Elevated MIR100HG promotes colorectal cancer metastasis and is associated with poor prognosis

WENHUA LI[1,2], FUKANG YUAN[3], XIN ZHANG[4], WANYUAN CHEN[4], XUEJUN TANG[2] and LUNGEN LU[1]

[1]Department of Gastroenterology, Shanghai General Hospital of Nanjing Medical University, Shanghai 201600;
[2]Department of Gastroenterology, The Affiliated Wuxi Second People's Hospital of Nanjing Medical University, Wuxi,
Jiangsu 214002; [3]Department of General Surgery, Xuzhou Central Hospital, Xuzhou Institute of Cardiovascular Disease,
Jiangsu, Xuzhou 221009; [4]Department of Pathology, Zhejiang Provincial People's Hospital, People's Hospital of
Hangzhou Medical College, Hangzhou, Zhejiang 310014, P.R. China

Abstract. mir-100-let-7a-2-mir-125b-1 cluster host gene (MIR100HG), which is located on chromosome 11q24.1, is a polycistronic microRNA host gene. MIR100HG overexpression in colorectal cancer (CRC) has been demonstrated to be associated with cetuximab resistance; however, the role of MIR100HG in CRC metastasis remains unclear. The present study aimed to investigate the impact of aberrant MIR100HG expression on metastasis and prognosis in patients with CRC. The results from reverse transcription-quantitative PCR demonstrated that MIR100HG expression was higher in CRC tissues compared with in corresponding normal mucosa tissues. In particular, MIR100HG expression was higher in advanced CRC compared with in early stage CRC. Furthermore, the results from Kaplan-Meier analysis followed by a log-rank test revealed that patients with CRC and high MIR100HG expression exhibited poorer disease-free survival and overall survival compared with patients with CRC and lower MIR100HG expression. Furthermore, results from *in vitro* Transwell assays and *in vivo* animal assays demonstrated that upregulated MIR100HG expression promoted CRC cell migration and invasion and the formation of liver metastatic colonies in mice. In conclusion, the present study demonstrated that MIR100HG overexpression may contribute to the progression of CRC and may predict a poorer prognosis in patients with CRC. MIR100HG may therefore be considered as a novel therapeutic target and a prognostic biomarker in patients with CRC.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed type of cancer worldwide, and represents a leading cause of mortality in men and women (1,2). Tumor invasion and distant metastasis are the main causes of cancer-associated mortality in patients with CRC (3). The liver is the most frequent site of metastatic spread in CRC, and 15-25% of patients with CRC present with liver metastases at the time of diagnosis (4). Although improvements in screening tests, chemotherapy, surgical techniques and multidisciplinary approaches have substantially decreased the morbidity and mortality rates of patients with CRC in the last decades (5,6), the prognosis of patients with advanced-stage CRC remains unsatisfactory due to frequent metastasis and CRC recurrence (7). Understanding the underlying mechanisms of CRC, particularly during colorectal liver metastasis, is therefore crucial to improve the clinical outcomes and overall survival of patients.

Long non-coding RNAs (lncRNAs) are defined as transcripts of >200 nucleotides in length that are not translated into proteins, and account for much of the transcribed genome (8). lncRNAs can promote or inhibit the expression of various protein-coding genes through interactions with other cellular macromolecules, including DNA, RNA and proteins (8). Numerous studies have reported that lncRNAs are crucial for the regulation of major physiological and pathological processes, including cell growth, apoptosis, stem-cell pluripotency, cancer invasion, metastasis, development, differentiation and the immune response, and serve a role in early diagnosis, targeted therapy and drug resistance of cancers (9-15). A previous study has demonstrated that the lncRNA mir-100-let-7a-2-mir-125b-1 cluster host gene (MIR100HG) is significantly associated with cetuximab resistance (15); however, other functional roles of MIR100HG in the development of cancer, particularly CRC, remain unknown and require further investigation.

In the present study, MIR100HG expression in CRC tissues and paired normal mucosae was assessed by reverse

Correspondence to: Dr Lungen Lu, Department of Gastroenterology, Shanghai General Hospital of Nanjing Medical University, 650 Xinsongjiang Road, Shanghai 201600, P.R. China
E-mail: lung_enlu1965@163.com

Dr Xuejun Tang, Department of Gastroenterology, The Affiliated Wuxi Second People's Hospital of Nanjing Medical University, 68 Zhongshan Road, Wuxi, Jiangsu 214002, P.R. China
E-mail: 13771118608@163.com

transcription-quantitative polymerase chain reaction (RT-qPCR). In addition, the association between MIR100HG expression and the clinicopathological characteristics of patients with CRC was assessed. Furthermore, Kaplan-Meier analysis with the log-rank test was used to evaluate the prognostic value of MIR100HG in patients with CRC. In addition, *in vitro* and *in vivo* cell function assays were performed to investigate the impact of MIR100HG on CRC cell migration and invasion and to determine the potential underlying mechanisms of CRC progression. The findings of the present study suggested that MIR100HG may be involved in CRC invasion and liver metastasis.

Materials and methods

Patients and specimens. A total of 116 paired CRC tissues and matched normal mucosae were collected from patients with CRC who underwent tissue biopsy at the Department of Gastroenterology, Shanghai General Hospital of Nanjing Medical University between September 2010 and March 2016. Patients comprised 60 men and 56 women (mean age, 64 years; age range, 32-89 years). All specimen were histopathologically confirmed as CRC. No patients had received local or other related anticancer therapies prior to tumor biopsy. The tumor grade and clinical stages were classified according to the guidelines of the American Joint Committee on Cancer (AJCC) (16). The present study was approved by the Ethics Committee of Shanghai General Hospital of Nanjing Medical University, and written informed consent was obtained from all patients prior to enrollment in the present study.

RNA extraction and RT-qPCR. Total RNA was isolated from fresh CRC and adjacent normal mucosae tissues using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). First strand cDNA was synthesized from 2 μg RNA using a Prime-Script PCR reagent kit (Takara Bio, Inc.) according to the manufacturer's protocol. By using the LightCycler®480 system (Roche Applied Science), the SYBR Premix Ex-Taq II kit (Takara Bio, Inc.) was applied to perform qPCR. The cycling conditions were as follows: Initial denaturation (2 min at 95˚C) followed by 40 cycles of denaturation (10 sec at 95˚C), annealing (30 sec at 59˚C), elongation (30 sec at 72˚C) and a final extension (30 sec at 72˚C). The amplified samples were then maintained at 4˚C. The primer sequences used for MIR100HG detection were as follows: MIR100HG forward, 5'-CCCAGTGCAAGGACAAAGA-3' and reverse, 5'-GCAGAGGAGGTGTCTTCAGG-3'; and GAPDH forward, 5'-GGGAAATTCAACGGCACAGT-3' and reverse, 5'-AGA TGGTGATGGGCTTCCC-3'. The relative expression levels of MIR100HG were normalized to the endogenous control GAPDH and expressed as $2^{-\Delta\Delta Cq}$ (17).

Cell culture and transfection. The human CRC LoVo, DLD-1, RKO, SW48, HCT8 and HCT116 cell lines, and the normal intestinal mucous epithelium FHC cell line were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences. All cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin and placed at 37˚C in a humidified incubator containing 5% CO_2.

The CRC cell line LOVo overexpressing MIR100HG was established by transfection with lentiviral vectors encoding human MIR100HG (PCDH-MIR100HG). In addition, MIR100HG-knockdown CRC cell line HCT116 were constructed by transfection with MIR100HG short hairpin RNA (shRNA). The sequences used for overexpression and knockdown were previously described (18).

In vitro Transwell assay. By using the two transfected CRC cell lines LoVo and HCT116 and their control groups, transwell assays were used to assess tumor cell migration and invasion. A Transwell 24-well Boyden chamber with a polycarbonate membrane (pore size, 8.0 μm; Corning Inc.) was used for cell migration (without Matrigel® coating; BD Biosciences) and invasion (with Matrigel® coating, prepared on ice) assays, according to the manufacturer's protocol. Briefly, 5x10⁵ cells/ml were seeded in 200 μl serum-free DMEM medium in the upper chamber, whereas DMEM medium supplemented with 500 μl 10% FBS was added to the lower chamber. Following incubation for 24 h, cells were fixed with 4% polyoxymethylene and stained with 0.1% crystal violet at room temperature. Cells that had moved to the lower side of the membrane were counted in 10 visual fields and the average value was calculated. Cells images were captured using a light microscope (Olympus Corporation; magnification, x200). Each experiment was performed independently three times.

In vivo metastatic assay. Nude mice were purchased from Shanghai Research Center for Model Organisms, Inc. Mice had an average weight of 20 g. Mice were placed in plastic cages with airtight air filter at the temperature of 18-22˚C, humidity of 40-60%, under a light/dark cycle of 10/14 h each day, and had free access to food and water. For the *in vivo* cell metastatic assay, following anesthesia with ether inhalation, MIR100HG-overexpressing CRC cells LoVo, sh-MIR100HG knockdown CRC cells HCT116 and their control groups were injected (200 μl at the density of 1x10⁶/ml in PBS) into the tail vein of nude mice (4 weeks old male BALB/C nude mice; n=3 for each group). After 4 weeks, mice were euthanized by cervical dislocation, and livers were collected and immediately fixed with 4% formaldehyde at room temperature for 24-48 h. After paraffin embedding, liver tissue was cut into 4-7 μm-thick sections. After hematoxylin-eosin staining at room temperature for 3-5 min, the tumor colonies formed in the livers were detected by light microscopy and quantified using GraphPad Prim 7 (GraphPad Software, Inc.). All experimental procedures and animal studies involving mice were in accordance with the Shanghai General Hospital of Nanjing Medical University Animal Care and Use guidelines. Ethical approval for the animal study was obtained from the Ethics Committee of Shanghai General Hospital of Nanjing Medical University.

Statistical analysis. Each experiment was performed independently three times. Data were expressed as the means ± standard deviation and were analyzed using SPSS v22.0 software (IBM Corp.). Student's t-test or one-way analysis of variance followed by Student-Newman-Keuls post hoc test were used to analyze the differences in MIR100HG expression among the continuous variables. A χ^2 test or Fisher's exact test was appropriately used to determine the statistical significance between

Table I. MIR100HG expression in paired normal colorectal mucosa and tumor tissues.

Tissue samples	Number	Relative MIR100HG expression		P-value
		Low, n (%)	High, n (%)	
Normal tissues	116	87 (75.0)	29 (25.0)	<0.001
Tumor tissue	116	37 (31.9)	79 (68.1)	

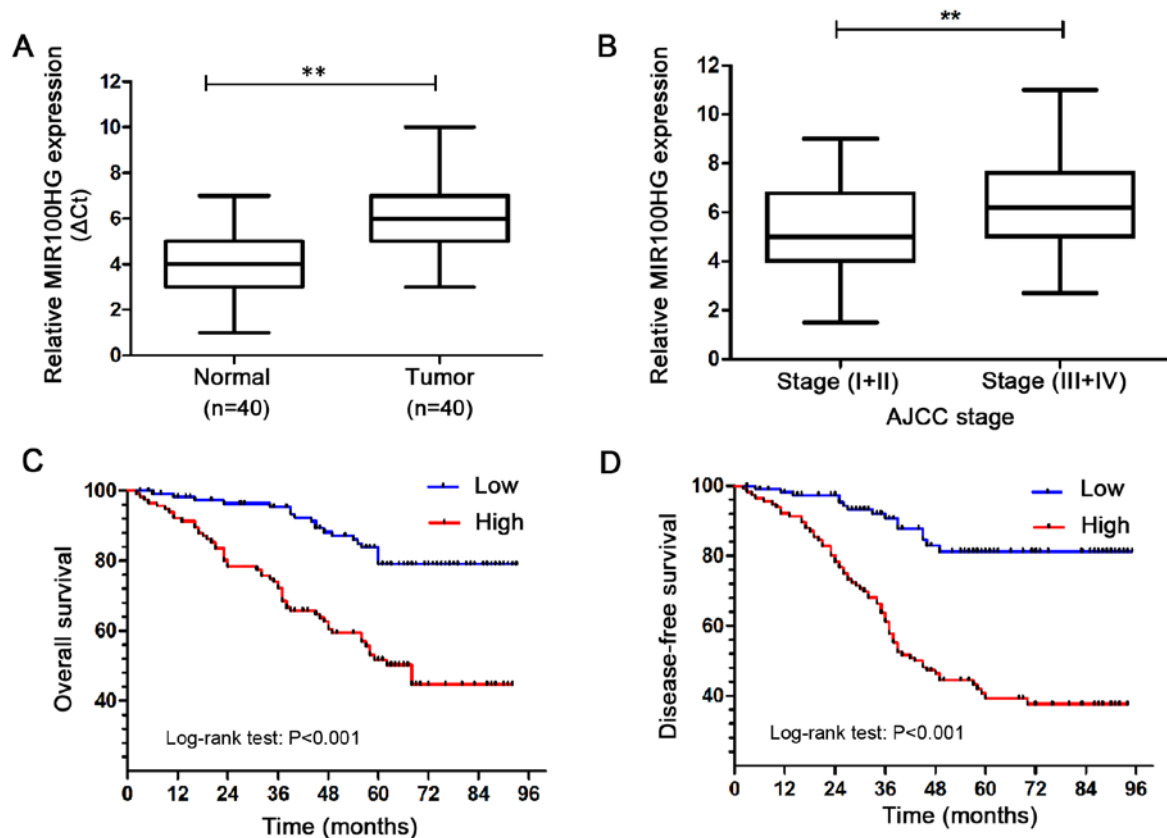MIR100HG, mir-100-let-7a-2-mir-125b-1 cluster host gene.

Figure 1. MIR100HG expression was significantly upregulated in CRC tissues and could predict a poor prognosis. (A) Expression levels of MIR100H in 40 paired CRC and adjacent noncancerous tissues. (B) MIR100HG expression level in low clinical stage (I-II) CRC tissues and advanced stage (III-IV) CRC tissues was analyzed. Kaplan-Meier analysis and log-rank test of the (C) overall survival and (D) disease-free survival in patients with CRC according to MIR100HG expression level. AJCC, American Joint Committee on Cancer; CRC, colorectal cancer; MIR100HG, mir-100-let-7a-2-mir-125b-1 cluster host gene. **P<0.01 vs. normal tissue.

MIR100HG expression and the patients' clinicopathological characteristics. Kaplan-Meier analysis and the log-rank test were used for the comparison of patients' survival curves. The hazard ratio with 95% confidence interval in the Cox proportional hazards model was calculated to measure the hazard risk of individual factors for disease-free survival (DFS) and overall survival (OS). All graphs were plotted using GraphPad Prism v5.0 software (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

High MIR100HG expression is associated with an aggressive phenotype in patients with CRC. A total of 40 paired CRC and normal specimen were randomly selected from the 116 patients to detect MIR100HG expression by RT-qPCR. The results demonstrated that MIR100HG expression was significantly increased in CRC tissues compared with adjacent normal colon mucosae (Fig. 1A). Furthermore, MIR100HG expression was examined in CRC tissues at different stages. The results revealed that MIR100HG expression was significantly higher in advanced-stage (III+IV) CRC tissues compared with low stage (I+II) CRC tissues (Fig. 1B). These results demonstrated that MIR100HG was significantly elevated in CRC and may be associated with an aggressive phenotype in patients with CRC.

Subsequently, the association between MIR100HG expression and TNM stage in the 116 patients with CRC was investigated (19). MIR100HG expression was first evaluated

Table II. Association between MIR100HG expression and clinicopathological characteristics of patients with colorectal cancer.

Variables	Number (%)	Relative MIR100HG expression		P-value
		Low, n (n=37, %)	High, n (n=79, %)	
Age, years				0.596
<65	54 (46.2)	20 (54.1)	34 (43.0)	
≥65	62 (53.8)	17 (45.9)	45 (57.0)	
Sex				0.629
Male	60 (51.7)	16 (43.2)	44 (55.7)	
Female	56 (48.3)	21 (56.8)	35 (44.3)	
Tumor size, cm				0.067
<3	62 (53.4)	25 (67.6)	37 (46.8)	
≥3	54 (46.6)	12 (32.4)	42 (53.2)	
Location				0.416
Right	29 (25.0)	6 (16.2)	23 (29.1)	
Transverse	27 (23.3)	17 (45.9)	10 (12.7)	
Left	60 (51.7)	14 (37.9)	46 (58.2)	
AJCC stage				0.024[a]
I-II	45 (38.8)	24 (64.9)	21 (26.6)	
III-IV	71 (61.2)	13 (35.1)	58 (73.4)	
pT stage				0.048[a]
T1	17 (14.6)	10 (27.0)	7 (8.9)	
T2	46 (39.7)	12 (32.4)	34 (43.0)	
T3	37 (31.9)	11 (29.8)	26 (32.9)	
T4	16 (13.8)	4 (10.8)	12 (15.2)	
pN stage				0.020[a]
N0	45 (38.8)	24 (64.9)	21 (26.6)	
N1-2	71 (61.2)	13 (35.1)	58 (73.4)	
pM stage				0.023[a]
M0	101 (87.1)	37 (100)	64 (81.0)	
M1	15 (12.9)	0 (0.0)	15 (19.0)	
Differentiation				0.035[a]
Well	31 (26.7)	19 (51.4)	12 (15.2)	
Moderate	40 (34.5)	10 (27.0)	30 (38.0)	
Poor	45 (38.8)	8 (21.6)	37 (46.8)	
Vascular invasion				0.862
Yes	3 (2.6)	0 (0.0)	3 (3.8)	
No	113 (97.4)	37 (100)	76 (96.2)	

[a]$P<0.05$. AJCC, American Joint Committee on Cancer; MIR100HG, mir-100-let-7a-2-mir-125b-1 cluster host gene.

in the 116 paired samples by RT-qPCR. A mean value of 5.55 was calculated. CRC samples with an expression level of MIR100HG ≥5.55 and <5.55 were classified in the high and low expression groups, respectively. Briefly, 75.0% (87/116) of specimens exhibited low MIR100HG expression in normal colorectal mucosa tissues. Furthermore, 25.0% (29/116) of normal colorectal mucosa tissues presented with high MIR100HG expression, whereas 68.1% (79/116) of CRC samples presented with high MIR100HG expression (Table I). In addition, high MIR100HG expression was positively associated with T stage, lymph node metastasis, distant metastasis, AJCC stage and histological differentiation in CRC samples (Table II). These results further indicated that MIR100HG overexpression may serve a role in CRC metastasis and in the prognosis of patients with CRC.

MIR100HG upregulation predicts an unfavorable prognosis and poor survival for patients with CRC. The prognostic value of MIR100HG expression in CRC was evaluated by assessing the DFS and OS of the 116 patients with CRC using Kaplan-Meier analysis and log-rank test. The results

Table III. Univariate and multivariate analyses of disease-free survival in patients with colorectal cancer.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
MIR100HG	3.658	1.956-6.893	0.001[a]	4.105	1.809-6.842	0.001[a]
Age	1.165	0.758-2.345	0.456	-	-	-
Sex	1.208	0.765-1.998	0.547	-	-	-
Tumor size	1.077	0.657-1.954	0.658	-	-	-
Location	0.895	0.384-1.848	0.506	-	-	-
AJCC Stage	4.924	1.264-5.831	0.002[a]	5.264	1.249-6.048	0.001[a]
T classification	1.216	1.851-2.536	0.035[a]	1.768	1.959-2.833	0.027[a]
N classification	1.659	1.181-2.611	0.032[a]	1.937	1.349-3.117	0.022[a]
M classification	3.786	1.854-4.594	0.003[a]	3.853	2.432-4.897	0.001[a]
Differentiation	1.821	1.125-2.543	0.021[a]	2.018	1.275-3.958	0.023[a]
Vascular invasion	1.354	0.872-1.685	0.183	-	-	-

[a]P<0.05 indicates that the 95% CI of the HR did not include 1. AJCC, American Joint Committee on Cancer; HR, hazard ratio; MIR100HG, mir-100-let-7a-2-mir-125b-1 cluster host gene.

Table IV. Univariate and multivariate analyses of overall survival in patients with colorectal cancer.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
MIR100HG	3.241	2.843-7.231	<0.001	3.533	1.877-5.958	<0.001
Age	1.072	0.694-2.017	0.442	-	-	-
Sex	1.128	0.911-1.333	0.526	-	-	-
Tumor size	0.987	0.733-2.142	0.083	-	-	-
Location	1.035	0.767-1.842	0.723	-	-	-
AJCC Stage	3.951	2.523-5.015	<0.001	4.017	2.349-3.483	<0.001
T classification	1.212	1.017-2.335	0.047[a]	2.012	1.954-2.874	0.031[a]
N classification	1.537	1.061-4.938	0.043[a]	1.977	1.575-3.213	0.044[a]
M classification	3.596	2.427-4.938	<0.001	3.672	2.731-5.376	<0.001
Differentiation	2.017	1.029-3.240	0.033[a]	1.996	1.233-2.841	0.042[a]
Vascular invasion	1.282	1.134-2.047	0.097	-	-	-

[a]P<0.05 indicates that the 95% CI of the HR did not include 1. AJCC, American Joint Committee on Cancer; HR, hazard ratio; MIR100HG, mir-100-let-7a-2-mir-125b-1 cluster host gene.

demonstrated that the DFS and OS of patients with CRC and high MIR100HG expression were shorter compared with those of patients with CRC and low MIR100HG expression (Fig. 1C and D). These results suggested that high MIR100HG expression may predict a poor prognosis and survival. Furthermore, the results from univariate and multivariate Cox regression analyses demonstrated that MIR100HG expression, AJCC stage, T classification, N classification, M classification and tumor differentiation may be considered as independent prognostic factors for DFS and OS (Tables III and IV). These findings suggested that high MIR100HG expression in CRC tissues may be considered as a prognostic factor for patients with CRC.

Association between MIR100HG expression and clinicopathological characteristics. To further confirm the clinical significance of MIR100HG expression in CRC, the association between MIR100HG expression and the clinicopathological characteristics of patients with CRC was assessed (Table II). The results demonstrated that high MIR100HG expression was significantly associated with AJCC stage (P=0.024), pathological T stage (P=0.048), N stage (P=0.020), M stage (P=0.023) and tumor differentiation (P=0.035). However, no association was identified between high MIR100HG expression and other clinical parameters, including age, sex, tumor size, tumor location and vascular invasion status (P>0.05; Table II). These findings suggested that
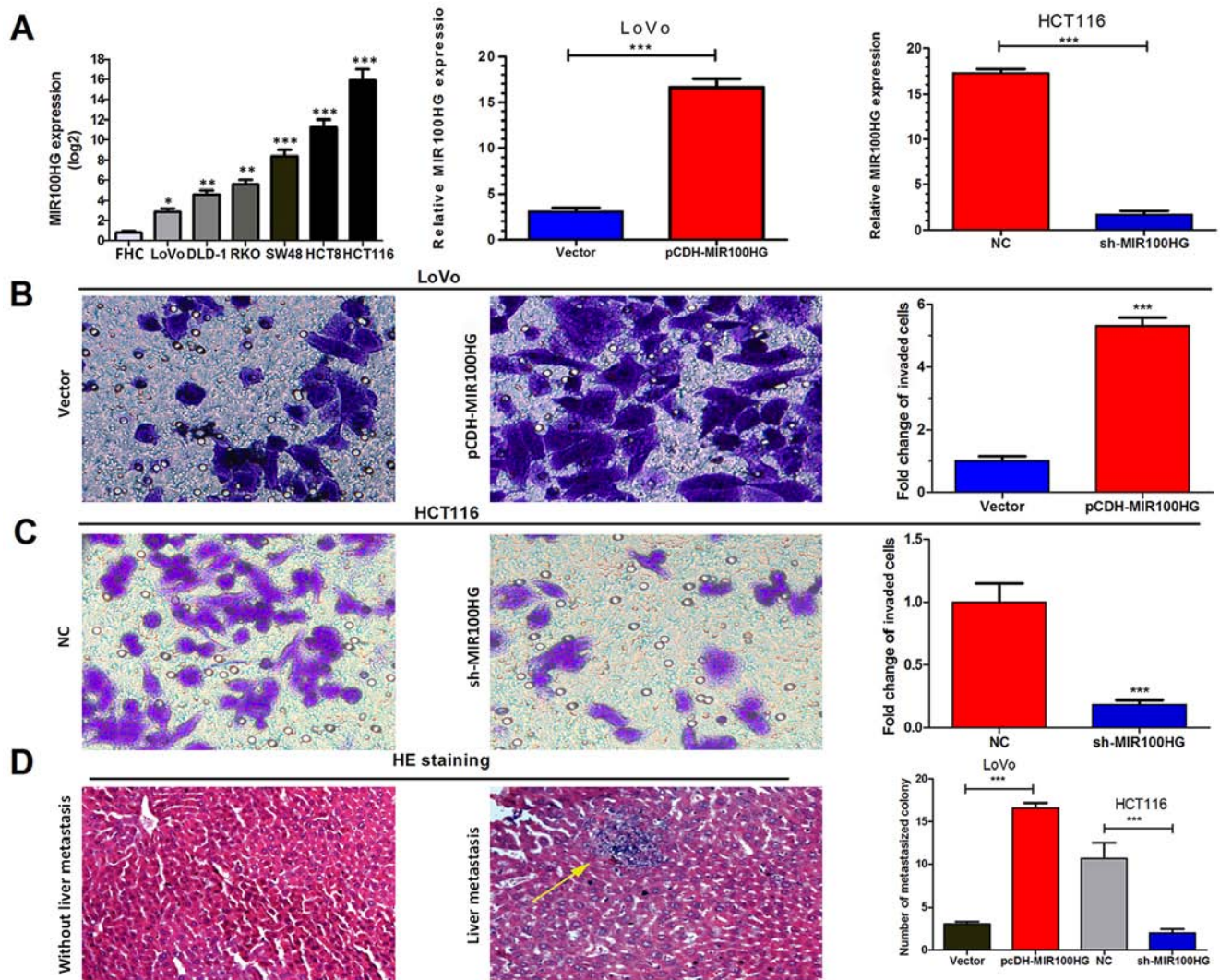
Figure 2. MIR100HG facilitated CRC cell migration and invasion *in vitro* and liver metastasis *in vivo*. (A) Relative expression levels of MIR100HG in six colorectal cell lines and the normal intestinal mucous epithelium cell line FHC. Transfection efficiency in LoVo and HCT116 cell lines. *P<0.05, **P<0.01 and ***P<0.001 vs. FHC. (B and C) Impact of MIR100HG overexpression or knockdown on CRC cell migration and invasion. Magnification, x200. (D) Impact of MIR100HG overexpression and knockdown on liver metastasis in nude mice *in vivo*. Left panel: Without liver metastasis. Right panel: With liver metastasis. HE staining of metastatic colonies formed in the liver. Magnification, x100. The yellow arrow indicates metastatic colonies. NC, negative control; sh, short hairpin RNA; MIR100HG, mir-100-let-7a-2-mir-125b-1 cluster host gene; HE, hematoxylin and eosin. ***P<0.001 vs. control.

that high MIR100HG expression may be associated with CRC migration and invasion.

Effect of MIR100HG on CRC cell migration and invasion in vitro and in vivo. To further investigate the role of MIR100HG in CRC cells, MIR100HG expression in various CRC cell lines (LoVo, DLD-1, RKO, SW48, HCT8 and HCT116) and the normal intestinal mucous epithelium FHC cell line was assessed by RT-qPCR. The results demonstrated that CRC cell lines exhibited higher MIR100HG expression compared with the FHC cell line (Fig. 2A). Subsequently, the HCT116 cell line, which exhibited the highest expression levels of MIR100HG, was selected to establish a cell line with knockdown of MIR100HG. Conversely, the LoVo cell line, which displayed the lowest MIR100HG expression level, was selected to establish an overexpression model for MIR100HG. The results of RT-qPCR analysis demonstrated that the transfections were successful (Fig. 2A).

In vitro Transwell assays were used to assess the function of MIR100HG in CRC progression. MIR100HG overexpression significantly enhanced the migration and invasion of LoVo cells (P<0.001; Fig. 2B), whereas MIR100HG-knockdown significantly inhibited the migration and invasion of HCT116 cells (P<0.001; Fig. 2C). These results suggested that MIR100HG overexpression may promote the aggressive phenotype of CRC cells *in vitro*.

Subsequently, by injecting CRC cells LoVo and HCT116 into tail veins of nude mice, the impact of MIR100HG on the migratory and invasive capacities of CRC cells was investigated. After 4 weeks, the results demonstrated that higher and lower numbers of liver metastatic colonies were formed in the MIR100HG-overexpression and knockdown groups, respectively, compared with their corresponding controls (P<0.001; Fig. 2D). MIR100HG overexpression may therefore enhance the migratory and invasive capacities of CRC cells *in vitro* and *in vivo*.

Discussion

Recent studies have reported that distant metastasis remains the main cause of mortality in patients with CRC, and that the liver is the most common metastatic site (4,6). In total, 15-25% of patients with CRC have liver metastases at the time of diagnosis (20). In addition, 30-50% of patients with CRC who undergo radical resection will have local and systemic recurrences, and their risk of recurrence following metastatic resection is ~75% (4-7). It is therefore crucial to understand the mechanism of CRC and metastasis development. The present study demonstrated that MIR100HG expression was higher in CRC tissues compared with adjacent normal mucosa, and that MIR100HG overexpression was associated with poor prognosis and survival in patients with CRC. In addition, MIR100HG overexpression enhanced the migratory and invasive capacities of CRC cells *in vitro* and liver metastasis *in vivo*, which indicated that MIR100HG expression may be crucial in CRC progression, particularly in colorectal liver metastasis.

MIR100HG, which is located on chromosome 11q24.1, is a polycistronic micro RNA host gene that is associated with the progression of several types of tumor (21-23). It has been reported that MIR100HG overexpression can predict a poor prognosis and is associated with metastasis in cervical cancer (21). In addition, MIR100HG overexpression is associated with OS in patients with oral cavity cancer (22). MIR100HG is also overexpressed in acute megakaryoblastic leukemia (23), and aberrant MIR100HG expression has been reported in patients with CRC and resistant to cetuximab (24). However, the clinical significance and prognostic value of MIR100HG expression in CRC remains unclear. The present study elucidated MIR100HG expression in CRC samples, examined its association with clinicopathological characteristics and distant metastasis in patients with CRC, and determined whether it could be considered as a prognosis factor.

The results of RT-qPCR demonstrated that MIR100HG expression was increased in CRC tissues compared with normal tissues. Furthermore, MIR100HG expression in advanced stage (III-IV) CRC tissues was significantly increased compared with low stage (I-II) CRC tissues, which was consistent with a previous study from The Cancer Genome Atlas data repository (24). Subsequently, the association between high MIR100HG expression and the clinicopathological characteristics of patients with CRC was investigated. Previous studies on acute megakaryoblastic leukemia, early-stage cervical cancer and head and neck squamous cell carcinoma have reported that MIR100HG overexpression accelerates tumor malignancy and is associated with poor prognosis and survival (21-23). Similarly, the present study reported that MIR100HG overexpression was associated with poor prognosis and survival in patients with CRC. Furthermore, univariate and multivariate Cox regression analyses demonstrated that MIR100HG expression, AJCC stage, N classification, M classification and tumor differentiation were significantly associated with DFS and OS. Subsequently, *in vitro* cell function assays investigated the impact of MIR100HG on the migratory and invasive capacities of CRC cells. The results demonstrated that MIR100HG upregulation enhanced CRC cell migration and

invasion *in vitro*, and liver metastasis *in vivo*. These findings suggested that MIR100HG may have crucial roles in CRC invasion and liver metastasis.

In conclusion, the present study reported the crucial roles of MIR100HG in CRC progression, and indicated that MIR100HG may serve as a novel prognostic biomarker for CRC. Furthermore, future manipulation of MIR100HG levels may represent a potential treatment strategy for patients with CRC, particularly those with colorectal liver metastasis. It has been reported that MIR100HG is the host gene of the microRNA (miR)-100/let-7a-2/miR-125b-1 cluster on chromosome 11, and that MIR100HG overexpression can upregulate miR-100 and miR-125b (24). The present study did not examine the impact of MIR100HG on miR-100 and miR-125b-1 expression levels. Future studies will therefore examine the effect of modulating MIR100HG expression on miR-100 and miR-125b expression levels, and will investigate how MIR100HG could promote CRC development and distant metastasis.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LL and XT conceived the study and wrote the manuscript. WL and FY performed the experiments. XZ and WC analyzed and interpreted the data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanghai General Hospital of Nanjing Medical University. All patients provided written informed consent prior to the present study. Animal studies involving mice were in accordance with the Shanghai General Hospital of Nanjing Medical University Animal Care and Use guidelines. Ethical approval was obtained from the Ethics Committee of Shanghai General Hospital of Nanjing Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2018. CA Cancer J Clin 68: 7-30, 2018.
3. Bhullar DS, Barriuso J, Mullamitha S, Saunders MP, O'Dwyer ST and Aziz O: Biomarker concordance between primary colorectal cancer and its metastases. EBioMedicine 40: 363-374, 2019.
4. Hur K, Toiyama Y, Okugawa Y, Ide S, Imaoka H, Boland CR and Goel A: Circulating microRNA-203 predicts prognosis and metastasis in human colorectal cancer. Gut 66: 654-65, 2017.
5. Cronin KA, Lake AJ, Scott S, Sherman RL, Noone AM, Howlader N, Henley SJ, Anderson RN, Firth AU, Ma J, *et al*: Annual report to the nation on the status of cancer, part I: National cancer statistics. Cancer 124: 2785-800, 2018.
6. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A and Jemal A: Colorectal cancer statistics, 2017. CA Cancer J Clin 67: 177-193, 2017.
7. van der Valk MJM, Hilling DE, Bastiaannet E, Meershoek-Klein Kranenbarg E, Beets GL, Figueiredo NL, Habr-Gama A, Perez RO, Renehan AG, van de Velde CJH and IWWD Consortium: Long-term outcomes of clinical complete responders after neoadjuvant treatment for rectal cancer in the International Watch & Wait Database (IWWD): An international multicentre registry study. Lancet 391: 2537-2545, 2018.
8. Kopp F and Mendell JT: Functional classification and experimental dissection of long noncoding RNAs. Cell 172: 393-407, 2018.
9. Leisegang MS, Fork C, Josipovic I, Richter FM, Preussner J, Hu J, Miller MJ, Epah J, Hofmann P, Gunther S, *et al*: Long noncoding RNA MANTIS facilitates endothelial angiogenic function. Circulation 136: 65-79, 2017.
10. Sahakyan A, Kim R, Chronis C, Sabri S, Bonora G, Theunissen TW, Kuoy E, Langerman J, Clark AT, Jaenisch R and Plath K: Human naive pluripotent stem cells model X chromosome dampening and X inactivation. Cell Stem Cell 20: 87-101, 2017.
11. Marin-Bejar O, Mas AM, Gonzalez J, Martinez D, Athie A, Morales X, Galduroz M, Raimondi I, Grossi E, Guo S, *et al*: The human lncRNA LINC-PINT inhibits tumor cell invasion through a highly conserved sequence element. Genome Biol 18: 202, 2017.
12. Grelet S, Link LA, Howley B, Obellianne C, Palanisamy V, Gangaraju VK, Diehl JA and Howe PH: A regulated PNUTS mRNA to lncRNA splice switch mediates EMT and tumour progression. Nature Cell Biol 19: 1105-15, 2017.
13. Jiang M, Zhang S, Yang Z, Lin H, Zhu J, Liu L, Wang W, Liu S, Liu W, Ma Y, *et al*: Self-Recognition of an inducible host LncRNA by RIG-I feedback restricts innate immune response. Cell 173: 906-19.e13, 2018.
14. Atianand MK, Caffrey DR and Fitzgerald KA: Immunobiology of long noncoding RNAs. Annu Rev Immunol 35: 177-98, 2017.
15. Li L, Lv G, Wang B and Kuang L: The role of lncRNA XIST/miR-211 axis in modulating the proliferation and apoptosis of osteoarthritis chondrocytes through CXCR4 and MAPK signaling. Biochem Biophys Res Commun 503: 2555-2562, 2018.
16. O'Connell JB, Maggard MA and Ko CY: Colon cancer survival rates with the new American Joint Committee on cancer sixth edition staging. J Natl Cancer Inst 96: 1420-1425, 2004.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
18. Wang S, Ke H, Zhang H, Ma Y, Ao L, Zou L, Yang Q, Zhu H, Nie J, Wu C and Jiao B: LncRNA MIR100HG promotes cell proliferation in triple-negative breast cancer through triplex formation with p27 loci. Cell Death Dis 9: 805, 2018.
19. Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, Lugli A, Zlobec I, Rau TT, Berger MD, *et al*: International validation of the consensus Immunoscore for the classification of colon cancer: A prognostic and accuracy study. Lancet 391: 2128-2139, 2018.
20. van der Geest LG, Lam-Boer J, Koopman M, Verhoef C, Elferink MA and de Wilt JH: Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis 32: 457-465, 2015.
21. Shang C, Zhu W, Liu T, Wang W, Huang G, Huang J, Zhao P, Zhao Y and Yao S: Characterization of long non-coding RNA expression profiles in lymph node metastasis of early-stage cervical cancer. Oncol Rep 35: 3185-3197, 2016.
22. Wilkins OM, Titus AJ, Salas LA, Gui J, Eliot M, Butler RA, Sturgis EM, Li G, Kelsey KT and Christensen BC: MicroRNA-related genetic variants associated with survival of head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 28: 127-136, 2019.
23. Emmrich S, Streltsov A, Schmidt F, Thangapandi VR, Reinhardt D and Klusmann JH: LincRNAs MONC and MIR100HG act as oncogenes in acute megakaryoblastic leukemia. Mol Cancer 13: 171, 2014.
24. Lu Y, Zhao X, Liu Q, Li C, Graves-Deal R, Cao Z, Singh B, Franklin JL, Wang J, Hu H, *et al*: lncRNA MIR100HG-derived miR-100 and miR-125b mediate cetuximab resistance via Wnt/beta-catenin signaling. Nat Med 23: 1331-1341, 2017.