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MiR-10b suppresses the growth and metastasis of colorectal cancer cell by targeting FGF13

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Abstract. – OBJECTIVE: MicroRNA (miRNA) plays important roles in the progression of different cancers. In this study, we investigated the precise role of miR-10b in the growth and metastasis of colorectal cancer (CRC) cell.

PATIENTS AND METHODS: The levels of miR-10b in CRC cell lines were detected using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) assay. A series of functional assays, including cell proliferation, colony formation, wound healing and transwell invasion were conducted using miR-10b transfected cells. The expressions of E-cadherin and N-cadherin were assessed by immunofluorescence staining. Xenograft model and lung metastasis model were constructed to investigate the impact of miR-10b on the growth and metastasis of CRC cell *in vivo*.

RESULTS: We revealed that miR-10b was markedly down-regulated in CRC. The up-regulation of miR-10b suppressed the growth, colony formation, migration and invasion of CRC cell. Furthermore, the xenograft model indicated that miR-10b inhibited CRC cell growth and lung metastasis *in vivo* by targeting fibroblast growth factor 13 (FGF13). In addition, we demonstrated that the overexpression of FGF13 rescued the suppressive effects of miR-10b on CRC cells growth, migration and invasion. Finally, the knockdown of FGF13 was able to mimic the inhibitory effects of miR-10b on the progression of CRC cells.

CONCLUSIONS: These results demonstrate that miR-10b plays an important role in growth, migration and invasion of CRC by targeting FGF13.

Key Words:
CRC, MiR-10b, FGF13, Migration, Invasion.

Introduction

Colorectal cancer (CRC) is one of the most common malignant cancers and remains the leading cause of cancer-related death worldwide[1-3]. Metastasis is one of the major causes of CRC-induced death. The epithelial-mesenchymal transition (EMT) process is an important event that is involved in tumor metastasis[4]. In the EMT process of cancer cell, the epithelial cell loses the epithelial phenotype (down-regulation of epithelial marker, E-cadherin), and acquires the mesenchymal phenotype (up-regulation of mesenchymal marker, N-cadherin and extracellular matrix (ECM) disruption).

MicroRNAs (miRNAs), which belong to a kind of small non-coding RNAs, regulate the expression of target genes in the post-transcriptionally dependent manner[5,6]. Importantly, the complicated regulatory network of miRNAs not only regulates the expression of target protein *via* one miRNA, but also allows the combination of multiple miRNAs to regulate target genes[7,8]. Scholars[9] have demonstrated that miRNAs are closely related with the development of cancer, including the growth, metastasis and angiogenesis. Meanwhile, miRNAs regulate the level of cancer-related genes, and serve as an oncogene or suppressor gene in the tumorigenesis and development of cancer[10,11].

Numerous miRNAs have been proved to be involved in the development of CRC, including miR-374b, has-miR-590-3p and miR-34a[12-14]. The miR-10 family consists of two members, miR-10b and miR-10a, which are located at chromosome 2 and 17, respectively[15]. Substantive evidence indicates that miR-10a and miR-10b functions as oncogene or anti-oncogene in several cancers, including cervical carcinoma, breast carcinoma, acute myeloid leukemia, hepatocellular carcinoma and glioblastoma[16-23]. All these results prove that both miR-10a and miR-10b are dysregulated in cancer and exert vital roles in its progression. In colorectal cancer, miR-10a inhibits the metastasis of colorectal cancer cell by regulating the EMT process[15]. However, the precise roles of miR-10b in the growth and metastasis of CRC remain unknown.

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In this work, we found that miR-10b is down-regulated in CRC. The over-expression of miR-10b inhibits the growth and metastasis of CRC cell HCT116. In addition, we demonstrate that fibroblast growth factor 13 (FGF13), which is the target of miR-10b, exerts crucial roles in the progression of CRC cell regulated by miR-10b.

Materials and Methods

Ethics Statement

The experiments conformed to the Animal Management Rule of the Chinese Ministry of Health (documentation 55, 2001), and the animal experiments were conducted in accordance with standard operating procedures approved by the Committee on the Use and Care of Animals at antai Municipal Laiyang Central Hospital.

Cell Culture

The CRC cell lines (HT-29, LOVO and HCT116) and normal colonic epithelial cell line (HcoEpiC) were obtained from Nanjing Cobioer biotechnology co. LTD (Nanjing, Jiangsu, China). HcoEpiC cell was maintained at 37°C in an atmosphere of 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% Fetal Bovine Serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA), 100 μg/ml penicillin, and 100 U/ml streptomycin (Beyotime, Shanghai, China). HCT116 and HT-29 cell was cultured in McCoy's 5a Modified Medium (Thermo Fisher Scientific, Waltham, MA, USA). LOVO was cultured in F-12K medium (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Transfections

MiR-10b mimics and its corresponding negative control (miR-ctr) were purchased from GenePharma (Shanghai, China). Two different short hairpin RNAs (shRNA) targeting FGF13 (shFGF13 #1 and shFGF13 #2) were purchased from GenePharma (Shanghai, China). HCT116 cell was transfected with miR-10b mimics or miR-ctr using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). The full-length open reading frame of FGF13 was cloned into pcDNA3.1(+) to generate FGF13 expression vector (pcDNA-FGF13). Co-transfection of miR-10b mimics and pcDNA-FGF13 was conducted using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA).

Western Blotting Assay

Whole-cell lysates were prepared with radioimmunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitors. Equal amounts of cell lysates (25 μg) were loaded on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After membranes were blocked using Tris-Buffered Saline and Tween 20 (TBST; TBS containing 0.1% Tween-20, 5% non-fat dry milk). The membranes were incubated with primary antibodies against FGF13 (13201-1-AP, Proteintech, Wuhan, Hubei, China) or GAPDH (60004-1-Ig, Proteintech, Wuhan, Hubei, China). T Next, PVDF membranes were incubated with horseradish peroxidase (HRP) conjugated IgGs (1:10000, Proteintech, Wuhan, Hubei, China). The target proteins were detected by the electrochemiluminescence system (ECL; Millipore, Billerica, MA, USA) and visualized with the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA).

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

The cell was cultured in 96-well plate for 1, 2, 3 or 4 days. After that, 5 mg/ml MTT (Sigma-Aldrich, St. Louis, MO, USA) was added into 96 well plates and cells were cultured in an incubator for 4 h. Then, the cell supernatant was removed and dimethyl sulfoxide (DMSO, 200 μl) was added into 96 well plates. Finally, the optical density (OD) was measured at 490 nm.

Wound Healing Assay

Cells (1×10^5 per well) were seeded into 6 well plates for 24 h. The cells were starved in a fetal bovine serum (FBS)-free culture medium for 12 h and then, a wound was made by scratching the cell monolayer using a 100 μl pipette tip. Next, the cell debris was removed and cells were incubated with complete medium. The wound was photographed at 0 h and 24 h. Scratch healing rate = (0 h width of scratch - 24 h width of scratch)/0 h width of scratch × 100%.

Transwell Invasion Assay

Cells (1×10^5 per well) were cultured into the upper chamber of transwell containing membrane (8 μm pore size) that was pre-coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The complete medium containing 20% FBS was added into the lower chamber. After 24 h, the invaded cells on the lower chamber were stained by

1% crystal violet and the number of the invaded cell was analyzed.

Colony Formation Assay

Cells (1×10^3 per well) were seeded into 25 mm culture dish, and cultured for 4 weeks. Finally, colonies were stained using 1% crystal violet and the number of cell colonies was counted.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Assay

Total RNA was extracted from cells using TRIzol (TaKaRa, Otsu, Shiga, Japan). 1 μg of RNA was reverse-transcripted to cDNA using PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan). To quantify the level of miR-10b, the stem-loop qRT-PCR was conducted using the TaqMan® miRNA Reverse Transcription Kit (Applied Biosystems, Franklin Lakes, NJ, USA) on the ABI 7500 sequence detection system (Applied Biosystems, Franklin Lakes, NJ, USA). U6 was the endogenous control. Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis of FGF13 was conducted using SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan) on the ABI 7500 sequence detection system (Applied Biosystems, Franklin Lakes, NJ, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the endogenous control. The primers were as follows (sense and antisense): GAPDH: AGGTCGGTGTGAACGGATTTG and GGGGTCGTTGATGGCAACA; FGF13: GTTACCAAGCTATACAGCCGAC and ACAGGGATGAGGTTAAACAGAGT;MiR-10b: GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCACAAA; U6: CGCTTCACGAATTTGCGTGTCAT

Immunofluorescence Staining

HCT116 cell was perforated with 10% Triton X-100 (Sangon, Shanghai, China). Then, the cells were incubated with primary antibodies against E-cadherin or N-cadherin overnight at 4°C. Next, cells were incubated with goat anti-rabbit IgG secondary antibody Alexa Fluor 488 (Beyotime, Shanghai, China) for 2 h. Cell nucleus was stained using DAPI (Beyotime, Shanghai, China). Image of immunofluorescence staining was taken with an inverted microscope (CarlZeiss, Hallbergmoos, Germany).

Luciferase Reporter Assay

The wild-type (wt) or mutate-type (mut) 3'-untranslated region (3'-UTR) of FGF13 gene was cloned into the pGL3 Luciferase reporter vector. The pGL3-FGF13-3'-UTR and the Renilla plasmid were cotransfected into miR-10b mimics or miR-ctr transfected HCT116 cell using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h, the Luciferase activity was assessed using the Luciferase assay system (Promega, Madison, WI, USA).

Tumorigenicity

Female nude mice were bought from the Shanghai Slake Laboratory Animal co. LTD (Shanghai, China). 100 μl of miR-ctr or miR-10b transfected HCT116 cells (1×10^7) were subcutaneously into nude mice. The tumor volume was measured each week and calculated as the following formula: $0.5 \times \text{Length} \times \text{Width}^2$. The animals were euthanized and the tumors were utilized for qRT-PCR analysis.

Experimental Metastasis Assay

Female nude mice were bought from the Shanghai Slake Laboratory Animal co. LTD (Shanghai, China). 100 μl of miR-ctr or miR-10b transfected HCT116 cells (1×10^5) were injected into nude mice through the lateral tail vein. After four weeks, nude mice were sacrificed and lung tissues were applied for hematoxylin-eosin staining (H&E) staining. The number of lung metastatic nodules was determined under an inverted microscope (CarlZeiss, Hallbergmoos, Germany).

Statistical Analysis

Data are presented as the mean ± SD of three repeated experiments. One-way ANOVA analysis of difference was used for comparisons among multiple groups, followed by Student's Post-Hoc two-tailed *t*-test. The Student's unpaired two-tailed tests were used for comparisons between the two groups. $p < 0.05$ was considered statistically significant.

Results

MiR-10b is Downregulated in CRC

To identify the potential miRNAs which were dysregulated in CRC, the expression pattern of miRNAs between primary CRC tissues *vs.* metastatic CRC tissues was analyzed using the GEO data set GSE44121. The heatmap that was generated with differential genes revealed that miR-10b was remarkably down-regulated (Fold change = -3.6 and $p < 0.01$) in the metastatic CRC tissues

(Figure 1A). Similarly, the level of miR-10b was generally lower in a panel of CRC cell lines than that in the human normal colonic epithelial cell, HcoEpiC (Figure 1B). Thus, the findings suggested that miR-10b was down-regulated in CRC.

Upregulation of MiR-10b Suppresses the Growth and Colony Formation of CRC Cell in vitro

To explore the effect of miR-10b on the growth of CRC cell *in vitro*, CRC cell HCT116 was transfected with miR-10b mimics to construct miR-10b over-expressing cell line. The qRT-PCR result indicated that the level of miR-10b was markedly increased in HCT116 cell that was transfected with miR-10b mimics (Figure 2A). Next, we detected the impact of the up-regulation of miR-10b on the proliferation of HCT116 cell and the results suggested that the up-regulation of miR-10b remarkably suppressed the proliferation of CRC cell *in vitro* (Figure 2B). The colony formation assay also indicated that the over-expression of miR-10b inhibited the colony formation of HCT116 cell (Figure 2C). All these observations indicated that miR-10b acted as an anti-oncogene in CRC.

Overexpression of MiR-10b Decreases the Migration and Invasion of HCT116 Cell

Then, we explored the morphology changes of HCT116 cell after miR-10b transfection. As shown in Figure 3A, HCT116 cells were changed from mesenchymal morphology to epithelial morphology once were transfected with miR-10b. The immunofluorescence staining assay using E-cadherin or N-cadherin antibody future confirmed the morphological alterations (Figure 3B), which suggests that the up-regulation of miR-10b induced the mesenchymal-epithelial transition (MET) of HCT116 cell. The expression of the mesenchymal marker, N-cadherin was decreased whereas the expression of E-cadherin was increased after HCT116 cell was transfected with miR-10b mimics. EMT always accompanies the alteration of the invasion and migration of cancer cell. Hence, we assessed the impact of miR-10b on the aggressiveness of CRC cell *in vitro*. As shown in Figure 3C-3D, the up-regulation of miR-10b dramatically suppressed the migration and invasion abilities of HCT116 cell *in vitro*.

Up-regulation of MiR-10b Suppresses the Tumor Growth and Metastasis of HCT116 Cell in vivo

In vitro, the up-regulation of miR-10b suppressed the growth and aggressiveness of HCT116 cell. Whether miR-10b suppressed the tumor growth and metastasis of CRC cell *in vivo* needed to be further explored. As shown in Figure 4A-B, the up-regulation of miR-10b suppressed the tumor growth of HCT116 *in vivo*, and the volume of

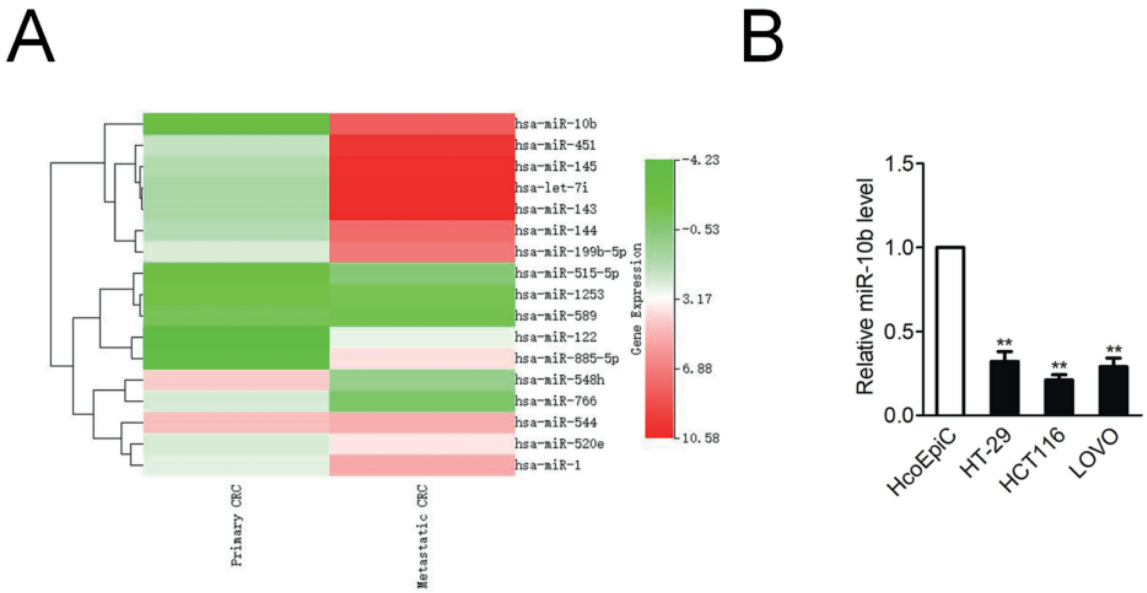

Figure 1. MiR-10b was down-regulated in CRC. ***A,*** Microarray analysis of miRNA expression in CRC tissues corresponding normal tissues. ***B,*** The levels of miR-10b in a panel of CRC cell lines were detected using qRT-PCR assay. U6 was used as loading control. $^{**}p < 0.01$ compared to HcoEpic cells.

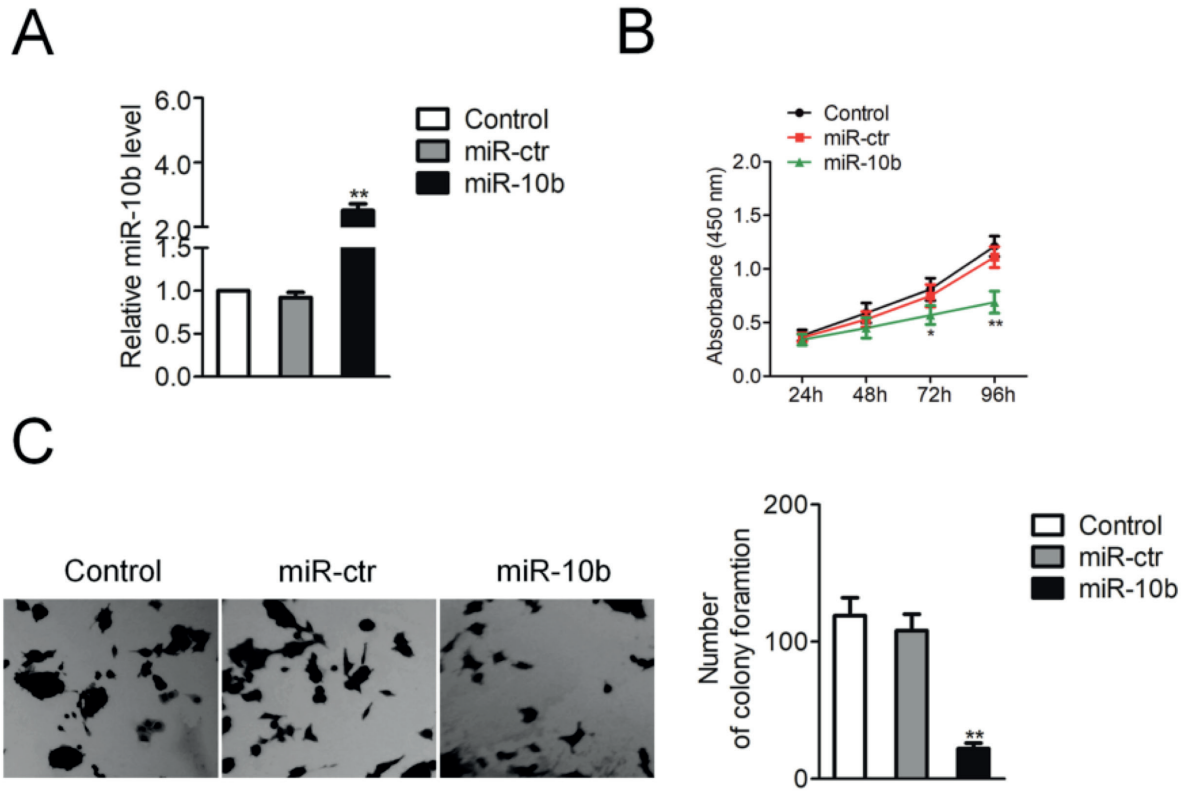

Figure 2. Overexpression of miR-10b inhibits the proliferation and colony formation of HCT116 cell *in vitro*. ***A,*** HCT116 cell was transfected with miR-10b mimics or miR-ctr. The level of miR-10b was measured using qRT-PCR assay. ***B,*** MTT assays were conducted using HCT116 cell that was transfected miR-10b. ***C,*** The colony formation assay was carried out using miR-ctr or miR-10b mimics transfected HCT116 cells. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to control.

tumor that was formed by miR-10b over-expressing cell was markedly smaller than the tumor that was formed by miR-ctr transfected HCT116 cell. The levels of miR-10b in the collected tumor tissues were also assessed using qRT-PCR assay, and the result indicated that the level of miR-10b was up-regulated in tumor derived from miR-10b over-expressing group (Figure 4C), which confirmed that miR-10b restrained the growth of CRC cell *in vivo*. Next, we explored the effect of miR-10b on the metastasis of CRC cell *in vivo* using experimental metastasis test. MiR-ctr or miR-10b transfected HCT116 cells were injected into nude mice *via* lateral tail vein. After four weeks, nude mice were sacrificed and metastasis foci were mainly found in the lungs. As shown in Figure 4D, the injection of miR-ctr transfected HCT116 cell produced numerous lung metastasis foci while the up-regulation of miR-10b significantly inhibited the lung metastasis of HCT116 cell. These findings demonstrated that miR-10b suppressed the tumor growth and metastasis of CRC cell *in vivo*.

FGF13 is the Target of MiR-10b

The bioinformatics online analysis tool Targetscan (http://www.targetscan.org/vert_72/) and miRBase (http://starbase.sysu.edu.cn/index.php) were selected to predict the target gene of miR-10b, and we found that FGF13 was the target gene of miR-10b (Figure 5A). To analyze whether FGF13 was the direct target of miR-10b, wild-type (wt) or mutate type (mut) 3'-UTR of FGF13 was inserted to the downstream of the Luciferase reporter vector. The Luciferase activity assay indicated that the up-regulation of miR-10b decreased the Luciferase activity in HCT116 cell that was transfected with wt 3'-UTR of FGF13 whereas miR-10b transfection had no significant inhibitory effect on the Luciferase activity in HCT116 cell that was transfected with mut FGF13 (Figure 5B). Furthermore, the expression of FGF13 was markedly decreased when miR-10b mimics was transfected into HCT116 cell (Figure 5C). Consistently, the immunohistochemistry (IHC) assay using tumor tissues that was formed by miR-10b transfected HCT116 cells or miR-ctr transfected HCT116 cells suggested that FGF13 was down-regulated

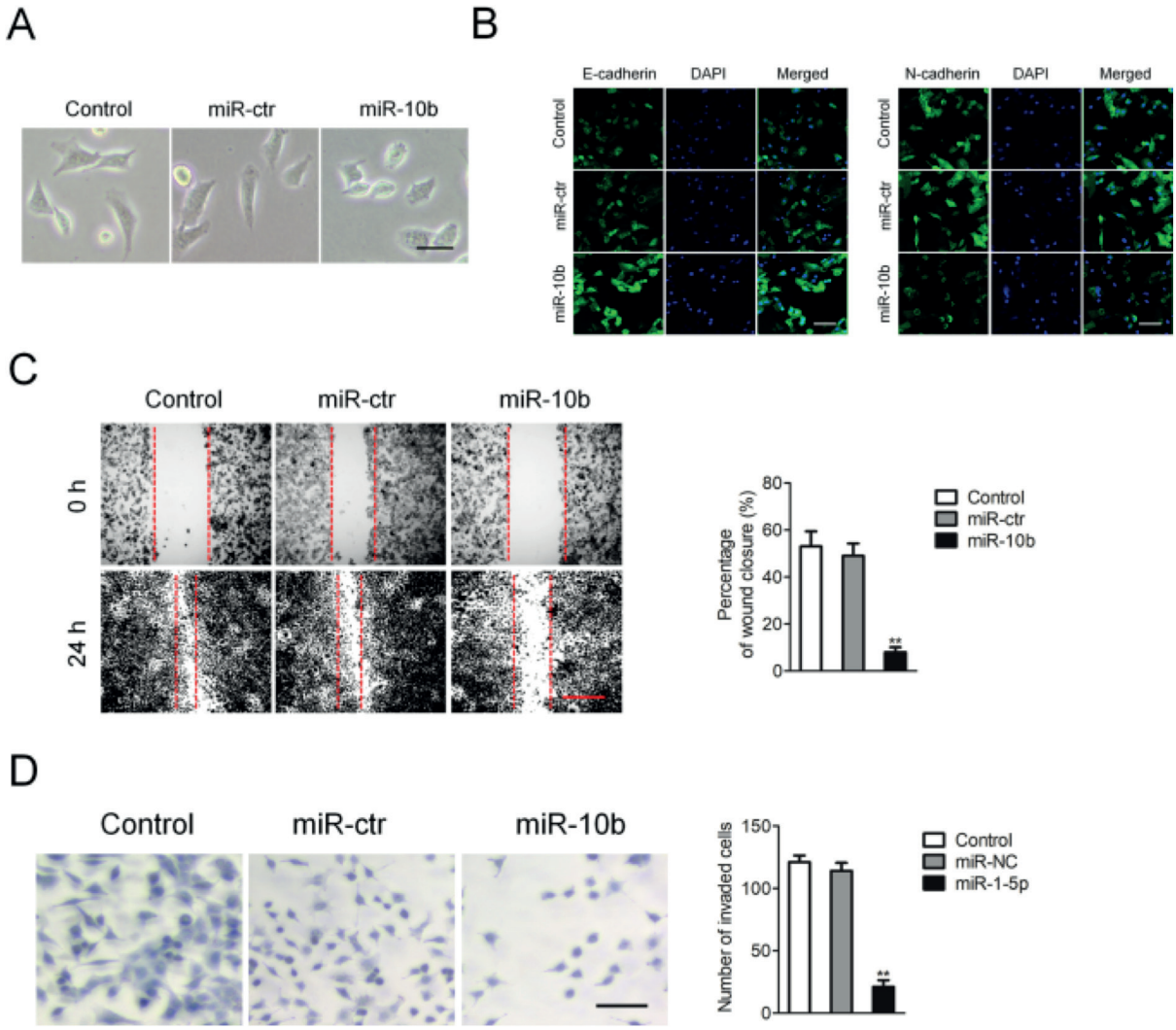

Figure 3. Overexpression of miR-10b inhibits the migration and invasion of HCT116 cell *in vitro*. ***A,*** Cell morphology was observed after HCT116 cell was transfected with miR-10b or miR-ctr for 48 h. ***B,*** Representative confocal images of immunofluorescence staining. Green, E-cadherin or N-cadherin. Blue, DAPI for nucleus. ***C,*** HCT116 cell was transfected with miR-10b mimics or miR-ctr. Then, the migration of HCT116 cell was determined using the wound healing assay. ***D,*** HCT116 cell was transfected with miR-10b mimics or miR-ctr. Then, the invasion of HCT116 cell was determined using the transwell invasion assay. $^{**}p < 0.01$ compared to control.

when miR-10b was up-regulated (Figure 5D). These findings demonstrated that FGF13 was the target gene of miR-10b in CRC.

The Inhibitory Effect of MiR-10b on CRC Cell is Neutralized by Overexpression of FGF13

To investigate whether miR-10b restrained the growth, migration and invasion of CRC cell *via* regulating FGF13, HCT116 cell was cotransfected with miR-10b and FGF13. The qRT-PCR and immunoblotting assay indicated that the expression of FGF13 was not decreased by miR-10b in HCT116 cell that was cotransfected miR-10b and FGF13 (Figure 6A-6B). Next, the proliferation assay and colony formation assay indicated that co-transfection of miR-10b and FGF13 increased HCT116 cell growth and colony formation when compared with the cell that was transfected with miR-200 alone, which confirmed that miR-10b inhibited HCT116 cell growth by down-regulating FGF13 (Figure 6C-6D). Consistently, the migration and invasion were promoted in HCT116 cell that was cotransfected with miR-10b and FGF13 when compared with the

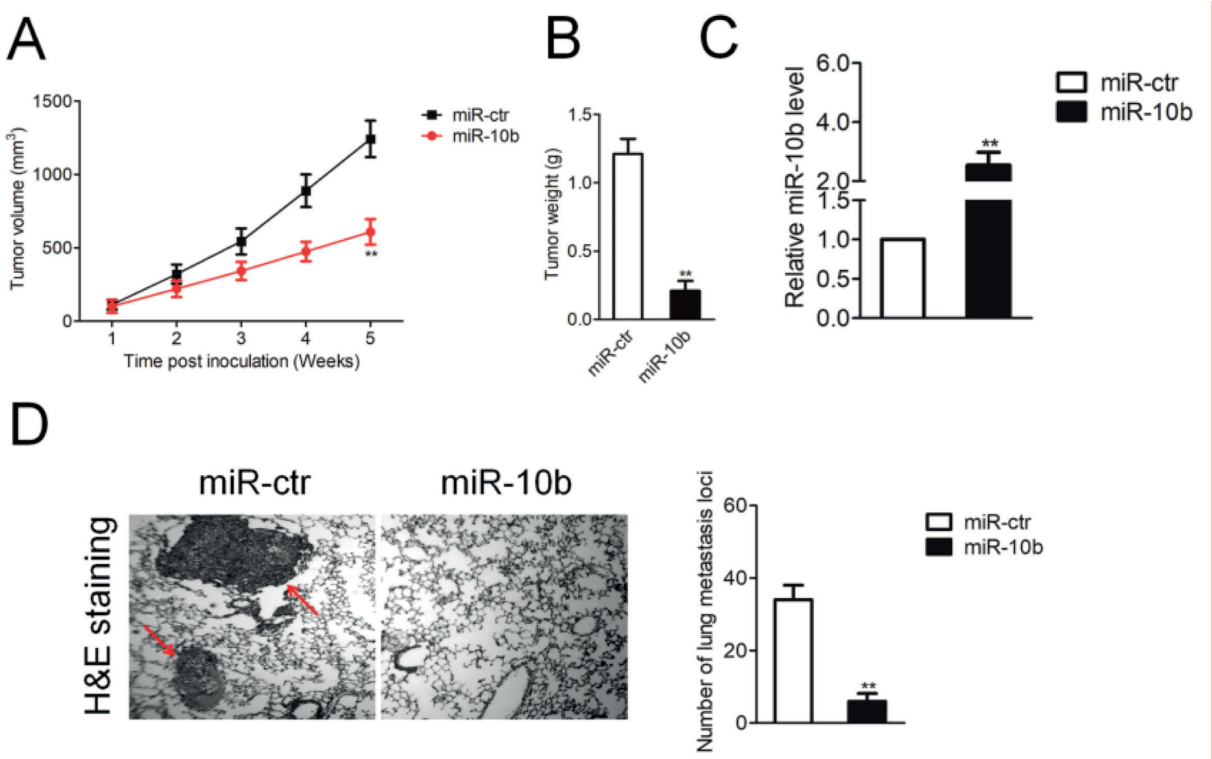

Figure 4. Overexpression of miR-10b inhibits the tumor growth and metastasis of HCT116 cells *in vivo*. ***A,*** HCT116 cells were implanted into nude mice. The volume of tumor was measured once a week. ***B,*** The tumors tissues in two groups were collected from nude mice at the end of experiment, and the tumor weight was shown, n = 6. ***C,*** The levels of miR-10b in tumor tissues were detected using qRT-PCR assay. ***D,*** Representative pictures of H&E stained using lungs from nude mice (left panel). The numbers of lung metastasis were quantified and showed by each data point (right panel). $^{**}p < 0.01$ compared to miR-ctr.

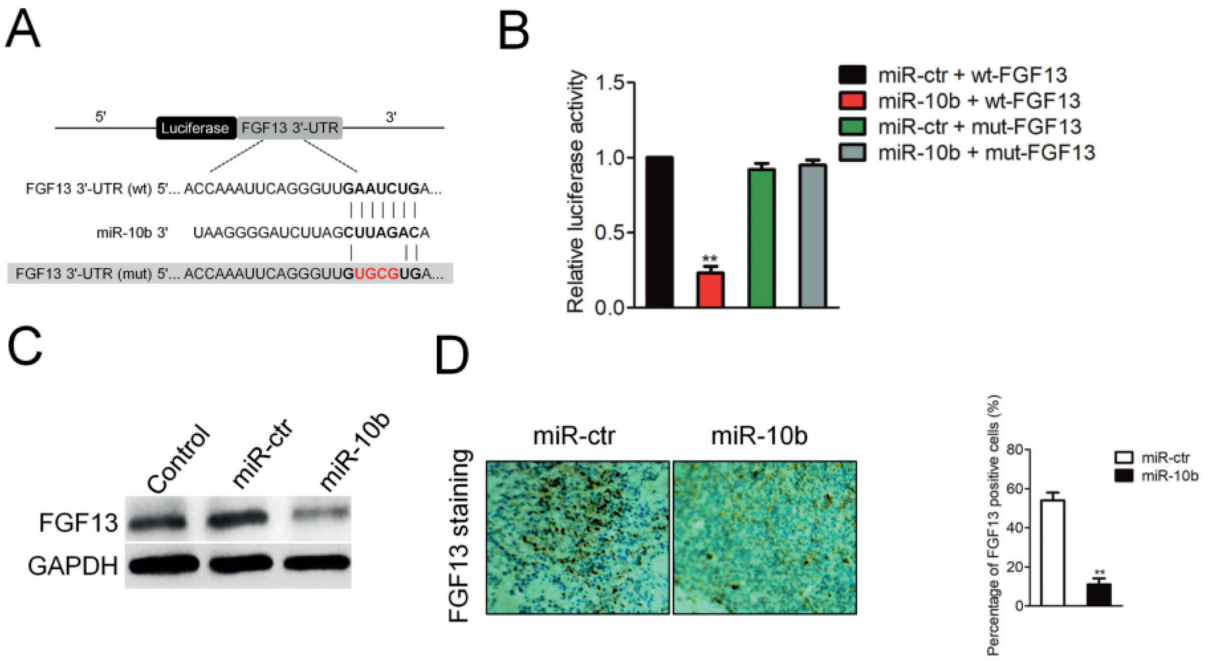

Figure 5. FGF13 is the direct target of miR-10b. ***A,*** Predicted miR-10b target sequences in 3'-UTR of FGF13. ***B,*** The pGL3-FGF13-3'-UTR vector and miR-10b were transfected into HCT116 and then cells were seeded in 96-well culture plates. The relative Luciferase activity was analyzed. $^{**}p < 0.01$ compared to miR-ctr + wt-FGF13. ***C,*** The expression level of FGF13 in HCT116 cell that was transfected with miR-10b was measured by Western blotting assay. ***D,*** The expression of FGF13 in tumor tissues was measured by immunohistochemical (IHC). $^{**}p < 0.01$ compared to miR-ctr.

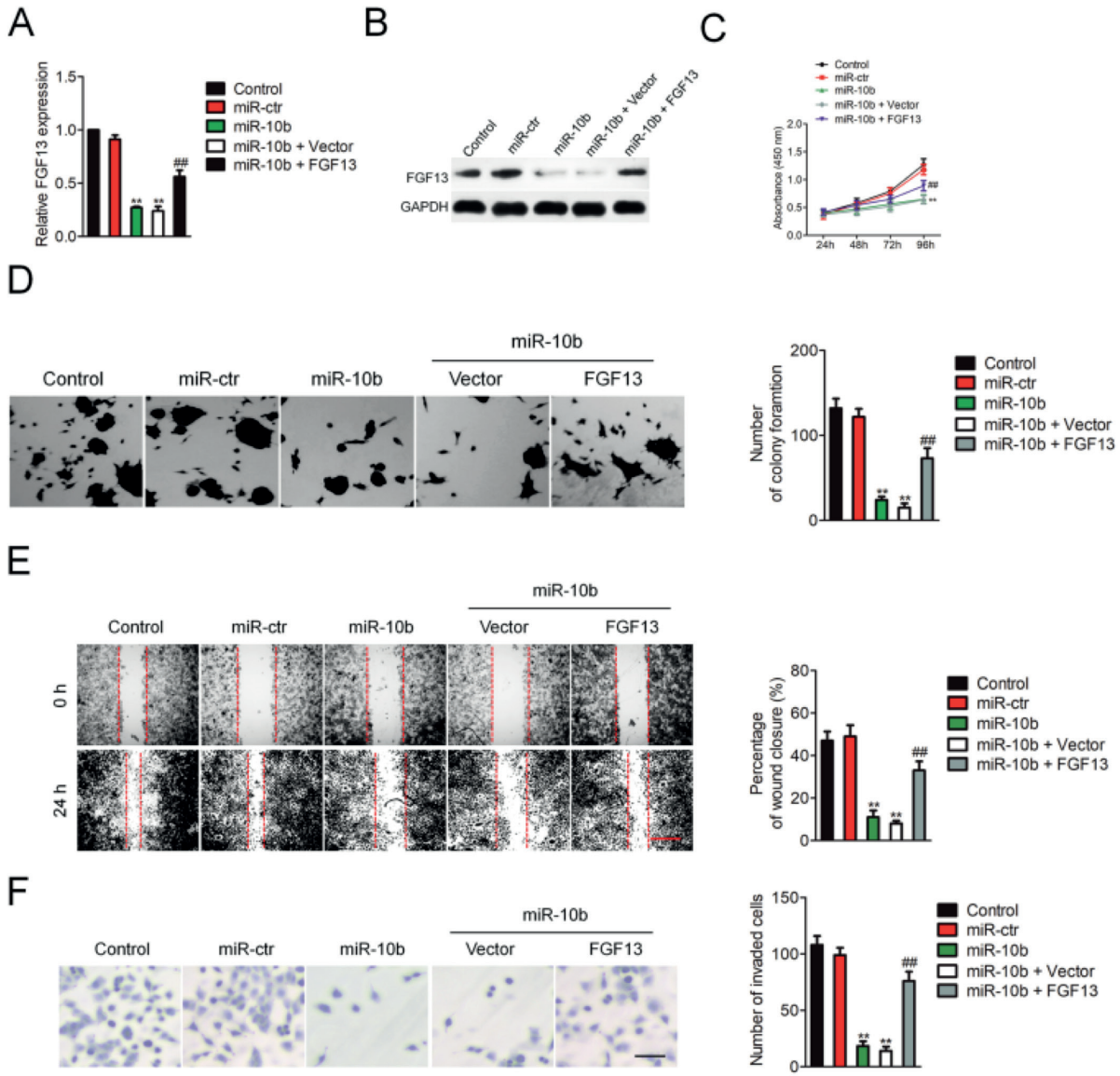

Figure 6. The inhibitory effect of miR-10b on HCT116 cell is rescued by the overexpression of FGF13. ***A,*** HCT116 cells were transfected with miR-10b alone or co-transfected with miR-10b and FGF13. The expression level of FGF13 was detected by qRT-PCR. ***B,*** The expression level of FGF13 was detected by Western blot. ***C,*** The proliferation of HCT116 cell was measured by MTT assay. ***D,*** HCT116 cells were transfected with miR-10b alone or co-transfected with miR-10b and FGF13. Colony formation assay was conducted. ***E,*** The migration of HCT116 cell was measured by the wound healing assay. ***F,*** Cell invasion ability was measured by the transwell invasion assay. $^{**}p < 0.01$ compared to control, $^{\#\#}p < 0.01$ compared to miR-10b.

cell that was transfected with miR-200 alone, which confirmed that the inhibitory effect of miR-10b on HCT116 cell aggressiveness could be recused by overexpression of FGF13 (Figure 6E-6F).

Downexpression of FGF13 Mimics the Suppressive Impact of MiR-10b on HCT116 Cell

The up-regulation of miR-10b inhibited the growth and aggressiveness of HCT116 cell by regulating FGF13, which indicated that FGF13 might play important roles in the progression of CRC. Nevertheless, whether FGF13 knocked-down could mimic the inhibitory impacts of miR-10b on the growth and metastasis of CRC cell was still not well investigated. Then, two different short hairpin RNAs (shRNA) targeting FGF13 were transfected into HCT116 cell to decrease the level of FGF13 (Figure 7A). The effects of FGF13 knocked-down on the growth and colony forma-

tion of HCT116 cells were analyzed. As shown in Figure 7B-7C, down-expression of FGF13 suppressed growth and colony formation of HCT116 *in vitro*. Consistently, FGF13 knockdown had similar impacts on the migration and invasion of HCT116 cell as the overexpression of miR-10b (Figure 7D-7E). All results suggest the probably oncogenic effect of FGF13 in CRC.

Discussion

Cancer cell metastasis is a complicated process, which consists of invasion, intravasation migration, extravasation and forms metastatic foci[24,25]. Numerous miRNAs have been demonstrated to participate in each step of tumor metastasis[24,26,27]. Investigation the precise molecular mechanism of the CRC me-

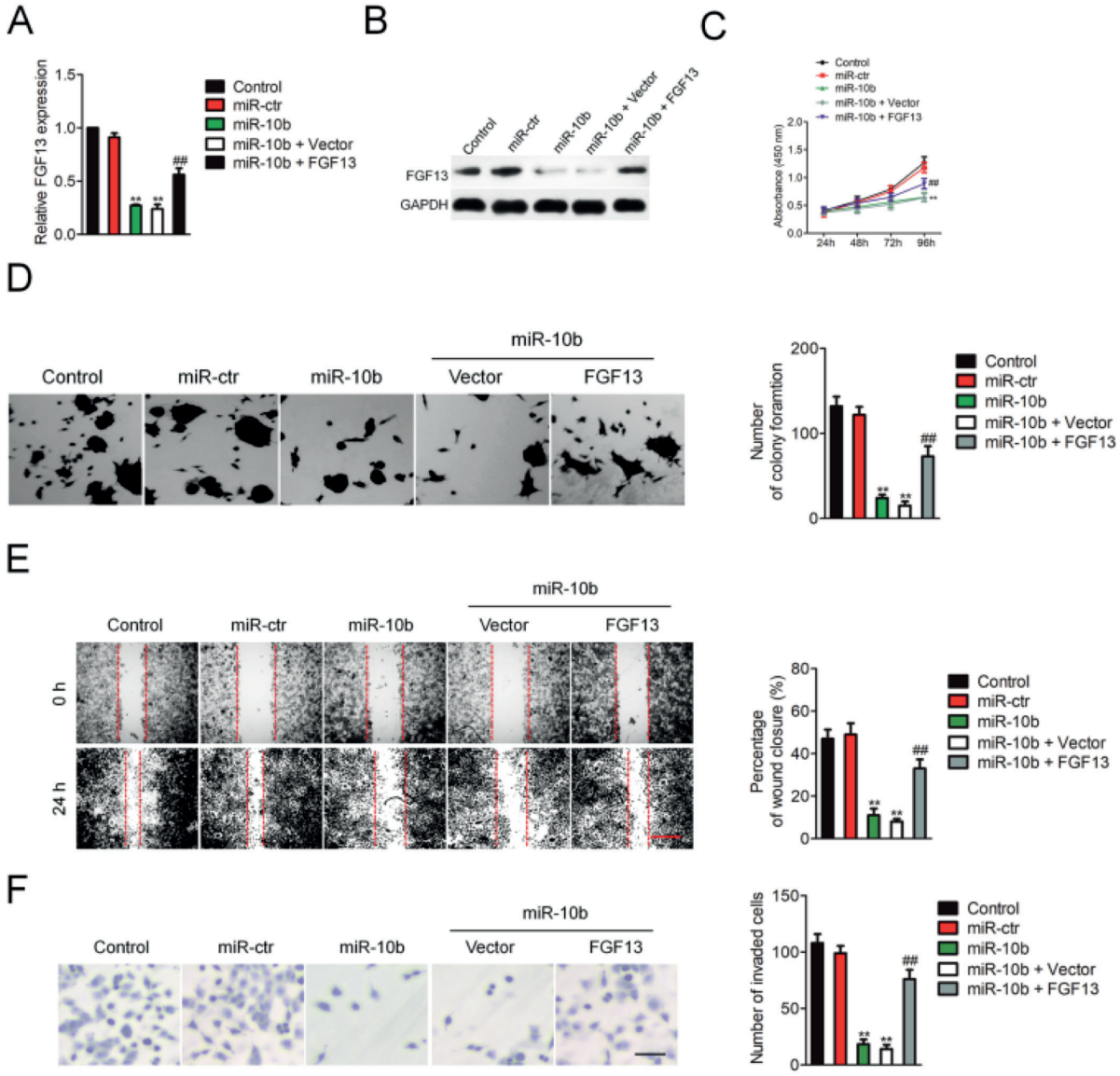

Figure 7. Down-regulation of FGF13 mimics the effects of miR-10b on the proliferation, migration and invasion of CRC cell. ***A,*** HCT116 cell was transfected with shFGF13 or shCTRL, and the expression of FGF13 was detected by Western blot assay. ***B,*** The proliferation of HCT116 cell was measured by MTT assay. ***C,*** Colony formation assay. The quantitative analysis of colonies was shown. ***D,*** The migration of HCT116 cell that was transfected with shCTRL or shFGF13 was detected using wound healing assay. ***E,*** Cell invasion ability was measured by the transwell invasion assay. $^{**}p < 0.01$ compared to control.

tastasis is very important for developing the effective therapy options for CRC. Our study showed that miR-10b regulated the growth and aggressiveness of CRC cells by targeting FGF13.

First, to explore the dysregulation pattern of miRNAs in CRC, we used an online miRNA array dataset to analyze the different miRNAs between CRC tissues and the corresponding normal tissues. Among the miRNAs, miR-10b was most down-regulated in CRC compared with normal tissues. Hence, we investigated the function of miR-10b in the progression of CRC. Previous studies reported that miR-10b directly targets some tumor suppressor or oncogenes, and the dysregulation of miR-10b leads to the alternation of these target genes, which play vital roles in the progression of cancers. Recently, the investigations about the functions of miR-10b in cancer development have been partly explored. MiR-10b induces the migration, invasion and metastasis of hepatocellular carcinoma cell by regulating the focal adhesion kinase/Protein kinase B (FAK/AKT) pathway[28]. In addition, miR-10b regulates the epithelial-mesenchymal transition (EMT) process of hepatocellular carcinoma cell by modulating Kruppel like Factor 4/Kruppel like factor 11/Smads (KLF4/KLF11/Smads)[29]. Furthermore, miRNA-10b suppresses the growth and metastasis of cervical cancer cell by targeting insulin-like growth factor-1 receptor (IGF-1R)[30]. However, the precise roles of miR-10b in CRC have not been well explored.

As the level of miR-10b is down-regulated in CRC, we speculated that miR-10a might be anti-oncogene in the progression of CRC. Then, the effects of miR-10a on CRC cell growth and metastasis were analyzed. The up-regulation of miR-10a suppressed the migration, invasion and the EMT of HCT116 cell *in vitro*. Consistently, the up-regulation of miR-10b suppressed the metastasis and tumor growth of CRC cell *in vivo*. To reveal the underlying mechanism by which miR-10b regulates the aggressiveness of CRC cell, we explored the potential target of miR-10b in CRC. Based on the bioinformatics analysis and Luciferase reporter assay, we validated the FGF13 gene was the target of miR-10b. Importantly, the expression of FGF13 was significantly decreased in HCT116 cell that was transfected with miR-10b, which suggested that FGF13 might play an important role in the regulation of miR-10b on CRC cell growth and metastasis.

FGF13 gene is conserved in vertebrates and is normally expressed abundantly in brain[31,32]. Previous studies[33-36] demonstrate that FGF13 is up-regulated in several cancers, including melanoma, pancreatic carcinoma, lung cancer and multiple myeloma. The current work suggests that FGF13 is over-expressed in CRC and plays a vital role in the migration and invasion of CRC cell. Although several important findings were revealed in this study, there were also some limits. First, the level of miR-10b in CRC tissues and its relationship with the prognosis of patients remained to be further explored. In the experiment metastasis assay, to intuitively measure the impact of miR-10b on HTC116 cell metastasis *in vivo*, fluorescence labeling might be inserted into cells to construct a metastasis model. In addition, as an oncogene, how FGF13 plays its important roles in the migration invasion of CRC cell by interacting with the downstream signaling pathway has not been investigated yet.

Conclusions

We demonstrated that miR-10b is down-regulated in CRC. The up-regulation of miR-10b suppresses the growth, migration, invasion and metastasis of CRC cell. FGF13, which is the target protein of miR-10b, plays a crucial role in the regulation of miR-10b on the progression of CRC cell. These observations might help us to deeper understand the underlying mechanisms of miR-10b in regulating the growth and metastasis of CRC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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