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Circ_0000554 is identified as a cancer-promoting circRNA in colorectal cancer by regulating the miR-1205/LASP1 axis

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Abstract

Background: Colorectal cancer (CRC) is a prevalent malignant tumor with poor prognosis. Circular RNAs (circRNAs) are key regulators in the progression of CRC. Our study aimed to disclose the role of circ_0000554 in CRC.

Methods: The expression of circ_0000554, miR-1205 and LIM and SH3 protein 1 (LASP1) was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation, invasion and migration were monitored using cell counting kit-8 (CCK-8) assay, EdU assay, transwell assay and wound healing assay respectively. The protein levels of C-myc, matrix metallopeptidase 2 (MMP-2) and LASP1 were detected by western blot. Tumor formation assay in nude mice was conducted to explore the role of circ_0000554 in vivo. The association between miR-1205 and circ_0000554 or LASP1 was identified by dual-luciferase reporter assay and RNA immunoprecipitation (RIP) assay.

Results: circ_0000554 was upregulated in CRC tissues and cells, high circ_0000554 expression was significantly linked to shorter overall survival. Downregulation of circ_0000554 restrained cell growth and metastasis while promoted apoptosis in vitro, and suppressed tumorigenesis of CRC in vivo. Furthermore, mechanism study and rescue experiments confirmed miR-1205 could be sponged by circ_0000554 and its inhibitor reversed the inhibitory effect of circ_0000554 silencing on CRC progression. LASP1 was a target gene of miR-1205 and the upregulation of LASP1 overturned miR-1205-induced effects on CRC cells. Circ_0000554 could elevate LASP1 expression via interacting with miR-1205.

Key points

- Circ_0000554 expression is upregulated in colorectal cancer tissues and cells.
- Circ_0000554 knockdown inhibits cell proliferation, migration and invasion of colorectal cancer cells.
- Circ_0000554 knockdown inhibits tumor formation in vivo.
- Circ_0000554 promotes colorectal cancer development by regulating the miR-1205/LASP1 pathway.

Keywords: circ_0000554, Colorectal cancer, miR-1205, LASP1

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Introduction

Colorectal cancer (CRC) is an aggressive malignancy [1]. Although great progress has been made in the treatment of CRC, the death rate among CRC patients remains frustratingly high [2, 3]. In recent years, the application

of targeted therapy and molecular marker technology in metastatic colorectal cancer has attracted great attention [4]. This provides a promising approach to the treatment of patients [5]. However, the mechanism of CRC is still unclear and further research is needed.

Recently, the role of circular RNAs (circRNAs) in cancer progression has been gradually revealed [6]. circRNAs are characterized by a covalently closed loop structure without 5'caps and 3'tails, featured by high stability and tissue specificity [7]. Current research has confirmed that circRNA was involved in the pathological development of CRC [8]. For instance, Huang et al. highlighted circ_0029803 deficiency restrained proliferation and metastasis by targeting the miR-216b-5p/SKIL axis [9]. Bai et al. identified circ_0007334 accelerated CRC tumorigenesis by sponging miR-577 [10]. Wang et al. illuminated that circDUSP16 enhanced the malignant development of CRC by elevating E2F6 [11]. Nevertheless, there were many abnormally expressed circRNAs in CRC tissues and cells, and their function remained unclear. Through the GEO database analysis (accession: GSE126094), we obtained numerous differently expressed circRNAs in CRC tumor tissues. Hsa_circRNA_001059, also termed as circ_0000554, was obviously increased in tumor tissues. However, the function and mechanism of circ_0000554 was hardly investigated in CRC until now. circ_0000554 has been reported to promote the malignant development of esophageal cancer [12]. Therefore, we hypothesized that circ_0000554 might also play a carcinogenic role in CRC.

When it comes to circRNAs, microRNAs (miRNAs) cannot be ignored because circRNAs are typical as miRNA sponges. MiRNAs is a small noncoding RNA with about 20 nucleotides [13]. MiRNAs mainly acts function by binding to the non-coding regions of target genes, and further leads to mRNA degradation or translation inhibition [14]. Bioinformatics database predicted that circ_0000554 harbored binding sites with miR-1205. Studies have shown that miR-1205 was involved in inhibiting the development of CRC [15]. However, it was unknown whether circ_0000554 played a role by targeting miR-1205 in CRC. In addition, the targetscan website predicts that miR-1205 binds to the 3′untranslated region (3′UTR) of LIM and SH3 protein 1 (LASP1), hinting that LASP1 might be a target of miR-1205. LASP1 was a well-known oncogene in various cancers, including CRC [16]. It was unclear whether miR-1205 affected the biological function of CRC by targeting LASP1. Therefore, the mechanism of action related to circ_0000554 and miR-1205 by LASP1 need to be studied and explored.

Hence, the purpose of our research was to explore the function of circ_0000554 in CRC tumorigenicity. Moreover, we also explored the action mode of circ_0000554 in regulating CRC development, which might provide a novel therapeutic target for CRC.

Materials and methods

Public database

CircRNA expression profile was obtained from GEO database (GSE126094: https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE126094). Bioinformatics tool (https://circinteractome.nia.nih.gov/index.html) and (http://www.targetscan.org/vert_71/) for target prediction was applied in this study.

Tissue specimens

CRC tissues and the para-cancerous tissues were collected from 33 postoperative CRC patients at Zigong Fourth People's Hospital. All tissues were stored at − 8 °C. The research was authorized by the Ethics Committee of Zigong Fourth People's Hospital, and each patient signed the written informed consents. The tumor tissues and the matched non-cancer tissues were embedded in paraffin for further immunohistochemical (IHC) analysis with Ki67 (anti-Ki67, ab15580, Abcam, Cambridge, UK).

Cell lines

CRC cell lines (LOVO, SW480 and HCT-116) and normal intestinal epithelial cell line (NCM460) cells were obtained from Bena culture collection (Beijing, China). Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Grand Island, NY, USA) was used to culture the cells at 37 °C with 5% CO_2. The medium was supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Gibco).

IHC assay

Treated tissue sections were incubated with anti-LASP1 (ab117806, Abcam), anti-C-myc (ab17355, Abcam), and anti-MMP2 (ab235167, Abcam), respectively. Then, goat-anti rabbit IgG-HRP (ab6721, Abcam) was used to incubate the tissue sections. Finally, it is photographed under a light microscope (Leica, Wetzlar, Germany).

Quantitative real-time polymerase chain reaction (QRT-PCR)

RNA was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) Then, RNA was reversely transcribed into cDNA using the PrimeScript RT reagent kit (Takara, Dalian, China) according to the instructions. QRT-PCR was conducted with SYBR Green (Takara). Relative expression was normalized by Glyceraldehyde-phosphate dehydrogenase (GAPDH) or U6 by using the $2^{-\Delta\Delta Ct}$ method. The sequences were displayed in Table 1.

Table 1 Primers sequences used for PCR

Name		Primers for PCR (5′-3′)
hsa_circ_0000554	Forward	GGCACTAGGGAGGGACTCAT
	Reverse	TGGATGTTCCCAGTAGAGCA
LASP1	Forward	TCGGAACCATGAACCCCAAC
	Reverse	GGACTGCTTGGGGTAGTGTG
miR-1205	Forward	GTATGAGTCTGCAGGGTTTGCT
	Reverse	CTCAACTGGTGTCGTGGAG
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG
U6	Forward	GCTTCGGCAGCACATATACTAA
	Reverse	AACGCTTCACGAATTTGCGT

Stability analysis of circ_0000554

Total RNA from CRC cells was exposed to RNase R (Epicentre, Madison, WI, USA) at a concentration of 3 U/μg RNA at 37 °C for 30 min. Then, RNA samples were used for qRT-PCR analysis.

Cell transfection

Small interfering RNA (siRNA) against circ_0000554 (si-circ_circ_0000554), mimic or inhibitor for miR-1205 and the negative controls were directly bought from GenePharma (Shanghai, China). The pcDNA and pcDNA-LASP1 plasmids were provided by Geneseed (Guangzhou, China). All transfections were performed using Lipofectamine 3000 (Invitrogen).

Cell counting kit-8 (CCK-8) assay

SW480 and HCT-116 cells with transfection were plated in 96-well plates. After culturing for 48 h, cells in each well were treated with 10 μL of CCK-8 (5 mg/mL) (Solarbio, Beijing, China) for 2 h. The absorbance at 450 nm was measured for each sample using a microplate reader (Thermo Fisher Scientific).

5-ethynyl-2′-deoxyuridine (EdU) assay

Cell proliferation was detected by using EdU staining kit (RiboBio, Guangzhou, China) in accordance with the protocol. SW480 and HCT-116 cells were seeded for 24 h, followed by cultivation with EdU solution for 2 h. Subsequently, the cells were incubated with Apollo staining reaction solution and DAPI successively. The number of EdU-positive cells was counted with a microscope (Leica).

Flow cytometry assay

Apoptosis was detected by flow cytometry. In brief, SW480 and HCT-116 cells transfected for 48 h were harvested and washed by PBS. Then, cells were treated with 5 μL Annexin V-FITC buffer and 10 μL propidium iodide (PI) solution (Solarbio) for 15 min. The apoptosis rate was measured by using a flow cytometer (BD Biosciences, San Jose, CA, USA).

Transwell assay

Matrigel (BD Biosciences)-coated transwell chambers (Corning Incorporated, Corning, NY, USA) were used for detecting invasive cell number. SW480 and HCT-116 cells in serum-free cell culture medium were inoculated into the upper chamber, medium containing 10% FBS was added to the lower chamber. After 24 h, cells in the lower membrane were fixed with methanol (Solarbio) and dyed with 0.1% crystal violet (Solarbio). The invasive cells were counted by using an inverted microscope (Leica).

Wound-healing assay

The migratory ability of SW480 and HCT-116 cells was further demonstrated by cell scratch test. Shortly, cells with transfection were grown to 100% cell confluence in 12-well plates. Then, scratch was made in the middle of the 12 well plate. The cell culture medium was then replaced with serum-free medium and cultured for 24 h. The images of migrated cells were captured using a microscope. Cell migratory ability was revealed by evaluating the width of the scratch gap.

Western blot

RIPA lysis buffer (Beyotime, Shanghai, China) was applied to extract total proteins. Total proteins were separated by a 10% polyacrylamide gel and then electrotransferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Darmstadt, Germany). The membrane was maintained overnight with primer antibodies including anti-c-myc (1/1000, ab32072), anti-MMP2 (1/1000, ab181286), LASP1 (1/10000, ab156872) or anti-GAPDH (1:10,000, ab181602), and then incubated with Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody. These antibodies were purchased from Abcam. Protein bands were observed using enhanced ECL kits (Solarbio).

Dual-luciferase reporter assay

The sequences of circ_0000554 or LASP1 3′UTR containing miR-1205 binding sites or mutate sites were cloned into pmirGLO vector (Promega, Madison, WI, USA) to construct the corresponding wild-type (WT) or mutated-type (MUT) vectors. Each reporter vector and miR-1205 mimic were co-transfected into SW480 and HCT-116 cells respectively, with miR-NC as the negative control. Luciferase activity was detected by using Dual-luciferase Reporter Kit (Solarbio).

RNA immunoprecipitation (RIP) assay

Imprint® RNA immunoprecipitation kit (Thermo Fisher Scientific) was used to measure enrichment of RNAs. Tumor cells were lysed by RIP buffer. After that, lysates was incubated with magnetic beads conjugated Ago2 or IgG antibodies at 4 °C for 24 h. After treating the proteinase K, immunoprecipitated RNA–protein complex was obtained in Lysis Buffer. RNA complexes were isolated by Trizol reagent (Takara) and analyzed by qPCR.

Animal experiment

The experimental mice (BALB/c, female, n = 12) were purchased from Vital River (Beijing, China). The mice were equally divided into sh-NC group and sh-circ_0000554 group. Then, nude mice were subcutaneously injected with the infected HCT-116 cells to induce tumor growth. Tumor volume was calculated. After 23 days, all mice were killed for subsequent analysis. The animal study was approved by Animal Care and Use Committee of Zigong Fourth People's Hospital.

Statistical analysis

All experiments contained 3 repetitions for each group. The data were analyzed by using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Kaplan–meier plot was used to prepare the patient survival curve, and log-rank test was used for analysis. The difference between two groups was analyzed by Student's *t*-test. Analysis of variance (ANOVA) was used to compare differences among multiple groups. $P < 0.05$ was indicative of statistical significance.

Results

Circ_0000554 was highly expressed in CRC tissues and cells, and associated with poor outcomes

The Ki67 IHC staining results showed that Ki67-positive cells were more in CRC tissues than in adjacent normal tissues (Fig. 1A). The dataset from GSE126094 (https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE126094) showed that hsa_circRNA_001059 (circ_0000554) were remarkably upregulated in CRC tissues compared to adjacent normal tissues (Fig. 1B). Further, we confirmed the upregulation of circ_0000554 in CRC tissues (Fig. 1C). Interestingly, Kaplan–Meier survival analysis revealed that patients in high expression of circ_0000554 group showed poor survival compared with low expression of circ_0000554 group (Fig. 1D). Subsequently, we also found circ_0000554 was significantly upregulated in the tissues of patients with advanced CRC and patients with lymph node metastasis (Fig. 1E, F). Similarly, we observed a high expression of circ_0000554 in CRC cells (LoVo, SW480 and HCT-116) compared with normal intestinal epithelial cell (NCM460) (Fig. 1G). It was subsequently found that there was little change in circ_0000554 expression after RNase R treatment, while the expression of linear GAPDH was significantly reduced (Fig. 1H, I). Collectively, these data suggested that circ_0000554 was upregulated in CRC tissues and cells, and high expression of circ_0000554 was associated with poor outcomes in CRC.

Circ_0000554 interference blocked cell proliferation, migration and invasion and induced cell apoptosis in SW480 and HCT-116 cells

To investigate the functions of circ_0000554 in tumorigenesis in CRC, the functional experiments regarding circ_0000554 were performed by respective transfection of si-NC and si-circ_0000554 in SW480 and HCT-116 cells. The expression of circ_0000554 in si-circ_0000554 group was significantly lower than that in the control group (Fig. 2A). Subsequently, data presented that circ_0000554 silencing repressed cell viability and reduced the number of EdU-positive in SW480 and HCT-116 cells (Fig. 2B, C). In the detection of apoptosis, we found that circ_0000554 deficiency could accelerate cell apoptosis (Fig. 2D). The data from Fig. 2E, F presented that circ_0000554 silencing repressed cell invasion and migration in CRC cells. In addition, western blot analysis showed that circ_0000554 knockdown downregulated the protein levels of C-myc and MMP2 in SW480 and HCT-116 cells (Fig. 2G–H). Taken together, knockdown of circ_0000554 suppressed cell proliferation and metastasis in CRC cells in vitro.

Circ_0000554 served as a sponge of miR-1205

To explore the mechanism of circ_0000554, the online circinteractome was used for predicting the targeted miRNAs of circ_0000554. We found that miR-1205 had binding sites with circ_0000554 (Fig. 3A). The expression of miR-1205 was effectively elevated in CRC cells transfected with miR-1205 (Fig. 3B). The dual-luciferase reporter experiments results showed that the luciferase activity of WT-circ_0000554 reporter was inhibited by miR-1205 overexpression. However, the luciferase activity of MUT-circ_0000554 reporter was almost unchanged (Fig. 3C, D). In order to further clarify the targeting relationship between the circ_0000554 and miR-1205, RIP experiment was carried out, and we found the obvious upregulation of miR-1205 and circ_0000554 by Ago2 protein in contrast with negative control IgG (Fig. 3E, F). We found that miR-1205 was decreased in CRC tissues compared with control groups (Fig. 3G). Moreover, a strong inverse correlation between miR-1205 and circ_0000554 levels was discovered in CRC tissues (Fig. 3H). Besides, the expression of miR-1205 was also decreased in SW480 and HCT-116 cells than that in NCM460 cells (Fig. 3I).

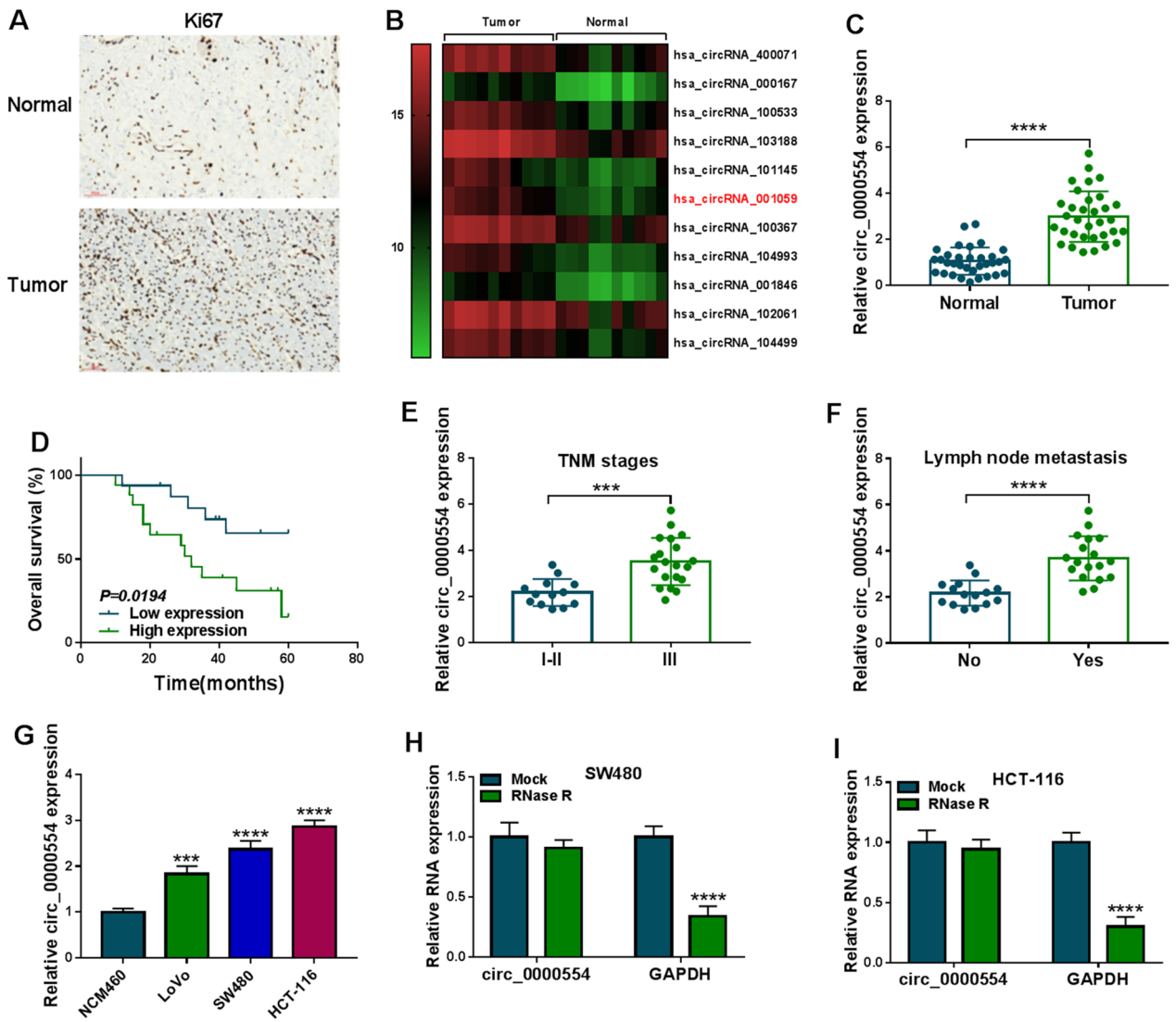
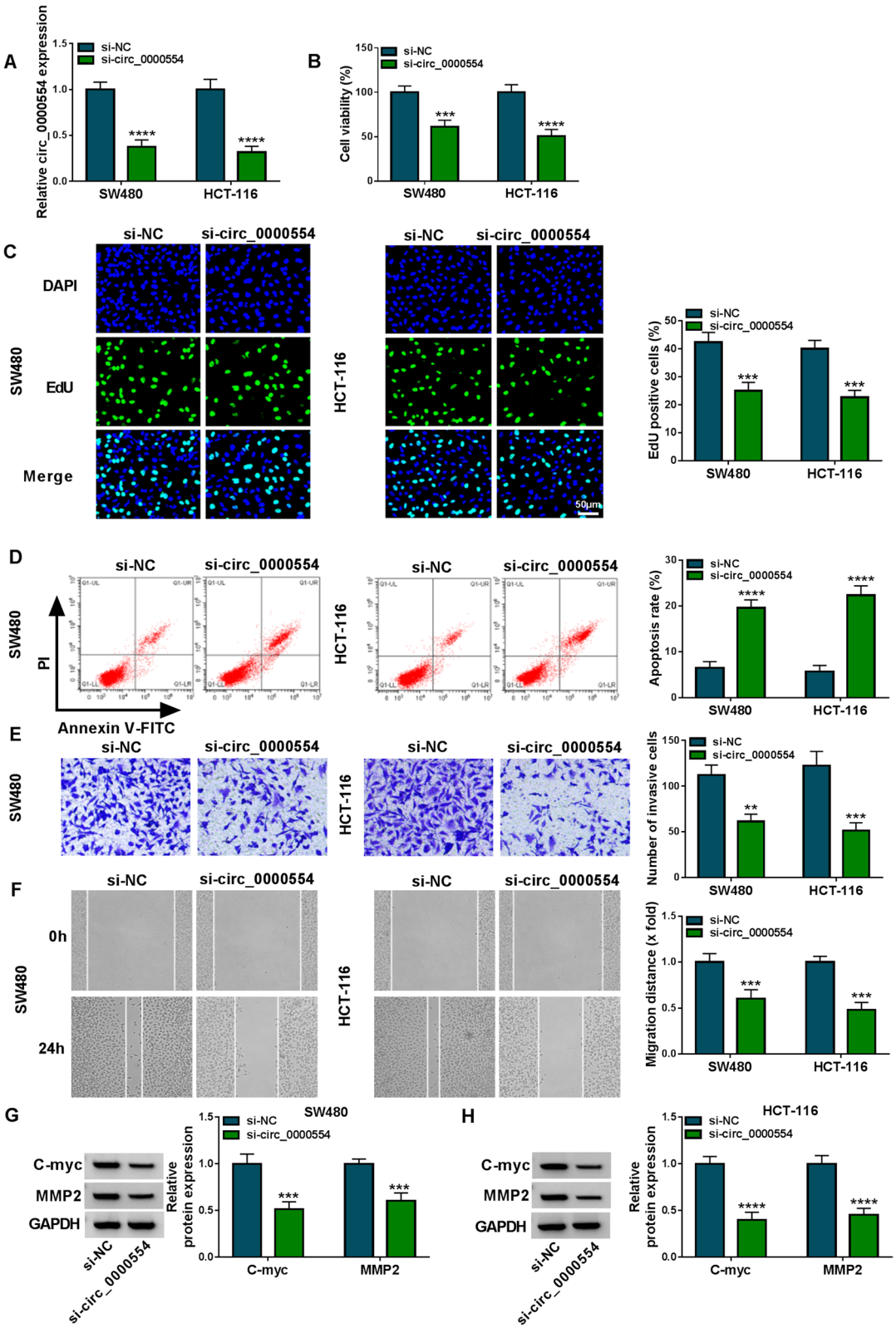


Fig. 1 Circ_0000554 was upregulated in CRC tissues and cells. **A** The histopathologic staining of Ki67 was detected by IHC assay. **B** The data of circRNA expression in CRC tissues were collected from a circRNA expression profile (GEO accession: GSE126094). **C** QRT-PCR was exploited to determine the expression of circ_0000554 in CRC tissues and normal tissues. **D** Overall survival was analyzed by Kaplan–Meier plot and log-rank test. **E** The expression of circ_0000554 in different TNM stages (I–II and III) of CRC patients was determined by using qRT-PCR. **F** QRT-PCR was used to detect the expression of circ_0000554 in CRC patients with (Yes) and without (No) lymph node metastasis. **G** The expression level of circ_0000554 was assessed by qRT-PCR assay in CRC cells and NCM460 cells. (**H**–**I**) QRT-PCR analysis of circ_0000554 expression in SW480 and HCT-116 cells treated with or without RNase R. ***$P < 0.001$, ****$P < 0.0001$

(See figure on next page.)

Fig. 2 Circ_0000554 knockdown inhibited CRC malignant progression. SW480 and HCT-116 cells were transfected with si-NC or si-circ_0000554. **A** Circ_0000554 expression was detected by qRT-PCR. **B** Cell viability was detected by CCK8 assay. **C** Cell proliferation was studied by EdU assays. **D** Cell apoptosis was investigated by flow cytometry analysis. **E**–**F** Transwell assay and wound-healing assay were performed to detect the invasion (**F**) and migration (**G**) capabilities. **G** and **H** The protein expression of C-myc and MMP2 was checked by western blot analysis. ***$P < 0.001$, ****$P < 0.0001$



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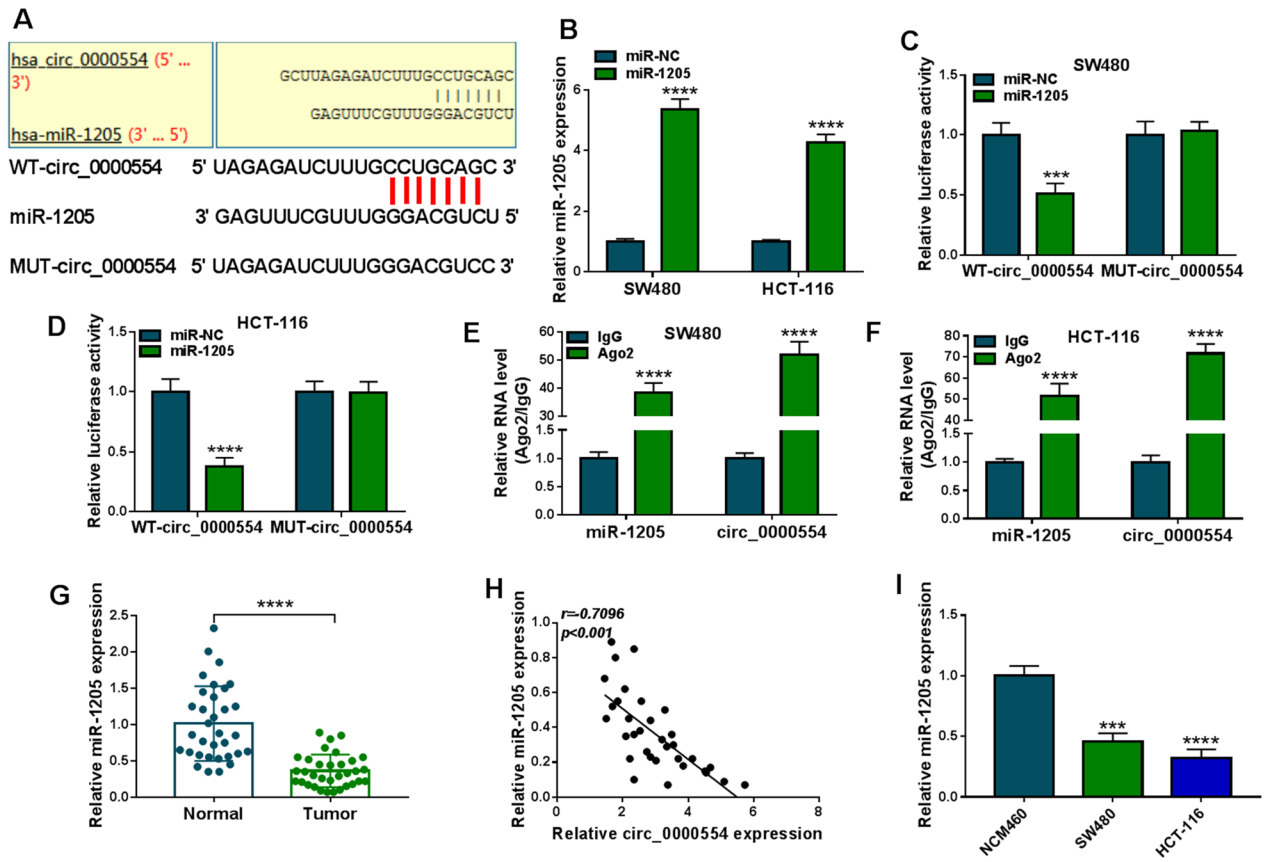

Fig. 3 MiR-1205 was a target of circ_0000554 in CRC cells. **A** The potential binding sites between circ_0000554 and miR-1205. **B** QRT-PCR analysis of miR-1205 expression in SW480 and HCT-116 cells transfected with miR-1205 mimic or miR-NC. **C**–**D** Dual-luciferase reporter assay was performed to confirm the relationship between circ_0000554 and miR-1205. **E**–**F** RIP was performed using Ago2 antibody in CRC cells, then the enrichment of miR-1205 and circ_0000554 was detected. **G** QRT-PCR analysis of miR-1205 level in CRC tissues and adjacent normal tissues. **H** The correlation between circ_0000554 and miR-1205 expression was analyzed by Pearson analysis. **I** QRT-PCR analysis of miR-1205 level in CRC cells and NCM460 cells. ***$P < 0.001$, ****$P < 0.0001$

These data showed that circ_0000554 could sponge miR-1205 in CRC.

MiR-1205 inhibition abrogated the effects of si-circ_0000554 on proliferation, migration, invasion and apoptosis in CRC cells

We have confirmed that circ_0000554 could target miR-1205, whether circ_0000554 modulated CRC process via sponging miR-1205 was further illustrated. The transfection of anti-miR-1205 strikingly restrained the augmentation of si-circ_0000554 on miR-1205 level in CRC cells (Fig. 4A). The cell viability and the number of EdU-positive of CRC cells was weakened by circ_0000554 knockdown but recovered by miR-1205 inhibition (Fig. 4B, C). The data from flow cytometry assay indicated that the apoptosis rate of SW480 and HCT-116 cell was augmented by circ_0000554 knockdown but recovered by miR-1205 inhibition (Fig. 4D). The capacities of invasion and migration were restrained in CRC cells transfected with si-circ_0000554 but partly elevated in cells transfected with si-circ_0000554 + anti-miR-1205 (Fig. 4E, F). Furthermore, the reduced expression of miR-1205 evidently reversed the suppressive effect of circ_0000554 knockdown on C-myc and MMP2 protein levels (Fig. 4G, H). Altogether, the effects of si-circ_0000554 on CRC cells were partially dependent on facilitating the expression of miR-1205.

LASP1 was a target of miR-1205

Targetscan was further used to explore the potential target mRNAs of miR-1205, and there was a special targeting site between miR-1205 and LASP1, indicating that LASP1 might be a target of miR-1205 (Fig. 5A). Overexpression of miR-1205 decreased the luciferase activity of LASP1 3'UTR-WT, while luciferase activity of LASP1 3'UTR-MUT was not affected by miR-1205 in CRC cells (Fig. 5B, C). Besides, LASP1 and miR-1205 were enriched by Ago2 antibody (Fig. 5D, E). Additionally, the results

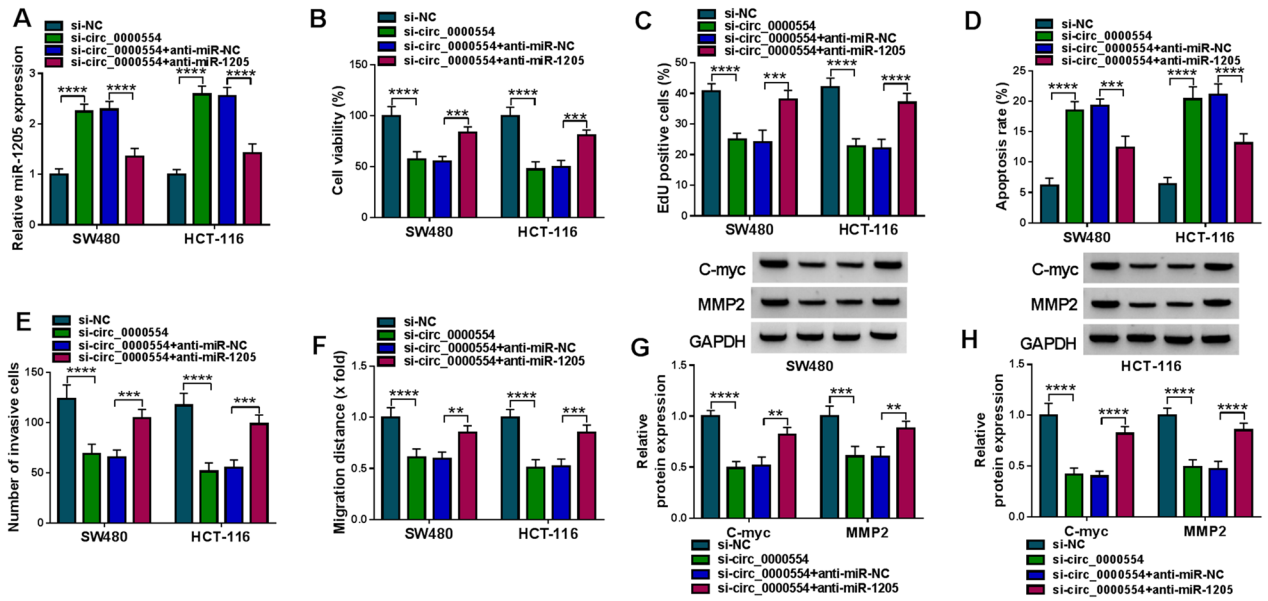

Fig. 4 MiR-1205 was involved in circ_0000554-mediated regulation in CRC cell progression in vitro. SW480 and HCT-116 cells were transfected with si-NC, si-circ_0000554, si-circ_0000554 + anti-miR-NC or si-circ_00005541 + anti-miR-1205. **A** MiR-1205 expression was determined by qRT-PCR. **B** Cell viability was detected by MTT assay. **C** The EdU assays were used to measure proliferation capability of SW480 and HCT-116 cells. **D** The apoptotic cells were monitored by flow cytometry assay. **E**–**F** Cell invaded and migrated abilities were assessed through transwell assay and wound-healing assay, respectively. **G**–**H** The protein levels of C-myc and MMP2 were performed by western blot. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

derived from GEPIA dataset showed that LASP1 expression was markedly higher in CRC tissues (Fig. 5F). In the meantime, the mRNA expression of LASP1 was notably promoted in CRC tissues compared with para-carcinoma tissue (Fig. 5G). LASP1 was negatively related to miR-1205 expression in CRC tissues (Fig. 5H). Moreover, protein levels of LASP1 were significantly upregulated in CRC tissues and cell lines compared with that in para-carcinoma tissue and NCM460 cells, respectively (Fig. 5I, J). The data suggested that LASP1 was a target of miR-1205.

LASP1 overexpression recovered miR-1205-blocked CRC cell proliferation, migration and invasion

Rescue experiments were conducted to determine whether miR-1205 modulated CRC process by binding to LASP1. SW480 and HCT-116 cells were transfected with miR-NC, miR-1205, miR-1205 + pcDNA and miR-1205 + LASP1, respectively. Results illustrated that miR-1205 mimic downregulated LASP1 protein expression, whereas LASP1 overexpression reversed this impact (Fig. 6A). Cell viability and number of EdU-positive were found to be suppressed but the apoptosis rate was increased by miR-1205 overexpression in SW480 and HCT-116 cells, whereas these effects were subsequently reversed by transfection of LASP1 (Fig. 6B, D). Transwell assay and wound-healing assay indicated that cell invasion and migration capabilities were blocked by miR-1205 overexpression but largely recovered by the reintroduction of LASP1, respectively (Fig. 6E, F). In addition, the protein levels of C-myc and MMP2 were reduced in SW480 and HCT-116 cells transfected with miR-1205 alone but restored in cells transfected with miR-1205 + LASP1 (Fig. 6G, H). These data revealed miR-1205 repressed CRC progression via suppressing LASP1.

Circ_0000554 facilitated the expression of LASP1 via miR-1205 sponging

Furthermore, whether circ_0000554 could regulate LASP1 via miR-1205 was investigated in SW480 and HCT-116 cells. Our results showed that transfection of si-circ_0000554 resulted in down-regulation of LASP1 at mRNA and protein levels, which was relieved after miR-1205 level was inhibited by anti-miR-1205 transfection (Fig. 7A, B). The data suggested that circ_0000554 regulated the expression of LASP1 by suppressing miR-1205.

Circ_0000554 knockdown inhibited tumor growth

HCT-116 cells were transfected with shRNA vector, and circ_0000554 level was significantly decreased by sh-circ_0000554 contraposed to sh-NC (Fig. 8A). After cell

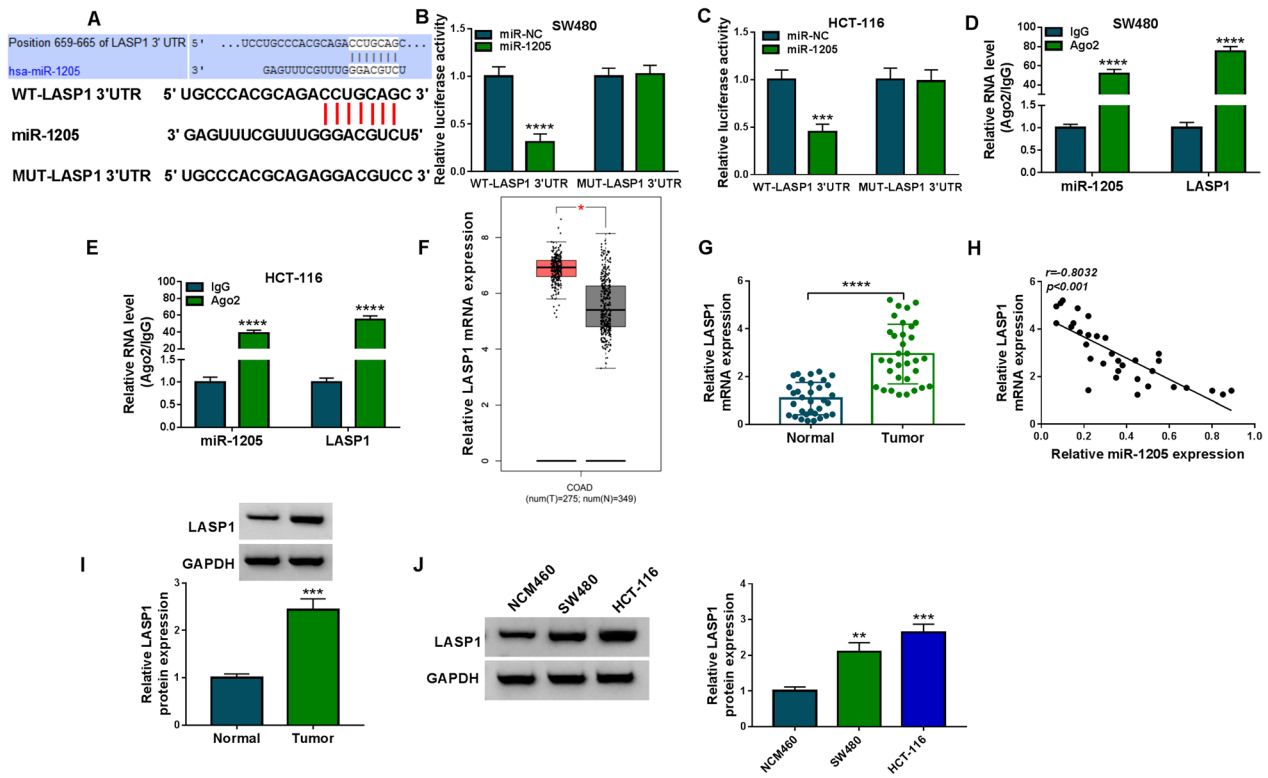

Fig. 5 LASP1 was associated with miR-1205 in CRC cells. **A** The binding sites between miR-1205 and LASP1. **B**–**E** The combination between miR-1205 and LASP1 was confirmed by dual-luciferase reporter assay (**B**, **C**) and RIP assay (**D**, **E**). **F** GEPIA dataset was employed to assess LASP1 expression in CRC tissues. **G** The expression of LASP1 mRNA in tumor tissues and normal tissues was measured by qRT-PCR. **H** The correlation between LASP1 expression and miR-1205 expression was analyzed by Pearson analysis. (**I**, **J**) The expression of LASP1 protein in CRC tissue and cells was measured by western blot. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

injection into mice, we observed that tumor volume and weight in sh-circ_0000554 group was markedly lower than that in sh-NC group (Fig. 8B, C). Furthermore, as exhibited in Fig. 8D–F, circ_0000554 knockdown caused the decrease of circ_0000554 and LASP1 expression levels and increase of miR-1205 expression in tumors. Additionally, IHC assay results showed that LASP1, C-myc and MMP2 levels were decreased in sh-circ_0000554 groups (Fig. 8G). Thus, tumor growth was inhibited after circ_0000554 deficiency in vivo.

Discussion

At present, multiple evidences suggest that circRNAs can regulate the development of CRC [17]. Research on biological function of circRNAs can provide important theoretical support for the treatment of CRC [18]. Although different circRNAs expression profiles summarized many upregulated or downregulated circRNAs in CRC, the functions and mechanisms of most circRNA remained unclear.

The biological role of circ_0000554 has not been studied in CRC. Therefore, the function and mechanism of circ_0000554 are explored in this paper. Results revealed upregulated circ_0000554 was found in CRC tissues and cell. In vitro functional experiments showed that circ_0000554 knockdown hindered CRC cell proliferation and metastasis as well as repressed the growth of transplanted tumor in nude mouse in vivo, which was attributed to the role of circ_0000554-mediated miR-1205/LASP1 pathway. Our results hinted that circ_0000554 silencing inhibited the malignant development of CRC, which was similar with the role of circ_0000554 in esophageal cancer [12].

CircRNA could regulate the expression of miRNA and then mediate the transcription and translation of downstream target genes, their miRNA sponging activity was widely reported [8]. To illustrate the functional mechanism of circ_0000554, we verified the target miRNAs of circ_0000554 by bioinformatics analysis. As a result, miR-1205 was ensured as a target of circ_0000554. Previous studies summarized that miR-1205 was down-regulated in many human cancers and was considered to be a regulator involved in the inhibition of tumor progression, such as breast cancer [19], ovarian cancer [20], glioma

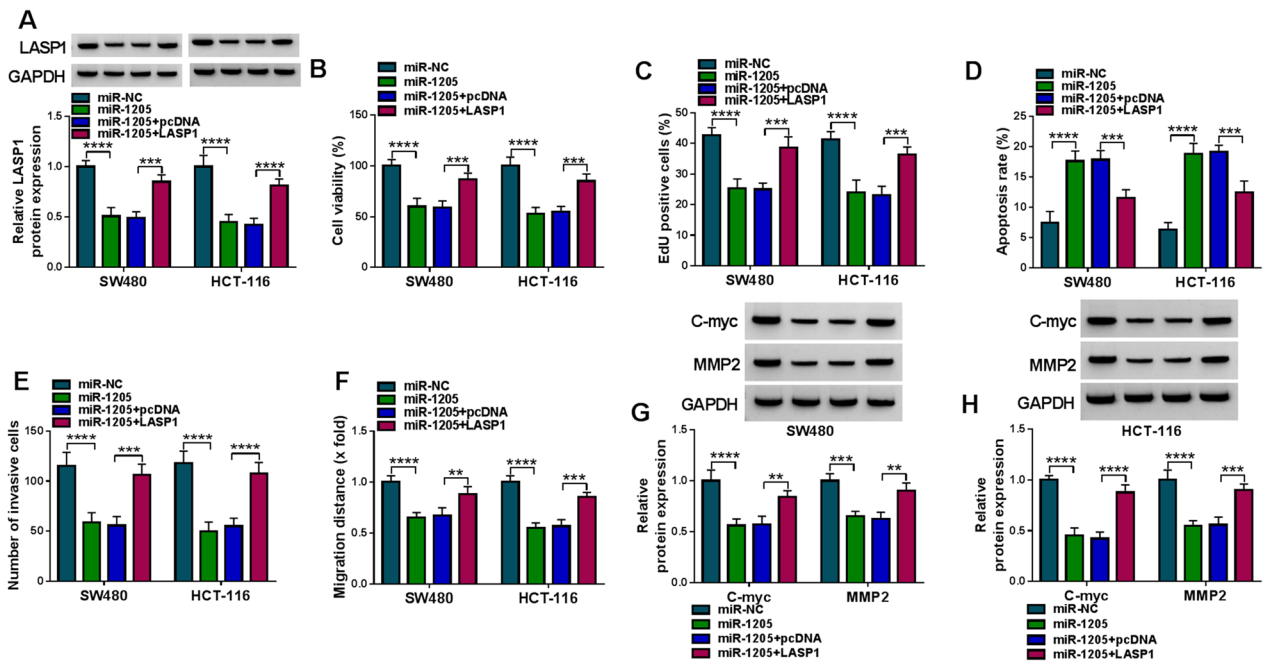

Fig. 6 LASP1 overexpression recovered CRC cell malignant behaviors suppressed by miR-1205 restoration. SW480 and HCT-116 cells were transfected with miR-NC, miR-1205, miR-1205 + pcDNA, or miR-1205 + LASP1. **A** The protein expression level of LASP1 was shown by western blot assay. **B**, **C** The proliferation ability was determined by MTT and EdU assays. **D** The apoptosis rate of CRC cells was shown by flow cytometry assay. **E**–**F** The invasion and migration were determined by transwell assay and wound-healing assay, respectively. **G**–**H** The protein levels of C-myc and MMP2 were measured by western blot. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

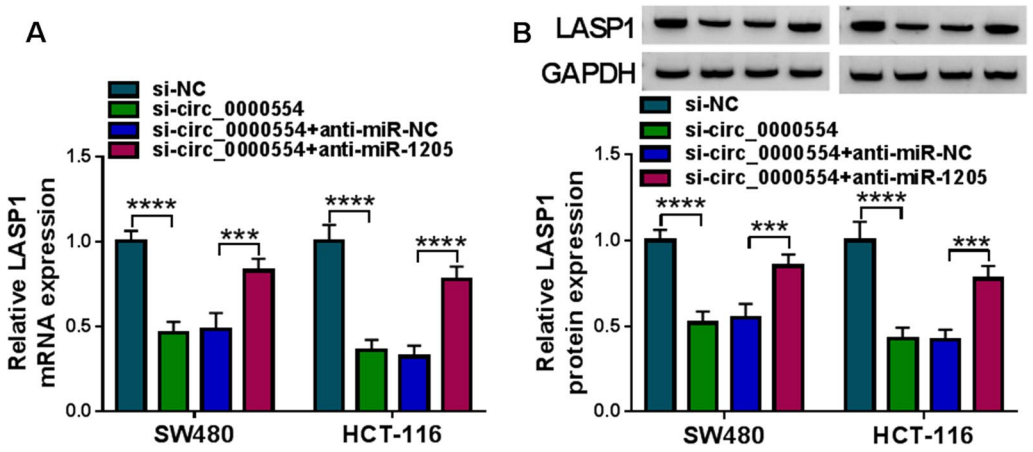

Fig. 7 Circ_0000554 regulated LASP1 expression through binding to miR-1205. **A**, **B** QRT-PCR and western blot analysis of LASP1 expression in SW480 and HCT-116 cells transfected with si-NC, si-circ_0000554, si-circ_0000554 + anti-miR-NC, si-circ_0000554 + anti-miR-1205. $^{***}P < 0.001$, $^{****}P < 0.0001$

[21] and gastric cancer [22]. Similarly, many current studies have revealed the anti-tumor effects of miR-1205 in CRC. For example, Fang et al. have already indicated the expression of miR-1205 was decreased in CRC tissues, and its mimic repressed CRC malignant progression [23]. Han et al. also showed miR-1205 suppressed cell proliferation and metastasis in CRC [15]. Additionally, Jiang et al. also presented miR-1205 was downregulated, and miR-1205 overexpression restrained cell proliferation, metastasis process [24]. In our study, we intend to further

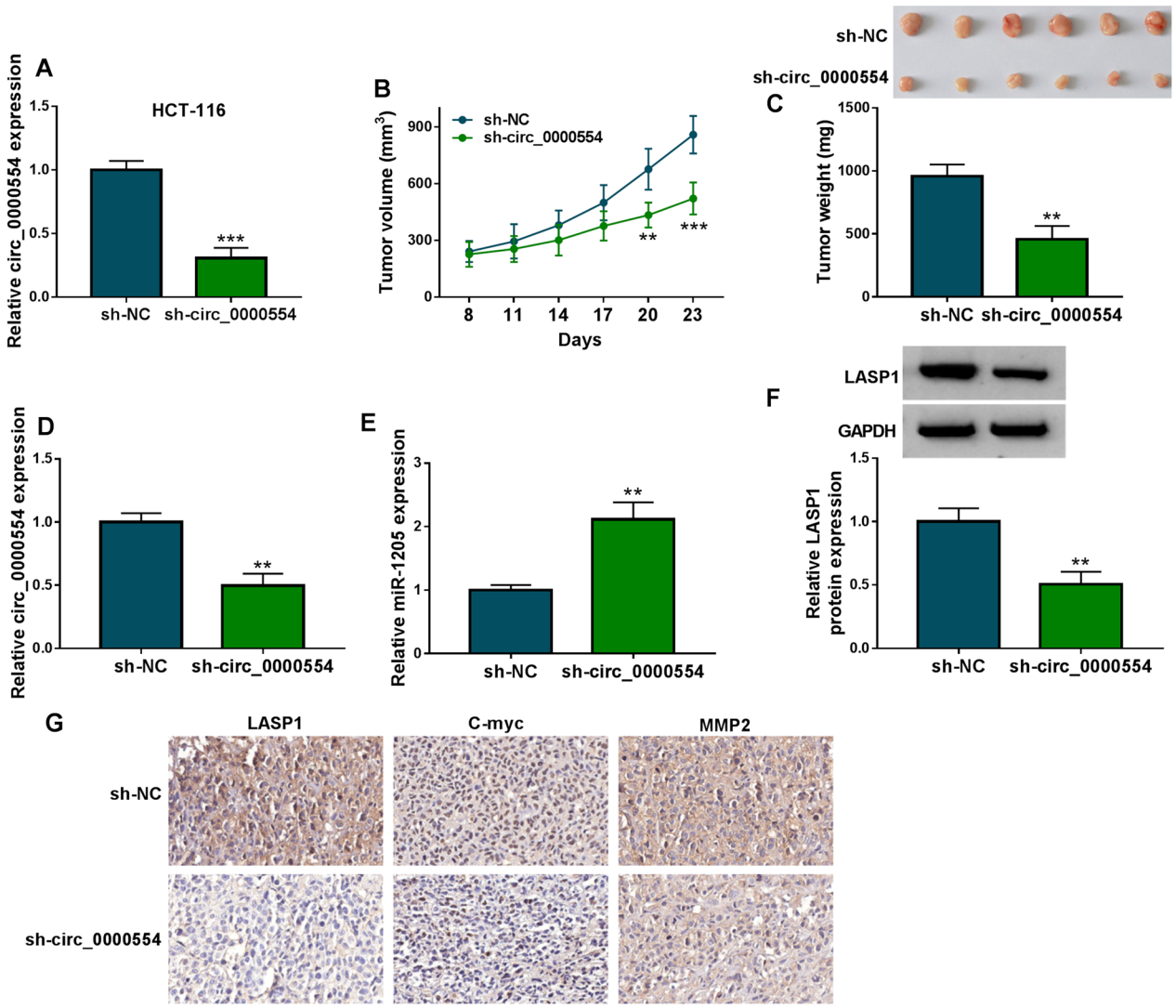

Fig. 8 Knockdown of circ_0000554 reduced tumor growth of CRC in vivo. **A** The expression of circ_0000554 was determined by QRT-qPCR assay in HCT-116 cells stably transfected with sh-NC or sh-circ_0000554. **B**–**C** The effects of circ_0000554 silencing on the volume and weight of the neoplasms were revealed. **D**–**F** The expression of circ_0000554, miR-1205 and LASP1 were determined by qRT-PCR or western blot analysis. **G** The effects of circ_0000554 knockdown on LASP1, C-myc and MMP2 expression in sh-circ_0025033 and sh-NC groups were investigated by IHC assay. $^{**}P < 0.01$ and $^{***}P < 0.001$

clarify the role of miR-1205 in CRC. As expected, miR-1205 was down-regulated in CRC. Molecular mechanism studies have shown that miR-1205 deficiency in our study counteracted the inhibitory effects on growth and metastasis of CRC cells caused by circ_0000554 knockdown. In exploring the downstream target genes of miR-1205, we found that miR-1205 could target the expression of LASP1.

Numerous studies have demonstrated that LASP1 expression was elevated in many human cancers and involved in the regulation of the development of malignant tumor [25, 26]. Most of studies have revealed that LASP1 was overexpressed in CRC and predicted poor prognosis, revealing the potential therapeutic value of LASP1 [27, 28]. Low expression of LASP1 inhibited CRC progression both in vivo and in vitro [16]. LASP1 expression was regulated by miR-431 [29], miR-326 [30] and miR-330-5p [16] suppressed CRC cell proliferation and metastasis by depleting LASP1. Considering the important role of LASP1 in CRC, we screened LASP1 as a target of miR-1205 in our study. In the present work, we demonstrated that miR-1205 suppressed CRC cell growth and metastasis. In addition, it also enhanced cell apoptosis. However, these effects were partially attenuated by

LASP1 overexpression. Collectively, circ_0000554 positively regulates LASP1 expression and miR-1205 negatively regulates LASP1 expression. Thus, the presence of the circ_0000554/miR-1205/LASP1 axis in the CRC was confirmed.

In conclusion, we demonstrated for the first time that circ_0000554 was closely associated with poor clinical outcome in CRC patient, and circ_0000554 upregulated LASP1 through sponging miR-1205 to promote CRC tumorigenicity, suggesting a potential prognostic predictor and therapeutic target for CRC.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00729-3.

Additional file 1. Has_circRNA_001059 (circ_0000554) was the most obviously upregulated in all circRNAs.

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Availability of data and materials
The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The present study was approved by the ethical review committee of Zigong Fourth People's Hospital. Written informed consent was obtained from all enrolled patients.

Consent for publication
Patients agree to participate in this work.

Competing interests
The authors declare that they have no competing interests.

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