

Original Article

miR19b-3p promotes the growth and metastasis of colorectal cancer via directly targeting ITGB8

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Abstract: MicroRNAs (miRNAs) are widely up-regulated or down-regulated in a variety of tumors, including lung cancer, liver cancer, and colorectal cancer (CRC). Furthermore, miRNAs can function as tumor suppressors or proto-oncogenes by controlling the growth and metastasis of cancer cells. In the present study, we found a significant increase in miR19b-3p levels in CRC compared to tumor tissue and revealed the role of miR19b-3p in CRC growth and metastasis. The exogenous overexpression of miR19b-3p induced the proliferation, migration, and invasion of CRC cells in vitro. In addition, the nude mouse xenograft model showed that miR19b-3p overexpression promoted CRC growth and lung metastasis in vivo, whereas silencing miR19b-3p showed opposite results. Mechanistic studies have shown that the integrin beta-8 (ITGB8) transcript is one of the direct targets of miR19b-3p, and the expression of ITGB8 in CRC specimens was positively correlated with miR19b-3p. Finally, ectopic expression of ITGB8 rescued cell proliferation and invasion, which was inhibited by down-regulation of miR19b-3p. In addition, knockdown of ITGB8 neutralized the effects of miR19b-3p overexpression on cell growth and metastasis in CRC cells. Together, these results suggest that the miR19b-3p/ITGB8 axis plays an important role in the growth and metastasis of CRC.

Keywords: Colorectal cancer, miR19b-3p, ITGB8, metastasis

Introduction

Colorectal cancer (CRC) is considered to be one of the most important causes of cancer-associated mortalities worldwide, and the incidence of CRC is expected to further increase [1]. Despite improvements in treatment strategies for CRC, the overall survival (OS) of patients with CRC after surgical resection remains unsatisfactory owing to its recurrence and metastasis [2]. In addition, the molecular mechanism of CRC metastasis has not been well elucidated. Hence, a better understanding of the molecular and cellular mechanism responsible for CRC metastasis is required to improve the prognosis of patients. Substantive studies have demonstrated that a variety of microRNAs (miRNAs) are associated with human cancer progression, including growth and metastasis [3]. MiRNAs are small non-coding RNAs that are involved in the post-transcriptional regula-

tion of genes. Importantly, its intricate regulatory network consists of a series of miRNAs that not only regulate the levels of multiple target genes, but also regulate genes via a combination of miRNAs [4]. For example, several miRNAs regulate the expression of tumor suppressor genes or oncogenes to play a functional role in the progression of cancer. In this study, we found that miR19b-3p was significantly up-regulated in CRC compared to that in adjacent non-tumor tissues [5].

MiR19b-3p belongs to both miR17-92 and miR106-363 clusters, which play significant roles in proliferation and cell survival [6]. A previous study revealed that miR19b-3p is up-regulated in nasopharyngeal carcinoma and served as an independent predictor for reduced patient survival [7]. MiR19b-3p overexpression resulted in decreased sensitivity of nasopharyngeal carcinoma cells to irradiation, whereas miR19b-3p down-regulation resulted in

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increased sensitivity to irradiation in vitro [8]. Mechanistically, we found that miR19b-3p increased nasopharyngeal carcinoma cell radio-resistance by activating the TNFAIP3/NF- κ B axis [9]. In addition, miR19b-3p may be a prospective biomarker for detecting gastric cancer and to assess its progression [10]. However, the roles of miR19b-3p in the progression of CRC have yet to be studied.

Integrins exist on the surface of cancer cells and promote the metastatic potential of cancer cells via mediating cell-cell adhesion and invasion [11]. The different integrin subfamilies are determined by the β subunit. Integrin β -8 (ITGB8) binds to the α v subunit [12], and has been shown to be up-regulated in many types of cancer, including lung cancer, breast cancer, laryngeal cancer, and gastric carcinoma [13]. In addition, ITGB8 showed higher expression in highly metastatic tumors and is considered a clinical metastasis-related gene and a potential target for the treatment of metastatic cancers [14]. However, the mechanism of ITGB8 in CRC metastasis and the relationship between ITGB8 and miR19b-3p is still not very clear.

In the present study, we demonstrated that miR19b-3p was up-regulated in CRC samples and cell lines. Overexpression of miR19b-3p induced the proliferation, colony formation, and tumor growth of CRC HCT116 and SW480 cells. In addition, overexpression of miR19b-3p significantly accelerated the metastasis of CRC cells in vitro and in vivo. However, silencing endogenous miR19b-3p in HCT116 and SW480 cells resulted in the opposite outcomes. In addition, we identified one of the miR19b-3p target genes, ITGB8, to play a functional role in the growth and metastasis of CRC cells regulated by miR19b-3p. Together, these results provide novel insights into the mechanism of the miR19b-3p/ITGB8 axis in CRC metastasis.

Materials and method

Colorectal cancer specimens and cell lines

The colorectal cancer samples and adjacent tissues were obtained from the Sixth Affiliated Hospital, Sun Yat-sen University. All informed consents from patients with colorectal cancer before surgery were obtained from the Regional Ethics Committee of the Sixth Affiliated Hospital, Sun Yat-sen University. Association of

miR-19b-3p expression with clinic-pathological characteristics was summarized in [Supplementary Table 2](#). Colorectal cancer cell lines, including HCT116, HT-29, SW480, LOVO and human colonic epithelial cells HCoEpiC were purchased from Guang Zhou Jennio Biotech Co.,Ltd. All cell lines were maintained at in the Dulbecco's Modified Eagle medium (DMEM) or RPMI-1640 supplemented with 10% FBS, at 37°C in 5% CO₂ atmosphere.

miRNA mimic, inhibitor and shITGB8 transfection

MiR-19b-3p mimics and negative control double helix (named miR-NC), miR-19b-3p inhibitor oligonucleotide (designated miR-19b-3p inhibitor) and the inhibitor of negative control oligonucleotide (named as inhibitor NC) were used for the transient gain of functional and loss of functional studies. Small interfering RNA duplex (shRNA) for ITGB8 (named shITGB8) was used for ITGB8 knock-down. Lipofectamine 2000 reagent (Invitrogen, USA) was used for transient transfection.

MTT proliferation assay and colony formation assay

3 x 10⁵ HCT116 or SW480 cells were plated into 96-well plates for 1, 2, 3 or 4 days. 100 μ l of MTT resolution was added to the wells, and the cells were incubated at 37°C for 3 h. Cell supernatant was removed and 200 μ l DMSO was added. Finally, the optical density (OD) value was measured at 490 nm. In colony formation assay, 1 x 10² HCT116 or SW480 cells were inoculated in complete medium and cultured for 2 weeks. The cell colonies were stained with 0.1% crystal violet and the number of colonies was analyzed.

Wound healing migration and Transwell invasion assays

1 x 10⁵ HCT116 or SW480 cells were seeded into 6-well plates. The confluent monolayers of cells were scratched with 100 μ l pipette tip, and the wound closures of cells was observed by microscopy, and by measuring the size of the initial wound and comparing the size of the wound after 24 h. 1 x 10⁵ HCT116 or SW480 cells were seeded into upper chamber of Transwell that coated 8 μ m pore size, and the Transwell chamber was placed into 24 well cul-

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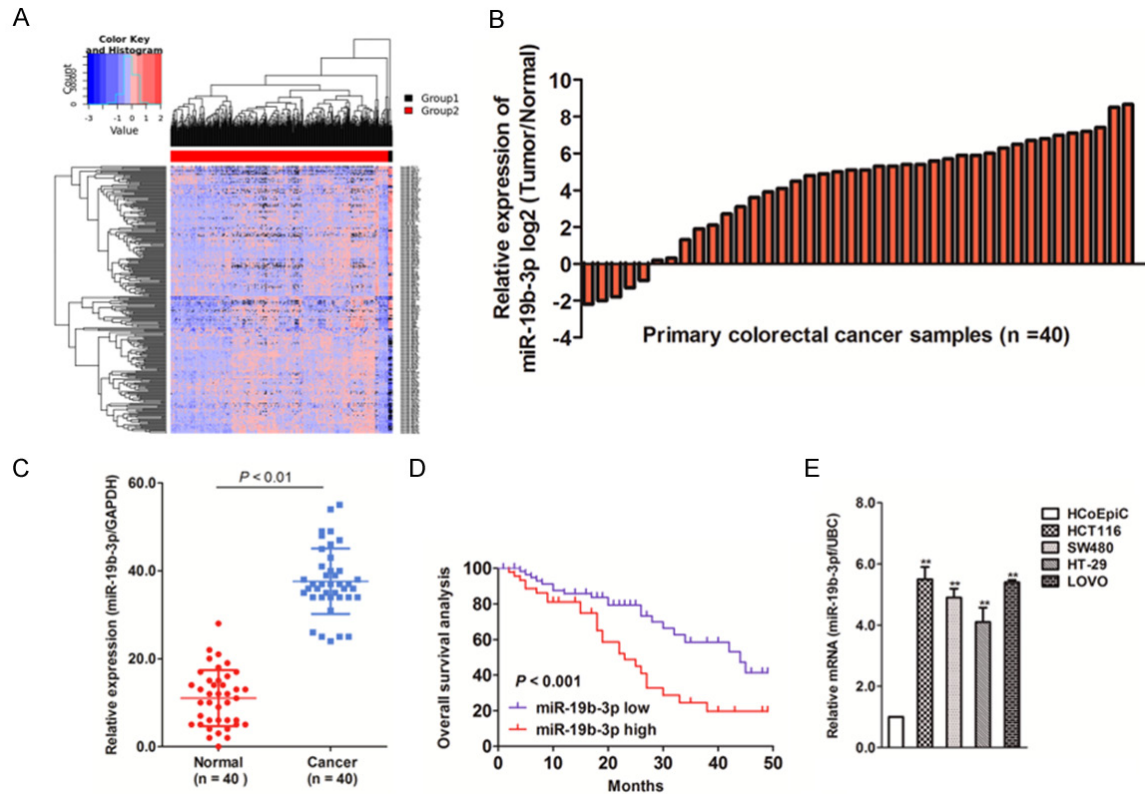


Figure 1. miR19b-3p expression up-regulated in colorectal cancer (CRC). A. MicroRNA (miRNA) meta-analysis in YM500v2 (<http://ngs.ym.edu.tw/ym500v2/index.php>) was performed to detect differentially expressed miRNAs in colon cancer compared to that in control solid tissue. Group 1: Normal tissues, Group 2: Colon cancer. B. Total RNA was extracted from CRC as well as normal tissues and then reverse transcribed into cDNA. miR19b-3p levels were detected by qRT-PCR. N represents the total number of patients with CRC. C. The levels of miR19b-3p in human colon cancer tissues and corresponding normal tissues were measured by qRT-PCR. D. A Kaplan-Meier survival analysis was performed to assess the prognostic value of miR19b-3p levels in the overall survival of patients with CRC. E. The levels of miR19b-3p in four different colorectal cancer cell lines were measured qRT-PCR. The PCR values were normalized to the levels of GAPDH. The data are expressed as the mean \pm SD of three independent measurements.

ture plate. After 24 h, the cells invaded into the lower chamber were stained with crystal violet and counted by microscopy [15].

Western blotting and quantitative RT-PCR

Cells were collected and split on the ice using cell lysis buffer for 15 minutes, and the supernatant was denatured in 5 x loading buffer containing SDS. PVDF membranes were blocked and antibody dilution using TBST. The following antibodies were prepared: MMP-2 (Cell Signaling Technology, 1:1000), MMP-9 (Cell Signaling Technology, 1:1000), TIMP-1 (Cell Signaling Technology, 1:1000), GAPDH (Cell Signaling Technology, 1:1000), ITGB8 (Cell Signaling Technology, 1:1000), goat anti-rabbit IgG-HRP (Santa Cruz, 1:5000). The target proteins were detected by ECL system (Millipore, Germany).

Total RNA was extracted from colorectal cancer cells or clinical tissues using Trizol. RNA (1 μ g) was reverse transcribed into cDNA by ReverTra Ace qPCR RT Kit. The qRT-PCR was performed to analysis mRNA of miR-19b-3p or ITGB8 using AceQ qPCR SYBR Green Master Mix. The formula for the relative expression value was calculated as $2^{-\Delta\Delta Ct}$. The primer sequences of GAPDH are 5'-CTCACCGGATGCACCAATGTT-3' and 5'-CGCGTTGCTCACAATGTTTCAT-3'; ITGB8 is 5'-CGTGACTTTCGTCTGGATTGG-3' and 5'-TCCTTTCGGGGTGGATGTAA-3'.

Luciferase reporter assay

Vector carrying the WT FOXL2 promoter sequence Luc-ITGB8 and the MUT Luc-ITGB8 were synthesized (GeneCopoeia). 1×10^5 cells/

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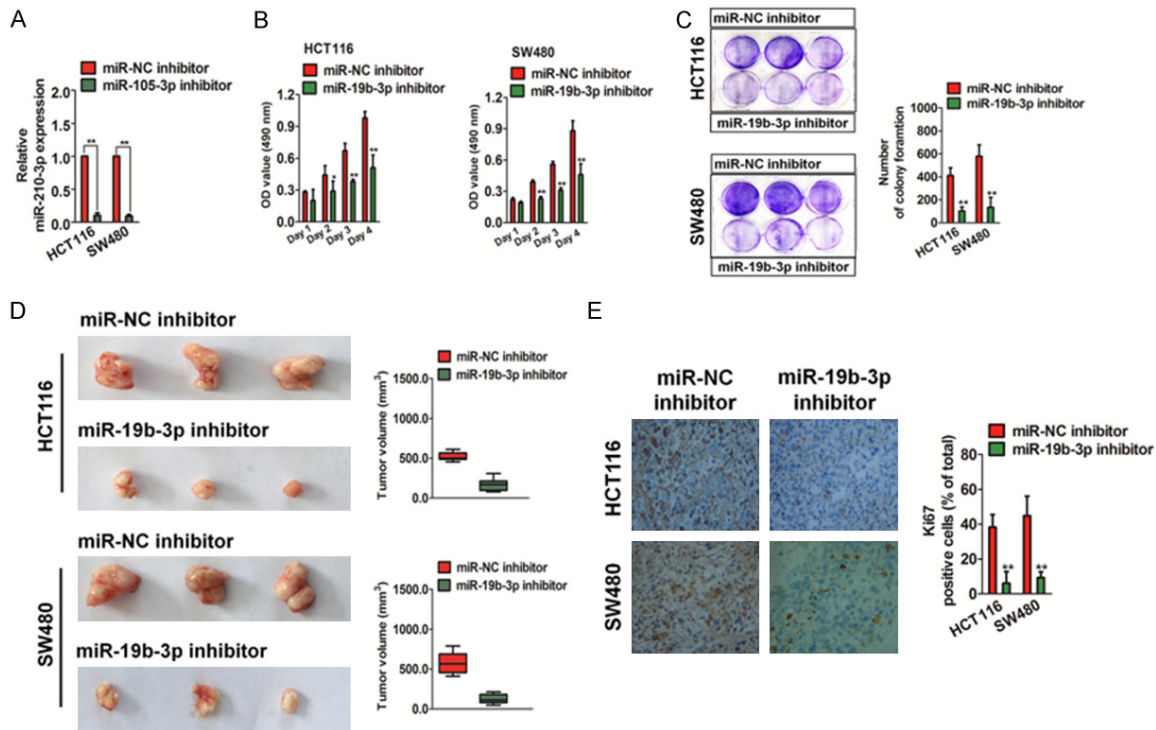


Figure 2. Down-regulation of miR19b-3p inhibits cell growth. A. Transfection of HCT116 and SW480 cells with a miR-NC inhibitor or miR19b-3p inhibitor. The total RNA was isolated and the mRNA level of miR19b-3p was detected by qRT-PCR. Data are expressed as mean \pm SD. **compared with miR-NC, $P < 0.01$. B. Colorectal cancer (CRC) cells transfected with the miR-NC inhibitor or miR19b-3p inhibitor were inoculated in 96-well plates and cultured for 24, 48, 72 or 96 h. MTT assay was performed and the viable cells were measured at 490 nm. C. The number of colonies in miR19b-3p inhibitor-transfected HCT116 and SW480 cells was significantly lower than that in miR-NC-inhibitor transfected cells. Data are expressed as mean \pm SD. ** $P < 0.01$, compared with miR-NC inhibitor. D. The miR-NC inhibitor- or miR19b-3p inhibitor-transfected CRC cells were implanted in the nude mice, and the tumor volume was measured once every three days. E. Xenotransplantation tumors were embedded in paraffin and preceded to IHC. Low Ki-67-positive expression was detected in tumors from CRC cells transfected with the miR19b-3p inhibitor. Data are expressed as mean \pm SD. ** $P < 0.01$, compared with miR-NC inhibitor.

well was seeded in 24-well plate. After 24 h, the cells were co-transfected with vector plasmids and miR-19b-3p using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. 24 h later, the transfected cells were lysed and the resulting lysates were centrifuged. Luciferase activity was measured by using luciferase assay system (E2920, Promega) [16].

In vivo tumorigenesis and metastasis

All mouse experiments were performed according to the standard procedure approved by the Sixth Affiliated Hospital, Sun Yat-sen University. 1×10^6 Control cells or miR-19b-3p overexpression/down-regulated cells was subcutaneously injection of into the lateral ventral. The tumor size was measured once a week with the caliper and calculated as tumor volume = $0.5 \times \text{length} \times \text{width}^2$. The tumor tissue from

mice was fixed with formalin and paraffin-embedded [17]. Sliced samples were performed immunohistochemistry staining for Ki67. In the lung metastasis assay, 1×10^6 HCT116 or SW480 cells were injected into BALB/c mice by tail vein. After 3 weeks, the nude mice were killed and the lungs were collected. H&E stains were performed on sections from embedded lung. Animal experiments were approved by the Animal Care Use Committee of the Sixth Affiliated Hospital, Sun Yat-sen University.

Statistical analysis

The results are expressed as the mean \pm SD deviation of at least three repetitions in each group, and the student's t test is performed to compare the differences between the two groups. $P < 0.05$ considered as significant.

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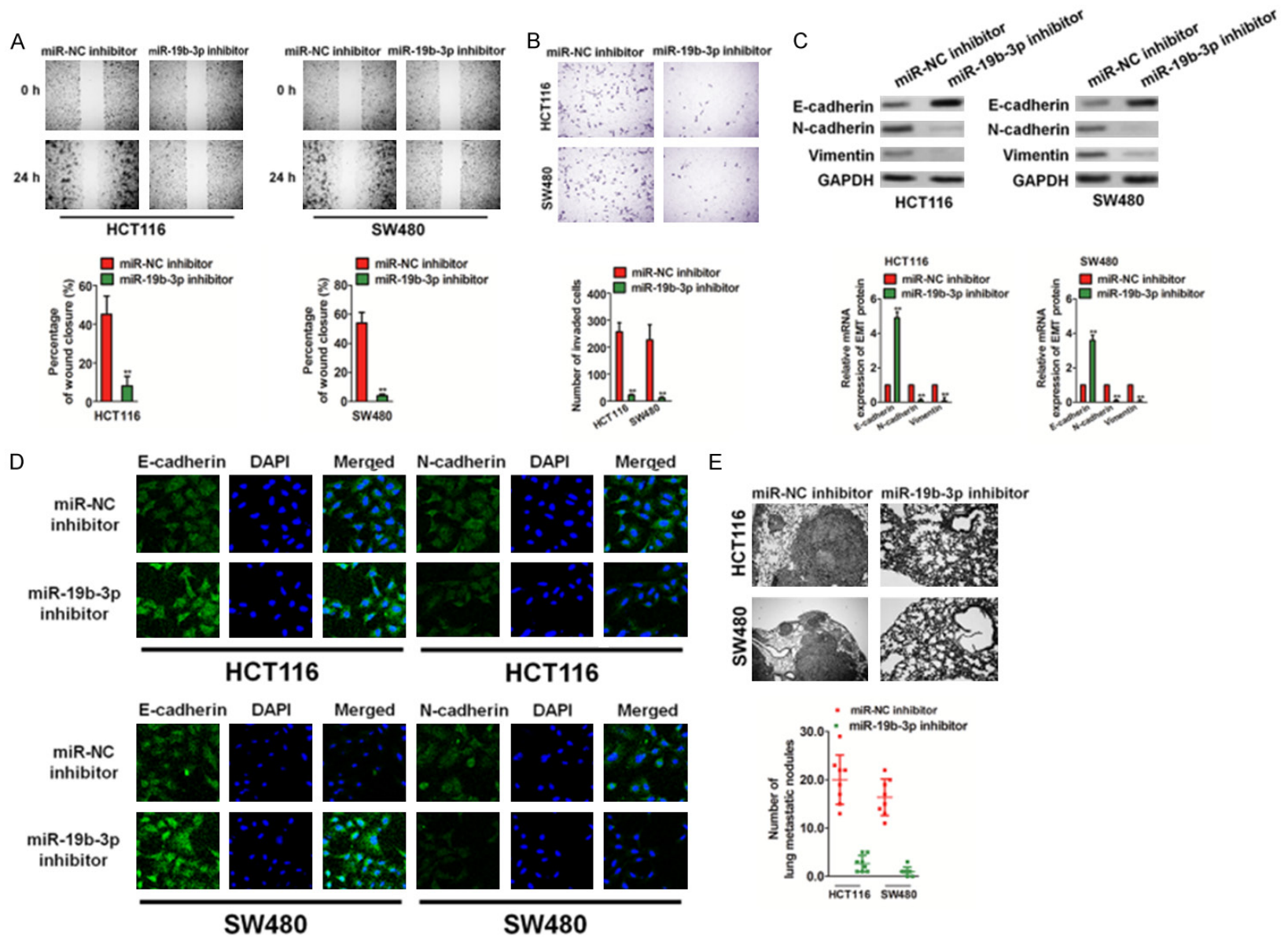


Figure 3. Down-regulation of miR19b-3p inhibits colorectal cancer (CRC) cell metastasis. A. HCT116 and SW480 cell transplantation with miR19b-3p inhibitor or miR-NC inhibitor was performed to evaluate the cell migration ability. Data are expressed as mean ± SD. **P < 0.01 compared with the miR-NC inhibitor. B. The

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invasive abilities of HCT116 and SW480 cells were assessed by the Transwell invasion assay. Quantitative statistics for invasive cells are shown on the right panel. Data are expressed as mean \pm SD. $**P < 0.01$ compared with miR-NC inhibitor. C. HCT116 and SW480 cells were transfected with the miR19b-3p inhibitor or miR-NC inhibitor, and western blotting (upper panel) and qRT-PCR (lower panel) assays were used to detect the expression of E-cadherin, N-cadherin, and vimentin. D. HCT116 and SW480 cells were incubated with E-cadherin and N-cadherin. Cells were immunized with anti-rabbit FITC-conjugated secondary antibody and then stained with DAPI. The core is shown in blue, and the target protein is shown in green. The scale bar represents 50 μ m. E. Representative hematoxylin and eosin staining of lung tissue from mice injected with miR-NC inhibitor cells and miR19b-3p inhibitor cells.

Results

The expression of miR19b-3p is down-regulated in CRC

YM500v2 meta-analysis [18] was performed to identify miRNAs that were differentially expressed in colonic adenocarcinoma tissue and normal solid tissue (**Figure 1A**). A total of 273 miRNAs were significantly altered in solid tumors (128 were up-regulated, 145 were down-regulated) compared to that in normal tissue (**Supplementary Table 1**). Of the 128 up-regulated miRNAs, 26 miRNAs were identified as up-regulated in infinitely increased levels of very low expression levels in the normal group of these miRNAs. For other miRNAs, we set a threshold of 42 for the basal mean of the primary solid tumor, which was the average of the basic mean of these miRNAs. Above the threshold, we found 22 significantly up-regulated miRNAs, among which miR19b-3p was ranked first in the list. To investigate the differences in miR19b-3p expression in CRC, we identified the levels of miR19b-3p in the clinical CRC tissues ($n = 40$) and adjacent non-tumor tissues by qRT-PCR. As shown in **Figure 1B**, compared with the normal counterparts, the levels of miR19b-3p were significantly up-regulated in 35 of the 40 CRC tissues (87%). When compared with the controls, miR19b-3p expression was increased by nearly three-fold in the CRC tissues (**Figure 1C**). We then performed a Kaplan-Meier survival analysis to assess the prognostic value of miR19b-3p expression in the OS of patient with CRC. The results showed that shorter OS was associated with higher expression of miR19b-3p (**Figure 1D**). Finally, we analyzed the miR19b-3p levels in several CRC cell lines, including HT-29, HCT116, SW-480, and LOVO, which were then compared to levels in human epithelial cell HCoEpiC cells by qRT-PCR. Similarly, the expression levels of miR19b-3p in the CRC cell lines were significantly higher than that in HCoEpiC cells (**Figure 1E**). These results suggest that miR19b-3p lev-

els were positively correlated with CRC malignancies.

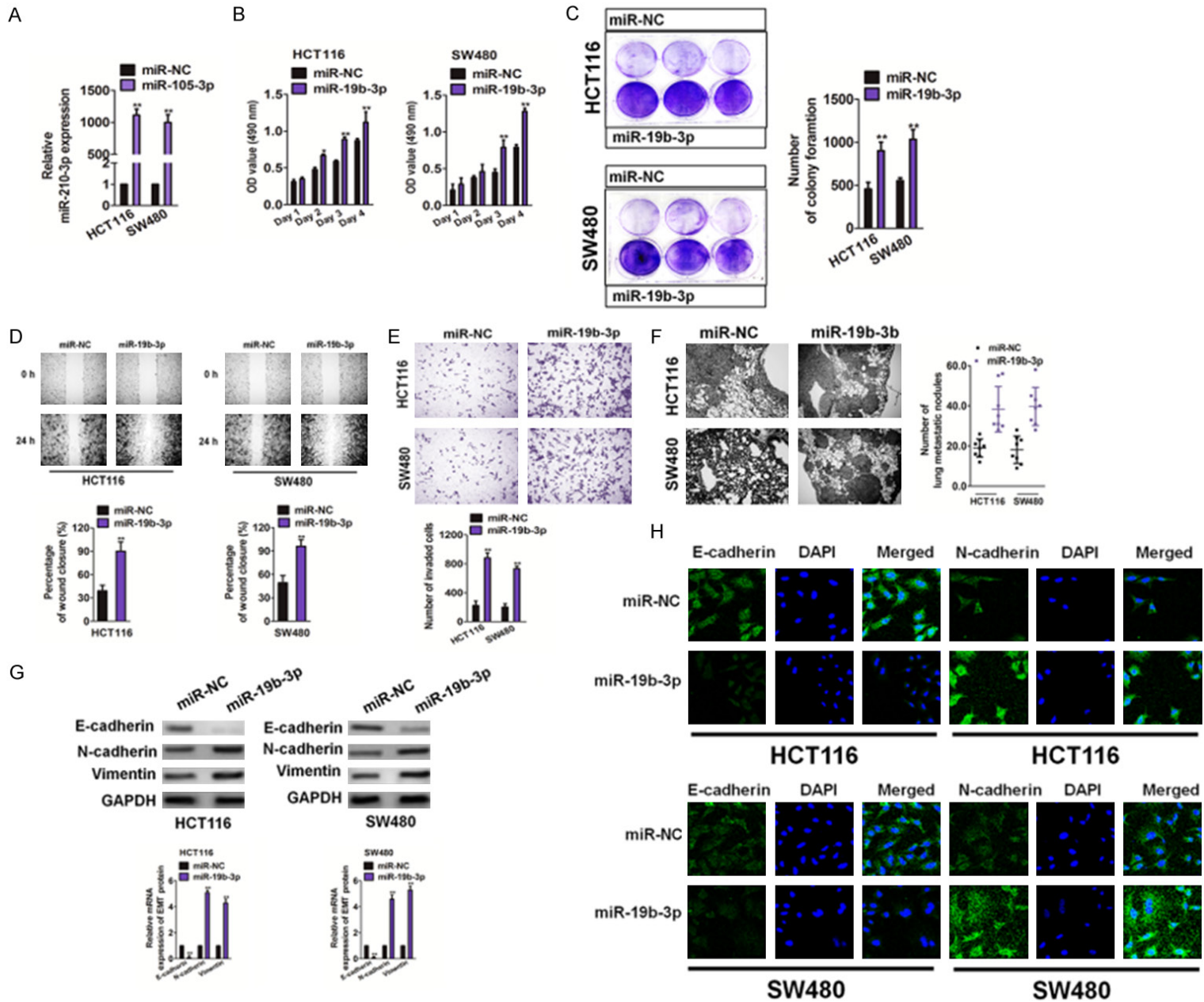
Down-regulation of miR19b-3p inhibits cell growth

In order to investigate the biological effects of miR19b-3p in CRC, we transfected HCT116 and SW480 cells with a miR19b-3p inhibitor to evaluate the effect of miR19b-3p down-regulation on CRC cell proliferation. MiR19b-3p was effectively inhibited in HCT116 and SW480 cells, as assessed by qRT-PCR (**Figure 2A**). Then, an MTT assay was performed and significant inhibition of CRC cell proliferation was observed in vitro (**Figure 2B**). miR19b-3p down-regulation also remarkably inhibited colony formation in HCT116 and SW480 cells compared to that in cells transfected with the control inhibitor (designated as miR-NC inhibitor) (**Figure 2C**). Next, we investigated the role of miR19b-3p in the growth of HCT116 and SW-480 CRC cells in vivo. The miR19b-3p-down-regulated HCT116 or SW480 cells were subcutaneously implanted into the nude mice. As shown in **Figure 2D**, miR19b-3p down-regulation inhibited tumor growth significantly in vivo, and the tumor volume derived from miR19b-3p-downregulated cells was remarkably smaller than tumors from control cells. An immunohistochemical staining assay indicated that the Ki-67 index of tumors from miR19b-3p-downregulated cells was lower than that of control cells (**Figure 2E**). In conclusion, these results indicated that the down-regulation of miR19b-3p inhibits CRC cell growth in vitro and in vivo.

Down-regulation of miR19b-3p inhibits CRC cell metastasis

We examined the functional role of miR19b-3p in the migration and invasion of CRC cells, since metastasis has been associated with the poor prognosis of CRC. Down-regulation of miR19b-3p significantly inhibited the migration of HCT116 and SW480 cells in a wound healing

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Figure 4. Overexpression of miR19b-3p promotes the growth and metastasis of colorectal cancer (CRC) cells. A. HCT116 and SW480 cells were transfected with miR19b-3p or miR-NC. The levels of miR19b-3p were detected by qRT-PCR. Data are expressed as mean \pm SD. $**P < 0.01$, compared with miR-NC. B. The relative cell viability of HCT116 and SW480 cells transfected with the miR19b-3p or miR-NC was evaluated by the MTT assays. C. A colony formation analysis was performed to evaluate the rates of cells transfected with miR19b-3p. Data are expressed as mean \pm SD. $**P < 0.01$, compared with miR-NC. D. Overexpression of miR19b-3p promoted the migration of CRC cells. Data are expressed as mean \pm SD. $**P < 0.01$, compared with miR-NC. E. Overexpression of miR19b-3p promoted the invasion of CRC cells in the Transwell invasion assays (left panel). Quantitative analysis of invasive cells was shown (right panel). Data are expressed as mean \pm SD. $**P < 0.01$, compared with miR-NC. F. Representative hematoxylin and eosin stains of lung tissue from mice injected with miR-NC and miR19b-3p overexpressing HCT116 and SW480 cells. Scale bar: 100 μ m. G. Cells were transfected with miR19b-3p or miR-NC, and western blotting (upper panel) and qRT-PCR (lower panel) assays were used to detect the expression of E-cadherin, N-cadherin and vimentin. H. Cells were incubated with E-cadherin and N-cadherin. Cells were immunized with anti-rabbit FITC-conjugated secondary antibody and then stained with DAPI. Blue depicted the core and green depicted the target protein.

analysis (**Figure 3A**). Similarly, the down-regulation of miR19b-3p dramatically suppressed the invasion of CRC cells in vitro (**Figure 3B**). Epithelial-mesenchymal transition (EMT) of cancer cells has been recognized to be associated with the initiation of metastasis. Immunoblotting analyses, qRT-PCRs, and immunofluorescence assays were performed to investigate whether miR-1296 down-regulation resulted in the inhibition of EMT in CRC cells. As shown in **Figure 3C** and **3D**, the miR19b-3p inhibitor resulted in increased expression of epithelial markers (E-cadherin) and decreased expressions of intermediate markers (N-cadherin and vimentin). Finally, we tested the effect of miR19b-3p on cancer cell metastasis in vivo. The miR19b-3p inhibitor or miR-NC inhibitor transfected cells were injected into nude mice via the tail vein. Hematoxylin and eosin (H&E) staining of pulmonary slices confirmed that the lung tissue of mice injected with the miR-NC inhibitor-transfected cells had a higher number of tumor lesions than those with the miR19b-3p inhibitor-transfected cells (**Figure 3E**). Based on the above evidence, we demonstrated that down-regulation of miR19b-3p leads to the inhibition of CRC cell metastasis in vitro and in vivo.

Overexpression of miR19b-3p promotes the growth and metastasis of CRC cells

We used precursor miR19b-3p clones to mimic overexpression of miR19b-3p in HCT116 and SW480 cells. After miR19b-3p was transfected into the cells, miR19b-3p mRNA levels were measured by qRT-PCR (**Figure 4A**). Both MTT and colony formation assay results (**Figure 4B** and **4C**, respectively) demonstrated that overexpression of miR19b-3p significantly increased CRC cell growth in vitro. Further-

more, overexpression of miR19b-3p effectively induced the migration (**Figure 4D**) and invasion (**Figure 4E**) of HCT116 and SW480 cells. MiR19b-3p-overexpressing HCT116 or SW480 cells were injected into mice via the tail vein. Histological analyses of the lung tissue showed that lung tissues of nude mice injected with miR19b-3p-overexpressing cells had a higher number of tumor lesions (**Figure 4F**). Finally, an immunoblotting analysis, qRT-PCR, and immunofluorescence assay were performed to reveal whether miR1296 overexpression accelerated EMT in CRC cells. As shown in **Figure 4G** and **4H**, miR19b-3p resulted in decreased expression of epithelial markers (E-cadherin) and increased expressions of intermediate markers (N-cadherin and vimentin). These results showed that overexpression of miR19b-3p accelerated colon cancer growth and metastases.

miR19b-3p binds to the 3'UTR of ITGB8

To investigate the target genes of miR19b-3p, three prediction websites (TargetsScan, MIRDB, and PicTar) were used to predict its target genes. Notably, 9 genes (ZBTB18, NFIB, RAP1B, BTF3L4, ARHGEF26, ITGB8, GOLGA8A, ZFPM2, and WNT10A) were present in the databases (**Figure 5A**). Among these candidates, we focused on ITGB8 because its role in CRC growth and metastasis was unknown. In order to validate the 3'-UTR of ITGB8 as a direct target of miR19b-3p, ITGB8 3'-UTR and wild-type miR19b-3p binding sites (WT) were inserted into the p-MIR report vector, and a vector with a corresponding mutation binding site (MUT) was also constructed. The relative luciferase activities in 293T cells co-transfected with miR19b-3p mimics and WT vectors were significantly inhibited, whereas the luciferase activity

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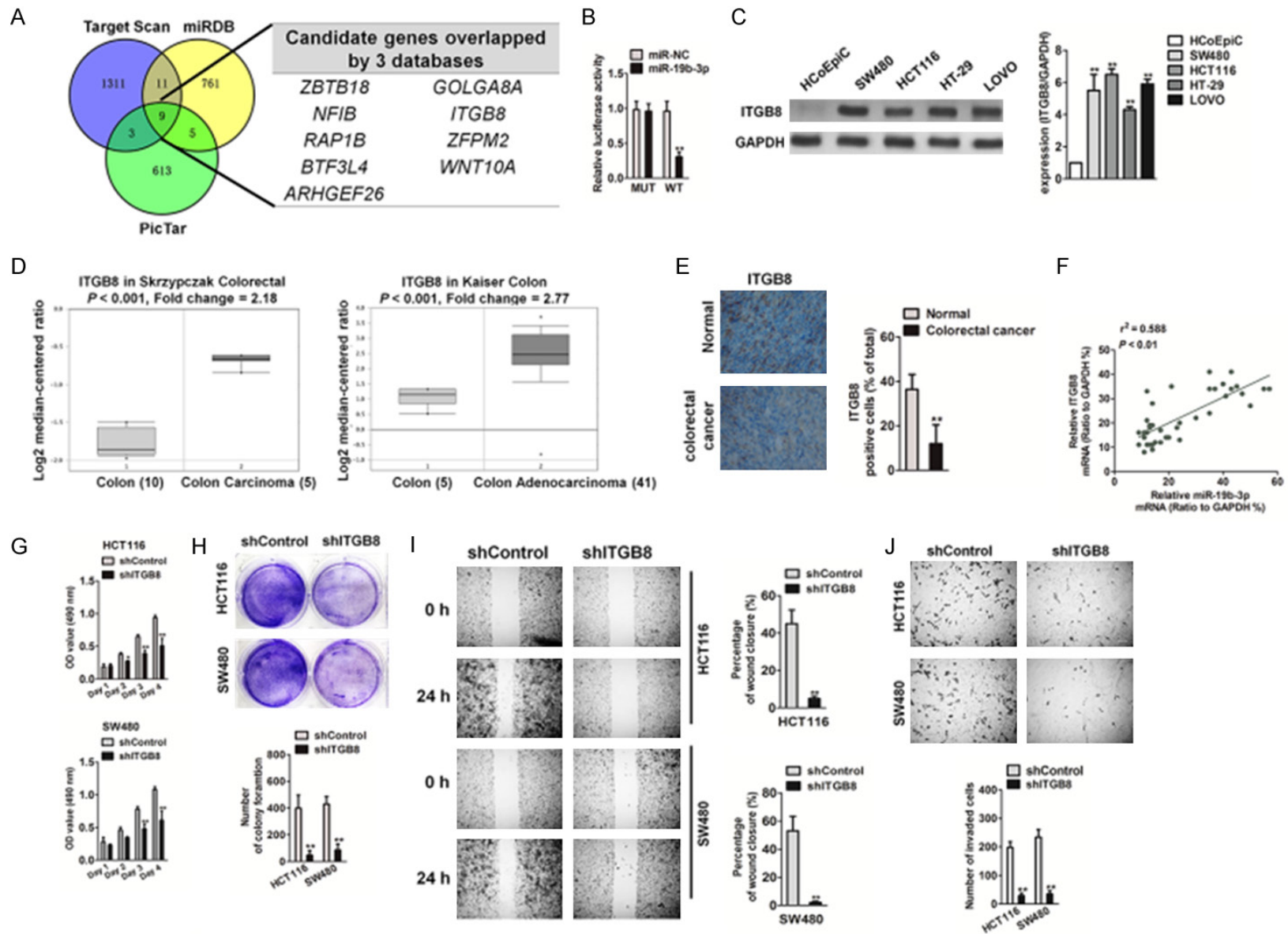


Figure 5. Effect of ITGB8 knockdown on CRC cells growth and metastasis. A. miR19b-3p target genes were predicted by in silico analyses. The Venn graph represented the number of candidate genes determined by three prediction algorithms. B. The relative luciferase activity in 293T cells co-transfected with the miR19b-3p

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mimic and WT vector or MUT vector was analyzed by luciferase reporter assay. C. RNA was extracted from CRC cells and the levels of ITGB8 were determined by qRT-PCR (upper panel). Data are expressed as mean \pm SD. $*P < 0.05$, $**P < 0.01$, compared with HCoEpiC cells. The expression of ITGB8 was detected by western blotting of CRC cell lines (lower panel). D. ITGB8 was overexpressed in CRC tissues compared to that in normal tissues, based on the datasets from Oncomine. E. ITGB8 levels in CRC tissues were measured by IHC. F. The positive correlation analysis of miR19b-3p and ITGB8 in CRC tissues. G. Cell proliferation activity was measured by MTT assay. H. The growth capacity was determined in the colony formation assay. I. HCT116 and SW480 cell migration was analyzed by the wound healing assay as described above. J. HCT116 and SW480 cell invasion capacity was analyzed by Transwell Matrigel invasion assay as described above. Data are expressed as mean \pm SD. $*P < 0.05$, $**P < 0.01$, compared with control cells.

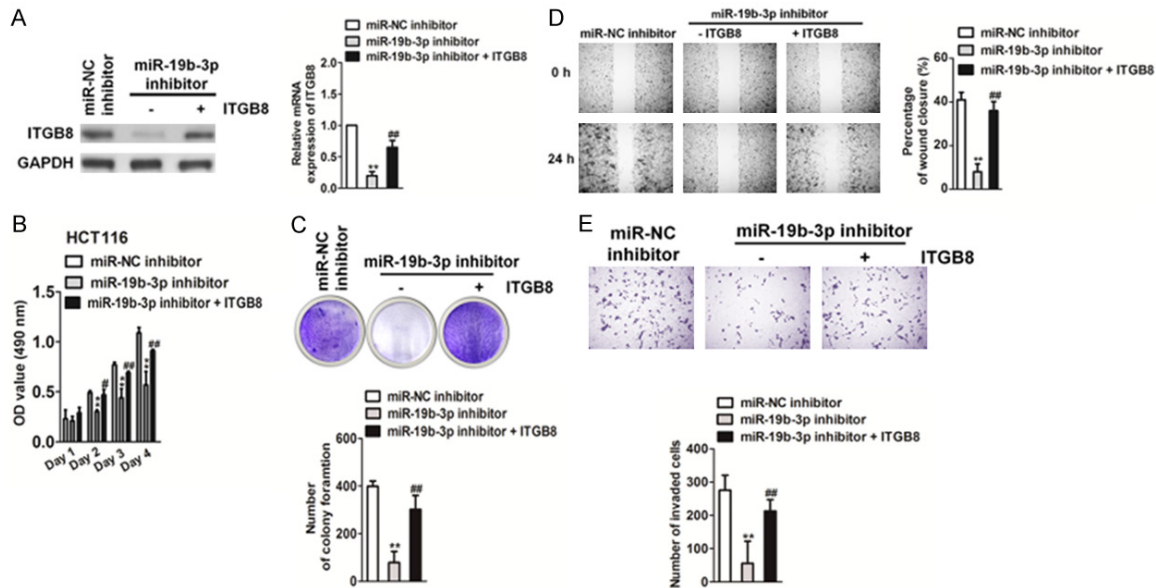


Figure 6. Effect of miR19b-3p inhibitor on colorectal cancer growth and metastasis is rescued by ITGB8 overexpression. A. The mRNA levels of ITGB8 were detected by qRT-PCR (right panel). Western blotting was performed to determine the expression levels of ITGB8 (left panel). B. HCT116 and SW480 cell proliferation was measured by MTT assay as described. C. Growth capacities of the indicated cells were detected by the soft agar colony formation assay as described above. Data are expressed as mean \pm SD. $**P < 0.05$ compared with miR-NC inhibitor, $##P < 0.01$ compared with miR19b-3p inhibitor. D. Wound healing assay as described was used to evaluate the cell migration ability in vitro. E. Cell invasion was assessed by Transwell Matrigel invasion assay. Data are expressed as mean \pm SD. $*P < 0.05$ compared with miR-NC inhibitor, $##P < 0.01$ compared with the miR19b-3p inhibitor.

in cells transfected with the miR19b-3p mimics and MUT vector were not affected (**Figure 5B**). In addition, ITGB8 expressions in several CRC cell lines were higher than that in HCoEpiC cells (**Figure 5C**). Meanwhile, the Oncomine microarray databases [19, 20] indicated that ITGB8 was substantially up-regulated in CRC compared to that in normal tissue (**Figure 5D**). We then attempted to analyze the correlation between ITGB8 and miR19b-3p expression in CRC and found that ITGB8 was overexpressed in CRC (**Figure 5E**) and was positively associated with miR19b-3p levels (**Figure 5F**). To determine whether ITGB8 was involved in the growth and metastasis of CRC, short hairpin RNA (shRNA) was used to knockdown ITGB8 expres-

sion in HCT116 and SW480 cells (**Supplementary Figure 1**). Then, we checked the effect of ITGB8 knockdown on tumor cell proliferation (**Figure 5G**), colony formation (**Figure 5H**), migration (**Figure 5I**), and invasion (**Figure 5J**). As expected, the stable knockdown of ITGB8 inhibited the proliferation, migration, and invasion of CRC cells in vitro, which suggests that ITGB8 functions as an oncogenic gene in CRC growth and metastasis.

Confirmation of the role of ITGB8 in miR19b-3p-induced colorectal cancer cell growth and metastasis

To investigate whether miR19b-3p regulates the proliferation and metastasis of CRC by tar-

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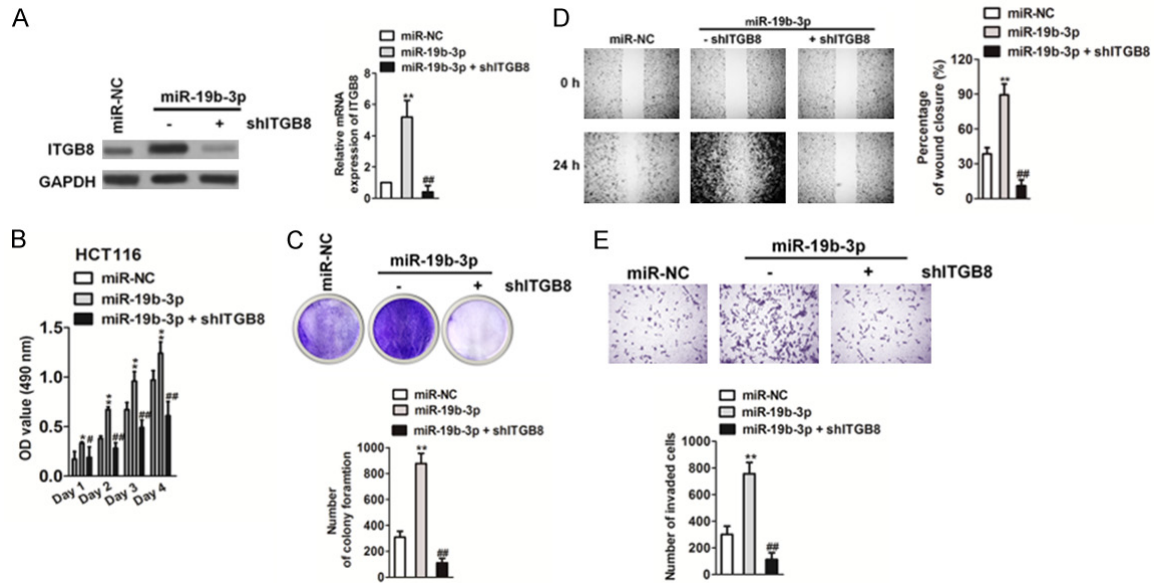


Figure 7. Effect of miR19b-3p on colorectal cancer (CRC) cell growth and metastasis is blocked by ITGB8 knock-down. A. The levels of ITGB8 in were detected by qRT-PCR (right panel) and western blotting analysis (left panel). B. Cell proliferation activity was measured by MTT assay as described. C. CRC growth was measured by the colony formation assay as described in vitro. The image (upper panel) and quantitative analysis of tumor cells colonies (lower panel) are shown. Data are expressed as mean \pm SD. ** $P < 0.05$ compared to miR-NC. ## $P < 0.01$ compared to miR19b-3p. D. Cell migration ability was measured by the wound healing assay. The image (left panel) and quantitative analysis of the percentage of closure (right panel) are shown. E. Cell invasion was analyzed by the Transwell Matrigel invasion assay. The image (upper panel) and quantitative analysis of the total invasive cells (lower panel) are shown. Data are expressed as mean \pm SD. ** $P < 0.05$ compared to miR-NC. ## $P < 0.01$ compared to miR19b-3p.

getting ITGB8, we constructed miR19b-3p-downregulated CRC cell lines that overexpressed ITGB8. Both qRT-PCR and western blot analysis showed that ectopic expression of ITGB8 rescued its levels in HCT116 and SW480 cells that were inhibited by the miR19b-3p inhibitor (Figure 6A). Both the MTT and soft agar growth assays (Figure 6B, 6C) showed that overexpression of ITGB8 promoted CRC cell proliferation when compared to that in cells transfected with the miR19b-3p inhibitor alone. Wound healing and Transwell invasion (Figure 6D, 6E) showed that overexpression of ITGB8 restored CRC cell migration and invasion compared with that in cells transfected with miR19b-3p inhibitor alone. To further confirm the function of ITGB8 in miR19b-3p-induced growth and metastasis, we co-transfected cells with shITGB8 and miR19b-3p (Figure 7A). As expected, ITGB8 knockdown counteracted the accelerated effects of miR19b-3p in CRC cell proliferation and growth (Figure 7B, 7C). Wound healing and Transwell invasion assays also showed that knockdown of ITGB8 reversed the promotion of cell migration and invasion caused

by miR19b-3p overexpression (Figure 7D, 7E). In summary, these results demonstrated the important role of ITGB8 in miR19b-3p-induced CRC cell growth and metastasis.

Discussion

CRC is the one of the most serious forms of human malignancies and is the main cause of global cancer-related deaths [21]. Although the treatments of CRCs have improved, the overall prognosis of patients with CRC remains poor. Consequently, in order to identify biomarkers and therapeutic targets for CRC, uncovering the molecular mechanism of CRC progression is crucial [22]. Recent studies have suggested that miRNAs regulate tumor cell processes, including cell proliferation, survival, invasion, and metastasis [23]. Thus, miRNAs are considered potential regulators in cancer progression. MIR19b has been implicated in a number of human cancers. MiR19a and miR19b (miR19a/b) promote the proliferation and migration of lung cancer cells by targeting microtubule-associated tumor suppressor 1

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(MTUS1) [24]. As members of the miR17-92 cluster, miR19b also functions as oncogenes in many types of cancer, including gastric cancer, pancreatic cancer, and breast cancer. Protein tyrosine phosphatase receptor type G (PTPRG) is an important tumor suppressor gene in multiple human cancers [25]. MiR19b could inhibit PTPRG expression to promote tumorigenesis in human breast cancer [25]. Collectively, these data indicate an oncogenic role of miR19b in cancer, though its clinical significance and potential roles in CRC remain uncovered. In the present study, overexpression of miR19b-3p was confirmed in CRC tissues. Our study demonstrated that miR19b-3p may serve an oncogenic role in CRC metastasis and as a potential biomarker for the prognostic prediction of CRC.

In order to test the hypothesis that miR19b-3p plays an important role in the growth and metastasis of CRC cells, we analyzed the expression levels of miR19b-3p in CRC and several cell lines. Compared with the normal tissue, we found that miR19b-3p was significantly increased in CRC. The gain-of-function and loss-of-function studies for miR19b-3p were performed in two CRC cell lines. The results showed that miR19b-3p down-regulation inhibited the proliferation and metastasis of CRC cells in vitro and in vivo. Nevertheless, overexpression of miR19b-3p resulted in opposite outcomes between the two lines, indicating that miR19b-3p was a tumor promoter in CRC. The molecular action of miR19b-3p in inducing cell growth and metastasis was partly due to the regulation of ITGB8. Furthermore, ITGB8 has been identified as a tumor promoter in several cancers. Herein, we identified ITGB8, a direct target gene for miR19b-3p, to play an important role in CRC cell metastasis. In this study, the complementary sequence of miR-19b-3p was identified to be in the 3'-UTR of ITGB8. In the luciferase reporter assay, miR-19b-3p resulted in a significant change in luciferase activity of WT ITGB8-3'-UTR without affecting the luciferase activity of MUT ITGB8-3'-UTR. Our current study showed that ITGB8 itself plays an important role in regulating tumor cell proliferation and metastasis. Our results also suggest that ITGB8 is up-regulated in all CRC cell lines, confirming that ITGB8 plays a broader role in regulating the development of CRC. In CRC cells, it was observed that ectopic

expression of ITGB8 significantly accelerated proliferation, migration, and invasion, while knockdown of ITGB8 inhibited CRC cell growth and metastasis.

In conclusion, we demonstrated that miR19b-3p is overexpressed in CRC tissues and verified that overexpression of miR19b-3p accelerated CRC cell growth, invasion, and metastasis both in vitro and in vivo. Functional studies also demonstrated that ITGB8 was a direct downstream target of miR19b-3p. Collectively, these results provide novel insights for better understanding the mechanisms of miR19b-3p/ITGB8 in the regulation of CRC metastasis and for identifying potential therapeutic targets for the clinical treatment of CRC metastasis.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Significant miRNA expression profiles in normal tissues compared to colorectal cancer

Name	id	Base Mean	Base Mean A	Base Mean B	Fold Change	log2 Fold Change	Pval	Padj
hsa-miR-3195	629	25.91255	701.4833	13.3145	0.01898	-5.71934	2.37E-05	0.000293
hsa-miR-486-5p	1454	296.997	7709.552	158.7675	0.020594	-5.60166	9.71E-15	7.46E-13
hsa-miR-139-3p	226	32.24175	781.4074	18.27129	0.023383	-5.41842	9.05E-19	1.31E-16
hsa-miR-328-3p	653	82.10105	1942.38	47.41052	0.024408	-5.35647	5.10E-39	1.26E-35
hsa-miR-139-5p	227	91.8201	1906.706	57.97607	0.030406	-5.03948	3.86E-30	2.37E-27
hsa-miR-6511b-3p	1978	5.226469	103.1142	3.401056	0.032983	-4.92212	1.10E-27	4.52E-25
hsa-miR-6511a-3p	1976	8.195837	156.1021	5.437679	0.034834	-4.84335	1.58E-30	1.30E-27
hsa-miR-197-3p	347	313.8414	5951.1	208.7177	0.035072	-4.83353	7.65E-38	9.41E-35
hsa-miR-129-5p	181	14.29499	248.3588	9.930166	0.039983	-4.64446	3.21E-06	4.84E-05
hsa-miR-1224-5p	65	6.915863	113.1099	4.935555	0.043635	-4.51837	0.005805	0.035522
hsa-miR-187-3p	304	7.170571	116.46	5.132541	0.044071	-4.50402	0.002746	0.018507
hsa-miR-766-3p	2443	25.10778	385.0006	18.39649	0.047783	-4.38736	1.46E-25	4.50E-23
hsa-miR-642b-5p	1943	6.529753	94.88033	4.88219	0.051456	-4.28051	9.16E-25	2.25E-22
hsa-miR-642a-5p	1941	7.166034	103.7212	5.365471	0.05173	-4.27286	6.56E-27	2.31E-24
hsa-miR-1306-5p	196	20.11679	284.5236	15.18613	0.053374	-4.22772	2.82E-23	5.78E-21
hsa-miR-194-3p	337	951.5085	13263.77	721.9092	0.054427	-4.19953	8.59E-28	4.22E-25
hsa-let-7d-3p	8	421.8525	5759.314	322.3194	0.055965	-4.15933	3.45E-25	9.42E-23
hsa-miR-149-5p	256	42.36008	561.6529	32.6763	0.058179	-4.10336	8.21E-14	5.61E-12
hsa-miR-4532	1153	24.01759	316.2576	18.5679	0.058711	-4.09022	0.000438	0.003859
hsa-miR-125a-5p	114	524.996	6907.581	405.9734	0.058772	-4.08872	1.32E-23	2.95E-21
hsa-miR-490-3p	1463	8.69307	112.9091	6.749647	0.059779	-4.06421	4.65E-05	0.00053
hsa-miR-133a-3p	209	47.40008	594.4515	37.19866	0.062576	-3.99824	2.23E-08	6.68E-07
hsa-miR-671-3p	2021	5.980782	73.26626	4.726041	0.064505	-3.95444	2.30E-18	3.14E-16
hsa-miR-195-3p	339	5.627187	68.02997	4.463498	0.065611	-3.92992	5.55E-17	6.20E-15
hsa-miR-3656	734	44.34642	521.7528	35.44374	0.067932	-3.87976	2.03E-06	3.25E-05
hsa-miR-378a-5p	827	403.4314	4719.868	322.9384	0.068421	-3.86942	3.47E-22	6.57E-20
hsa-miR-193b-5p	336	8.04803	92.21883	6.478411	0.07025	-3.83135	6.89E-18	8.47E-16
hsa-miR-1180-3p	41	33.60934	385.0688	27.05532	0.070261	-3.83113	2.52E-19	4.04E-17
hsa-miR-1226-3p	69	3.509023	39.5984	2.836027	0.07162	-3.8035	7.34E-14	5.16E-12
hsa-miR-1296-5p	183	14.1093	159.0752	11.40597	0.071702	-3.80185	6.65E-15	5.45E-13
hsa-miR-145-5p	239	8152.049	86575.07	6689.615	0.07727	-3.69396	1.81E-11	8.39E-10
hsa-miR-150-5p	258	1386.199	14495.9	1141.729	0.078762	-3.66635	3.54E-21	6.22E-19
hsa-miR-3615	705	6.401324	66.1447	5.287229	0.079934	-3.64504	3.11E-15	2.84E-13
hsa-miR-181a-5p	286	2146.552	21790.22	1780.237	0.081699	-3.61354	2.63E-19	4.04E-17
hsa-miR-744-5p	2431	58.42131	589.8287	48.51162	0.082247	-3.60389	1.94E-16	1.99E-14
hsa-miR-150-3p	257	7.160982	72.06157	5.950715	0.082578	-3.5981	6.95E-15	5.52E-13
hsa-miR-574-3p	1816	91.68594	918.7596	76.26265	0.083006	-3.59064	6.01E-17	6.43E-15
hsa-miR-6724-5p	2037	3.759868	36.64197	3.146682	0.085876	-3.54159	3.26E-12	1.67E-10
hsa-let-7b-5p	5	13437.72	129370.9	11275.8	0.087159	-3.52021	9.80E-18	1.15E-15
hsa-miR-423-3p	911	179.8016	1605.537	153.2144	0.095429	-3.38943	9.94E-16	9.40E-14
hsa-miR-146b-3p	245	89.75184	782.0266	76.84229	0.09826	-3.34725	5.48E-16	5.39E-14
hsa-miR-7706	2454	4.894643	42.47602	4.193825	0.098734	-3.34031	1.67E-12	8.94E-11
hsa-miR-193a-5p	334	369.2766	3179.979	316.8626	0.099643	-3.32709	2.70E-13	1.75E-11
hsa-let-7b-3p	4	109.8896	942.4216	94.36452	0.10013	-3.32006	1.02E-14	7.63E-13
hsa-miR-532-3p	1632	72.79012	618.8993	62.60627	0.101157	-3.30533	6.66E-14	4.82E-12
hsa-miR-324-3p	648	28.83194	242.7887	24.84207	0.10232	-3.28884	9.08E-14	6.03E-12
hsa-miR-326	652	82.30884	692.3833	70.93216	0.102446	-3.28706	1.14E-12	6.53E-11
hsa-miR-1307-3p	197	3169.658	26535.96	2733.923	0.103027	-3.2789	6.54E-15	5.45E-13

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hsa-miR-92b-3p	2548	87.95365	725.5736	76.0633	0.104832	-3.25385	3.60E-13	2.27E-11
hsa-miR-1268b	131	12.62176	102.4199	10.94721	0.106886	-3.22586	1.17E-12	6.54E-11
hsa-miR-3127-5p	530	22.13094	177.5766	19.23218	0.108304	-3.20685	9.46E-13	5.68E-11
hsa-miR-342-5p	676	9.220269	73.88078	8.014479	0.108479	-3.20452	2.61E-12	1.37E-10
hsa-miR-2110	389	3.965436	31.06439	3.460094	0.111385	-3.16638	6.02E-09	2.03E-07
hsa-miR-3150b-3p	563	6.166556	47.54828	5.394869	0.113461	-3.13973	6.31E-08	1.63E-06
hsa-miR-1273h-3p	145	8.64038	65.41483	7.581649	0.115901	-3.10903	1.51E-12	8.27E-11
hsa-miR-375	816	73470.47	549550.1	64592.53	0.117537	-3.08881	9.95E-13	5.83E-11
hsa-miR-1268a	130	20.97387	155.2252	18.47035	0.118991	-3.07108	1.42E-11	6.73E-10
hsa-miR-133b	211	5.816792	42.2219	5.137909	0.121688	-3.03874	0.000843	0.006873
hsa-miR-5010-3p	1506	4.134032	29.62536	3.658669	0.123498	-3.01744	6.28E-08	1.63E-06
hsa-miR-7641	2441	953.8713	6822.903	844.4255	0.123763	-3.01434	2.45E-06	3.81E-05
hsa-miR-3142	551	4.206985	29.93022	3.727297	0.124533	-3.0054	0.000258	0.002409
hsa-let-7c-5p	7	14017.04	98695.44	12437.95	0.126024	-2.98823	1.18E-11	5.82E-10
hsa-miR-99b-5p	2578	20468.29	143837.3	18167.71	0.126307	-2.98499	8.49E-11	3.60E-09
hsa-miR-296-5p	469	9.962774	69.17026	8.858672	0.128071	-2.96499	1.89E-06	3.09E-05
hsa-miR-23a-5p	440	10.68558	73.77321	9.509122	0.128897	-2.95571	3.36E-08	9.49E-07
hsa-miR-4286	952	59.13546	405.8757	52.66944	0.129767	-2.946	2.08E-11	9.50E-10
hsa-miR-7704	2451	13.09205	88.93473	11.67774	0.131307	-2.92899	0.000201	0.001929
hsa-miR-3653	731	23.66718	159.3201	21.13752	0.132673	-2.91405	3.36E-06	5.01E-05
hsa-miR-361-3p	702	233.5454	1571.488	208.5954	0.132737	-2.91335	2.99E-11	1.31E-09
hsa-miR-193b-3p	335	30.08676	199.3063	26.93115	0.135124	-2.88764	5.50E-10	2.11E-08
hsa-miR-4492	1092	4.672774	30.0988	4.198629	0.139495	-2.84172	0.002814	0.018865
hsa-miR-432-5p	996	13.99142	88.79458	12.59649	0.141861	-2.81745	2.38E-08	6.96E-07
hsa-miR-6892-5p	2374	5.212119	32.87253	4.696308	0.142864	-2.80728	1.64E-06	2.72E-05
hsa-let-7e-3p	10	8.306276	49.83342	7.531877	0.151141	-2.72603	3.39E-07	6.89E-06
hsa-miR-484	1450	169.4388	1006.272	153.8335	0.152875	-2.70958	1.46E-09	5.19E-08
hsa-miR-1260a	118	164.1607	962.1947	149.279	0.155144	-2.68832	3.55E-08	9.80E-07
hsa-miR-874-3p	2517	53.64413	306.5116	48.92865	0.159631	-2.64719	1.51E-08	4.69E-07
hsa-miR-125b-5p	117	472.2057	2693.844	430.7765	0.159911	-2.64465	9.48E-08	2.24E-06
hsa-miR-6842-3p	2277	8.302347	46.86734	7.583186	0.161801	-2.62771	1.14E-06	1.98E-05
hsa-miR-30c-2-3p	506	24.48187	137.9223	22.36643	0.162167	-2.62445	2.75E-07	5.88E-06
hsa-miR-219a-1-3p	416	5.247507	29.44282	4.796312	0.162903	-2.61792	2.83E-07	6.01E-06
hsa-miR-574-5p	1817	56.7917	317.971	51.92123	0.163289	-2.6145	4.79E-08	1.29E-06
hsa-miR-99b-3p	2577	44.13884	246.4456	40.36622	0.163794	-2.61005	8.47E-08	2.05E-06
hsa-miR-378a-3p	826	2876.293	15754.58	2636.138	0.167325	-2.57927	1.87E-08	5.74E-07
hsa-miR-941	2563	22.55735	123.3881	20.67706	0.167577	-2.5771	1.97E-07	4.28E-06
hsa-miR-15b-5p	278	460.7649	2518.988	422.3831	0.16768	-2.57622	1.91E-08	5.81E-07
hsa-miR-3605-3p	690	9.706167	52.24242	8.91295	0.170608	-2.55125	5.48E-07	1.05E-05
hsa-miR-3928-3p	874	3.592234	18.99619	3.304981	0.173981	-2.523	2.41E-06	3.77E-05
hsa-miR-1468-5p	241	12.3878	65.02337	11.40625	0.175418	-2.51113	2.89E-07	6.08E-06
hsa-miR-1260b	119	156.0927	813.6991	143.8296	0.17676	-2.50013	1.03E-06	1.82E-05
hsa-miR-370-3p	798	8.619851	44.65858	7.947801	0.177968	-2.49031	3.44E-06	5.10E-05
hsa-miR-7977	2474	5.347183	27.6041	4.932136	0.178674	-2.4846	0.000437	0.003859
hsa-miR-654-5p	1997	6.679729	34.15161	6.167433	0.18059	-2.46921	1.11E-06	1.94E-05
hsa-miR-1273h-5p	146	4.899958	24.67781	4.53114	0.183612	-2.44527	1.20E-06	2.04E-05
hsa-miR-589-5p	1840	132.8953	665.4246	122.9646	0.184791	-2.43603	3.35E-07	6.87E-06
hsa-miR-629-3p	1926	15.18984	74.7693	14.0788	0.188296	-2.40892	1.84E-07	4.08E-06
hsa-miR-937-3p	2556	9.457574	45.24184	8.790268	0.194295	-2.36368	7.29E-06	0.000101
hsa-miR-377-5p	825	4.245566	20.08042	3.950277	0.196723	-2.34576	0.000149	0.001505
hsa-miR-92a-3p	2547	23315.85	108179	21733.32	0.200901	-2.31544	1.41E-06	2.38E-05
hsa-miR-29b-2-5p	477	35.25288	162.902	32.87247	0.201793	-2.30905	1.36E-06	2.31E-05

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hsa-miR-939-5p	2560	5.091265	23.45382	4.74884	0.202476	-2.30418	3.40E-05	0.0004
hsa-miR-3614-5p	704	10.10058	46.34806	9.424637	0.203345	-2.298	1.31E-05	0.000168
hsa-miR-132-3p	206	253.1919	1082.849	237.7204	0.219532	-2.18749	5.93E-06	8.33E-05
hsa-miR-3074-5p	500	18.39725	78.06426	17.28458	0.221415	-2.17518	7.45E-06	0.000102
hsa-miR-140-3p	228	709.1641	3006.666	666.3203	0.221614	-2.17388	7.15E-06	9.93E-05
hsa-miR-125b-2-3p	116	4.776723	19.96946	4.493408	0.225014	-2.15191	0.000894	0.007213
hsa-miR-652-3p	1992	60.75837	253.0108	57.17324	0.225972	-2.14579	3.98E-06	5.75E-05
hsa-miR-185-3p	300	6.561655	27.23486	6.17614	0.226773	-2.14068	0.00018	0.001757
hsa-miR-4746-5p	1352	8.553894	35.20079	8.056982	0.228886	-2.1273	3.43E-05	0.000401
hsa-miR-1247-5p	96	120.5349	486.6075	113.7084	0.233676	-2.09742	0.000139	0.001423
hsa-miR-877-5p	2524	6.236984	24.64999	5.893618	0.239092	-2.06436	0.000253	0.002376
hsa-miR-331-3p	659	31.4319	122.9842	29.72464	0.241695	-2.04874	4.22E-05	0.000483
hsa-miR-431-3p	981	23.75834	92.71209	22.47249	0.24239	-2.0446	0.00019	0.001845
hsa-miR-423-5p	912	55.97321	216.4412	52.9808	0.244782	-2.03043	0.000171	0.001687
hsa-miR-339-5p	668	109.532	417.2508	103.7937	0.248756	-2.0072	2.45E-05	0.000301
hsa-miR-320a	638	627.8297	2339.304	595.9141	0.25474	-1.9729	6.76E-05	0.000749
hsa-miR-491-5p	1466	3.95618	14.57491	3.758161	0.257851	-1.95539	0.002638	0.017829
hsa-miR-323a-3p	643	3.999895	14.58794	3.802449	0.260657	-1.93978	0.001316	0.010076
hsa-miR-191-3p	325	18.89524	67.29973	17.9926	0.26735	-1.9032	5.01E-05	0.000565
hsa-miR-128-1-5p	156	17.58859	62.45578	16.75191	0.26822	-1.89851	0.000227	0.002144
hsa-miR-503-3p	1514	3.869819	13.68829	3.686724	0.269334	-1.89253	0.00408	0.02607
hsa-miR-330-3p	657	7.363408	25.82285	7.019175	0.27182	-1.87927	0.000925	0.007366
hsa-miR-200c-3p	364	9833.096	33831.85	9385.567	0.277418	-1.84987	0.00018	0.001757
hsa-miR-214-3p	403	19.15192	64.97926	18.29733	0.281587	-1.82835	0.000937	0.007416
hsa-miR-505-5p	1520	16.71789	56.0949	15.98359	0.284938	-1.81128	0.000516	0.004483
hsa-miR-324-5p	649	43.30729	145.0309	41.41035	0.285528	-1.8083	0.000193	0.001861
hsa-miR-127-3p	147	1096.213	3635.633	1048.858	0.288494	-1.79339	0.000632	0.005344
hsa-miR-4326	997	19.57168	63.8683	18.74564	0.293505	-1.76855	0.000774	0.006429
hsa-miR-378i	835	376.7219	1170.792	361.9141	0.309119	-1.69377	0.000711	0.005993
hsa-miR-1266-5p	128	63.23403	195.9807	60.75857	0.310023	-1.68955	0.000593	0.005029
hsa-miR-18a-3p	308	5.806211	17.96418	5.579489	0.31059	-1.68692	0.001665	0.012414
hsa-miR-1301-3p	188	10.1356	30.62769	9.753463	0.318452	-1.65085	0.002951	0.019621
hsa-miR-378c	829	376.6414	1122.943	362.7244	0.323012	-1.63034	0.001257	0.009663
hsa-let-7i-3p	17	128.09	381.4153	123.366	0.323443	-1.62842	0.000745	0.006235
hsa-miR-338-5p	666	16.426	48.38367	15.83005	0.327178	-1.61185	0.005305	0.033041
hsa-miR-342-3p	675	96.49705	283.6436	93.00714	0.327901	-1.60867	0.000796	0.00657
hsa-miR-320b	639	530.7052	1552.19	511.6566	0.329635	-1.60106	0.001873	0.013438
hsa-miR-181b-5p	288	340.1672	988.4352	328.0783	0.331917	-1.59111	0.00117	0.009038
hsa-miR-505-3p	1519	120.2167	346.5526	115.996	0.334714	-1.579	0.001748	0.012835
hsa-miR-3934-5p	878	3.780528	10.5751	3.653822	0.345512	-1.53319	0.00804	0.047314
hsa-miR-106b-3p	31	992.1591	2760.605	959.1811	0.347453	-1.52511	0.004111	0.026133
hsa-miR-3130-5p	535	11.5858	32.17941	11.20177	0.348103	-1.52241	0.007197	0.043076
hsa-miR-200b-5p	363	226.4931	616.7961	219.2148	0.355409	-1.49245	0.003173	0.020851
hsa-miR-455-3p	1165	622.7586	1554.322	605.3868	0.389486	-1.36036	0.006517	0.039391
hsa-miR-664a-3p	2012	34.88704	86.04483	33.93305	0.394365	-1.3424	0.007215	0.043078
hsa-miR-30e-5p	511	10047.43	1979.507	10197.88	5.151726	2.365056	0.005255	0.032808
hsa-miR-218-5p	415	39.07111	6.802227	39.67286	5.832335	2.544074	0.003597	0.023164
hsa-miR-10b-5p	37	137003.2	23387.21	139121.9	5.948633	2.572558	0.002965	0.019657
hsa-miR-152-3p	262	349.014	59.45517	354.4137	5.961023	2.57556	0.002072	0.014608
hsa-miR-451a	1130	695.1483	118.347	705.9045	5.964704	2.57645	0.002367	0.016401
hsa-miR-10a-5p	35	146523	24891.25	148791.2	5.977652	2.579579	0.002945	0.019621
hsa-miR-338-3p	665	1146.718	189.9488	1164.56	6.130916	2.616103	0.002411	0.016613

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hsa-miR-21-3p	402	2293.771	362.673	2329.782	6.423918	2.683453	0.002401	0.016589
hsa-miR-10a-3p	34	20.4245	3.106106	20.74745	6.679571	2.739755	0.004843	0.030395
hsa-miR-22-5p	430	56.88366	8.417513	57.78746	6.865147	2.779291	0.001833	0.013344
hsa-miR-183-5p	298	21744.03	3172.543	22090.35	6.962978	2.799704	0.001172	0.009038
hsa-miR-192-5p	332	181691	25831.69	184597.5	7.146163	2.837169	0.001853	0.013438
hsa-miR-26b-5p	459	2041.818	289.8145	2074.49	7.157992	2.839555	0.000813	0.006692
hsa-miR-3677-3p	764	19.89858	2.738012	20.21859	7.384407	2.884482	0.005695	0.034939
hsa-miR-337-3p	663	32.54284	4.428856	33.06711	7.466287	2.900391	0.003332	0.021628
hsa-miR-598-3p	1851	34.95979	4.754345	35.52306	7.471704	2.901437	0.002317	0.0161
hsa-miR-185-5p	301	124.7783	16.93777	126.7893	7.485595	2.904117	0.001439	0.010928
hsa-miR-199a-3p	351	3749.697	480.6404	3810.659	7.928294	2.98701	0.000917	0.00735
hsa-miR-199b-3p	353	3749.697	480.6404	3810.659	7.928294	2.98701	0.000917	0.00735
hsa-miR-30b-5p	504	810.229	93.21965	823.5998	8.835045	3.143237	0.000456	0.004007
hsa-miR-193a-3p	333	19.1093	2.147505	19.4256	9.045662	3.177226	0.001678	0.012466
hsa-miR-223-5p	426	9.867993	0.962526	10.03406	10.42471	3.381936	0.003026	0.020009
hsa-miR-195-5p	340	80.54483	7.849763	81.90045	10.43349	3.38315	0.000311	0.00288
hsa-miR-455-5p	1166	38.05734	3.500182	38.70176	11.05707	3.466898	0.000286	0.002655
hsa-miR-126-3p	123	1800.668	159.6419	1831.269	11.4711	3.519932	0.000112	0.001182
hsa-miR-17-5p	283	1076.097	92.82846	1094.433	11.78985	3.559473	0.000165	0.001639
hsa-miR-7-1-3p	2402	89.55108	7.625556	91.07882	11.94389	3.578201	0.000182	0.001771
hsa-miR-660-5p	2007	112.3921	9.467646	114.3115	12.07391	3.593821	0.000139	0.001423
hsa-miR-15b-3p	277	47.42316	3.960854	48.23364	12.17758	3.606156	0.000353	0.00318
hsa-miR-148a-3p	251	90921.89	7544.823	92476.71	12.25698	3.615531	0.000145	0.001469
hsa-miR-106a-5p	30	738.3902	59.79972	751.0445	12.55933	3.650688	0.000137	0.001413
hsa-miR-199b-5p	354	252.6002	20.34823	256.9312	12.62671	3.658407	7.11E-05	0.000781
hsa-miR-127-5p	149	596.8146	48.06226	607.0477	12.63045	3.658834	9.93E-05	0.001057
hsa-miR-217	412	90.86857	7.164997	92.42947	12.90014	3.689315	0.000759	0.006329
hsa-miR-188-5p	307	15.98609	1.251859	16.26085	12.98937	3.699259	0.000924	0.007366
hsa-miR-200a-3p	360	800.157	59.29697	813.9726	13.72705	3.77895	7.53E-05	0.000824
hsa-miR-140-5p	229	104.068	7.184831	105.8746	14.73586	3.881259	6.17E-05	0.000687
hsa-miR-376a-3p	818	7.232895	0.481263	7.3588	15.29059	3.934572	0.003342	0.021637
hsa-miR-379-5p	838	1867.398	121.8397	1899.949	15.59385	3.962905	1.88E-05	0.000236
hsa-miR-552-3p	1730	101.9181	6.587911	103.6959	15.74032	3.976393	5.15E-05	0.000578
hsa-miR-374b-5p	813	167.3001	10.75684	170.2193	15.82429	3.984069	2.97E-05	0.000358
hsa-miR-16-5p	281	1160.917	72.34482	1181.216	16.32759	4.02924	3.40E-05	0.0004
hsa-miR-301a-5p	483	17.04604	1.058429	17.34418	16.38672	4.034455	0.001428	0.010873
hsa-miR-29a-5p	475	32.53858	1.884352	33.11023	17.57114	4.135136	0.000167	0.001651
hsa-miR-16-1-3p	279	10.54332	0.599242	10.72876	17.90389	4.162201	0.00252	0.017219
hsa-miR-19b-1-5p	357	32.25181	1.81228	32.81945	18.10948	4.178673	0.000153	0.001532
hsa-miR-24-2-5p	445	61.72163	3.402578	62.80916	18.45929	4.206275	2.80E-05	0.000339
hsa-miR-889-3p	2531	19.89042	1.07995	20.2412	18.74271	4.228258	0.00012	0.001253
hsa-miR-215-5p	406	5175.577	272.1843	5267.016	19.35091	4.27433	7.54E-06	0.000103
hsa-miR-411-5p	906	21.6218	1.117696	22.00416	19.68707	4.299177	0.000398	0.003563
hsa-miR-2355-5p	437	75.57241	3.818884	76.91047	20.13951	4.331957	2.32E-05	0.000289
hsa-miR-203a	368	33469.95	1635.819	34063.6	20.82358	4.380146	8.23E-06	0.000112
hsa-miR-335-3p	661	308.211	14.46693	313.6888	21.68316	4.438503	1.15E-05	0.000152
hsa-miR-1307-5p	198	1383.651	63.24947	1408.274	22.26539	4.476731	8.74E-06	0.000118
hsa-miR-16-2-3p	280	11.92617	0.531814	12.13866	22.82498	4.512542	0.001542	0.011598
hsa-miR-29b-3p	478	2310.667	102.8291	2351.839	22.87134	4.515469	3.68E-06	5.40E-05
hsa-miR-101-5p	23	15.11131	0.636226	15.38124	24.17576	4.595489	0.000496	0.004339
hsa-miR-452-5p	1145	258.9823	10.63358	263.6135	24.79066	4.631725	5.15E-06	7.28E-05
hsa-miR-106b-5p	32	713.1934	27.68836	725.9767	26.21956	4.712572	1.95E-06	3.16E-05
hsa-miR-503-5p	1515	23.74346	0.858469	24.17022	28.15503	4.815321	0.000136	0.00141

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hsa-miR-340-5p	674	32.00443	1.15468	32.57972	28.21537	4.81841	6.06E-05	0.000678
hsa-miR-582-5p	1830	26.33238	0.871829	26.80717	30.74818	4.942429	8.71E-05	0.00094
hsa-miR-20a-5p	384	1245.153	40.48084	1267.618	31.31401	4.968736	1.16E-06	1.99E-05
hsa-miR-141-5p	231	1790.025	57.23668	1822.339	31.83865	4.992707	9.56E-07	1.72E-05
hsa-miR-136-3p	220	10.04082	0.318113	10.22213	32.13366	5.006013	0.000888	0.007187
hsa-miR-4677-3p	1240	8.270769	0.259227	8.420168	32.48184	5.021562	0.002282	0.015902
hsa-miR-182-5p	295	39010.38	1219.708	39715.1	32.56115	5.02508	5.52E-07	1.05E-05
hsa-miR-141-3p	230	868.1016	26.8161	883.7899	32.95743	5.042532	1.06E-06	1.87E-05
hsa-miR-651-5p	1989	8.469031	0.259227	8.622127	33.26092	5.055756	0.001969	0.013961
hsa-miR-1	19	67.9064	2.001746	69.1354	34.53755	5.110094	0.002587	0.017578
hsa-miR-552-5p	1731	342.3273	9.635283	348.5313	36.1724	5.176817	6.79E-07	1.25E-05
hsa-miR-96-5p	2572	47.27156	1.272451	48.12935	37.82411	5.241234	1.04E-05	0.000138
hsa-miR-708-5p	2382	56.49566	1.516964	57.5209	37.91844	5.244828	3.87E-06	5.63E-05
hsa-miR-136-5p	221	64.73582	1.716176	65.91101	38.40574	5.26325	2.61E-06	4.03E-05
hsa-miR-222-5p	424	33.0569	0.872917	33.65706	38.55699	5.26892	1.52E-05	0.000195
hsa-miR-101-3p	22	9467.777	241.9745	9639.82	39.83816	5.316079	1.58E-07	3.56E-06
hsa-miR-708-3p	2381	83.74956	2.116858	85.27185	40.28227	5.332073	1.94E-06	3.16E-05
hsa-miR-144-5p	237	164.1136	4.122318	167.0971	40.53475	5.341087	3.00E-07	6.22E-06
hsa-miR-582-3p	1829	930.3941	23.0351	947.3145	41.12482	5.361937	1.06E-07	2.45E-06
hsa-miR-4791	1425	10.51081	0.259227	10.70198	41.28422	5.367518	0.00096	0.007565
hsa-let-7a-3p	2	93.25915	2.227938	94.9567	42.62089	5.413489	2.06E-06	3.25E-05
hsa-miR-429	956	327.0786	7.663613	333.0351	43.45667	5.441506	3.56E-07	7.17E-06
hsa-miR-203b-3p	369	539.8223	11.16712	549.6806	49.22312	5.621264	1.12E-07	2.58E-06
hsa-miR-33b-5p	672	15.93549	0.318113	16.22672	51.00932	5.672689	0.000856	0.006951
hsa-miR-126-5p	126	425.5089	8.423291	433.2867	51.43912	5.684794	8.56E-08	2.05E-06
hsa-miR-374a-5p	811	86.49797	1.620654	88.08076	54.34889	5.764179	4.35E-07	8.64E-06
hsa-miR-153-5p	265	102.3651	1.408396	104.2477	74.0188	6.20982	3.01E-06	4.59E-05
hsa-let-7f-2-3p	13	23.48096	0.318113	23.9129	75.17111	6.232106	1.73E-05	0.00022
hsa-miR-301a-3p	482	20.45777	0.259227	20.83444	80.37142	6.328611	4.02E-05	0.000463
hsa-miR-33a-5p	670	145.295	1.673133	147.9733	88.44081	6.46664	1.94E-07	4.26E-06
hsa-miR-542-3p	1638	357.7569	3.923463	364.3551	92.86571	6.537074	6.59E-09	2.19E-07
hsa-miR-21-5p	407	484310.7	4983.408	493249.2	98.97828	6.62904	2.17E-09	7.64E-08
hsa-miR-142-3p	232	6616.125	50.08807	6738.568	134.5344	7.071831	5.46E-10	2.11E-08
hsa-miR-135a-5p	217	126.4526	0.871829	128.7945	147.729	7.206809	3.54E-08	9.80E-07
hsa-miR-577	1821	279.2024	1.709484	284.3771	166.3526	7.378101	2.89E-06	4.45E-05
hsa-miR-424-5p	914	333.4213	1.989921	339.6018	170.6609	7.414989	7.48E-10	2.83E-08
hsa-miR-590-5p	1842	47.23186	0.272588	48.10756	176.4848	7.4634	6.51E-07	1.20E-05
hsa-miR-142-5p	233	243.3581	1.403263	247.87	176.6383	7.464654	2.26E-09	7.83E-08
hsa-miR-374a-3p	810	1178.285	6.223592	1200.142	192.8375	7.591241	6.67E-11	2.88E-09
hsa-miR-135b-5p	219	79.31846	0.340015	80.79125	237.6109	7.892457	3.88E-08	1.06E-06
hsa-miR-32-5p	651	68.66026	0.272588	69.93555	256.5618	8.003163	7.16E-08	1.80E-06
hsa-miR-19b-3p	359	311.6285	0.889942	317.4231	356.6786	8.478481	1.01E-10	4.21E-09
hsa-miR-135b-3p	218	5.73676	0	5.843739	Inf	Inf	0.004109	0.026133
hsa-miR-144-3p	236	21.04329	0	21.43571	Inf	Inf	8.25E-05	0.000898
hsa-miR-153-3p	264	8.694724	0	8.856863	Inf	Inf	0.000314	0.002896
hsa-miR-181b-3p	287	18.32387	0	18.66558	Inf	Inf	1.30E-05	0.000168
hsa-miR-18a-5p	309	30.3168	0	30.88215	Inf	Inf	8.44E-07	1.54E-05
hsa-miR-18b-5p	311	21.42392	0	21.82344	Inf	Inf	5.08E-06	7.23E-05
hsa-miR-190a-5p	317	11.78169	0	12.0014	Inf	Inf	8.78E-05	0.000943
hsa-miR-19a-3p	355	154.1403	0	157.0147	Inf	Inf	1.93E-10	7.79E-09
hsa-miR-26a-1-3p	455	10.0573	0	10.24484	Inf	Inf	0.00052	0.004503
hsa-miR-26a-2-3p	456	11.46325	0	11.67701	Inf	Inf	0.000109	0.001158

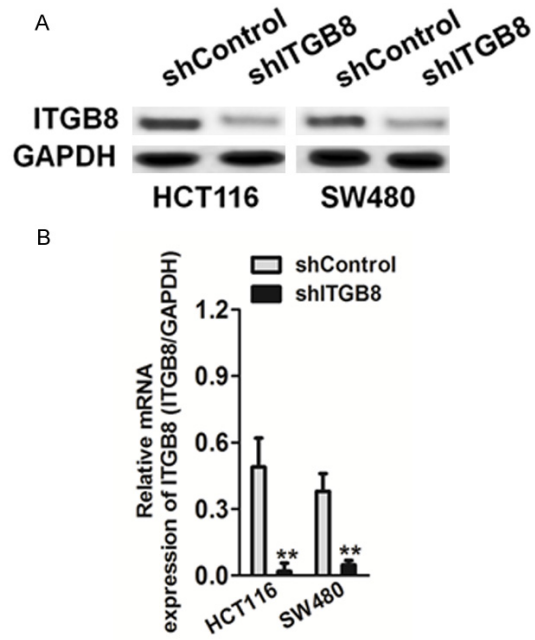
MiR-19b-3p promotes colorectal cancer growth and metastasis

hsa-miR-335-5p	662	44.96798	0	45.80655	Inf	Inf	1.13E-07	2.58E-06
hsa-miR-3613-5p	701	34.84435	0	35.49412	Inf	Inf	4.65E-07	9.14E-06
hsa-miR-369-3p	796	7.090954	0	7.223186	Inf	Inf	0.000818	0.006707
hsa-miR-374b-3p	812	6.022013	0	6.134312	Inf	Inf	0.001608	0.01202
hsa-miR-376c-3p	822	5.01555	0	5.109081	Inf	Inf	0.002867	0.019163
hsa-miR-450a-5p	1117	14.24286	0	14.50846	Inf	Inf	3.25E-05	0.000387
hsa-miR-450b-5p	1119	23.11534	0	23.5464	Inf	Inf	3.19E-06	4.84E-05
hsa-miR-454-3p	1163	16.50982	0	16.81769	Inf	Inf	1.70E-05	0.000217
hsa-miR-495-3p	1472	6.233747	0	6.349994	Inf	Inf	0.001319	0.010076
hsa-miR-548f-3p	1694	4.419817	0	4.502238	Inf	Inf	0.005104	0.031949
hsa-miR-590-3p	1841	10.82339	0	11.02523	Inf	Inf	0.000136	0.00141
hsa-miR-592	1844	93.57421	0	95.31919	Inf	Inf	1.02E-07	2.38E-06
hsa-miR-628-5p	1925	54.68006	0	55.69974	Inf	Inf	6.87E-08	1.74E-06
hsa-miR-671-5p	2025	4.938473	0	5.030566	Inf	Inf	0.003276	0.021321
hsa-miR-7-5p	2436	36.8128	0	37.49929	Inf	Inf	3.01E-07	6.22E-06
hsa-miR-98-3p	2573	11.95185	0	12.17473	Inf	Inf	8.44E-05	0.000915

Supplementary Table 2. Association between miR-19b-3p expression and clinic-pathological characteristics of colorectal cancer

Clinical Variables	miR-19b-3p		
	Low	High	<i>P</i>
Age, years			0.399
≥ 70	11	15	
< 70	9	5	
Sex			0.274
Female	8	14	
Male	11	7	
Tumor size, cm			0.102
≥ 6	6	14	
< 6	7	13	
Differentiation			0.062
Well	3	11	
Moderate	5	8	
Poor	2	11	
TNM stage			0.128
I and II	4	16	
III and IV	15	5	

MiR-19b-3p promotes colorectal cancer growth and metastasis



Supplementary Figure 1. To knock down ITGB8, shITGB8 and shControl was transfected HCT116 and SW480 cells. The ITGB8 expressing level was detected by western blotting (A) and qRT-PCR (B).