binding site for miR-2467-3p from starBase (Figure 4b). In addition, a notable decrease in the luciferase activity of pGL3-MMP14-WT induced by miR-2467-3p upregulation was observed in RKO and LOVO cells. However, the luciferase activity of pGL3-MMP14-Mut demonstrated no evident change (Figure 4c). RIP assay suggested that MMP14 and miR-2467-3p were aggregated in Ago2 antibody group instead of IgG antibody group (Figure 4d). Moreover, MMP14 expression was remarkably upregulated in CRC cell lines in comparison with normal human colonic epithelial cell line (Figure 4e). RT-qPCR and western blot assays confirmed that hsa_circ_0053277 knockdown or miR-2467-3p mimics conspicuously reduced mRNA and protein expression of MMP14 (Figure 4f–g). To sum up, MMP14 can bind with miR-2467-3p and hsa_circ_0053277 positively regulates MMP14

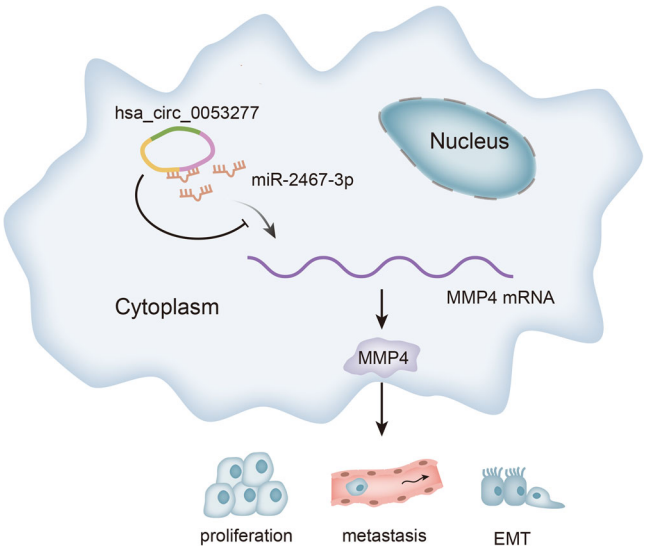

FIGURE 6 A graphical abstract was generated to illustrate the role of circ_0053277/miR-2467-3p/MMP14 axis in CRC progression [Color figure can be viewed at wileyonlinelibrary.com]

expression whereas miR-2467-3p negatively regulates MMP14 expression in CRC.

3.5 | Hsa_circ_0053277 facilitates CRC progression via miR-2467-3p/MMP14 axis

Rescue assays were conducted to test the regulatory mechanism of hsa_circ_0053277 in CRC. MMP14 downregulation reversed the promoting function of miR-2467-3p inhibitor on the proliferation of RKO cells transfected with sh-hsa_circ_0053277#1 according to the results of CCK-8 and colony formation assays (Figure 5a,b). Similarly, knockdown of MMP14 countervailed the effects of miR-2467-3p suppression on the migration ability of sh-hsa_circ_0053277#1-transfected cells (Figure 5c). Due to the results of western blot assay that MMP14 deficiency recovered the protein expression of E-cadherin and N-cadherin caused by miR-2467-3p downregulation in RKO cells transfected with sh-hsa_circ_0053277#1, we confirmed that MMP14 knockdown offset the EMT process induced by miR-2467-3p inhibitor in sh-hsa_circ_0053277#1-transfected cells (Figure 5d). All the findings suggest that hsa_circ_0053277 promotes CRC development via miR-2467-3p/MMP14 axis (Figure 6).

4 | DISCUSSION

CRC has been reported to be a malignant tumor, with high incidence and mortality (Torre et al., 2015). Abundant evidence has confirmed that circRNAs exert their crucial effects on the development of various cancers. For example, Increased expression of circRNA_102231 promotes the progression of lung cancer (Zong et al., 2018). CircRNA_001569 promotes the proliferation of gastric cancer cells by absorbing miR-145 (Shen et al., 2019). CircRNA-000284 promotes cell proliferation and invasion in cervical cancer by acting as a sponge for miR-506 (Ma et al., 2018). The hsa_circRNA_101996 promotes cell proliferation and invasion in cervical cancer by restraining miR-8075 to activate TPX2 expression (Song et al., 2019). The hsa_circ_0053277 was a novel circRNA in cancer research and the role of it in CRC needs to be explored. In this study, the expression of hsa_circ_0053277 was upregulated in CRC tissues and cells. Silencing hsa_circ_0053277 suppressed cell proliferation, migration, and EMT process in CRC.

miRNAs, which belong to the noncoding single-stranded small-molecule RNAs with a length of about 22 nucleotides, have been reported to participate in tumor onset and progression (Acunzo, Romano, Wernicke, & Croce, 2015; Gajos-Michniewicz, Duechler, & Czyz, 2014). For instance, miR-99a modulates the expression of E2F2 and EMR2 and suppresses stemness in lung cancer (Feliciano et al., 2017). Serum miR-16 acts as an underlying biomarker for the diagnosis of human cancer (Huang et al., 2019). miR-190 facilitates endocrine therapy sensitivity by modulating SOX9 expression in breast cancer (Yu et al., 2019). miR-193b modulates colon cancer progression by targeting RAB22A (Fang, Li, & Li, 2019). miR-2467-3p is a new miRNA that has not been studied in cancer. In our study, miR-2467-3p was found to have a binding site for hsa_circ_0053277 and could bind with hsa_circ_0053277 in CRC.

Previous studies have indicated that MMP14 plays an important role in tumor growth and metastasis. For example, miR-22 suppresses gastric cancer progression by targeting MMP14 and Snail (Zuo et al., 2015). Human astrocytes secrete IL-6 to facilitate cell migration and invasion in glioma by upregulating cytomembrane MMP14 (Chen et al., 2016). MMP14 acts as a tumor promoter in CRC (Cui, Cai, Ding, & Gao, 2019). Our study demonstrated that MMP14 expression was upregulated in CRC cells and MMP14 was a downstream target gene of miR-2467-3p. Besides, hsa_circ_0053277 positively regulated MMP14 expression while miR-2467-3p negatively regulated MMP14 expression. Furthermore, rescue assays testified that MMP14 downregulation offset the function of miR-2467-3p inhibitor on cell proliferation, migration, and EMT process in CRC.

In other words, hsa_circ_0053277 facilitated the development of CRC by targeting miR-2467-3p/MMP14 axis, indicating that hsa_circ_0053277 could be treated as a therapeutic target for CRC patients.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

Authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Hongqi Xiao: manuscript and experiments. Ming Liu: figures and data analysis.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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