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Long non-coding RNA Fer-1-like protein 4 suppresses oncogenesis and exhibits prognostic value by associating with miR-106a-5p in colon cancer

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Key words

colon cancer, FER1L4, MiR-106a-5p, prognosis, tumor marker

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Novel long non-coding RNA Fer-1-like protein 4 (FER1L4) has been confirmed to play crucial regulatory roles in tumor progression. It exerts an impact on tumor suppression and functions as a competing endogenous RNA (ceRNA) by sponging miR-106a-5p in gastric cancer. However, its clinical significance in colon cancer is completely unknown. The aim of the present study was to annotate the role of FER1L4 and its clinical value in colon cancer. The results showed the aberrant expression of FER1L4 and miR-106a-5p in colon cancer tissues. In addition, significant negative correlation between FER1L4 and miR-106a-5p expression levels was observed. Among the colon cancer cell lines, FER1L4 levels were relatively lower, with concurrent high levels of miR-106a-5p. Restoration of FER1L4 decreased the expression of miR-106a-5p, and had a significant influence on colon cancer cell proliferation, migration and invasion. The FER1L4 expression was correlated with depth of tumor invasion, lymph node metastasis, vascular invasion and clinical stage. Moreover, striking differences in overall survival and disease-free survival were observed for the cases with both low FER1L4 expression and high miR-106a-5p expression compared with cases with high FER1L4 expression and low miR-106a-5p expression. Circulating FER1L4 and miR-106a-5p levels were decreased and increased, respectively, in colon cancer patients after surgery. Our findings indicated that FER1L4 could exert a tumor suppressive impact on colon cancer, which at least, in part, through suppressing miR-106a-5p expression, and depletion of FER1L4, alone or combined with overexpression of miR-106a-5p, is predictive of poor prognosis in colon cancer and may play a crucial role in cancer prevention and treatment.

As one of the most frequently diagnosed tumors in the world, colon cancer is the third leading cause of fatal malignancy worldwide and incidence has remained high over the past 20 years.[1,2] At present, the gold standard for early detection of colon cancer is colonoscopy. However, given its invasive nature, new non-invasive methods are urgently required. Moreover, clinicopathologic staging is considered as the main risk assessment for the recurrence and metastasis of colon cancer. Better understanding of the relationship between clinical outcomes and novel biomarkers is the key to early diagnosis and improved prognosis of patients with colon cancer. Therefore, it is vital to continue to reveal the molecular mechanism of the progression of colon cancer, and identification of potential new targets is needed.

Long non-coding RNA (lncRNA) are a class of mRNA-like transcripts longer than 200 nucleotides.[3] They lack protein-coding ability and are believed to be involved in various kinds of biological processes.[4,5] Increasing evidence suggests that lncRNA are frequently aberrantly expressed in plenty of cancers; thus, the roles of dysregulated functional lncRNA in human malignant tumors have attracted considerable scientific interest.[6,7] LncRNA have been shown to regulate gene expression by a series of mechanisms, including not only transcription and post-transcription processing, but also genomic imprinting, chromatin modification and regulation of protein function.[8,9] Accumulating studies indicate that the deregulated lncRNA play a critical role in the pathogenesis of various human cancers, such as colorectal cancer, breast cancer, gastric cancer, bladder cancer, esophageal cancer, and so on.[10–12] For instance, as an oncogene, Malat1 can promote the growth and metastasis of small cell lung cancer and might be used as a new biomarker to determine the prognosis of patients.[13] HOX transcript antisense intergenic RNA (HOTAIR) reprograms the chromatin state and its overexpression in tumor tissues is correlated with cancer metastasis.[14] However, up to now, the characteristics and functions of lncRNA in tumorigenesis have remained largely elusive.[15] It is important to seek novel lncRNA as new biomarkers and targets for therapeutic intervention.

Tian *et al.* report that long non-coding RNA Fer-1-like protein 4 (FER1L4) and miR-106a-5p function as competing endogenous RNA (ceRNA) that miR-106a-5p could modulate FER1L4 expression levels through targeting RB1 in gastric cancer.[16] Although it is clear that FER1L4 and miR-106a-5p

are one pair of ceRNA that show reciprocal repression to each other, there are currently no published investigations on the possible association of their expression with colon cancer progression. In the present study, the expression levels of FER1L4 and miR-106a-5p in colon cancer tissues and cell lines were determined. Then, the colon cancer cells were treated with pcDNA3.1-FER1L4, and cell proliferation, migration and invasion were analyzed. The correlations between FER1L4 and miR-106a-5p and their clinicopathological significance in colon cancer were investigated. Finally, in human plasma, the expression level of FER1L4 was examined.

Material and Methods

Specimens. A total of 176 tissue samples were collected from Shanghai Jiao Tong University Affiliated First People's Hospital, China, from October 2005 to June 2007. The 70 fresh colon cancer tissues and matched adjacent nontumorous tissues and another 36 lymph node metastatic tissues were obtained from 31 males and 39 females with ages ranging from 40 to 85 years. All tissues were stored at −80°C immediately following removal until use. Peripheral blood was collected from 100 volunteers between December 2013 and June 2014, including 50 preoperative colon cancer blood samples, 50 postoperative colon cancer blood samples after surgery in 1 month and 50 healthy blood samples. All blood samples were centrifuged for 30 min after collection. The plasma was stored at −80°C until use. The diagnosis of all specimens was histopathologically confirmed by a pathologist. We staged tumors according to the cancer staging criteria of the 7th edition staging American Joint Committee On Cancer (AJCC). None of the patients had received any treatment before surgery. The study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated First People's Hospital and informed consent was obtained from each patient enrolled in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1995 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Cell culture and the treatment of cell lines. A human colon mucosal epithelial cell line (NCM460) and five colon cancer cell lines (RKO, Lovo, HCT116, SW480 and SW620) were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM (Gibco BRL, Grand Island, NY, USA), containing 10% FBS (Invitrogen, Camarillo, CA, USA). Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. For functional assays, gene-specific pcDNA3.1-FER1L4 or control pcDNA3.1 vector was transfected into colon cancer cells.

Total RNA isolation and quantitative real-time PCR. Total RNA from all tissues and cells was extracted by Trizol Reagent (Ambition, Carlsbad, CA, USA). For FER1L4, RNA was reverse transcribed into cDNA using the PrimeScript RT-PCR Kit (TaKaRa, Dalian, China) following the manufacturer's instructions. For miR-106a-5p, RNA was reverse transcribed using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was used to detect FER1L4 and miR-106a-5p expression levels using the SYBR Premix Dimmer Eraser Kit (Takara). The following primer sequences were used for qRT-PCR: for FER1L4, 5′-ACACA GTCCT TGTGG GTTCC-3′ (forward) and 5′-CCTGT CTCCT CCATC TCTCC-3′ (reverse); for miR-106a-5p, 5′-AAAAG TGCTT ACAGT GCAGG TAG-3′ (forward) and 5′-GAAAA GTGCT TACAG TGCAG GT-3′ (reverse); for GAPDH, 5′-GGAGC GAGAT CCCTC CAAAA T-3′ (forward) and 5′-GGCTG TTGTC ATACT TCTCA GG- 3′ (reverse). The conditions of thermal cycling were as follows: 95°C at 10 min, 40 cycles at 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The quantitative PCR reaction was repeated in triplicate. The relative expression of FER1L4 and miR-106a-5p was calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation assay. A cell proliferation assay was performed using the Cell Counting Kit-8 (Byotime, Haimen, China) according to the manufacturer's protocol, and detected at 24, 48, 72 and 96 h.

Cell migration and invasion assays. According to the manufacturer's protocol, 48 h after transfection, cells in 200 μL serum-free media were placed into the upper Transwell chamber (8.0-μm pore size, BD Biosciences, Franklin Lakes, NJ, USA) for migration assay (without Matrigel) or for invasion assay (with Matrigel). The chambers were incubated in media with 10% FBS in the bottom chambers for 48 h. Cells that migrated and invaded to the reverse side of chamber inserts were fixed and stained with methanol and 0.1% crystal violet. Finally, the stained cells were counted under microscope and images were captured. Experiments were independently carried out in triplicate.

Serological tumor marker analysis. Serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were analyzed using an Elecsys 2010 machine (Roche Diagnostics, Basel, Switzerland). The cutoff values for CEA and CA19-9 were 5 ng/mL and 35 U/mL, respectively.

Statistical analysis. All statistical analyses were set with a significance level of $P < 0.05$. Data were performed using Statistical Program for Social Sciences (SPSS) 19.0 software (SPSS, Chicago, IL, USA). The paired *t*-test, the two-independent sample *t*-test, one-way ANOVA, the χ^2-test and the Kruskal–Wallis test were used as appropriate. All graphs were plotted using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

Expression of Fer-1-like protein 4 and miR-106a-5p in colon cancer. The expression levels of FER1L4 and miR-106a-5p were examined in 70 pairs of colon cancer and matched adjacent normal tissues by qRT-PCR, and the results showed that FER1L4 was downregulated in 60.3% (44/70) of colon cancer tissues compared with the matched adjacent normal tissues ($P < 0.001$, Fig. 1a). Inversely, the levels of miR-106a-5p were increased in 60.1% (43/70) of cancer tissues. Furthermore, miR-106a-5p levels were increased with concurrent decreased levels of FER1L4 in 31 paired colon cancer tissues ($P < 0.001$, Fig. 1a). An inverse correlation between FER1L4 and miR-106a-5p was also observed in colon cancer tissues ($P < 0.01$, $R^2 = 0.318$, Fig. 1b). In addition, FER1L4 and miR-106a-5p expression levels were assayed in five colon cancer cell lines, RKO, Lovo, HCT116, SW480 and SW620, and all the levels were normalized to the level in NCM460, a normal colon mucosal epithelial cell line. Among all the cancer cell lines, FER1L4 levels were lower with concurrent high levels of miR-106a-

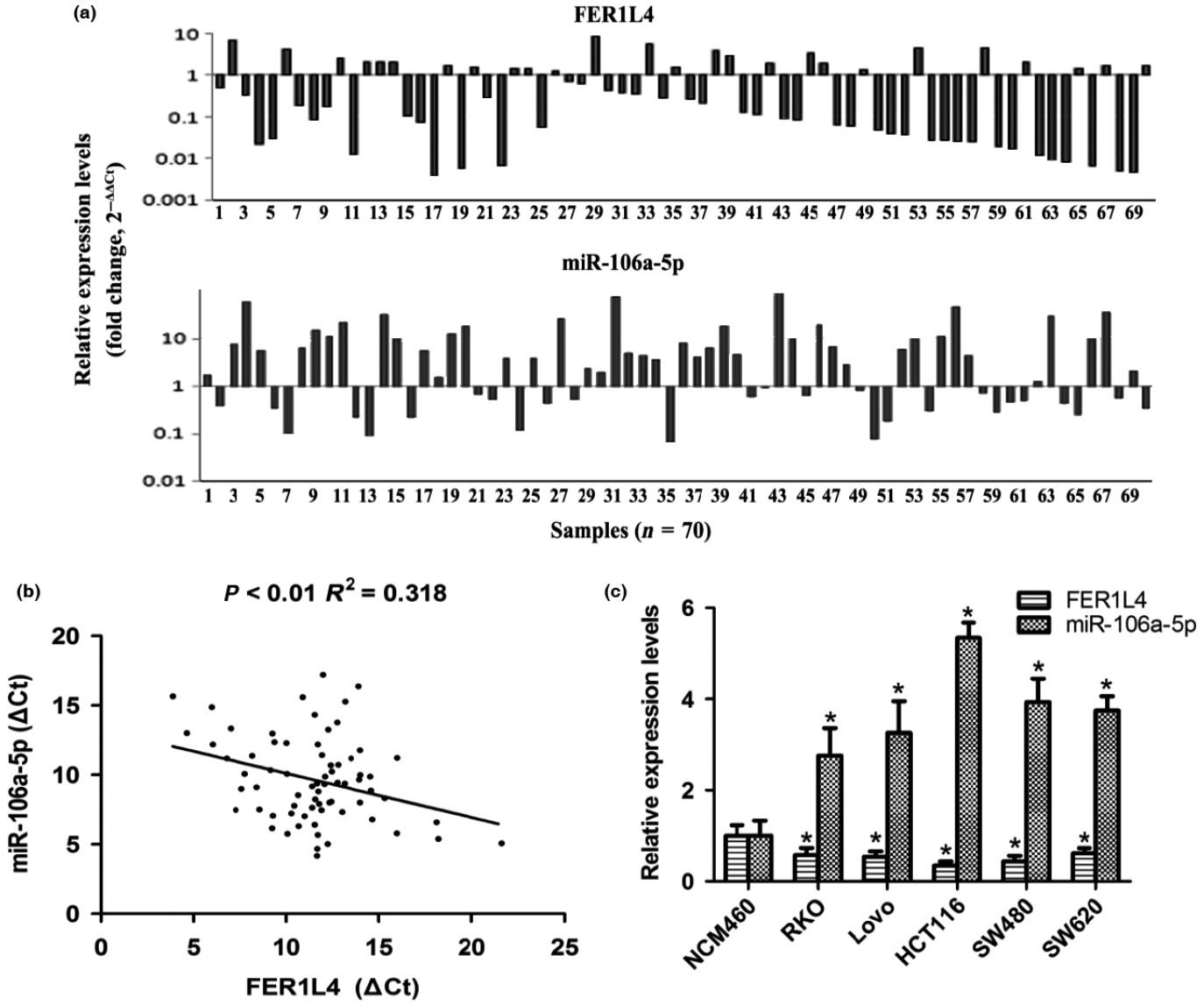

Fig. 1. Relative expression levels and the correlation of FER1L4 and miR-106a-5p in colon cancer. A series of 70 matched colon cancer tissues compared with adjacent normal mucosa and five colon cancer cell lines (RKO, Lovo, HCT116, SW480 and SW620) compared with normal colon mucosal epiyhelial cell line (NCM460) were used in the study. A logarithmic scale of $2^{-\Delta\Delta Ct}$ or ΔCt were, respectively, used to represent the fold change or the relative quantity in qRT-PCR detection. (a) Downregulation of FER1L4 in 60.3% (44/70) and upregulation of miR-106a-5p in 60.1% (43/70) of colon cancer tissues compared with the matched adjacent normal tissues. MiR-106a-5p levels were increased with concurrent decreased levels of FER1L4 in 31 paired colon cancer tissues. (b) Negative correlation between the FER1L4 levels and the miR-106a-5p levels in 70 matched tissues of colon cancer patients ($P < 0.01$, $R^2 = 0.318$). (c) The lower levels of FER1L4 with concurrent higher miR-106a-5p levels in colon cancer cell lines compared with normal colon mucosal epithelial cell line. *$P < 0.05$. qRT-PCR, quantitative RT-PCR.

5p (Fig. 1c), consistent with the expression of FER1L4 and miR-106a-5p in colon cancer tissues. It is noteworthy that FER1L4 was downregulated in 86.1% (31/36) of lymph node metastatic tissues, with significant means between lymph node metastatic tissues and primary cancer tissues (Table 1).

Fer-1-like protein 4 inhibits proliferation, migration and invasion of colon cancer cells. According to the findings that

Table 1. Expression of FER1L4 and miR-106a-5p in colon cancer tissues and lymph node metastatic tissues

	n	FER1L4		miR-106a-5p	
		High *n* (%)	Low *n* (%)	High *n* (%)	Low *n* (%)
Cancer tissue	70	26 (37.1)	44 (62.9)	43 (61.4)	27 (38.6)
Lymph node metastatic tissue	36	5 (13.9)	31 (86.1) $P = 0.013$*	29 (80.6)	7 (19.4) $P = 0.046$*

*$P < 0.05$ indicates a significant difference in the expression of Fer-1-like protein 4 (FER1L4) and miR-106a-5p between primary colon cancer and lymph node metastatic tissues.

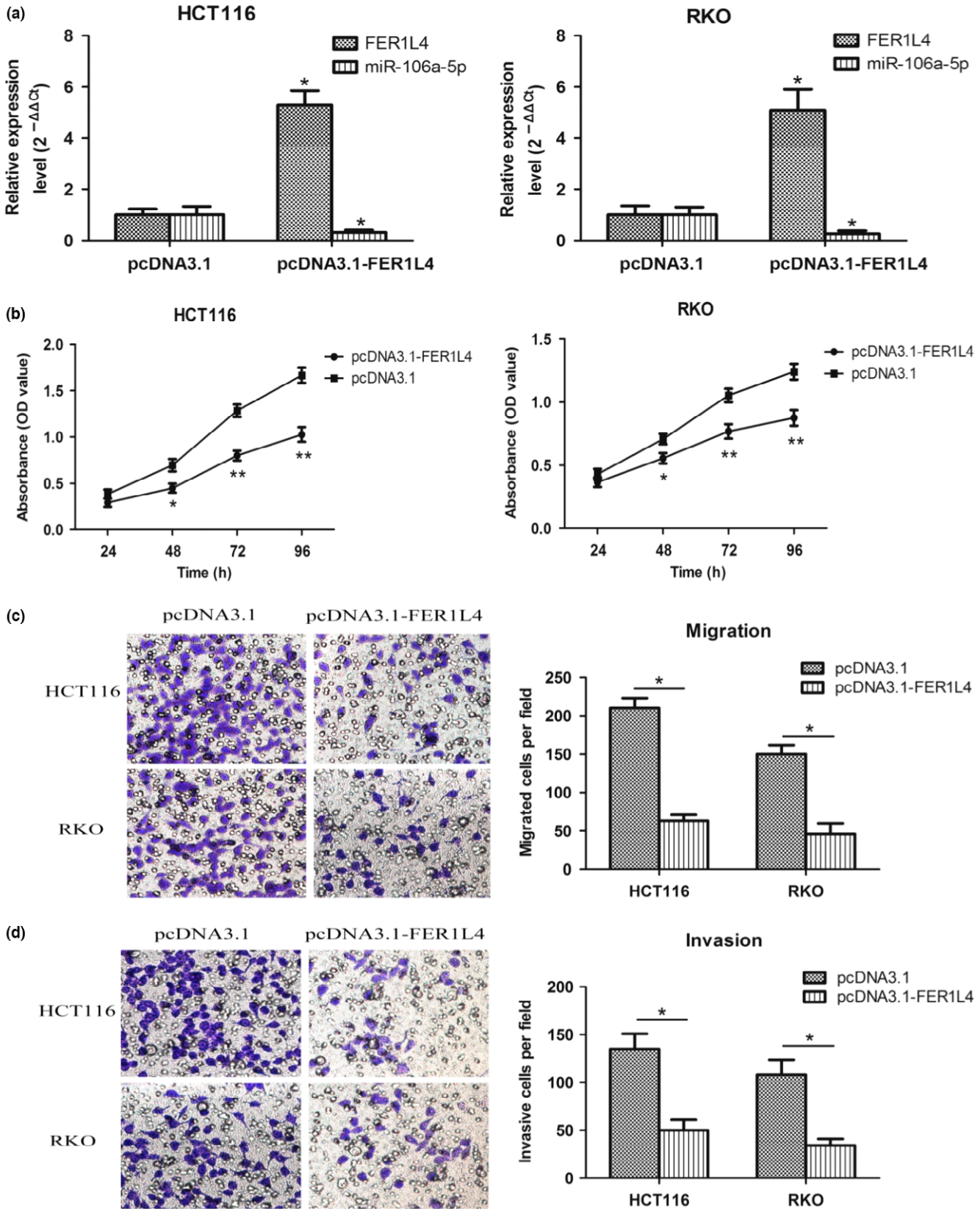

Fig. 2. Confirmation of FER1L4 transfection and its effect on colon cancer cell proliferation, migration and invasion. (a) FER1L4 transfection was validated by quantitative RT-PCR and the empty vector pcDNA3.1 transfected cells were used as controls. Reduction of MiR-106a-5p was consequent upon FER1L4 reintroduction. (b) Compared with control, FER1L4 exhibited a significant inhibition of colon cancer cell proliferation by CCK-8 assay, (c) migration and (d) invasion by Transwell assays. The experiments were repeated in triplicate. Data represent means and SD. Differences among the two groups were analyzed by ANOVA. Graphics were the representative presentations of cell migration and invasion from three independent experiments. *$P < 0.05$, **$P < 0.01$.

FER1L4 expression levels in HCT116 and RKO cells were more significantly downregulated than in the other colon cancer cell lines, the HCT116 and RKO cells were treated with pcDNA3.1-FER1L4, respectively. As a result, FER1L4 expression levels were effectively restored; meanwhile, the FER1L4 reintroduction-induced reduction of miR-106a-5p was also observed (Fig. 2a). Using the Cell Counting Kit-8 assay, FER1L4-enhanced cells exhibited a significant proliferation inhibition compared with the control (Fig. 2b). Moreover, in the Transwell migration assay, pcDNA3.1-FER1L4 impeded the migratory ability of HCT116 and RKO cells effectively when compared to cells treated with pcDNA3.1 normal control (Fig. 2c). Similar results were observed in the invasion assay (Fig. 2d).

Correlation between Fer-1-like protein 4 expression and clinicopathological characteristics in colon cancer. Based on the above findings, whether FER1L4 and miR-106a-5p expression levels were associated with the clinicopathological features of patients with colon cancer were further analyzed. As in a previous report in which lncRNA FENDRR in tumor tissues were categorized as high or low according to the median value of FENDRR expression,[17] in the present study, the colon cancer patients of this study were divided into two groups in relation to the median value of relative FER1L4 and miR-106a-5p expression in tissues. As shown in Table 2, the FER1L4 expression level demonstrated a negative association with depth of tumor invasion (pT stage, $P = 0.011$), lymph node metastasis (pN stage, $P = 0.003$), vascular invasion ($P = 0.019$) and AJCC stage ($p < 0.001$). MiR-106a-5p was positively associated with pT stage ($P = 0.013$), pN stage ($P = 0.009$), AJCC stage ($P < 0.001$) and vascular invasion ($P = 0.010$).

Downregulation of Fer-1-like protein 4 alone or combined with overexpression of miR-106a-5p predicts poor prognosis. A total of 48 of the 70 (68.6%) patients who underwent curative operations experienced recurrent disease. The Kaplan–Meier plot showed that striking differences in OS and DFS were observed between the low FER1L4 expression group and the high FER1L4 expression groups (Fig. 3a). Meanwhile, miR-106a-5p showed no correlation with OS but was significantly associated with DFS (Fig. 3b). Notably, the patient group with both low FER1L4 and high miR-106a-5p expression exhibited a significant difference in prognosis compared with the patient group with high FER1L4 and low miR-106a-5p expression (Fig. 3c). Univariate and multivariate analysis demonstrated that decreased tumor FER1L4 expression was a significant independent prognostic factor for decreased survival and increased disease recurrence. In contrast, miR-106a-5p alone was not a prognostic indicator; however, it appeared to be an independent prognostic factor for OS and DFS when combined with FER1L4 in colon cancer (Table 3).

Expression of Fer-1-like protein 4 and miR-106a-5p in human plasma. Using the blood samples, the existence of FER1L4 and miR-106a-5p in human plasma was observed in the present study, and then the relationship between their expression levels with colon cancer patients was analyzed. From a total of 150 blood samples, including 50 preoperative colon cancer blood samples, 50 postoperative colon cancer blood samples one month after surgery and 50 healthy blood samples, we found that there was no difference of circulating FER1L4 between preoperative patients and healthy persons, and decreased levels of circulating FER1L4 in 70% (35/50) of colon cancer patients one month after surgery ($P < 0.01$, Fig. 4a). Similarly, comparing with healthy blood samples, circulating miR-106a-5p revealed no statistical difference in preoperative patients, but its levels had increased after surgery in 56% (28/50) of postsurgical patients ($P < 0.01$, Fig. 4b). Nevertheless, the circulating FER1L4 and miR-106a-5p had no significant relationship with colon cancer patients' clinicopathological features (Table S1).

Table 2. Association between clinicopathologic features and FER1L4 or miR-106a-5p expression

	Expression of FER1L4		*P*-value	Expression of miR-106a-5p		*P*-value
	High ($n = 26$)	Low ($n = 44$)		High ($n = 43$)	Low ($n = 27$)	
Age (years)						
<65	12	23	0.621	24	11	0.220
≥65	14	21		19	16	
Gender						
Male	17	25	0.480	23	19	0.160
Female	9	19		20	8	
Location						
Right	16	20	0.193	21	15	0.584
Others	10	24		22	12	
pT stage						
T1	9	5	0.011*	4	10	0.013*
T2	8	7		8	7	
T3	6	14		14	6	
T4	3	18		17	4	
pN stage						
N0	14	8	0.003*	10	12	0.009*
N1	7	15		11	11	
N2	5	21		22	4	
AJCC stage						
I	14	4	<0.001*	5	13	<0.001*
II	5	9		6	8	
III	4	18		19	3	
IV	3	13		13	3	
Differentiation						
Well	11	13	0.371	12	12	0.350
Moderate	8	12		13	7	
Poor	7	19		18	8	
Vessel invasion						
No	17	16	0.019*	15	18	0.010*
Yes	9	28		28	9	
Serum CA19-9						
Negative	13	26	0.459	22	17	0.333
Positive	13	18		21	10	
Serum CEA						
Negative	15	19	0.241	19	15	0.354
Positive	11	25		24	12	

*$P < 0.05$ indicates a significant association among the variables.
AJCC, American Joint Committee On Cancer; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; FER1L4, Fer-1-like protein 4.

Discussion

In the present study, we verified for the first time the association of FER1L4 and miR-106a-5p expression with colon cancer progression. As a tumor suppressor, FER1L4 exhibits its clinical significance in colon cancer. We find that FER1L4

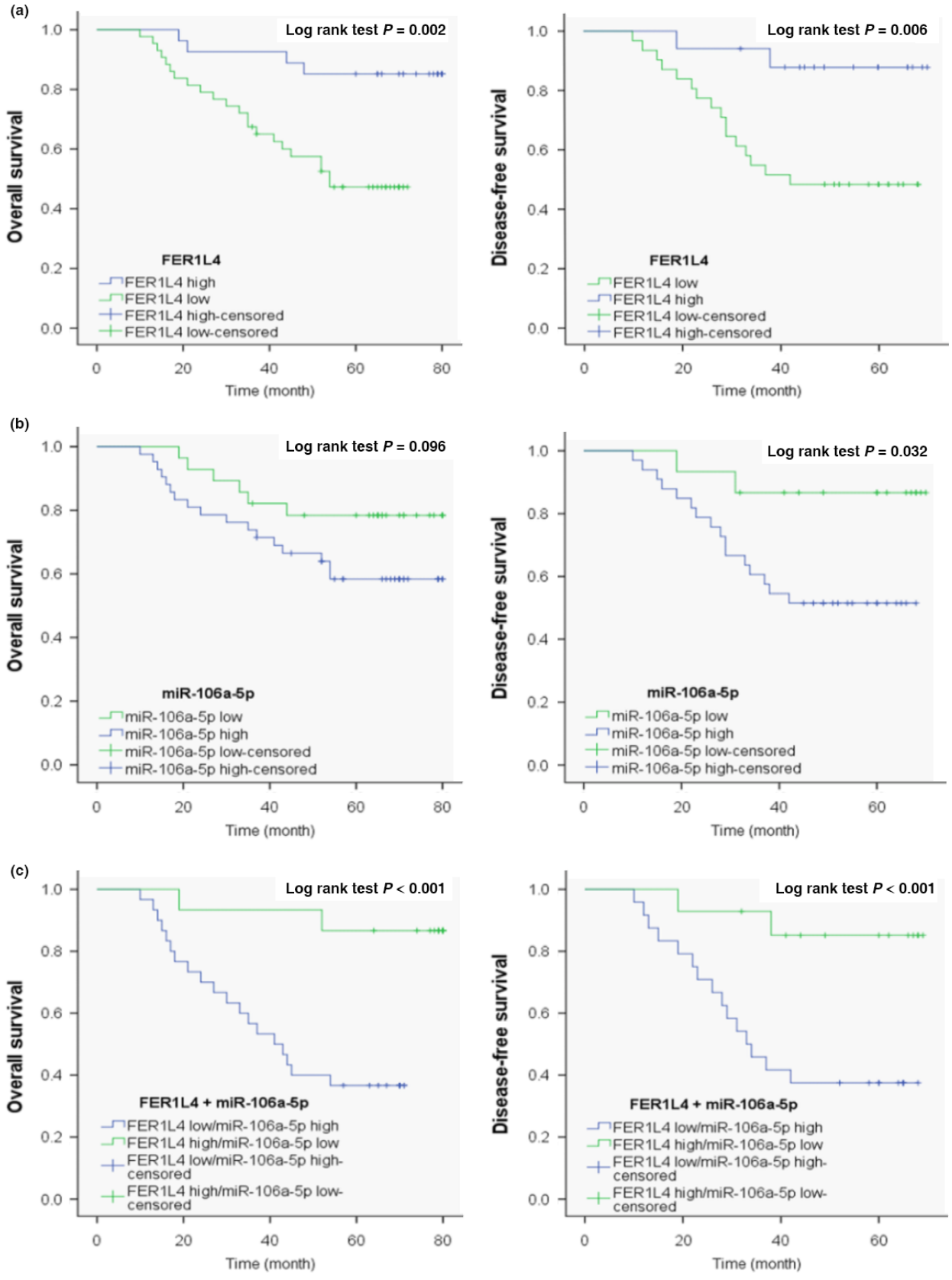

Fig. 3. Kaplan–Meier curves based on FER1L4, miR-106a-5p and their combined expression levels of 70 colon cancer patients. (a) The overall survival (OS) and disease-free survival (DFS) of the FER1L4 low group (n = 44) was significantly shorter than that of the high expression group (n = 26). (b) The DFS of the miR-106a-5p high group (n = 43) was significantly shorter than that of the low expression group (n = 27), but the OS was no different. (c) The OS and DFS of the FER1L4 low combined with the miR-106a-5p high expression group (FER1L4 low/miR-106a-5p high, n = 30) was significantly shorter than that of the FER1L4 high/miR-106a-5p low group (n = 15).

Table 3. Univariate and multivariate analysis of overall survival and disease-free survival after surgery

	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age								
<65	–				–			
≥65	0.83 (0.44, 1.55)	0.558			1.06 (0.52, 2.07)	0.611		
Gender								
Male	–				–			
Female	0.95 (0.51, 1.80)	0.684			1.16 (0.63, 1.98)	0.475		
Location								
Right	–				–			
Other	1.03 (0.81, 1.96)	0.711			1.21 (0.68, 1.92)	0.536		
T stage								
T1	1.16 (0.81, 1.68)	0.419			1.27 (0.73, 1.81)	0.504		
T2	0.91 (0.54, 1.46)	0.206			0.94 (0.49, 1.51)	0.323		
T3	0.68 (0.29, 1.19)	0.015*			0.72 (0.31, 1.35)	0.032*		
T4	–				–			
N stage								
N0	–		–		–		–	
N1	3.28 (1.97, 5.48)	<0.001*	2.82 (0.81, 4.83)	0.007*	3.05 (1.65, 4.96)	<0.001*	2.34 (0.63, 4.26)	0.019*
N2	14.37 (6.83, 25.88)	<0.001*	7.25 (2.52, 33.89)	<0.001*	14.64 (7.44, 28.67)	<0.001*	5.56 (1.72 30.88)	<0.001*
AJCC stage								
I	–		–		–		–	
II	3.32 (0.75, 11.01)	0.404	2.59 (0.66, 9.98)	0.357	3.01 (0.63, 9.25)	0.335	2.38 (0.51, 8.40)	0.406
III	9.12 (2.28, 37.46)	0.009*	7.81 (1.97, 32.69)	0.024*	8.27 (1.84, 30.55)	0.006*	6.41 (1.56, 29.77)	0.029*
IV	26.21 (11.32, 101.43)	<0.001*	21.83 (8.95, 89.24)	<0.001*	22.56 (9.57, 98.27)	<0.001*	20.55 (8.18, 82.40)	<0.001*
Differentiation								
Well	–				–			
Moderate	1.17 (0.75, 1.93)	0.703			0.89 (0.40, 1.75)	0.682		
Poor	1.59 (1.03, 2.98)	0.425			1.13 (0.87, 2.59)	0.303		
Vascular invasion								
No	–				–			
Yes	3.06 (1.54, 5.85)	0.014*			3.48 (1.77, 6.12)	0.004*		
FER1L4								
Low	10.25 (5.09, 24.04)	<0.001*	3.99 (1.67, 9.01)	0.021*	8.87 (4.39, 19.65)	<0.001*	4.51 (1.99, 9.02)	0.032*
High	–		–		–		–	
miR-106a-5p								
Low	–				–			
High	2.07 (1.22, 3.85)	0.073			2.21 (1.46, 4.11)	0.034*		
FER1L4/miR-106a-5p								
High/Low	–		–		–		–	
Low/High	13.31 (4.86, 37.23)	<0.001*	7.39 (3.13, 18.45)	<0.001*	12.30 (4.96, 33.55)	<0.001*	9.09 (3.75, 25.88)	<0.001*

*$P < 0.05$ indicated that 95% CI of HR was not including. HR, hazard ratio; 95% CI, 95% confidence interval.

exerts tumor suppressive effects on colon cancer by mediating miR-106a-5p repression, and might serve as a novel biomarker for prognosis of colon cancer when evaluated with miR-106a-5p expression.

Although over the past decade research on microRNA in maintaining malignant disorders has dominated the field of non-coding RNA regulation,[18] the effects of lncRNA on the tumorigenesis of colon cancer are still not completely known. A growing number of reports suggest that plenty of lncRNA could be used as diagnostic biomarkers and therapeutic targets in human cancers and play oncogenic or tumor suppressor roles in human cancer pathogenesis. For instance, colon cancer-associated transcript-1 (CCAT 1) was upregulated in gallbladder cancer tissues and cell lines, and suppression of CCAT

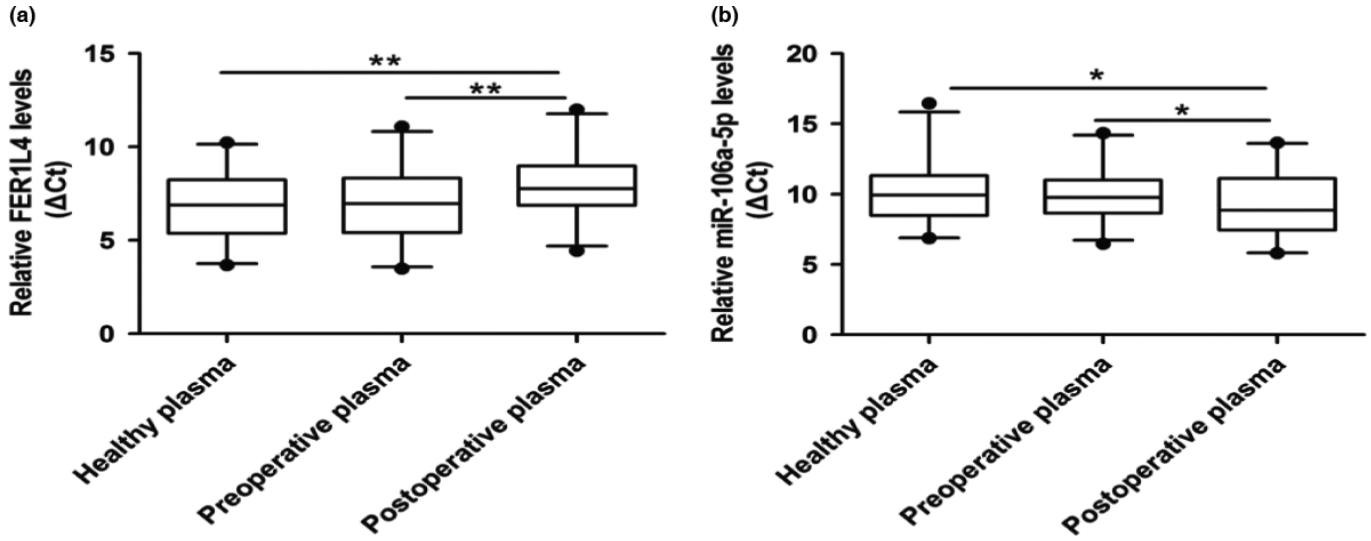

Fig. 4. The comparison of FER1L4 and miR-106a-5p expression in plasma of preoperative and postoperative colon cancer patients (n = 50) and healthy controls (n = 50). (a) Plasma FER1L4 was significantly decreased (70%, 35/50) and (b) plasma miR-106a-5p was significantly increased (56%, 28/50) in postoperative blood samples compared with the matched preoperative ones. The different levels of plasma FER1L4 and miR-106a-5p were also obvious between the postoperative groups and healthy controls, but there were no differences between preoperative groups and healthy controls. The quantitation was calculated by using the ΔCt method and higher ΔCt means lower expression levels. *P < 0.05, **P < 0.01.

1 by RNAi impaired the proliferation and invasion of gallbladder cancer cells.[19] Yuan JH *et al.* confirm that lncRNA activated by TGF-β (lncRNA-ATB), a mediator of TGF-β signaling, was upregulated in hepatocellular carcinoma metastases and might be applicable as a candidate biomarker for the diagnosis of hepatocellular carcinomas. LncRNA-ATB induced EMT and invasion through upregulated ZEB1 and ZEB2 by binding the miR-200 family.[20] Homeobox transcript antisense intergenic RNA (HOTAIR) involved in colon cancer progression and its expression was significantly correlated with lymph node metastasis, the depth of tumor invasion and vascular invasion. Moreover, increased HOTAIR expression had lower metastasis-free and overall survival and higher recurrence rates in colon cancer patients.[21] Liu *et al.* show that as a p53-regulated tumor suppressor, lncRNA loc285194, inhibits colon cancer cell growth, which acts in part via negative modulation of miR-211. There exists a competitive endogenous RNA regulatory network where lncRNA loc285194 may exert functions through targeting miR-211.[22] All these findings suggest that lncRNA as regulators influence various biological processes and play a crucial regulatory function in carcinogenesis. Thus, identification of new lncRNA and understanding the potential molecular mechanisms would facilitate the progression of lncRNA-directed diagnostics and therapeutics against cancers.

Fer-1-like protein 4 is a novel long non-coding RNA which was first published as occurring in gastric cancer. It was downregulated in gastric cancer tissues compared with matched adjacent normal tissues.[23] To date, a great deal of investigations have suggested the existence of widespread lncRNA–miRNA–mRNA interaction network about competing endogenous RNA (ceRNA), where lncRNA could function as a molecular sponge to modulate mRNA by binding microRNA.[24,25] In other words, the ceRNA network showed a reciprocal repression between lncRNA and microRNA.[26–28] Similarly, FER1L4 and miR-106a-5p have also been validated as ceRNA. FER1L4 could interact with some mRNA mediated by miR-106a-5p, and FER1L4 knockdown could result in more miR-106a-5p free to bind to other targets, such as RB1 mRNA.[16] However, whether there exist some other target genes except for RB1 participating in the FER1L4–miR-106a-5p ceRNA network in colon cancer needs to be further explored.

In the current study, attenuation of FER1L4 was a frequent event in colon cancer tissues. It is noticeable that miR-106a-5p has recently been reported in several cancers; however, it has a controversial role and exerts oncogenic or suppressive impacts on different tumors.[29–32] Our findings indicated that increased expression of miR-106a-5p was assessed in 60.1% (43/70) of colon cancer tissues, and its levels were found to be negatively correlated with FER1L4 expression. To our knowledge, it is the first study analyzing miR-106a-5p levels in colon cancer tissues. In addition, FER1L4 levels were lower with concurrent upregulation of miR-106a-5p in colon cancer cell lines, which is consistent with the results in cancer tissues. Currently, there are no published reports on the correlation between FER1L4 and tumor node metastasis; our further validation suggested that FER1L4 expression was considerably lower in invaded lymph nodes than in primary colon cancers. These data suggest that tumor FER1L4 depletion may be involved in the metastatic process of colon cancer. Consistent with the above findings, our functional studies confirmed that restoration of FER1L4 led to the reduction of miR-106a-5p expression, and increased FER1L4 levels in colon cancer cells coincided with decreased cell proliferation, migration and invasion.

Recent evidence demonstrated that some clinicopathological characteristics such as tumor clinical stage, histologic grade and distant metastasis can be used to predict tumor progression and as an independent prognostic factor on survival;[33,34] however, optimal prognostic biomarkers for colon cancer have not been established until now.[35] Therefore, the relationships among FER1L4, miR-106a-5p expression levels and clinicopathological characteristics were further explored. In our study, FER1L4 expression was significantly associated with tumor invasion depth, lymph node metastasis, distant metastasis and AJCC stage. Moreover, we found that colon cancer patients

with low tumor FER1L4 expression were strongly linked to increased risk of poor survival and tumor recurrence. Univariate and multivariate analysis indicated that FER1L4 expression alone or combined with miR-106a-5p expression could be served as an independent prognostic factor for OS and DFS in colon cancer.

Recent studies demonstrated that some biomarkers, including lncRNA and microRNA, exist in human plasma.[36–39] Therefore, FER1L4 and miR-106a-5p expression levels were detected in human plasma, among preoperative patients, postoperative patients and healthy persons. Although there was no difference in circulating FER1L4 between preoperative patients and healthy persons, it is noteworthy that circulating FER1L4 was significantly decreased in 70% (35/50) of colon cancer patients in the 1 month following surgery. In contrast, circulating miR-106a-5p revealed no statistical difference between healthy blood samples and preoperative blood samples; however, its levels had increased after surgery in 56% (28/50) of postsurgical patients. Based on this truth, we assumed that circulating FER1L4 expressed poorly in postsurgical colon cancer patients and its attenuation may be due to some oncogenic factors which could be secreted by micrometastatic circulating tumor cells (CTC), and miR-106a-5p, which might be released into peripheral blood by CTC mainly rather than primary tumor cells in view of the level changes among preoperative patients, postoperative patients and healthy persons. All these findings underscored the predictive potential of circulating FER1L4 and miR-106a-5p in colon cancer. It is possible that attenuation of circulating FER1L4 in colon cancer correlates with disease recurrence and metastasis, which needs to be verified in a larger prospective clinical investigation.

Collectively, based on the evidence that FER1L4 suppresses carcinogenesis via interaction with miR-106a-5p in colon cancer, we confirmed that colon cancer patients who had low FER1L4 expression and high miR-106a-5p expression tended to have a poor prognosis. It is possible that the reciprocal modulation of FER1L4 and miR-106a-5p may also involve other factors and signaling pathways. Thus, further investigations are warranted to advance our understanding of their effects in colon cancer. FER1L4 plays a crucial regulatory role in colon cancer, at least in part, by suppressing miR-106a-5p expression, and restoration of FER1L4 may provide a promising therapeutic option for suppressing colon cancer progression.

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Disclosure Statement

The authors have no conflict of interest to declare.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Relationship of circulating FER1L4 and miR-106a-5p level changes (ΔΔCt) after surgery with clinicopathological factors of colon cancer patients.