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Tumor-derived CircRNA_102191 promotes gastric cancer and facilitates M2 macrophage polarization

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ABSTRACT

Background: Gastric cancer is a common malignant tumor of the digestive tract and the fourth leading cause of death from cancer-related diseases. In recent years, many studies have found that circular RNAs play an important role in cancer. Tumor-associated macrophages (TAMs) are also critical for tumor progression. Objective: This study examined the role of circRNA_102191 in gastric cancer progression. Methods: The relative mRNA levels were determined by qRT-PCR. Western blotting and ELISA were used to detect the protein levels. In vitro proliferation was assessed using CCK8 and clonogenic assays. The migration and invasion of cell lines were assessed by transwell-based assays. The interactions between molecules were detected using a luciferase reporter assay. M0 macrophages were induced with PMA. M1 macrophages were induced with LPS and IFN-γ, and M2 macrophages were induced with IL-4. Results: The expression of circRNA_102191 was enhanced significantly in gastric cancer cell lines and clinical tumor tissues. CircRNA_102191 promotes gastric cancer cell progression by regulating miR-493-3p and its downstream target gene XPR1. CircRNA_102191 can enhance the EMT process of gastric cancer cells by promoting the M2 polarization of macrophages. Conclusion: CircRNA_102191 promotes the biological function of gastric cancer cells by regulating the miR-493-3p/XPR1 axis and M2 macrophage polarization.

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Introduction

Gastric cancer is a common malignant tumor of the digestive tract and the fourth leading cause of death from cancer-related diseases. In recent years, many scientific studies have proposed new insights into gastric cancer [1]. On the other hand, the prevention and treatment of gastric cancer and research into its mechanism still face many challenges. Therefore, it is very important to find new molecular markers of gastric cancer to monitor and intervene in its occurrence and development.

Circular RNAs are a new class of endogenous non-coding RNAs. Different from linear RNAs, circRNAs have closed circular structures. Studies have shown that circRNAs are present in various tissues [2–5] and play an important role in cancer [6–8]. For example, Xince Huang studied the circular RNA circERBB2 to promote gallbladder cancer progression by regulating PA2G4-dependent rDNA transcription [9]. Ying Yu reported that CircCEIP promotes anti-anoikis by enhancing the protective autophagy in prostate cancer cells [10]. Xiaodan Chong examined the PIK3CA mutation-mediated downregulation of circLHFPL2 to inhibit colorectal cancer progression by upregulating PTEN [11]. The communication link between gastric cancer cells and the tumor microenvironment is also an important factor in the occurrence and development of gastric cancer [12,13].

The tumor microenvironment contains cytokines, growth factors, and a variety of tumor-induced immune cells, which play an important role in immunosuppression. Tumor-associated macrophage TAMs can polarize into two phenotypes, M1 and M2, according to environmental

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stimuli and their activation state. In general, M1-type macrophages are characterized by secreting pro-inflammatory cytokines to eliminate tumor cells, while M2-type macrophages can express high levels of anti-inflammatory cytokines to promote tumor cell progression [14]. On the other hand, the molecular mechanism of TAM polarization in gastric cancer is unclear. In this study, an analysis of the gastric cancer-related circRNA chips in the GEO database revealed significant differences in circRNA_102191 expression in gastric cancer chips GSE141977and GSE83521. CircRNA_102191 was highly expressed in gastric cancer cells and had significant differences. The mechanism of circRNA_102191 regulating the growth and proliferation of gastric cancer cells was explored. Gastric cancer cells induce macrophage polarization through circRNA_102191, thereby promoting the proliferation of gastric cancer cells. Therefore, this study examined the regulatory mechanism of circRNA_102191 on the biological function of gastric cancer cells and its regulatory effect on tumor-associated macrophage differentiation, which is expected to provide a theoretical basis for the clinical diagnosis and treatment of gastric cancer to find new tumor markers.

Materials and methods

Cell culture, differentiation, transfection and RNA FISH

The macrophage cell line THP-1 and human gastric cancer cell line, and gastric normal mucosa epithelial cells were obtained from Jiangsu University School of Medicine. The cells were cultured in RPMI1640 medium containing 10% inactivated fetal bovine serum and passaged every two days. When macrophage cells were differentiated, PMA with a final concentration of 100 nmol/L was added to the THP-1 cell culture medium. The cells were induced in a 37°C incubator for 24 h to obtain M0 macrophages. M0 macrophage medium was then added with 20 ng/ml IFN-γ and 10 ng/ml LPS, and cultured for 24 h to differentiate into M1 macrophages. In another experiment, 20 ng/ml IL-4 was added and cultured for 24 h to induce differentiation into M2 macrophages. After discarding the differentiation medium, fresh complete medium was added to continue the culture for 24 h. The vector was derived from the School of Medicine of Jiangsu University, the plasmid was synthesized by Sangon Biotech (Shanghai) Co., Ltd. and the siRNA was synthesized by GenePharma (Suzhou, China). Vector, circRNA_102191 plasmid, and nc, si-circRNA_102191 were transfected into M0 or M2 macrophages using lipofectamine 3000. After 6 h of transfection, the old medium was discarded. Fresh complete medium was added, and the culture was continued for 48 h. The RNA FISH kit and probe were provided by GenePharma (Suzhou, China). We prepared reagents according to the kit instructions and carried out RNA fluorescence in situ hybridization

Co-culture of macrophages and gastric cancer cells

The experiments were performed using a 0.4um Transwell cell. Macrophages were added to the 24-well Transwell plate chamber at a density of 1 × 105 cells/well, and gastric cancer cells were added to the lower chamber at the same density for three days of co-culture.

Real-time fluorescent quantitative PCR

The cells were collected, rinsed twice with PBS, lysed with Trizol, and reverse transcribed to obtain cDNA, which was detected and analyzed by qRT-PCR using SYRB Super Mix. The primer was synthesized by Sangon Biotech (Shanghai) Co., Ltd. and the primer sequence is shown as Table S1.The reaction conditions were: 95°C, 10 s; 60°C, 30 s; 40 cycles. GAPDH was used as an internal reference control, and the relative expression level of each marker was calculated using the 2-ΔΔCt method.

ELISA assay

The peripheral blood of tumor-bearing mice was collected in anticoagulation tubes and centrifuged at 2000 rpm to obtain peripheral blood supernatant, which was stored at 4°C.

M0 macrophage medium and M1 and M2 macrophage medium after differentiation for 24 h were filtered through a 45 μm filter to remove cell debris and stored at 4°C. The standard of the

indicator to be tested was diluted into the corresponding concentration gradients. A 100 µl aliquot of the standard and medium to be tested was added to the 96 blank and incubated at 4°C for 2 h. The diluted detection antibody was then added and incubated at 4°C for 2 h. Finally, a chromogenic substrate was added for color development at room temperature for 15 min, and the stop solution was then added to quench the color development. A microplate reader was used to read the absorbance value A at the wavelength of 450 nm in each well, and a standard curve was drawn according to the standard. The content of each index in the sample was calculated from the standard curve.

Detection of apoptosis by flow cytometry

The cells were collected, resuspended in Annexin V incubation solution, and stained with FITC-Annexin V and PI at room temperature for 15 min. Subsequently, the FITC-Annexin V and PI-positive cells were detected by flow cytometry. The Annexin V single positive cells were regarded as early apoptosis. AnnexinV/PI double positive cells were the late apoptotic cells.

Dual luciferase assay

The wild-type and mutant-type dual luciferase vectors were constructed and co-transfected with nc and miR-493-3p in 293T cells with a fusion degree of 60–80%. After cells were treated with a Vazyme detection kit, the firefly and Renilla luciferase reporter gene activity were detected using a Promage instrument.

Nude mouse subcutaneous tumor experiment

The cells were collected, resuspended in PBS, and counted. Each mouse was injected with 2×10^6 cells/200 µl cell suspension into the back of nude mice. The subcutaneous tumors were assessed every three days using Vernier calipers. The nude mice were weighed, and the growth curve was drawn.

Western blot experiment

The cells were collected, resuspended in 150 µl RIPA cell lysate, placed on ice for 30 min, shaken every 10 minutes, and centrifuged at 12 000 r/min for 10 min after lysis. A 150 µl aliquot of the supernatant was added to 50 µl of 4×SDS loading buffer. The protein samples were heated in a boiling water bath at 100°C for 7 min and separated by SDS-PAGE gel electrophoresis. The protein samples in the gel were then transferred to a PVDF membrane. The PVDF membrane was blocked with nonfat milk powder, incubated with antibody, incubated with HRP-conjugated goat anti-rabbit secondary antibody, and finally added with a chemiluminescent substrate. The protein bands were obtained using a gel imager.

Determination of cell proliferation ability by CCK8 assay

Transwell lower chamber gastric cancer cells were adherently cultured for 48 h, and the proliferation of gastric cancer cells was detected using the CCK8 method. After the cells were digested, they were fully resuspended, and after cell counting, the 96-well plates were plated with 1,000 cells per well, and three duplicate wells were set up. The CCK8 reaction solution was added to the ultra-clean bench in the dark. After incubation for 1 h, the absorbance at a wavelength of 450 nm was detected.

Plate colony formation assay

Cells were collected, resuspended in PBS, and counted. One thousand cells were seeded in 6-well plates with 2 ml of complete medium. Macroscopic cell clones were formed after 14 days of culture. The culture medium was discarded; PBS was added to wash the cells; 4% paraformaldehyde was added to fix the cells at room temperature for 30 minutes; crystal violet stain was then added for 30 minutes. After washing three times, the cells were photographed and stored.

Transwell cell transfer assay

Gastric cancer cells in each treatment group were digested with 0.25% trypsin and resuspended in a serum-free medium. A suspension containing

1 × 105 cells was added to the upper chamber of an 8.0 μm transwell chamber, and a complete medium was added to the lower chamber. After culturing in a cell incubator for 36 hours, the chambers were removed, fixed, stained, and photographed under a microscope for preservation

Statistical analysis

Statistical analysis was performed using GraphPad software. An independent samples t-test was used to compare the samples between groups. P values < 0.05 were considered significant.

Results and discussion

Identification and characterization of circRNA_102191 in gastric cancer

Two expression chips, GSE141977and GSE83521, from the GEO database were integrated and analyzed to identify circRNAs critical for gastric cancer progression. There were 54 circRNAs with p- values less than 0.05 and logFold changes greater than 1, among which 38 circrna expressions were up-regulated and 16 down-regulated (Figure 1a, Table S2). By gene chip ID conversion, circRNA_102191 also known as hsa_circ_0045602, in the circbank database, it was found to originate from the ontology gene HN1, which is circularized by reverse splicing of exon 7 and exon 10 (Figure 1b). CircRNA_102191 remained more stable than linear HN1 after treatment with RNase R and actinomycin D (Figure 1c–e). In addition, RNA-FISH showed that circRNA_102191 was distributed in the cytoplasm and nucleus of gastric cancer cells (Figure 1f). The above results suggest that circRNA_102191 is overexpressed in gastric cancer patient tissues and resistant to RNase R.

CircRNA_102191 promotes the proliferation of gastric cancer cells

The circRNA_102191 RNA levels were first measured in normal gastric mucosal epithelial cells GES-1 and

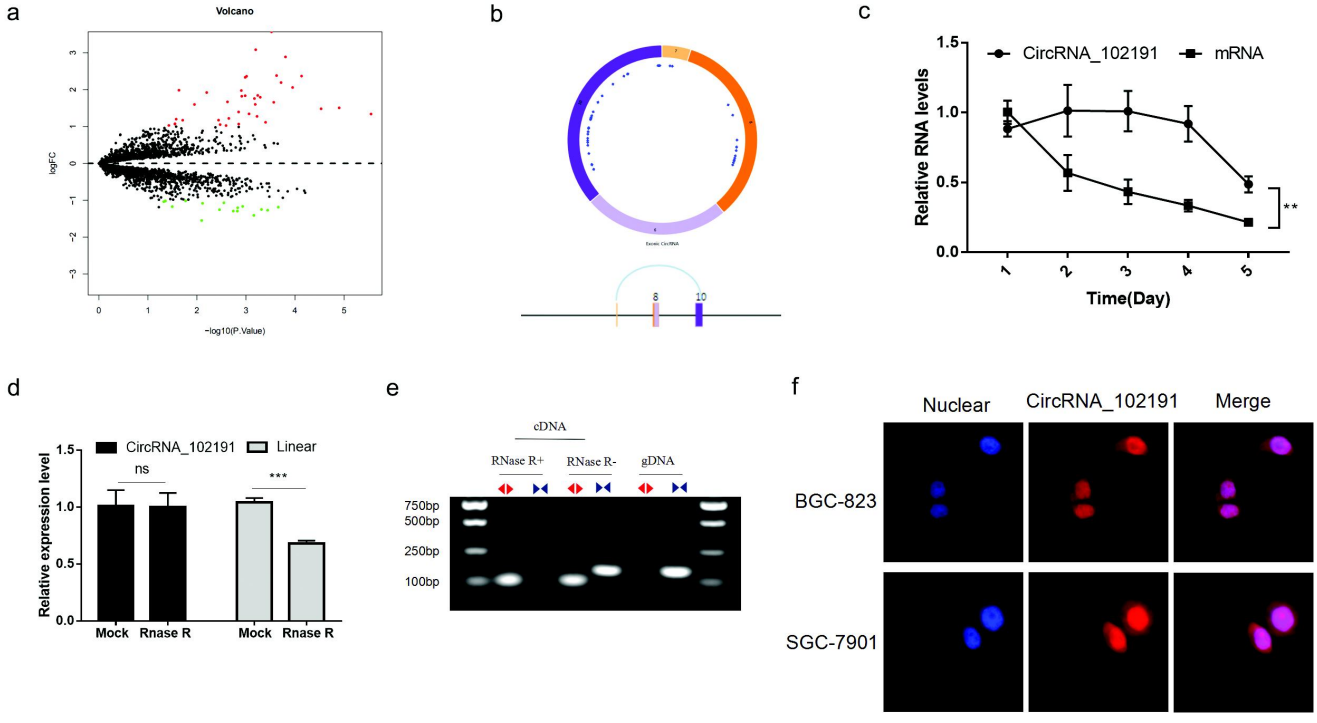

Figure 1. Identification and characterization of CircRNA_102191 in gastric cancer. (a) scatter plot of differentially expressed circular RNAs in gastric cancer microarrays. (b) schematic diagram of the structure of CircRNA_102191. (c) actinomycin D treatment to assess the stability of CircRNA_102191 and HN1 mRNA in gastric cancer cells over time. (d) qRT-PCR analysis to confirm the resistance of CircRNA_102191 and linear HN1, with and without RNase R treatment, in gastric cancer cells. (e) detection of CircRNA_102191 resistance to RNase R by agarose gel electrophoresis f RNA-FISH assay to detect the localization of CircRNA_102191 in SGC-7901 cells and BGC-823 cells.

Data represent means ± SD of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$

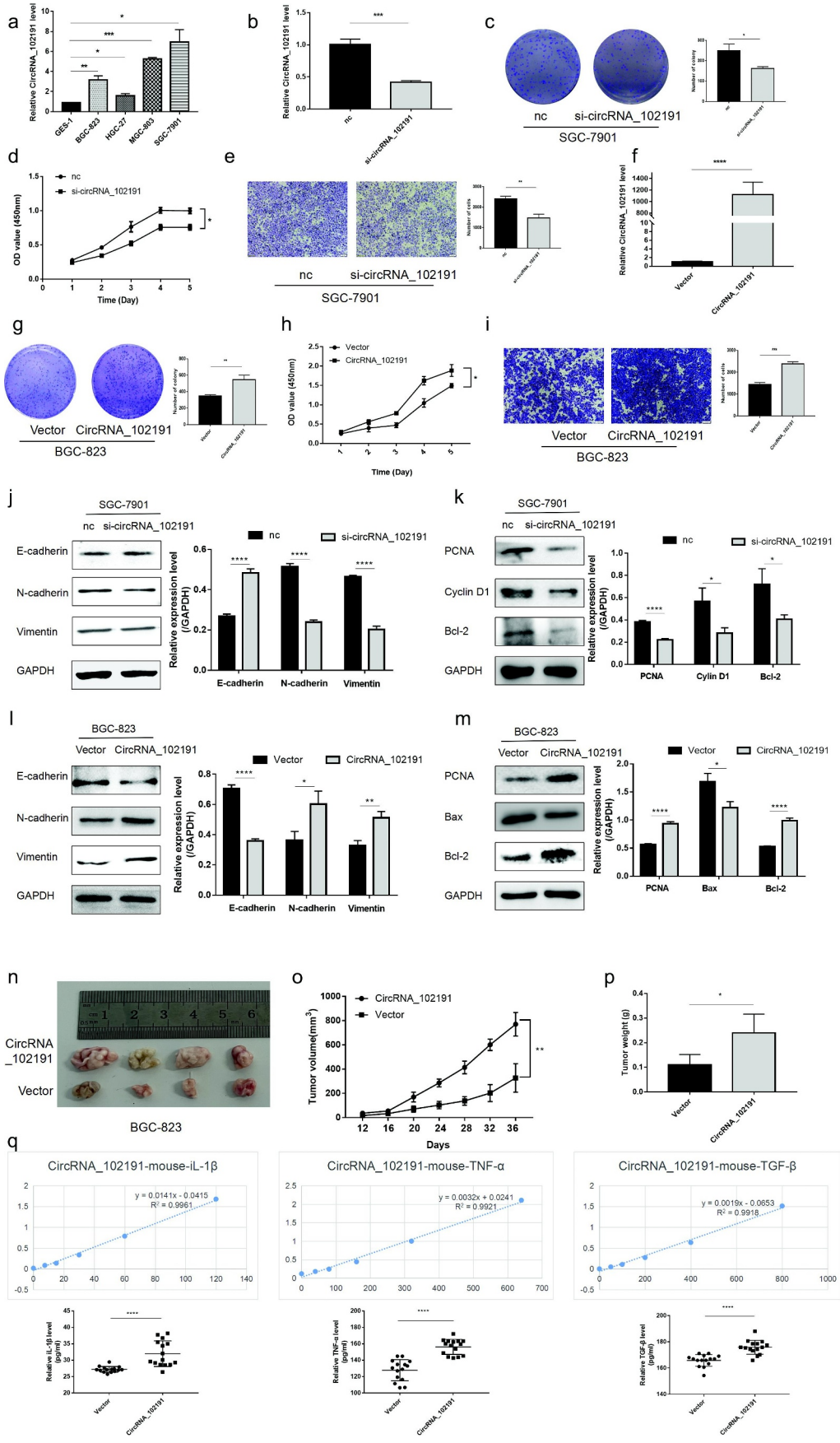

Figure 2. CircRNA_102191 promotes the proliferation of gastric cancer cells. A expression of CircRNA_102191 in normal gastric mucosal cells and gastric cancer cell lines. B,F qRT-PCR to detect the silencing efficiency or overexpression efficiency of CircRNA_102191 in SGC-7901 cells and BGC-823 cells. C,G plate clone formation assay to analyze the effect of silencing or

four gastric cancer cell lines (BGC-823, HGC-27, MGC-803, and SGC-7901). The expression of circRNA_102191 in these cells differed because of its different origin, morphology, differentiation, grade, and tumorigenicity. The expression level of circRNA_102191 in SGC-7901 cells was the highest, while the expression level of circRNA_102191 in the other four cell lines was relatively low. The expression level of circRNA_102191 in gastric cancer cell lines was higher than that in normal gastric mucosa epithelial cells GES-1 (Figure 2a). Therefore, SGC-7901 and BGC-823 cells were selected for further analysis. The biological properties of circRNA_102191 in gastric cancer cells were investigated using small interfering RNA (siRNA) to down-regulate circRNA_102191 in SGC-7901 cells and overexpress circRNA_102191 in BGC-823 cells. Real-time fluorescence quantitative experiments were conducted to detect the knockdown and overexpression efficiency (Figure 2b,f). The counting kit-8 (CCK-8) and colony formation assays showed that down-regulation of circRNA_102191 inhibited the proliferation of SGC-7901 cells (Figure 2c,d). In contrast, circRNA_102191 overexpression enhanced the proliferation ability of BGC-823 cells (Figure 2g,h). Subsequently, the downregulation of circRNA_102191 reduced the migration rate of SGC-7901 cells (Figure e), while overexpression of circRNA_102191 promoted the migration rate of BGC-823 cells (Figure 2i). The EMT process-related indicators and indicators of cell proliferation and apoptosis were detected by western blotting. The knockdown of circRNA_102191 inhibited the EMT process and cell proliferation and promoted cell apoptosis(Figure 2j,k). On the other hand, overexpression of circRNA_102191 yielded opposite results (Figure 2l,m). A subcutaneous xenograft tumor model was established using BGC-823 cells overexpressing circRNA_102191 or transfected with an empty vector to evaluate the tumor-promoting activity
of circRNA_102191 in vivo. As shown in Figure 2n, the tumor weight and volume in the circRNA_102191-overexpressing group were much higher than those in the control group. The ELISA results showed that the contents of il-1β, TNF-α, and TGF-β in peripheral blood of the circRNA_102191 overexpression group were higher than those of the control group (Figure 2o). Overall, these findings suggest that circRNA_102191 has a tumor-promoting role in gastric cancer cells.

CircRNA_102191 binds to miR-493-3p in gastric cancer cells

To investigate the downstream target miRNAs further, the downstream of circRNA_102191 in circbank was predicted, and five candidate targets were detected. Next, the relative expression levels of candidate targets were examined in the overexpression circRNA_102191 experimental group and the circRNA_102191 knockdown experimental group. miR-493-3p was associated with circRNA_102191 in gastric cancer cells (Figure 3a,b). Furthermore, miR-493-3p reduced the luciferase activity in gastric cancer cells transfected with the circLIFR wild-type (WT) reporter gene, but the circRNA_102191-MUT group showed no significant change in luciferase activity (Figure 3c). We used the Ct values of CircRNA_102191 and miR-493-3p to analyze their expression correlation in gastric cancer tissues. The results showed that the expression of CircRNA_102191 was negatively correlated with the expression of miR-493-3p in gastric cancer tissues (Figure 3d). The effects of miR-493-3p on the biological behavior of gastric cancer cells were first examined using a CCK8, transwell assay and colony formation assay to determine if circRNA_102191 promotes gastric cancer progression by adsorbing miR-

overexpression CircRNA_102191 on the proliferation of SGC-7901 cells and BGC-823 cells. D,H CCK8 assay to analyze the effect of silencing or overexpression CircRNA_102191 on the proliferation of SGC-7901 cells and BGC-823 cells. E,I transwell cell transfer assay analysis of the effect of silencing or overexpression CircRNA_102191 on the transfer ability of SGC-7901, the scale bars are 100 μm. J-M Western Blot to detect the changes of proliferation-related proteins and apoptosis-related proteins after silencing or overexpression of CircRNA_102191. N-P effects of control group and overexpressed CircRNA_102191 group with BGC-823 cells on subcutaneous tumors in nude mice. Q the secretion of iL-1β, TFN-α and TGF-β in serum of tumor-bearing nude mice.

Data represent means ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

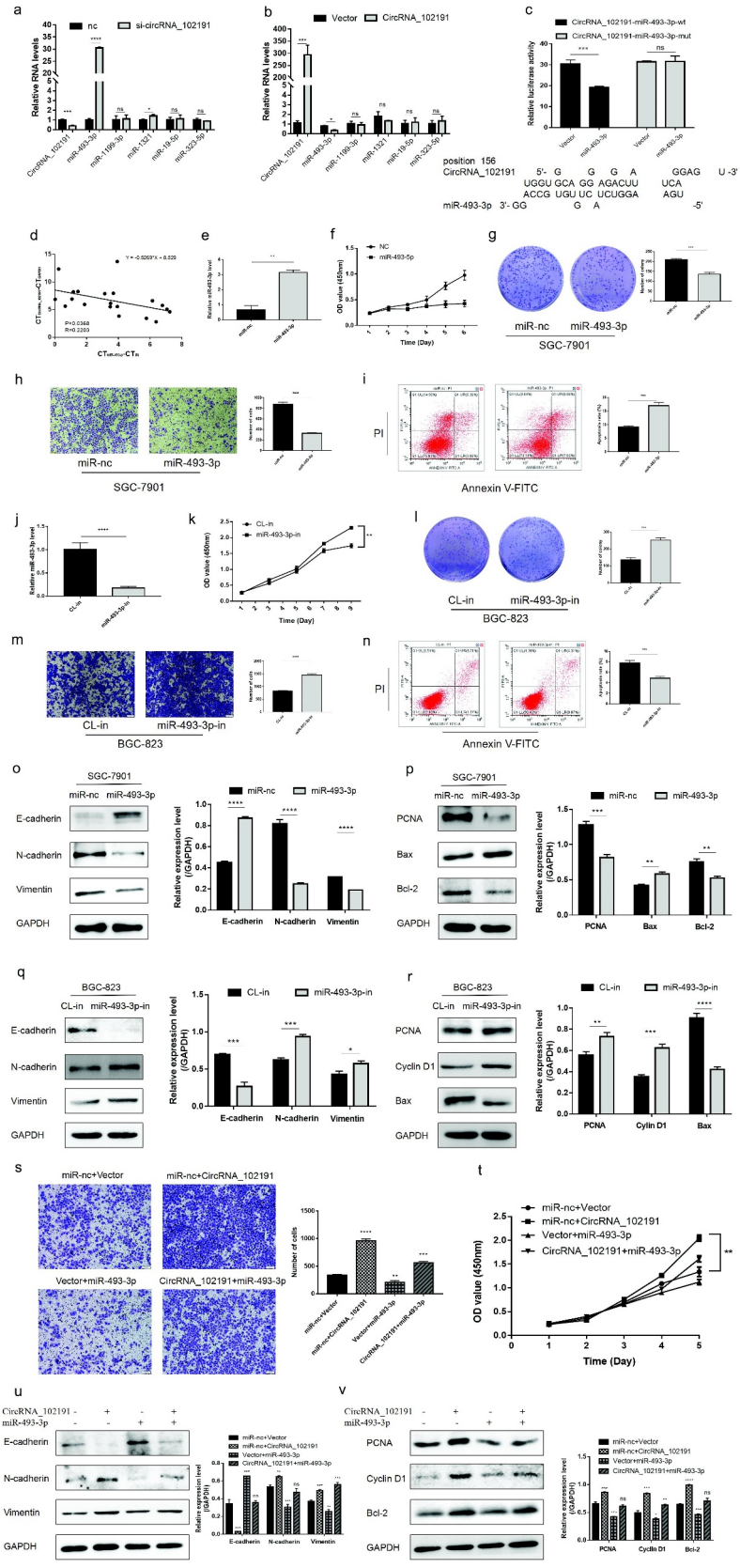

Figure 3. CircRNA_102191 binds to miR-493-3p in gastric cancer cells. A,B qRT-PCR analysis of the expression changes of potential miRnas after abnormal expression of CircRNA_102191. C dual-luciferase assay to determine the binding site of CircRNA_102191 to miR-493-3p. D the co-expression of CircRNA_102191 and miR-493-3p was analyzed by qRT-PCR analysis in 20 paired of GC tissues. The *P* value was estimated using Pearson correlation test. E,J qRT-PCR to detect the silencing efficiency or overexpression efficiency

493-3p. The miR-493-3p mimic inhibited the proliferation and migration ability of SGC-7901 cells and promoted apoptosis (Figure 3e–i). In contrast, the miR-493-3p inhibitor promoted the proliferation and migration of BGC-823 cells and inhibited apoptosis (Figure 3j–n). The WB experimental results confirmed this conclusion (Figure 3o–r). Rescue experiments showed that the miR-493-3p mimics could reverse circRNA_102191 overexpression-mediated cell proliferation and migration promotion, which was also supported by the WB results (Figure 3s–v). These results suggest circRNA_102191 promotes gastric cancer progression through sponge adsorption of miR-493-3p.

CircRNA_102191 promotes gastric cancer progression via the circRNA_102191-miR-493-3p-XPR1 axis

The downstream targets of miRNAs were determined because circRNA_102191 can act as a ceRNA against miR-493-3p. The potential targets of miR-493-3p were based on three databases (miRDB (http://mirdb.org), miRTarbase(http://mirtarbase.mbc.nctu.edu.tw), and Targetscan (https://www.targetscan.org). The analysis showed that miR-493-3p shared 11 common candidate genes (Figure 4a, Table S3). As an indispensable player in tumor progression [15–17], XPR1 was confirmed to be a target of miR-493-3p, and the expression of miR-493-3p at the molecular level was significantly negatively correlated with the expression of XPR1 (Figure 4b,c). Binding to XPR1 mRNA in wild-type at the putative binding site of miR-493-3p was detected in 293T cells but not in mutants, as determined by a dual-luciferase reporter gene (Figure 4d). We analyzed the expression correlation of CircRNA_102191 and XPR1. The results showed that the expression of CircRNA_102191 was positively correlated with the expression of XPR1 in gastric cancer tissues (Figure 4e).

The role of XPR1 on gastric cancer progression was next examined by performing transwell experiments and colony formation assays. The knockdown of the target gene XPR1 inhibited the proliferation and migration ability of SGC-7901 cells and promoted apoptosis (Figure 4f–j). By contrast, the overexpression of XPR1 promoted the proliferation and migration of BGC-823 cells and inhibited apoptosis (Figure 4k–o). The WB experimental results proved this conclusion (Figure 4p–s). The rescue experiments showed that the target gene XPR1 rescued miR-493-3p-mediated cell proliferation promotion and migration promotion, which was also supported by the WB results (Figure 4t–w). Overall, these results suggest that XPR1 promotes gastric cancer progression downstream of miR-493-3p.

CircRNA_102191 regulates the biological function of gastric cancer cells by promoting macrophage polarization

This study next whether gastric cancer cells induce macrophage polarization through circRNA_102191, thereby promoting gastric cancer cell proliferation. Human THP-1 cells were induced into M0, M1, and M2 cells with drugs. The cell morphological changes were observed under a microscope (Figure 5a), and qRT-PCR was used to verify that the induction was successful (Figure 5b,c). The expression of circRNA_102191 in the macrophages at various differentiation stages was detected. CircRNA_102191 was expressed strongly in M2 cells with cancer-promoting function (Figure 5d). The circRNA_102191 plasmid in M0 cells was transfected and co-cultured with untreated SGC. The results showed that compared to the control group, the levels of CD206 and CD163 expression in macrophages in

of miR-493-3p in BGC-823 cells and SGC-7901 cells. F,K CCK8 assay to analyze the effect of miR-493-3p on the proliferation of gastric cancer cells. G,L plate clone formation assay to analyze the effect of miR-493-3p on the proliferation of gastric cancer cells. H,M transwell cell transfer assay analysis of the effect of miR-493-3p on the transfer ability of gastric cancer cells, the scale bars are 100 μm. I,N cell apoptosis assay to analyze the effect of miR-493-3p on the apoptosis ratio of gastric cancer cells. O-R Western Blot to detect the changes of proliferation-related proteins and apoptosis-related proteins after silencing or overexpression of miR-493-3p. T transwell cell transfer assay analysis of the effects of co-transfection of miR-493-3p mimics and CircRNA_102191 plasmids on the metastatic ability of gastric cancer cells. S CCK8 assay to analyze the effects of miR-493-3p mimics and CircRNA_102191 plasmid co-transfection on the proliferation of gastric cancer cells. U,V Western Blot detected the changes of proliferation-related proteins and apoptosis-related proteins after co-transfection of miR-493-3p mimics and CircRNA_102191 plasmids.

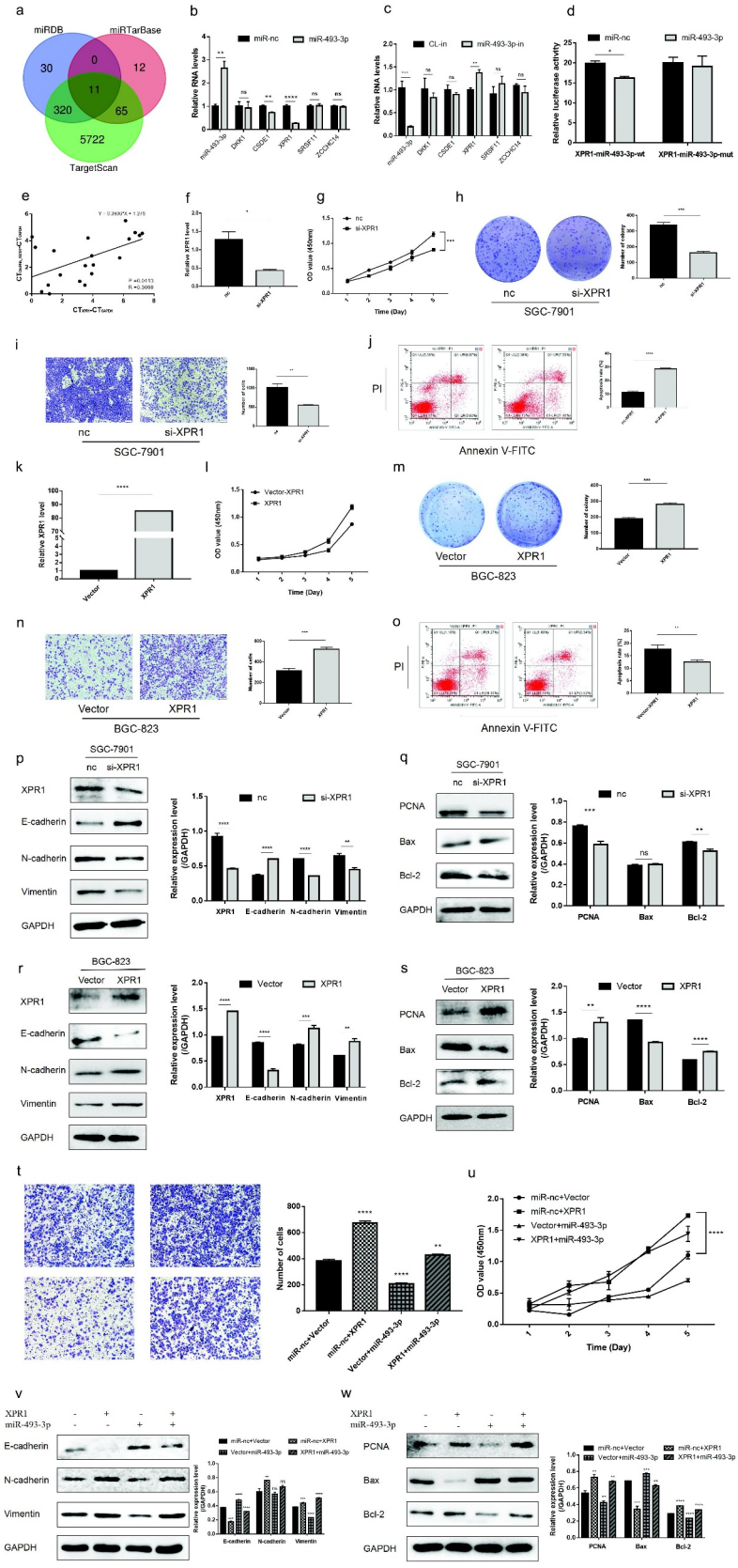

Figure 4. CircRNA_102191 promotes gastric cancer progression via the CircRNA_102191-miR-493-3p-XPR1 axis. A database screening of downstream target genes of miR-493-3p. B,C qRT-PCR analysis of the expression changes of potential target genes after abnormal expression of miR-493-3p. D dual-luciferase assay to determine the binding site of XPR1 to miR-493-3p. E the co-expression of CircRNA_102191 and XPR1 was analyzed by qRT-PCR analysis in 20 paired of GC tissues. The *P* value was estimated using Pearson correlation test. F,K qRT-PCR to detect

the experimental group increased significantly. The migration and proliferation of SGC in the experimental group were enhanced, and apoptosis was reduced (Figure 5e–i). CircRNA_102191 promoted the polarization of macrophages M0 to M2 and the biological function of gastric cancer cells SGC.

Similarly, the same conclusion was made when circRNA_102191 was knocked down in M0 (Figure 5j–n). Next, this study examined whether circRNA_102191 can promote the differentiation of M2 macrophages. CircRNA_102191 was over-expressed in M2 macrophages, and the expression levels of M2 macrophage markers in each group of cells were analyzed by qRT-PCR. Compared to the M0 macrophages, the expression levels of related marker molecules CD206 and CD163 in M2 macrophages were increased significantly. Moreover, the expression levels of the marker molecules CD206 and CD163 in the M2 macrophages in the over-CircRNA_102191 group were higher than those in the M2 macrophages in the nc group (Figure 5o). The M0 and treated M2 macrophages were then co-cultured with SGC. The experimental results showed that compared to the M0 control group, the migration ability of SGC cells in the M2-Vector group and the M2-over-CircRNA_102191 group was enhanced, and apoptosis was reduced. Compared with the M2-Vector group, the M2-over-CircRNA_102191 group had a more obvious enhancement of cell migration ability and less apoptosis (Figure 5p–s). Similarly, SGC were co-cultured after knocking down circRNA_102191 in M2. Compared to the M0 control group, the migration ability of SGC cells in the M2-nc group was enhanced, and apoptosis was reduced. The migration ability of the M2-si-circRNA_102191 group was weakened, and the number of cells was increased (Figure 5t–w).

Discussion

With the rapid development of bioinformatics and high-throughput sequencing technologies, there are many circRNAs in organisms, which are a new type of endogenous non-coding RNAs that are widely present and stably expressed in organisms [18–20]. Studies have shown that a variety of circRNAs are involved in the pathogenesis of gastric cancer, including circ-HuR [21], circ-DONSON [22], and circRanGAP1 [23]. The dysregulated circRNAs allow a clear identification of the mechanism and provide help for further gastric cancer treatment. The current study first focused on the expression of circRNA_102191 in gastric cancer cell lines. The results showed that the expression of circRNA_102191 in SGC-7901, BGC-823, and MGC-803 cells was higher than that in normal gastric mucosal epithelial cells GES-1, suggesting that circRNA_102191 is an important biomarker for the occurrence and development of gastric cancer.

Subsequently, through bioinformatics prediction, it was found that miRNAs that circRNA_102191 may act on included miR-493-3p, miR-1199-3p, and miR-1321. Studies have found that miR-493-3p plays a tumor suppressor role in ovarian cancer, non-small cell lung cancer, laryngeal squamous cell carcinoma, and glioma [24–27]. Kaishuai Zhan et al. found that miR-493-3p can be induced by propofol and inhibit the growth and invasion of gastric cancer by inhibiting the activation of Wnt/β-Catenin signal mediated by DKK1 [28]. We found that circRNA_102191 sponge adsorbed miR-493-3p through qRT-PCR and dual luciferase experiments, and miR-493-3p could reverse the effect of circRNA_102191 on the biological behavior of gastric cancer cells. Then we selected the downstream target gene XPR1 of miR-493-3p in the three

the silencing efficiency or overexpression efficiency of XPR1 in BGC-823 cells and SGC-7901 cells. G,L CCK8 assay to analyze the effect of XPR1 on the proliferation of gastric cancer cells. H,M plate clone formation assay to analyze the effect of XPR1 on the proliferation of gastric cancer cells. I,N transwell cell transfer assay analysis of the effect of XPR1 on the transfer ability of gastric cancer cells, the scale bars are 100 μm. J,O cell apoptosis assay to analyze the effect of XPR1 on the apoptosis ratio of gastric cancer cells. P-S Western Blot to detect the changes of proliferation-related proteins and apoptosis-related proteins after silencing or overexpression of XPR1. T transwell cell transfer assay analysis of the effects of co-transfection of miR-493-3p mimics and XPR1 plasmids on the metastatic ability of gastric cancer cells. U CCK8 assay to analyze the effects of miR-493-3p mimics and XPR1 plasmid co-transfection on the proliferation of gastric cancer cells. V,W Western Blot detected the changes of proliferation-related proteins and apoptosis-related proteins after co-transfection of miR-493-3p mimics and CircRNA_102191 plasmids.

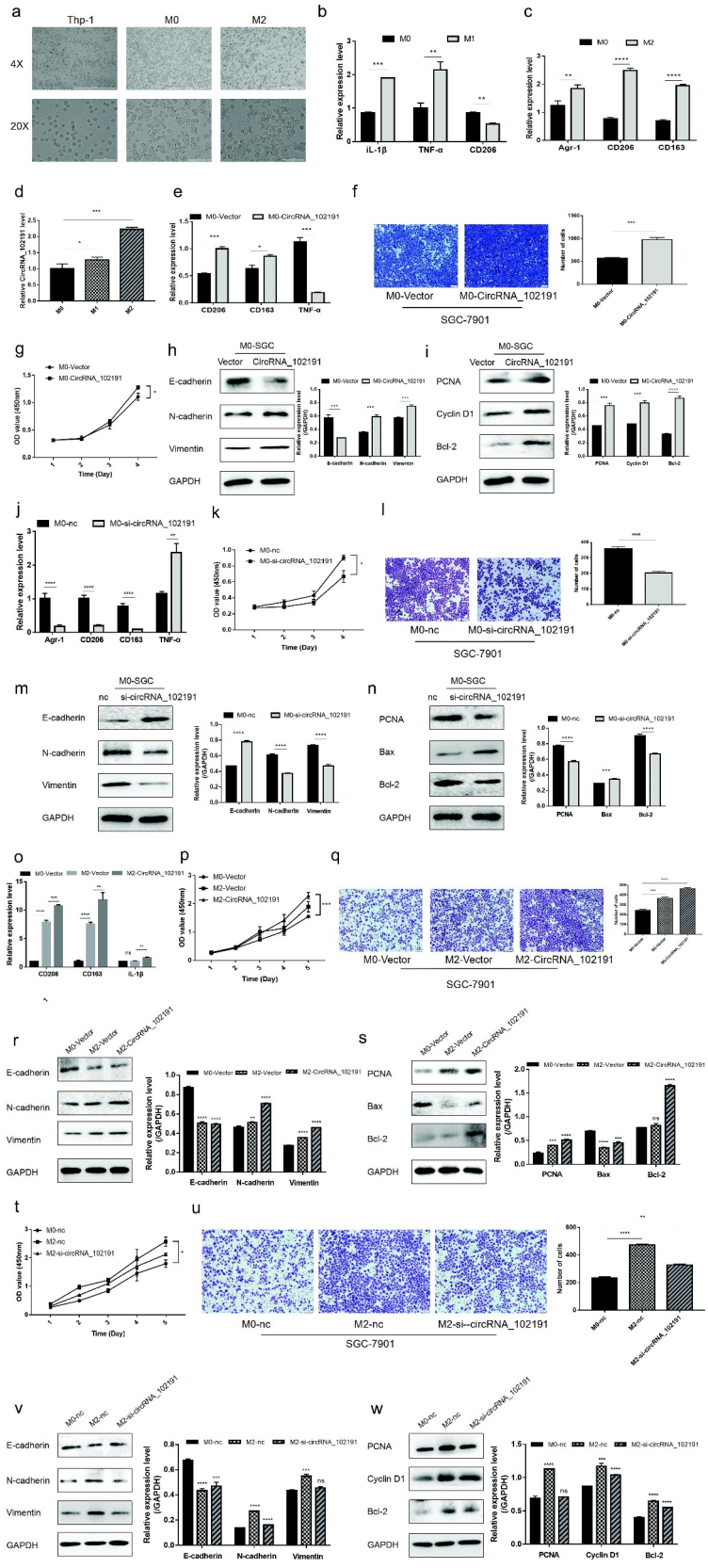

Figure 5. CircRNA_102191 regulates the biological function of gastric cancer cells by promoting macrophage polarization. A morphology of thp-1, M0, M1 cells under microscope. B,C qRT-PCR analysis of the expression of M1 and M2 macrophage markers. D the expression of CircRNA_102191 in macrophage of different differentiation states. E,J,O quantitative analysis of macrophage marker molecules in cells of each group by RT-qPCR. F,L,Q,U transwell cell metastasis assay was used to analyze the effect of macrophages in each group on the metastasis ability of gastric cancer cells, the scale bar is 100 μm. G,K,P,T CCK8 assay to analyze the effect of macrophages in each group on the proliferation of gastric cancer cells. H,I,M,N,R,S,V,W Western Blot detected the changes of proliferation-related proteins and apoptosis-related proteins of macrophages in each group.

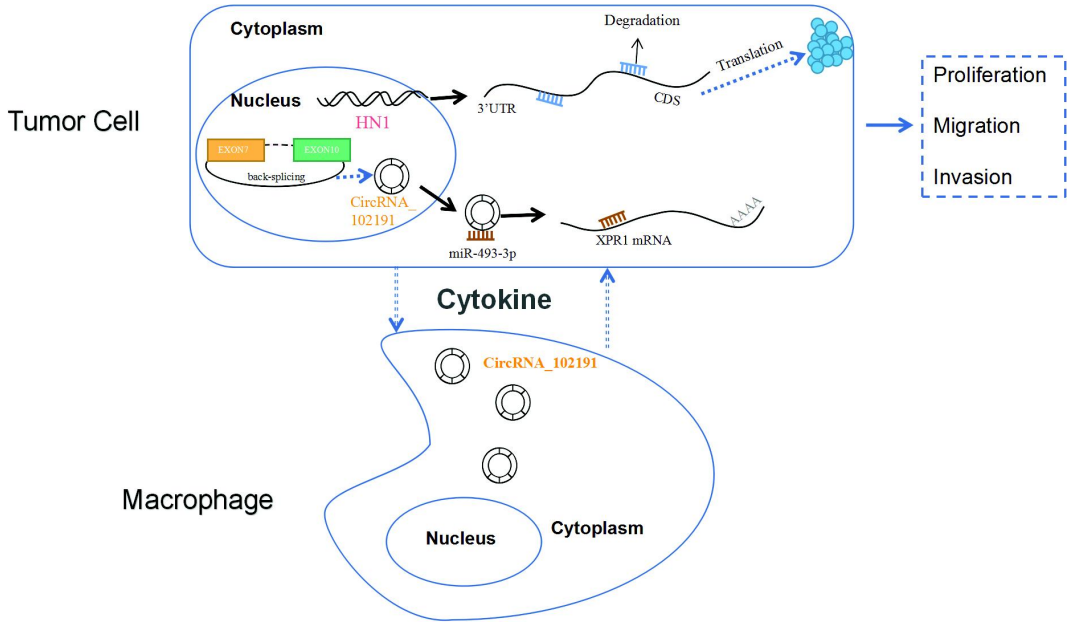

Figure 6. Graphical summary of CircRNA_102191-mediated gastric cancer progression. CircRNA_102191 regulates gastric cancer cell progression by acting as a ceRNA to competitively bind to miR-493-3p, affecting the expression of the target gene XPR1. CircRNA_102191 promotes macrophage polarization, and the polarized macrophages further promote the biological function of gastric cancer cells.

prediction databases as the research object. Previous studies have found that XPR1 is involved in the progression of tongue squamous cell carcinoma, lung adenocarcinoma and esophageal squamous cell carcinoma [29–31]. Our study found that XPR1 can promote the proliferation and metastasis of gastric cancer cells and inhibit cell apoptosis.

As an important member of the monocyte-macrophage system of immune cells, macrophages participate in the innate immune response and play an important role in the body [32,33]. TAMs generally have two differentiation states, the M1 type and the M2 type. Among them, M1-type macrophages have strong pro-inflammatory functions. They can secrete many pro-inflammatory factors, such as IL-6, IL-12, TNF-α, and iNOS [34]. In addition, type 2 macrophages can inhibit the process of inflammation, development, help cancer cells evade immune surveillance, and promote cancer cell proliferation and metastasis [35]. The epithelial-mesenchymal transition (EMT) is a process in which tumor epithelial cells lose epithelial characteristics and acquire mesenchymal characteristics, resulting in the destruction of tight junctions between cells and the remodeling of the cytoskeleton [36,37]. TAMs can secrete various cytokines to induce EMT and secrete EGF-like ligands or factors, thereby activating the EGFR pathway of tumor cells and promoting EMT [38–40]. Studies have shown that many non-coding RNAs play an important role in the differentiation and maintenance of macrophage activity, but the specific mechanism of action is unclear [41–43]. This study found that the level of intracellular circRNA_102191 expression was upregulated significantly during the polarization of IL-4/stimulated M2-type tumor-associated macrophages, and the up-regulation of circRNA_102191 promoted the polarization of macrophages to M2 and also promoted the expression of CD206 and CD163 in M2 macrophages. In addition, M2 macrophage conditioned medium was used to culture gastric cancer cell line SGC-7901 in vitro, and an in vitro co-culture system was established. M2 macrophage conditioned culture promoted the EMT process of SGC-7901 cells and inhibited the apoptosis of SGC. In contrast, the knock-down of circRNA_102191 in M0 and M2 macrophages suppressed the tumor biological function of SGCs. CircRNAs exist in tumor cells and

tumor-associated macrophages, and a large number of circRNAs are released into the tumor microenvironment by macrophages through the exosome pathway and act on tumor cells [44]. Therefore, in this study, in addition to affecting the polarization of M2 macrophages and the secretion of related cytokines, circRNA_102191 may also act directly on gastric cancer SGC-7901 cells through exosomes.

In summary, we found that circRNA_102191 can promote gastric cancer progression and M2 macrophage polarization through miR-493-3p.

Conclusions

In conclusion, this study showed that circRNA_102191 mediates the biological functions of gastric cancer cells by regulating the miR-493-3p/XPR1 axis and inducing macrophage polarization Figure 6.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Authors' contributions

Min Yao, Yue Xi and Haining Gan made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

Xuhua Mao and Zherui Zhang drafted the work or revised it critically for important intellectual content;

Feilun Cui approved the version to be published;

Shihe Shao agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics statement

The research has been approved by Jiangsu University and Jiangsu Provincial Science and Technology Commission. Each experiment was conducted in accordance with the specific protocols of SYXK(su)2018–0053 as well as internal biosafety and bioethics guidelines, following the legal requirements or guidelines of Jiangsu Province regarding the care and use of animals. The maximum tumor size allowed by the Ethics Committee is 2000 mm^3, which was not exceeded in this study.

Data availability statement

The GSE141977and GSE83521 data used to support the findings of this study have been deposited in the Gene Expression Omnibus repository.

References

[1] Assumpção Monica B, Moreira Fabiano C, Hamoy Igor G, et al. High-throughput miRNA sequencing reveals a Field effect in gastric cancer and suggests an Epigenetic Network mechanism Epigenetic Network mechanism. Bioinform Biol Insights. 2015;9:111–117. doi: 10.4137/BBI.S24066

[2] Wanying W, Jinyang Z, Xiaofei C, et al. Exploring the cellular landscape of circular RNAs using full-length single-cell RNA sequencing. Nat Commun. 2022;13:3242. doi: 10.1038/s41467-022-30963-8

[3] Bolisetty Mohan T, Graveley Brenton R. Circuitous route to transcription regulation. Mol Cell. 2013;51:705–706. doi: 10.1016/j.molcel.2013.09.012

[4] Sebastian M, Marvin J, Antigoni E, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495:333–338. doi: 10.1038/nature11928

[5] Jeck William R, Sorrentino Jessica A, Kai W, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013;19:141–157. doi: 10.1261/rna.035667.112

[6] Lingdong M, Yihan Z, Pengfei W, et al. CircSTX6 promotes pancreatic ductal adenocarcinoma progression by sponging miR-449b-5p and interacting with CUL2. Mol Cancer. 2022;21:121. doi: 10.1186/s12943-022-01599-5

[7] Xiuhui S, Jingxuan Y, Mingyang L, et al. Circular RNA ANAPC7 inhibits tumor growth and muscle wasting via PHLPP2-AKT-TGF-β signaling axis in pancreatic cancer. Gastroenterology. 2022;162:2004–2017.e2. doi: 10.1053/j.gastro.2022.02.017

[8] Hui L, Tian L, Hailing L, et al. IL-6-induced cGGNBP2 encodes a protein to promote cell growth and metastasis in intrahepatic cholangiocarcinoma. Hepatology. 2022;75:1402–1419. doi: 10.1002/hep.32232

[9] Xince H, Min H, Shuai H, et al. Correction to: circular RNA circERBB2 promotes gallbladder cancer progression by regulating PA2G4-dependent rDNA transcription. Mol Cancer. 2022;21:122. doi: 10.1186/s12943-022-01563-3

[10] Ying Y, Yarong S, Lulin C, et al. CircCEMIP promotes anoikis-resistance by enhancing protective autophagy in prostate cancer cells. J Exp Clin Cancer Res. 2022;41:188. doi: 10.1186/s13046-022-02381-7

[11] Xiaodan C, Jingde C, Nanxin Z, et al. PIK3CA mutations-mediated downregulation of circLHFPL2 inhibits colorectal cancer progression via upregulating PTEN. Mol Cancer. 2022;21:118. doi: 10.1186/s12943-022-01531-x

[12] Mahshid Deldar Abad P, Maliheh E, Sepideh M, et al. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. J Hematol Oncol. 2022;15:83. doi: 10.1186/s13045-022-01305-4

[13] Ceu F, Camargo MC, Marina L, et al. Erratum to: pathogenesis of gastric cancer: genetics and molecular Classification. Curr Top Microbiol Immunol. 2017;400:E1.

[14] Hirotake T, Koji F, Azusa M, et al. Combined blockade of IL6 and PD-1/PD-L1 signaling abrogates mutual regulation of their immunosuppressive effects in the tumor microenvironment. Cancer Res. 2018;78:5011–5022. doi: 10.1158/0008-5472.CAN-18-0118

[15] Wenbin W, Yangmei Z, Li X. Li Xiaowu et al. miR-375 inhibits the proliferation, migration and invasion of esophageal squamous cell carcinoma by targeting XPR1. Curr Gene Ther. 2021;21:290–298. doi: 10.2174/1566523220666201229155833

[16] Yoko A-N, Tomoatsu H, Ayako K, et al. PHOSPHATE exporter XPR1/SLC53A1 is required for the tumorigenicity of epithelial ovarian cancer. Cancer Sci. 2022;113:2034–2043. doi: 10.1111/cas.15358

[17] Wei-Chao C, Qiu-Li L, Qimei P, et al. Xenotropic and polytropic retrovirus receptor 1 (XPR1) promotes progression of tongue squamous cell carcinoma (TSCC) via activation of NF-κB signaling. J Exp Clin Cancer Res. 2019;38:167. doi: 10.1186/s13046-019-1155-6

[18] Li D, Shen L, Zhang X, et al. LncRNA ELF3-AS1 inhibits gastric cancer by forming a negative feedback loop with SNAI2 and regulates ELF3 mRNA stability via interacting with ILF2/ILF3 complex. J Exp Clin Cancer Res. 2022 Dec 2;41(1):332. PMID: 36457025; PMCID: PMC9716751. doi: 10.1186/s13046-022-02541-9

[19] Li D, She J, Hu X, et al. The ELF3-regulated lncRNA UBE2CP3 is over-stabilized by RNA-RNA interactions and drives gastric cancer metastasis via miR-138-5p/ITGA2 axis. Oncogene. 2021 Sep;40(35):5403–5415. Epub 2021 Jul 17. Erratum in: Oncogene. 2023 Apr 28: PMID: 34274947; PMCID: PMC8413130. doi: 10.1038/s41388-021-01948-6

[20] Li D, Xu M, Wang Z, et al. The EMT-induced lncRNA NR2F1-AS1 positively modulates NR2F1 expression and drives gastric cancer via miR-29a-3p/VAMP7 axis. Cell Death Dis. 2022 Jan 26;13(1):84. PMID: 35082283; PMCID: PMC8791943. doi: 10.1038/s41419-022-04540-2

[21] Feng Y, Anpei H, Dan L, et al. Circ-HuR suppresses HuR expression and gastric cancer progression by inhibiting CNBP transactivation. Mol Cancer. 2019;18:158. doi: 10.1186/s12943-019-1094-z

[22] Lixian D, Yuying Z, Shuwei D, et al. Circular RNA circ-DONSON facilitates gastric cancer growth and invasion via NURF complex dependent activation of transcription factor SOX4. Mol Cancer. 2019;18:45. doi: 10.1186/s12943-019-1006-2

[23] Jun L, Yao-Hui W, Changhwan Y, et al. Circular RNA circ-RanGAP1 regulates VEGFA expression by targeting miR-877-3p to facilitate gastric cancer invasion and metastasis. Cancer Lett. 2020;471:38–48. doi: 10.1016/j.canlet.2019.11.038

[24] Liu Q, Yang H, Hua H. Overexpression of miR-493-3p suppresses ovarian cancer cell proliferation, migration and invasion through downregulating DPY30. Reprod Biol. 2022 Jun;22(2):100610. Epub 2022 Mar 18. PMID: 35313261. doi: 10.1016/j.repbio.2022.100610

[25] Ding D, Yang F, Chen Z, et al. Circ_0007385 regulates cell proliferation, apoptosis and stemness via targeting miR-493-3p/RAB22A axis in non-small cell lung cancer. Thorac Cancer. 2022 Feb;13(4):571–581. Epub 2022 Jan 5. PMID: 34989145; PMCID: PMC8841703. doi: 10.1111/1759-7714.14300

[26] Wang XY, Wang L, Xu PC, et al. LINC01605 promotes the proliferation of laryngeal squamous cell carcinoma through targeting miR-493-3p. Eur Rev Med Pharmacol Sci. 2019 Dec;23(23):10379–10386. PMID: 31841192. doi: 10.26355/eurrev_201912_19677

[27] Liu Y, Chen S, Peng G, et al. CircRNA NALCN acts as an miR-493-3p sponge to regulate PTEN expression and inhibit glioma progression. Cancer Cell Int. 2021 Jun 10;21(1):307. PMID: 34112159; PMCID: PMC8194043 10.1186/s12935-021-02001-y

[28] Zhan K, Song X, Zhang Q, et al. Propofol-induced miR-493-3p inhibits growth and invasion of gastric cancer through suppression of DKK1-mediated Wnt/β-catenin signaling activation. Dis Markers. 2023 Jan 31;2023:1–8. PMID: 36762306; PMCID: PMC9904924. 10.1155/2023/7698706

[29] Chen WC, Li QL, Pan Q, et al. Xenotropic and polytropic retrovirus receptor 1 (XPR1) promotes progression of tongue squamous cell carcinoma (TSCC) via activation of NF-κB signaling. J Exp Clin Cancer Res. 2019 Apr 17;38(1): :167. PMID: 30995931; PMCID: PMC6469095 10.1186/s13046-019-1155-6

[30] Hu Y, Bai J, Zhou D, et al. The miR-4732-5p/XPR1 axis suppresses the invasion, metastasis, and epithelial-mesenchymal transition of lung adenocarcinoma via the PI3K/Akt/GSK3β/Snail pathway. Mol Omics. 2022 Jun 13;18(5):417–429. PMID: 35388387 10.1039/d1mo00245g

[31] Wu W, Zhang Y, Li X, et al. miR-375 inhibits the proliferation, migration and invasion of esophageal squamous cell carcinoma by targeting XPR1. Curr Gene Ther. 2021;21(4):290–298. PMID: 33372873. doi: 10.2174/1566523220666201229155833

[32] McGrath KE, Frame JM, Palis J. Early hematopoiesis and macrophage development. Semin Immunol. 2015 Dec

;27(6):379–387. Epub 2016 Mar 25. PMID: 27021646; PMCID: PMC4856633. doi: 10.1016/j.smim.2016.03.013

[33] Michela M, Roberta C, Federica P, et al. Lipid-loaded tumor-associated macrophages sustain tumor growth and invasiveness in prostate cancer. J Exp Med. 2022;219:undefined. doi: 10.1084/jem.20210564

[34] Atri C, Guerfali FZ, Laouini D. Role of human macrophage polarization in inflammation during infectious diseases. Int J Mol Sci. 2018 Jun 19;19(6):1801. PMID: 29921749; PMCID: PMC6032107. doi: 10.3390/ijms19061801

[35] Senlin Z, Yushuai M, Bingjie G, et al. Tumor-derived exosomal miR-934 induces macrophage M2 polarization to promote liver metastasis of colorectal cancer. J Hematol Oncol. 2020;13:156. doi: 10.1186/s13045-020-00991-2

[36] Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010 Apr 2;141 (1):39–51. PMID: 20371344; PMCID: PMC4994190. doi: 10.1016/j.cell.2010.03.014

[37] Li D, Xia L, Huang P, et al. Heterogeneity and plasticity of epithelial-mesenchymal transition (EMT) in cancer metastasis: focusing on partial EMT and regulatory mechanisms. Cell Prolif. 2023 Feb;19:e13423. Epub ahead of print. PMID: 36808651. doi: 10.1111/cpr.13423

[38] Suarez-Carmona M, Lesage J, Cataldo D, et al. EMT and inflammation: inseparable actors of cancer progression. Mol Oncol. 2017 Jul;11(7):805–823. Epub 2017 Jun 26. PMID: 28599100; PMCID: PMC5496491. doi: 10.1002/1878-0261.12095

[39] Qin S, Wang Z, Huang C, et al. Serine protease PRSS23 drives gastric cancer by enhancing tumor associated macrophage infiltration via FGF2. Front Immunol. 2022 Sep 15;13: 955841. PMID: 36189305; PMCID: PMC9520605. 10.3389/fimmu.2022.955841

[40] Li D, Xia L, Huang P, et al. Cancer-associated fibroblast-secreted IGFBP7 promotes gastric cancer by enhancing tumor associated macrophage infiltration via FGF2/FGFR1/PI3K/AKT axis. Cell Death Discov. 2023 Jan 21;9(1):17. PMID: 36681667; PMCID: PMC9867714 10.1038/s41420-023-01336-x

[41] Jingwei Z, Liming C. Long non-coding RNA CCAT1/miR-148a axis promotes osteosarcoma proliferation and migration through regulating PIK3IP1. Acta Biochim Biophys Sin (Shanghai). 2017;49:503–512. doi: 10.1093/abbs/gmx041

[42] Zhixin L, Changjiang F, Junhong G, et al. GNAS-AS1/miR-4319/NECAB3 axis promotes migration and invasion of non-small cell lung cancer cells by altering macrophage polarization. Funct Integr Genomics. 2020;20:17–28. doi: 10.1007/s10142-019-00696-x

[43] Fuquan Z, Yonghua S, Donglai C, et al. M2 macrophage-derived exosomal long non-coding RNA AGAP2-AS1 enhances radiotherapy immunity in lung cancer by reducing microRNA-296 and elevating NOTCH2. Cell Death Dis. 2021;12:467. doi: 10.1038/s41419-021-03700-0

[44] Zhou Z, Jiang R, Yang X, et al. circRNA mediates silica-induced macrophage activation via HECTD1/ZC3H12A-Dependent ubiquitination. Theranostics. 2018 Jan 1;8(2):575–592. Erratum in: Theranostics. 2022 Apr 26;12(7):3574. PMID: 29290828; PMCID: PMC5743568. doi: 10.7150/thno.21648