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30-OCT-2025 08:44AM

PAPER ID

118814981

Name of Journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 113524

Manuscript Type: ORIGINAL ARTICLE

Basic Study

Exosomal miR-191 promotes colorectal cancer progression by inducing M2 macrophage polarization and inhibiting ferroptosis

Functions of Exosomal miR-191 in CRC

Qingyun Zhao, Shou-Jiang Wei

Abstract

BACKGROUND

Multiple exosomal miRNAs were reported to have a significant role in colorectal cancer (CRC) cells. The function and mechanism of exosomal miR-191 in CRC have not been clearly elucidated.

AIM

To explore the roles of miR-191 in CRC.

METHODS

Supernatant exosomes from CRC cells were extracted and identified. After coculture, macrophage polarization was determined using flow cytometry for the markers CD68 and CD163, ELISA for the cytokines interleukin (IL)-4 and IL-10, western blotting for YM1 and Arg-1 expression, and immunofluorescent staining for PKH26. Reactive oxygen species (ROS) level, ferroptosis-related proteins (SLC7A11 and GPX4), and apoptosis were determined with flow cytometry, western blotting, and TUNEL staining. We performed in vivo experiments to determine the function of exosomal miR-191 and M2 macrophage polarization.

RESULTS

We successfully isolated exosomes from CRC cells. Inhibition of miR-191 in CRC cells suppressed M2 polarization of macrophages. After coculture of macrophages, inhibition of miR-191 induced ROS production, ferroptosis, and apoptosis of CRC cells. Silencing of exosomal miR-191 from CRC cells prevented M2 polarization of macrophages, and weakened CRC development by inducing ferroptosis. Exosomal miR-191 accelerated cancer progression in CRC nude mice by promoting M2 polarization of macrophages.

CONCLUSION

Inhibition of exosomal miR-191 attenuated CRC progression by inducing ferroptosis in macrophages. This study revealed a novel mechanism by which exosomal miR-191 modulates the tumor microenvironment.

Key Words: colorectal cancer; miR-191; exosome; macrophages; ferroptosis; M2 polarization

Zhao Q, Wei SJ. Exosomal miR-191 promotes colorectal cancer progression by inducing M2 macrophage polarization and inhibiting ferroptosis. *World J Gastrointest Oncol* 2025; In press

Core Tip: We successfully isolated exosomes from colorectal cancer (CRC) cells. Inhibition of miR-191 in CRC cells suppressed M2 polarization of macrophages. After coculture of macrophages, inhibition of miR-191 induced reactive oxygen species production, ferroptosis, and apoptosis of CRC cells. Silencing of exosomal miR-191 from CRC cells prevented M2 polarization of macrophages, and weakened CRC development by inducing ferroptosis in macrophages. Exosomal miR-191 also accelerated cancer progression in CRC nude mice by promoting M2 polarization of macrophages.

1 INTRODUCTION

Colorectal cancer (CRC) is a frequent gastrointestinal malignancy worldwide. There were 1.9 million new cases and 935 000 deaths in 2020, accounting for 10% of all cancers[1, 2]. Most cases of CRC are not caused by a single factor[3]. Most patients have no obvious symptoms in the early stage, and 40%-50% of patients are already at an advanced stage when diagnosed, with distant metastases and a 5-year survival rate of only 12.5%[4]. The most familiar sites of metastasis are the liver, peritoneum and lung[5]. Currently, therapy is based on radical surgery and radiotherapy[2, 6].

Recurrence and metastasis rates are high after surgery. Therefore, studying the mechanism of CRC development is beneficial for accurate diagnosis, precise treatment and early prevention of the disease, which is also an effective way to reduce CRC mortality.

Exosomes are small extracellular vesicles, 40-150 nm in diameter, derived from intranuclear bodies[7]. All types of cells in the tumor microenvironment are capable of releasing exosomes and regulating the microenvironment through the delivery of bioactive molecules in exosomes[8]. Exosomes have immunostimulatory and immunosuppressive effects in tumor immunity[9]. Research confirmed that tumor cells can secrete intracellular noncoding RNAs with specific functions into the tumor microenvironment in an exosomal manner, thus affecting tumor cell proliferation, apoptosis and metastasis[10, 11]. miRNAs are one class of noncoding RNA molecules consisting of 18 - 28 nucleotides[12, 13]. miRNAs can participate in multiple pathophysiological processes in cells through post-transcriptional regulation[14]. Therefore, studying the role and mechanism of tumor-cell-derived exosomal miRNAs in cancer progression can guide the search for better therapy. miR-191 has been reported to have a role in the induction several types of cancer, including prostate cancer[15, 16], hepatocellular carcinoma[17], colorectal cancer[18] and endometrial cancer[19]. However, the action and mechanism of exosomal miR-191 in CRC is not clear; therefore, we selected exosomal miR-191 as the subject of this study.

CRC progression is a complex process caused by a combination of genetic modifications that accumulate in cancer cells and the surrounding microenvironment[20]. Success of CRC chemotherapy is associated with a durable tumor-targeted immune response[21]. Tumor-associated macrophages (TAMs) have been reported to affect the prognosis and efficacy of chemotherapy and immunotherapy[22]. In many cases, TAMs can also directly influence tumor progression, suggesting that they are associated with CRC progression[23]. Therefore, exploring the relationship between TAMs and CRC may provide ideas for new immune target studies. Studies of TAMs in CRC have focused on basic experimental

investigations and are dominated by M2 TAMs[24, 25]. Current evidence suggests that M2 TAMs are key immunosuppressive cells that induce tumor growth, angiogenesis and epithelial mesenchymal transition (EMT)[26, 27]. Therefore, M2 TAMs are a potential target for immunotherapy. However, whether CRC-derived exosomal miR-191 can affect CRC progression by altering macrophage polarization has not been reported.

In this study, we extracted and identified exosomes from CRC cells, and investigated the influence of miR-191 inhibition on M2 polarization of macrophages. We explored the roles of miR-191 inhibition in CRC-cell-related functions by coculturing with macrophages. More importantly, we verified the role and mechanism of exosomal miR-191 from CRC cells on the M2 polarization of macrophages and CRC progression. It is hoped that our study will provide an experimental basis for immunotherapy of CRC.

MATERIALS AND METHODS

Cell culture

Fetal human colon epithelial cells and CRC cells (SW620, SW480, HCT116 and LoVo) were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) at 37°C with 5% CO₂.

Isolation and induction of macrophages

Peripheral blood of volunteers was collected in heparin anticoagulation tubes. After centrifugation, the supernatant was collected. The supernatant was added to twice the volume of phosphate-buffered saline (PBS), followed by the addition of 2/3 volume of lymphatic separation solution. After centrifugation, the white film layer was collected as peripheral blood mononuclear cells. After PBS cleaning, the LS sorting column was placed in the sorting field and cleaned by adding MACS sorting buffer (#130-091-376,

Miltenyi Biotec). After counting, 10⁷ cells were added to 20 μ L of sorting magnetic beads and 80 μ L of MACS buffer at 4^o C for 15 min. After MACS buffer treatment, the samples were added to the LS sorting column. Cells in the column were collected and cultured in RPMI 1640. The CD14-positive monocytes were inoculated in 12-well plates. M-CSF was added to positive monocytes. After 5 days, the unadhered cells were discarded and the remaining adhered cells were macrophages.

Cell treatment

miR-191 inhibitor and negative control (NC) were provided by Genepharma (Shanghai, China). CRC cells or macrophages were transfected with miR-191 inhibitor or NC with Lipofectamine 3000 reagent (Invitrogen) for 48 h. CRC cells or macrophages were exposed to exosomes from CRC cells for 48 h, using a concentration of 10⁷ exosomes/mL. Macrophages were also processed with 5 μ M ferrostatin-1. CRC cells were cocultured with the treated macrophages.

qRT-PCR

Total RNA was extracted with Trizol reagent (Invitrogen). Nanodrop spectrophotometry was used to measure the concentration of RNA. Reverse transcription was conducted to produce cDNAs using BestarTM qPCR RT kit (DBI Bioscience, China). Polymerase chain reaction (PCR) amplification was performed with SYBR Green qPCR Mix kit (Sparkjade, China) on the QuantStudioTM6 Realtime-PCR instrument. The relative level was calculated by 2^{- $\Delta\Delta$ CT}.

Extraction of exosomes

When CRC cells reached 80% confluence, the original ⁶ culture medium was replaced with serum-free medium and the culture was continued for 48 h. The supernatant was collected and centrifuged to remove impurities. Subsequently, the centrifuged supernatant was filtered through a 0.22- μ m filter and centrifuged at 4^o C to extract exosomes (6000 \times g for 40 min and 10 000 \times g for 1 h).

NanoSight particle-tracking analysis

Exosomes were diluted with PBS and placed on a cuvette. The particle size was analyzed by dynamic light scattering (Litesizer 500, Anton Parr, Austria).

Transmission electron microscope (TEM)

The extracted exosomes were fixed with 100 μ L 2% paraformaldehyde at 4° C. Five microliters of exosome suspension was added dropwise to a copper grid for 20 min at room temperature. After PBS washing, the samples were ⁵ fixed with 1% glutaraldehyde for 5 min, rinsed 10 times with double distilled water, and negatively stained with 4% dioxin acetate for 5 min. The residual solution was blotted off on filter paper and dried at room temperature. Exosomes were observed by transmission electron microscopy (TEM).

Western blot

The transplanted tumor tissue from the mice were grounded, and groups of cells and exosomes were collected. Total protein was extracted with RIPA lysis buffer. The proteins (40 μ g) were ⁴ separated by SDS-PAGE, and transferred to polyvinylidene difluoride membranes (Merck, Billerica, MA, USA). After blocking with 5% skimmed milk for 1 h, the samples were added to primary antibody at 4° C overnight, followed by secondary antibody (1:2000; Abcam, Cambridge, MA, USA) for 1 h. Each group was exposed and photographed on the ChemiDoc MP Imager System (#12003153, Bio-Rad) after uniformly adding ECL reagents (Thermo, Waltham, MA, USA). The primary antibodies included anti-CD9 ³ (1:1000, ab236630; Abcam), anti-TSG101 (1:1000; Abcam), anti-YM1 (1:1000, ab125011; Abcam), anti-Arg-1 (1:1000, ab281603; Abcam), anti-SLC7A11 (1:1000, ab307601; Abcam), anti-GPX4 (1:1000, ab262509; Abcam), and anti-GAPDH (1:1000, ab9485; Abcam).

ELISA

The levels of interleukin (IL)-4 (CSB-E04633h; Cusabio, Wuhan, China) and IL-10 (CSB-E04593h-IS; Cusabio) in cell supernatant or mouse serum were assessed with the corresponding ELISA kits. OD 450 nm was measured and the standard curve was plotted. The corresponding concentration of samples was obtained according to the standard curve.

Flow cytometry for CD68 and CD163+

Macrophages were digested with 0.25% trypsin without EDTA and collected after termination of digestion. Macrophages were resuspended by adding 200 μ l PBS and incubated with the corresponding antibodies (CD68 and CD163) for 30 min at 4° C. After centrifugation, macrophages were resuspended with 200 μ l PBS. CD68 and CD163 were analyzed by flow cytometry (BD Biosciences, San Jose, CA, USA).

Uptake of exosomes

The exosomes were labeled with PKH26. After ultracentrifugation (100 000 \times g for 1 h), PKH26-labeled exosomes were collected. The macrophages were inoculated in six-well plates and cocultured with PKH26-labeled exosomes. The co-incubated cells were fixed with 4% formaldehyde, and the nuclei were labeled with 4',6-diamidino-2-phenylindole (DAPI; Sigma). Uptake of exosomes by macrophages was observed under fluorescence microscopy.

Flow cytometry for ROS

The collected cells were added to 500 μ l PBS and cell homogenates were prepared. The level of intracellular ROS was determined using the DCFH-DA (2',7'-Dichlorodihydrofluorescein diacetate) probe. The cells were stained with 20 μ M DCFH-DA for 30 min in the dark. Fluorescence intensity was monitored by flow cytometry.

Experimental animals

Fifteen sterile-grade healthy BALB/c nude mice (5 weeks, 18-20 g) were housed in the SPF-grade animal house at the Animal center of North Sichuan Medical College. ¹⁰ The experimental protocol was approved by the Ethics Committee of North Sichuan Medical College (approval no. 20240520). Each nude mouse was inoculated by subcutaneous injection of 5.0×10^7 CRC cells in a volume of 0.2 mL. Mice in the exosome group were given 50 μ L exosomes (1 mg/mL) via the tail vein every 2 days for five times. The mice were also treated with M2 type macrophage inhibitor erastin (10mg/kg.) After inoculation for 7 days, tumor size was measured using vernier calipers. At 28 days after implantation, the tumors were photographed and weighed. The mice were killed by 200 mg/kg intraperitoneal injection of sodium pentobarbital.

H&E staining

Based on previous research, nude mouse tumors were fixed in 4% paraformaldehyde (Solarbio), dehydrated, waxed, embedded, and sectioned. The hematoxylin and eosin (H&E) stain was baked for 1 h before staining, and dewaxed with graded ethanol and xylene until hydrated. The a ⁸ were stained with hematoxylin for 5 min and eosin for 3 min. Finally, the sections were dehydrated, transparented and sealed. The tissue structure was observed under light microscope.

TUNEL analysis

CRC cells were inoculated at 5×10^5 /well in 24-well plates at 37° C for 24 h. When the cell density was suitable, the cells were fixed with 4% paraformaldehyde. After washing, the samples were immersed in the closure solution for 15 min, permeabilized for 2 min, and stained with TUNEL solution for 1 h. The cells were stained with DAPI (Sigma). Finally, the fluorescence signal was monitored under fluorescence microscopy.

Statistical analysis

All data were presented as mean \pm SD from three independent repetitions. SPSS 22.0 (SPSS, Chicago, IL, USA) was used to analyze the data. Data between two groups were analyzed using the t test, and the quantitative data in multiple groups were tested by one-way analysis of variance. Differences were defined as statistically significant at $P < 0.05$.

RESULTS

Identification of exosomes derived from CRC cells

We verified the expression difference of miR-191 in FHC and CRC cell lines (SW620, SW480, HCT116 and LoVo) by quantitative reverse transcription PCR (qRT-PCR). Expression of miR-191 was increased in CRC cell lines compared with that in the FHC group. CRC cell lines SW620 and LoVo with the highest expression of miR-191 were selected for subsequent experiments (Figure 1A). Exosomes were collected from SW620 and LoVo cell supernatants. Through NanoSight particle - tracking analysis, the size of SW620 exosomes was mainly around 70 nm, and that of LoVo exosomes was mainly around 80 nm (Figure 1B). TEM showed that the exosome particles were circular and similar in size to the above results (Figure 1C). Western blotting showed that exosomal markers CD9 and TSG101 were highly expressed in SW620 and LoVo exosomes (Figures 1D and S1A). We transfected miR-191 inhibitor into CRC cells and isolated the exosomes. qRT-PCR showed that inhibition of miR-191 markedly reduced miR-191 expression in CRC cells and CRC-derived exosomes (Figure 1E). We successfully knocked down miR-191 by transfection.

Inhibition of miR-191 in CRC cells prevents M2 polarization of macrophages

To confirm whether macrophages successfully took up exosomes, CRC cells transfected with miR-191 inhibitor were cocultured with M0 macrophages. We analyzed the polarization of M2 macrophages by flow cytometry to analyze the expression of macrophage markers CD68 and CD163. Expression of CD68/CD163 in macrophages

was dramatically reduced in the miR-191 inhibitor group with respect to that in the control group (Figure 2A). We measured the cellular inflammatory factors ¹¹IL-4 and IL-10. The levels of IL-4 and IL-10 were reduced in the miR-191 inhibitor group relative to the NC group (Figure 2B). Western blotting demonstrated that miR-191 inhibition downregulated YM1 (chitinase-like protein 3) and Arg-1 (Arginase 1) in macrophages (Figures 2C and S1B). We labeled exosomes with PKH red dye and macrophage nuclei with DAPI blue dye. Immunofluorescence staining indicated red fluorescence near the blue fluorescently labeled nuclei, indicating successful uptake of exosomes by macrophages (Figure 2D). More importantly, we found that the intracellular miR-191 content of macrophages was reduced after uptake of exosomes from CRC cells transfected with miR-191 inhibitor (Figure 2E). We proved that macrophages had successfully taken up exosomes.

Downregulation of miR-191 promotes ROS production, ferroptosis, and apoptosis of CRC cells after co-culture of macrophages

CRC cells transfected with miR-191 inhibitor were cocultured with M0 macrophages. qRT-PCR showed that miR-191 was markedly downregulated in CRC cells after miR-191 inhibitor transfection (Figure 3A). Flow cytometry revealed that ROS level was markedly higher in the miR-191 inhibitor group than in the NC group (Figure 3B). Western blotting also demonstrated that the levels of ferroptosis marker proteins SLC7A11 and GPX4 were notably ¹⁴lower in the miR-191 inhibitor group than that in the NC group (Figures 3C and S1C). We used TUNEL staining to confirm the change in apoptosis and found that miR-191 inhibition increased the rate of apoptosis in CRC cells (Figure 3D).

Silencing of exosomal miR-191 from CRC cells attenuate inflammation and M2 polarization of macrophages by inducing ferroptosis

We verified whether exosomal miR-191 from CRC cells affected M2 polarization of macrophages by altering ferroptosis. Macrophages were processed with exosomes from

CRC cells, and treated with miR-191 inhibitor and/or ferrostatin-1. Flow cytometry showed that macrophage markers CD68 and CD163¹⁶ were lower in the miR-191 inhibitor group than in the NC group, and the decrease in CD68/CD163 positivity was reversed by ferrostatin-1 (Figure 4A). ELISA showed that inhibition of exosomal miR-191 decreased the concentrations of IL-4 and IL-10 in macrophages, which was also partly restored by ferrostatin-1 (Figure 4B). Western blotting revealed that inhibition of exosomal miR-191 downregulated YM-1 and Arg-1 expression in macrophages, while ferrostatin-1 treatment markedly reduced the downregulation of YM-1 and Arg-1 mediated by miR-191 inhibitor (Figures 4C and S1D). qRT-PCR confirmed that inhibition of exosomal miR-191 reduced IL-4 and IL-10 levels in macrophages by inducing ferroptosis (Figure 4D).

Inhibition of exosomal miR-191 from CRC cells enhance ROS production, ferroptosis, and apoptosis of CRC cells through accelerating ferroptosis in macrophages

We investigated whether ferroptosis of macrophages was related to the influence of exosomal miR-191 from CRC cells on CRC-related functions. CRC cells were incubated with exosomes from CRC cells and miR-191 inhibitor or ferrostatin-1-treated macrophages. qRT-PCR showed that inhibition of exosomal miR-191 downregulated miR-191 in CRC cells, which was reversed by ferrostatin-1 (Figure 5A). Inhibition of exosomal miR-191 enhanced ROS production in CRC cells, and this was partially attenuated by ferrostatin-1 (Figure 5B). Ferrostatin-1 treatment also markedly reversed the reduction in SLC7A11 and GPX4 expression mediated by miR-191 inhibitor in CRC cells (Figures 5C and S1E). TUNEL staining indicated that ferrostatin-1 reduced the enhancement of CRC cell apoptosis in the miR-191 inhibitor group (Figure 5D).

Exosomal miR-191 from CRC cells promotes growth, and alters pathological structure of subcutaneous tumors in CRC nude mice by inducing M2 polarization of macrophages

We validated the role of exosomal miR-191 from CRC cells in nude mice. After injection with exosomal miR-191 and/or erastin, nude mice were continued to be fed until 28 days. ² At the end of the experiment, the mice were killed and the subcutaneous tumors were removed and photographed (Figure 6A). Tumor weight was notably heavier in the ExomiR-191 group than in the ExoNC group, and the ExomiR-191-mediated increase in tumor weight was partly attenuated by erastin (Figure 6B). Tumor volume was also higher in the ExomiR-191 group than in the ExoNC group, and the ExomiR-191-mediated increase in tumor volume was partly attenuated by erastin (Figure 6C). H&E staining revealed that in the ExoNC group, the tumor tissues of nude mice exhibited oval, long oval or polygonal epithelial-like cells, which were closely arranged, and occasionally the cancer foci were distributed in clusters or cords. In the ExomiR-191 group, the tumor tissues demonstrated obvious clusters of cancer foci with disorganized cell arrangement and decreased inter-tissue polarity. In the ExomiR-191 + erastin group, the tumor tissue tended to be similar to that of the ExoNC group (Figure 6D). ELISA showed that ExomiR-191 dramatically elevated the concentration of IL-4 and IL-10 in the serum of mice, which was reversed by erastin (Figure 6E). Erastin downregulated miR-191 expression in tumor tissues, which had been upregulated by ExomiR-191 (Figure 6F). ExomiR-191 increased the levels of SLC7A11, GPX4, YM1 and Arg-1 in tumor tissues, while upregulation of these four proteins was inhibited by erastin (Figures 6G and S1F).

DISCUSSION

CRC is a malignant tumor with high morbidity and mortality[28]. Early-stage CRC patients have a good postoperative prognosis, but some patients still develop recurrence and metastasis[29]. TAMs are relevant to the progression and metastasis of CRC[30], and have become a hot research topic as new therapeutic targets[31]. In our

study, we investigated the effects of exosomal miRNAs on TAMs with the aim of providing new therapeutic approaches for CRC.

Macrophages are heterogeneous and plastic after being recruited to the tumor microenvironment[32]. TAMs can be polarized into different phenotypes under the regulation of various signaling factors in the microenvironment[33]. TAMs are mainly classified into M1 and M2 types[34]. M1 macrophages can prevent tumor progression by mediating Th1-type immune responses. M2 macrophages can induce tumor cell infiltration and metastasis[33]. CD68 is a marker for M1 and M2 macrophages, while CD186 is a marker that specifically identifies M2 macrophages[35, 36]. Therefore, our current study adopted CD68 and CD163 to identify macrophages and determined M2 polarization of macrophages by detecting phenotypic markers YM1 and Arg-1[37, 38].

Exosomes play a key role in tumor growth, angiogenesis and metastasis, and are ideal diagnostic and prognostic markers[7]. Exosome-mediated therapy has also been considered as a potential strategy for comprehensive treatment[39]. Exosomes secreted by tumor cells carrying specific small molecules or RNAs can influence the biological functions of other cells[40]. Exosome-delivered bioactive molecules have been confirmed to be critical in the CRC process. miRNAs, as cancer markers, are associated with cancer cell proliferation, differentiation and apoptosis[12, 13]. Therefore, miRNAs can act as an effective molecular targeting drug for tumor treatment[41]. Exosome-encapsulated miRNAs can also remain stable in plasma[42]. Therefore, exosomal miRNAs may be applied as a noninvasive, convenient and sensitive marker to assist in the diagnosis, therapy and prognosis of CRC.

miR-191 has procancer effects in several types of cancer[15, 17, 18, 43, 44]. For instance, miR-191 induces radiation resistance of prostate cancer[15], and miR-191 inhibition can prevent tumorigenicity of breast cancer[44]. Triptolide can prevent colon carcinoma progression by downregulating miR-191[45], and β -elemene can enhance the chemosensitivity of CRC cells to 5-fluorouracil by decreasing miR-191 expression[46]. These data suggest that miR-191 has an accelerating effect on the malignant process of CRC cells. Therefore, we chose exosomal miR-191 for further research. Through cell

screening, miR-191 was highly expressed in SW620 and LoVo cells, which were also selected as the target cells for the current study. Subsequently, we extracted exosomes from each of these two cell lines. The expression of exosomal markers CD9 and TSG101 was tested by western blotting, and the extracted exosomes were identified. TEM identified the exosomes. We demonstrated that inhibition of miR-191 by transfection downregulated the expression of miR-191 in CRC cells and exosomes. Therefore, we speculate that exosomal miR-191 may make a significant contribution in the CRC process. Our data verified that inhibition of miR-191 or exosomal miR-191 attenuated M2 polarization of macrophages.

Ferroptosis is relevant to tumor progression, therapy and drug resistance[47]. Ferroptosis, as a form of cellular immunogenic death, releases a series of damage-related molecules that trigger an inflammatory response[48]. The inflammatory response also can exert antitumor effects by activating immune cells, such as TAMs[49]. Tumor cells are more sensitive to ferroptosis than normal cells. SLC7A11, a key regulatory protein of ferroptosis, is responsible for transporting cystine from the extracellular to the intracellular compartment[50]. GPX4 is the only enzyme known to be effective in reducing lipid peroxidation in biological membranes[51]. Inhibition of GPX4 activity can cause lipid ROS accumulation and ferroptosis[52]. In our study, we revealed that miR-191 inhibition induced ROS production, ferroptosis and apoptosis of CRC cells. Ferroptosis may play a role in mediating the inhibitory effect of exosomal miR-191 on M2 macrophage polarization. We also showed that inhibition of exosomal miR-191 prevented the malignant properties of CRC cells by ferroptosis in macrophages. In vivo, exosomal miR-191 facilitated tumor growth and suppressed apoptosis of subcutaneous tumors in CRC nude mice by inducing M2 polarization of macrophages.

Although the present study has provided preliminary insights into the functional role of miR-191 in CRC, some limitations should be acknowledged. Despite the high prevalence of CRC, the limited timeframe for patient recruitment at our single institution restricted the acquisition of a sufficiently large cohort for robust statistical analysis. As a result, it was not possible to conduct further evaluations regarding the

association between miR-191 expression and clinicopathological parameters or survival outcomes. To more comprehensively assess the clinical relevance of miR-191, we intend to perform larger-scale, multicenter collaborative studies involving systematically collected CRC specimens in future research.

CONCLUSION

The present study confirms that exosomal miR-191 from CRC cells has a potent regulatory effect on macrophage M2 polarization, which may participate in the malignant process of CRC via ferroptosis. Therefore, exosomal miR-191 might be a latent therapeutic target characterized by increased activation of M2 macrophages.

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