

Clinical value of microRNA-135a and MMP-13 in colon cancer

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Abstract. The aim of the present study was to investigate the expression and prognostic value of microRNA-135a (miR-135a) and matrix metalloproteinase-13 (MMP-13) in serum of colon cancer (CC). A total of 117 cases of patients admitted to Sheng Li Oil Field Central Hospital from May 2015 to May 2017 were enrolled in the research group (RG), and 120 cases of subjects undergoing normal health examination were included in the control group (CG). The expression of miR-135 and MMP-13 in peripheral blood of the two groups were compared, and their values were analyzed. It was found that miR-135a was decreased and MMP-13 was increased in the RG ($P<0.050$), both of which were closely related to the pathological features and prognosis of CC ($P<0.050$), and was also significantly correlated with CEA ($P<0.001$). ROC curve analysis showed that both of them had great predictive value for the occurrence, prognosis and death of CC. In conclusion, miR-135a was low expressed in CC, while MMP-13 was increased in CC, suggesting that the combined detection of the two had a good diagnostic effect on the occurrence of CC, and was closely related to the prognosis of CCC patients, which might be an excellent potential indicator for the diagnosis and treatment of CC in the future.

Introduction

Colon cancer (CC) is one of the most common malignancies of the digestive system, with a high incidence worldwide (1). According to statistics, the incidence of CC worldwide is approximately 6.1%, and the incidence rate in North America, Australia and other regions is significantly higher than other countries (2). Moreover, in recent years, with the improvement of living standards and changes in dietary structure, the incidence of CC has been on the rise (3). CC tends to occur at the junction of rectum and rectum sigmoid colon, and its

male patients are significantly more prominent than female patients (4). CC, not only has a high incidence, but also poses a great threat to human health (1). Previous findings have shown that in 2012, 1.4 million new CC cases resulted in almost 700,000 deaths (5). One of the main reasons for the poor prognosis of CCC patients is that it is difficult to conduct early screening in clinic, and there is no obvious special clinical disease in the early stage of CC. Therefore, patients are often deprived of the early optimal treatment period due to the lack of medical and health knowledge, and CC has developed to the middle and late stage when diagnosed (6). At this time, the tumor is usually accompanied by metastasis and invasion, and the commonly used clinical treatment scheme (surgery or combined chemoradiotherapy) has generally failed to achieve the optimal effect of tumor lesion removal (7). In the face of the increasingly serious challenges brought by CC to the clinic, finding an effective, convenient and accurate tumor marker is a hot and difficult point in the research of CC.

With the deepening of research, it has been suggested that the occurrence of tumor diseases is closely related to microRNAs (8-10). MicroRNAs are conservative non-coding RNAs that regulate gene expression at the post-transcriptional level and have certain effects on regulating cell proliferation, apoptosis and differentiation (11). MicroRNA-135a (miR-135a) is a member of the miR-135 family discovered in recent years, located at chromosome 3p21.1. Currently, miR-135a has been confirmed to have abnormal expression in prostate and pancreatic cancer (12,13). A study suggested that lncRNA FOXD3-AS1 affects the development of CC via regulating miR-135a (14), which indicates that miR-135a is also correlated with the occurrence of CC. However, there are few studies on the role of miR-135a in CC. We speculated that miR-135a may be the key to the potential diagnosis and treatment of future CC. However, the detection of microRNA alone may have low specificity, which requires the combination of other detection indicators to improve its application value. Matrix metalloproteinase-13 (MMP-13), as a member of MMPs family, has an important role in degrading and remodeling the dynamic balance of extracellular matrix, and is involved in the occurrence of numerous diseases (15). In addition, it was found that MMP-13 has a certain influence on the occurrence of stomach cancer (16). Consequently, MMP-13 may also have some potential clinical significance in CC. Moreover, with advances in research, MMP-13 has been confirmed to play an important role in colorectal cancer

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with liver metastasis (17). Rath *et al* (18) also suggested that MMP-13 has the potential to be a marker of intestinal disease in the future. CC is a malignant tumor with extremely high morbidity and mortality in the clinic. Finding an effective blood marker can, not only effectively improve the diagnosis rate of early CC, but also assist clinical judgment of the patient's disease development, and timely and effective intervention measures can be carried out to assess the prognosis of patients by evaluating changes in markers, which is of great significance for the clinical treatment and prognosis judgment of CC patients. Therefore, by analyzing the diagnostic and therapeutic significance of miR-135a and MMP-13 on CC, this study aims to provide a new reference for the clinical diagnosis and treatment of CC.

Materials and methods

General data. A total of 117 CCC patients admitted to Sheng Li Oil Field Central Hospital from May 2015 to May 2017 and 120 normal physical examination subjects were selected for prospective analysis. Of these, CC patients were taken as the research group (RG) and healthy physical subjects were taken as the control group (CG).

This study was approved by the ethics committee of Sheng Li Oil Field Central Hospital, and all the subjects mentioned above signed informed consent.

Inclusion and exclusion criteria. Inclusion criteria for the study were: Patients whose symptoms met the clinical manifestations of CC and was diagnosed as CC after biopsy by the pathology department of our hospital, patients received follow-up treatment in our hospital after diagnosis, patients aged 30-70 years, patients with complete case data, patients who agreed to co-operate with the investigation of our hospital, patients without adjuvant treatment prior to admission, patients treated with radical resection after admission, and the patients who could not achieve complete resection were treated with postoperative chemotherapy. Exclusion criteria were: Patients complicated with other tumors, cardio-cerebral vascular disease, chronic diseases, mental diseases or autoimmune diseases, organ failure, hepatic and renal insufficiency, or drug allergy, patients with long-term physical disability or bedridden and unable to take care of themselves, patients transferred to other hospitals, patients who died during treatment. Inclusion and exclusion criteria of the CG: Subjects without disease, subjects whose examinations were normal according to physical examination results, subjects aged 30-70 years. All the subjects agreed to participate in the investigation of our hospital and provided informed consent.

Materials and methods

Treatment methods. All the patients in the RG received tumor removal surgery (or postoperative chemotherapy) in our hospital. The surgery was completed by senior digestive surgeons. Postoperative chemotherapy regimen: Based on 5-FU, tetrahydrofolate was used as a regulator to enhance the efficacy of 5-FU.

Enzyme linked immunosorbent assay (ELISA) determination. Fasting venous blood (4 ml) was extracted from subjects in

the RG (before and after treatment) and the CG, placed at room temperature for 30 min and then centrifuged for 10 min (2,504 x g, 4°C) to obtain the serum. The concentration of MMP-13 in serum was determined by ELISA. The kit was purchased from Shanghai Jingkang Biotechnology Co., Ltd., Jk-(a)-5224. The operation was carried out in a sterile environment in strict accordance with the kit instructions.

Electrochemiluminescence immunoassay (ECLI) determination. Fasting venous blood (3 ml) was extracted from subjects in the RG (before and after treatment) and the CG, placed at room temperature for 30 min and then centrifuged for 10 min (2,504 x g, 4°C) to obtain the serum. ECLI was applied to determine the concentration of tumor marker CEA in serum. The kit was purchased from Shanghai Yaji Biotechnology Co., Ltd.: No. CL01236. The assay was completed by the laboratory department of our hospital.

PCR determination. Fasting venous blood (4 ml) was extracted from subjects in the RG (before and after treatment) and the CG, placed at room temperature for 30 min and then centrifuged for 10 min (2,504 x g, 4°C) to obtain the serum. EasyPure miRNA Kit (Beijing TransGen Biotech Co., Ltd.: No. ER601-01) was used for total RNA extraction. The purity of serum extracted RNA was determined by UV spectrophotometer and agarose gel electrophoresis. TransScript Green miRNA Two-Step RT-qPCR SuperMix (Beijing TransGen Co., Ltd.: No. AQ202-01) was used for reverse transcription of the extracted total RNA. The steps were carried out according to the kit instructions, and cDNA was collected for PCR amplification. Primer sequences are shown in Table I. qPCR amplification system was as follows: cDNA 1 µl, sense primer 0.4 µl, reverse primer 0.4 µl, 2X TransTaq® Tip Green qPCR SuperMix 10 µl, Passive Reference Dye (50X) 0.4 µl, and ddH₂O was added to complement to 20 microns, qPCR amplification conditions were as follows: Pre-denaturation at 94°C for 30 sec, denaturation at 94°C for 5 sec, annealing and extension at 60°C for 30 sec, for a total of 40 cycles. Each sample was set with 3 duplicate holes, and the experiment was carried out 3 times. In this study, U6 was taken as the internal parameter and 2^{-ΔΔC_q} was used to analyze the data (19). ELISA was applied to detect the concentration of MMP-13 in serum. The kit was purchased from Shanghai Jingkang Bioengineering Co., Ltd., JK-(a)-5224. The operation process was conducted in a sterile environment in strict accordance with the kit instructions. CEA concentration of tumor markers in serum was detected by electrochemical immunoluminescence. The kit was purchased from Shanghai Yaji Biotechnology Co., Ltd., CL01236, and the detection was completed by the clinical laboratory of our hospital.

Patient follow-up. Patients in the RG were followed up for 3 years. Patient prognosis and 3-year survival were recorded in the form of hospital review.

Observational indicators. Main indicators were miR-135a and MMP-13 in the RG and CG before and after treatment. The diagnostic value of miR-135a and MMP-13 for CC was assessed. Secondary indicators included the correlation of miR-135a and MMP-13 with the pathological features of

Table I. Primer sequences.

	Upstream (5'-3')	Downstream (5'-3')
miR-135a	ACACTCCAGCTGGGTATGGCTTTTTATTCT	GGTGTCTGTCGGAGTCGGCAA
U6	GCTCTGTCACCAACACTCACT	GCTGCCTTTCTTGTGTCGTT

Table II. Comparison of clinical data of patients in the two groups [n (%)].

	Research group (n=117)	Control group (n=120)	t/ χ^2	P-value
Age (years)	54.3±8.2	55.1±7.6	0.437	0.779
BMI (KG/cm ²)	24.62±2.84	24.78±3.38	0.694	0.394
Sex			1.133	0.287
Male	78 (66.67)	72 (60.00)		
Female	39 (33.33)	48 (40.00)		
Smoking			0.318	0.573
Yes	64 (54.70)	70 (58.33)		
No	53 (45.30)	50 (41.67)		
Drinking			1.193	0.275
Yes	81 (69.23)	75 (62.50)		
No	36 (30.77)	45 (37.50)		
Exercise habit			0.830	0.362
With	42 (35.90)	50 (41.67)		
Without	75 (64.10)	70 (58.33)		
Place of residence			1.537	0.215
Cities	92 (78.63)	86 (71.67)		
Countryside	25 (21.37)	34 (28.33)		
Nationality			1.404	1.185
Han Chinese	112 (95.73)	118 (98.33)		
Minority	5 (4.27)	2 (1.67)		
Treatment mode				
Curative resection	48 (41.03)			
Adjuvant or palliative chemotherapy	69 (58.97)			

CC. The correlation of miR-135a and MMP-13 with CEA and the effect of miR-135a and MMP-13 on the prognosis of CC patients were investigated.

Statistical analysis. The data results were processed by SPSS 22.0 statistical software, and the data results were graphically drawn by Graphpad8. The enumeration data were expressed by (rate), the comparison in groups was performed by the Chi-square test. The measurement data are expressed by mean ± standard deviation. The data conforming to the normal distribution were qualified by the independent sample t-test, the data conforming to the non-normal distribution were qualified by the rank sum test. Paired t-test was used for comparison before and after treatment. The comparison between groups was analyzed by univariate analysis of variance and the LSD back testing. The correlation was analyzed by Pearson's correlation coefficient. The diagnosis was analyzed by ROC curve. Binary Logistic Regression Analysis

was used to calculate the combined formula and then conduct ROC curve analysis. Survival rate was calculated using the Kaplan-Meier method, and was compared using the log-rank test. P<0.05 was considered statistically significant.

Results

Comparison of general data. There was no difference in age, BMI, sex, smoking, drinking, exercise habits, place of residence and ethnicity between the two groups (P>0.05) as is evident in Table II.

Comparison of miR-135a and MMP-13 in the two groups. Prior to treatment, miR-135a in the RG was lower than that in the CG, and MMP-13 was higher than that in the CG (P<0.050, Fig. 1A and B). After treatment, miR-135a in the RG was increased compared with that before treatment, while MMP-13 was decreased (P<0.050, Fig. 1C and D).

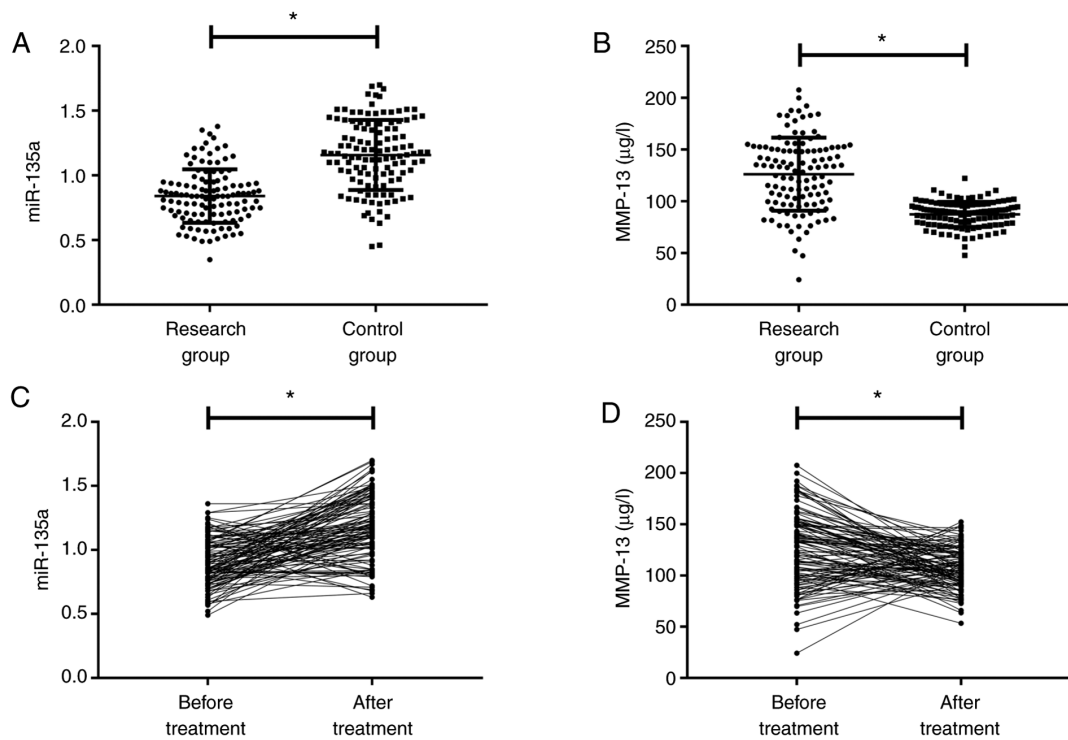


Figure 1. miR-135a and MMP-13 in the two groups. (A) Comparison of expression level of miR-135a between the RG and the CG. (B) Comparison of concentration of MMP-13 between the RG and the CG. (C) Comparison of expression level of miR-135a in the RG before and after treatment. (D) Concentration of MMP-13 in the RG before and after treatment. * $P<0.005$.

Diagnostic value of miR-135a and MMP-13 for CC. Among hospital CCC patients, 42 cases were in clinical stage I or II. ROC curve analysis revealed that the diagnostic sensitivity and specificity of miR-135a for early CC were 69.05 and 67.50%, respectively, and the diagnostic sensitivity and specificity of MMP-13 for early CC were 45.24 and 99.17%, respectively. Binary logistic regression analysis with miR-135a and MMP-13 as two independent variables was performed to obtain the joint detection model $\text{Log (P)}=1.799+ -0.043 \times \text{miR-135a} + 3.254 \times \text{MMP-13}$, when taking a cut-off value of 0.703, the model had a diagnostic sensitivity of 61.90% for CC, with a specificity of 85.00% (Fig. 2A, Table III, $P<0.001$). Then, we drew the ROC curve based on the detection results of miR-135a and MMP-13 of CCC patients in stage I and II prior to treatment. In addition, miR-135a had a sensitivity of 76.92% and a specificity of 71.43% for predicting CC development into the late stages of CC, and MMP-13 had a sensitivity of 73.33% and a specificity of 66.67% for predicting CC development into the late stage. As above, the Log (P) of miR-135a combined with MMP-13 formula model $=27.545 + -0.081 \times \text{miR-135a} + -19.548 \times \text{MMP-13}$ showed a sensitivity of 76.92% and a specificity of 88.10% in predicting the development of CC in the middle and later stages (Fig. 2B and Table IV, $P<0.001$).

The correlation of miR-135a and MMP-13 with the pathological features of CC before treatment. miR-135a was not related to patient age, BMI, sex or intestinal inflammation history ($P>0.05$), but was closely related to T stage, N stage, clinical stage, tumor type, tissue type, lymphatic metastasis and differentiation degree ($P<0.05$) (Table V). MMP-13 was

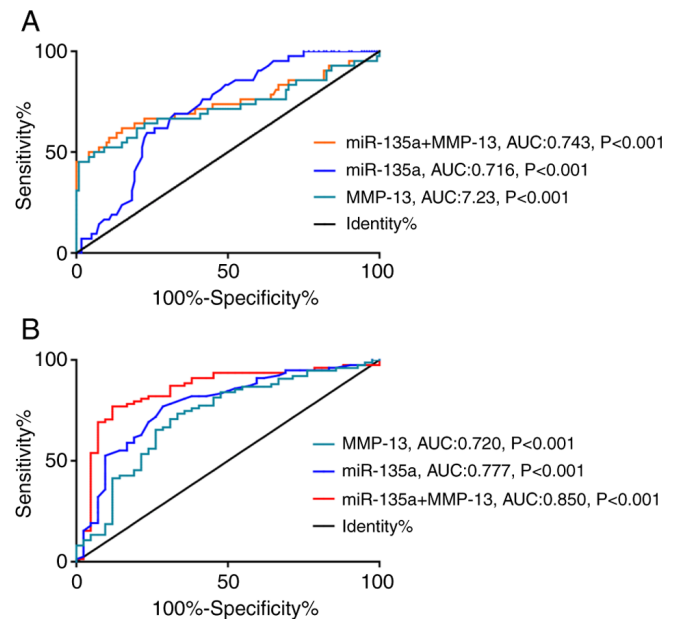


Figure 2. The diagnostic value of miR-135a and MMP-13 for CC. (A) ROC curve analysis of diagnosis of miR-135a for CC. (B) ROC curve analysis of diagnosis of MMP-13 for CC.

not related to the patient age, BMI or gender ($P>0.05$), but was closely related to the intestinal inflammation history, T stage, N stage, clinical stage, tumor type, tissue type, lymphatic metastasis, differentiation degree, and invasion ($P<0.05$) (Table V).

The correlation of miR-135a and MMP-13 with CEA before treatment. According to Pearson's correlation coefficient

Table III. The diagnostic value of miR-135a and MMP-13 for CC.

Item	miR-135a	MMP-13	Joint detection
AUC	0.716	0.723	0.743
Standard error	0.04142	0.05454	0.05342
95% CI	0.6355 to 0.7979	0.6161 to 0.8299	0.6383 to 0.8477
Cut-off	<1.045	>111.000	<0.703
Sensitivity (%)	69.05	45.24	61.90
Specificity (%)	67.50	99.17	85.00
P-value	<0.001	<0.001	<0.001

Table IV. miR-135a and MMP-13 in the middle and late stage of CC development.

Item	miR-135a	MMP-13	Joint detection
AUC	0.777	0.720	0.850
Standard error	0.04531	0.05061	0.03917
95% CI	0.6884 to 0.866	0.6208 to 0.8192	0.7737 to 0.9272
Cut-off	<0.865	>117.00	>0.713
Sensitivity (%)	76.92	73.33	76.92
Specificity (%)	71.43	66.67	88.10
P-value	<0.001	<0.001	<0.001

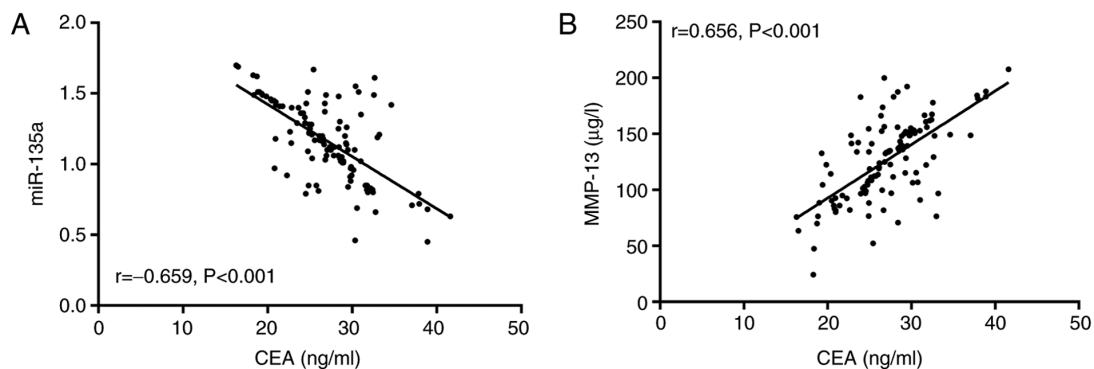


Figure 3. Correlation of miR-135a and MMP-13 with CEA before treatment. (A) Correlation analysis between miR-135a and CEA before treatment in the RG. (B) Correlation analysis between MMP-13 and CEA before treatment in the RG.

analysis, miR-135a was negatively correlated with CEA in the study group before treatment ($r = -0.659$, $P < 0.001$, Fig. 3A), while MMP-13 was negatively correlated with CEA ($r = 0.656$, $P < 0.001$, Fig. 3B).

Effect of miR-135a and MMP-13 on prognosis of CCC patients after treatment. A total of 117 patients in the RG were successfully followed up for 3 years, and 112 patients were successfully followed up, with a follow-up success rate of 95.73%. According to the median expression levels of miR-135a after treatment, the patients were divided into high-miR-135a group (miR-135a > 0.91 , $n = 60$) and low-miR-135a group (miR-135a ≤ 0.91 , $n = 52$). According to the median expression levels of MMP-13 after treatment, the patients were divided into high-MMP-13 group (MMP-13 > 104.24 $\mu\text{g/l}$, $n = 63$) and low-MMP-13 group

(MMP-13 ≤ 104.24 $\mu\text{g/l}$, $n = 49$). Their prognosis of survival were compared, the prognosis of the group with high miR-135a was better than that of the group with low miR-135a ($P = 0.025$, Fig. 4A), and the prognosis of the group with low MMP-13 was better than that of the group with high MMP-13 ($P = 0.042$, Fig. 4B).

Predictive value of miR-135a and MMP-13 levels in CCC patients after treatment. The 3-year prognosis of the patients in the RG was 23.21% (26/112). ROC curve analysis of levels of miR-135a and MMP-13 of patients after treatment showed that the sensitivity and specificity of miR-135a in predicting the prognosis of CCC patients was 65.38 and 65.93% when the cut-off value was 1.215. When the cut-off value was 123.000, the sensitivity and specificity of MMP-13 in predicting the prognosis of CCC patients were

Table V. Relationship of miR-135a and MMP-13 with the pathological features of CC.

Item	n	miR-135a	t/F	P-value	MMP-13 ($\mu\text{g/l}$)	t/F	P-value
Age (years)			0.365	0.716		0.645	0.520
<54.3	49	0.84 \pm 0.25			125.98 \pm 27.38		
\geq 54.3	68	0.82 \pm 0.32			122.12 \pm 34.85		
BMI (KG/cm ²)			0.181	0.857		1.208	0.230
<24.62	34	0.81 \pm 0.22			135.83 \pm 35.54		
\geq 24.62	83	0.80 \pm 0.29			127.13 \pm 35.30		
Sex			0.192	0.849		0.199	0.843
Male	78	0.81 \pm 0.29			125.41 \pm 38.27		
Female	39	0.80 \pm 0.21			126.77 \pm 26.56		
Intestinal inflammation history (have suffered from colitis or proctitis)			0.433	0.666		5.476	<0.001
With	65	0.83 \pm 0.22			149.62 \pm 28.97		
Without	52	0.81 \pm 0.28			121.16 \pm 26.58		
T stage			15.140	<0.001		8.504	<0.001
T1+T2	49	1.04 \pm 0.18			118.62 \pm 24.62		
T3+T4	68	0.62 \pm 0.12			156.21 \pm 22.82		
N stage			4.288	0.001		8.374	<0.001
N0	32	1.08 \pm 0.25			115.42 \pm 25.62		
N1+N2+N3	85	0.68 \pm 0.16			153.80 \pm 20.63		
Clinical stage			64.390	<0.001		30.880	<0.001
I-II	42	1.02 \pm 0.16			113.32 \pm 28.62		
III	49	0.79 \pm 0.20			142.96 \pm 35.83		
IV	26	0.54 \pm 0.12			172.62 \pm 21.52		
Tumor type			34.080	<0.001		5.300	0.002
Protrud type of polyps	14	1.13 \pm 0.22			118.62 \pm 21.52		
Flat protrud type	16	1.10 \pm 0.24			121.42 \pm 20.52		
Flat protrude with ulcer type	12	1.11 \pm 0.14			128.62 \pm 18.62		
Mass type	31	0.61 \pm 0.24 ^{a-c}			136.24 \pm 26.32 ^{a-c}		
Ulcerative type	28	0.63 \pm 0.16 ^{a-c}			146.16 \pm 25.62 ^{a-c}		
Infiltrating type	16	0.51 \pm 0.21 ^{a-d}			153.16 \pm 28.16 ^{a-c}		
Pattern of organization			29.170	<0.001		5.262	0.007
Adenocarcinoma	62	0.96 \pm 0.21			124.62 \pm 24.16		
Mucinous carcinoma	36	0.95 \pm 0.16 ^e			138.62 \pm 28.41 ^e		
Undifferentiated carcinoma	19	0.64 \pm 0.25 ^e			142.62 \pm 26.87 ^e		
Lymph node metastasis			13.240	<0.001		4.150	<0.001
With	26	0.58 \pm 0.25			152.13 \pm 25.60		
Without	91	1.12 \pm 0.16			122.41 \pm 33.81		
Grade of Differentiation			8.444	<0.001		4.620	<0.001
Middle, high	85	0.94 \pm 0.22			118.37 \pm 32.14		
Low	32	0.58 \pm 0.16			148.62 \pm 29.96		
Invasion			6.058	0.001		5.474	0.001
Yes	22	0.62 \pm 0.18			152.62 \pm 24.86		
No	95	0.95 \pm 0.24			116.62 \pm 28.41		

^aComparison with the protrude type of polyps, P<0.05. ^bComparison with the flat protrusion type, P<0.05. ^cComparison with flat protrusion with ulcer type, P<0.05. ^dComparison with mass type, P<0.05. ^eComparison with adenocarcinoma, P<0.05.

50.00 and 86.81%, respectively. With miR-135a and MMP-13 being regarded as two independent variables, binary Logistic

regression analysis was performed to obtain the joint detection model $\text{Log(P)} = 8.739 + -0.412 \times \text{miR-135a} + -1.298 \times \text{MMP-13}$.

Table VI. Predictive value of miR-135a and MMP-13 on death in patients with CC after treatment.

Item	miR-135a	MMP-13	Joint detection
AUC	0.665	0.687	0.786
Standard error	0.061	0.062	0.046
95% CI	0.544-0.785	0.564-0.809	0.695-0.877
Cut-off	>1.215	>123.000	>0.205
Sensitivity [n (%)]	65.38	50.00	73.08
Specificity (%)	65.93	86.81	66.18
Youden index (%)	31.31	36.81	51.47
P	0.005	0.004	<0.001

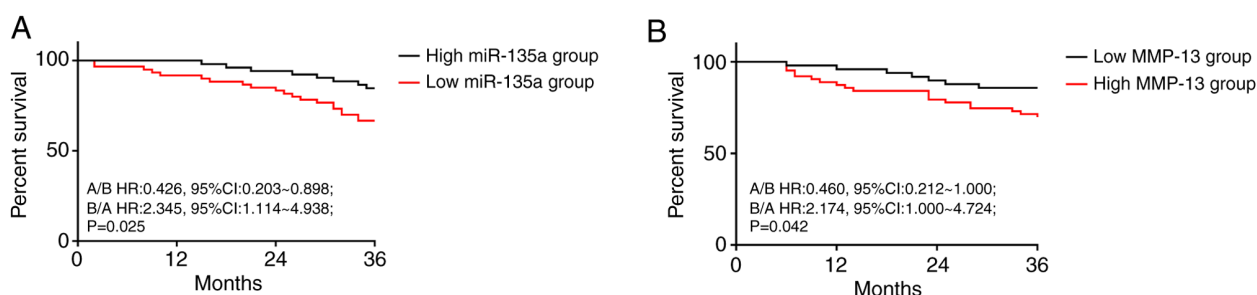


Figure 4. The 3-year survival curve of CCC patients. (A) The 3-year survival curve of prognosis of the high and low miR-135a groups. (B) The 3-year survival curve of prognosis of the high and low MMP-13 groups.

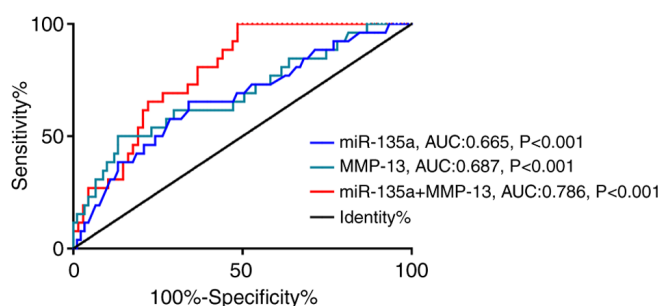


Figure 5. ROC curve of miR-135a and MMP-13 in predicting death for CCC patients after treatment.

When the cut-off value was 0.205, the sensitivity and specificity of the model for predicting the prognosis of CCC patients were 73.08 and 66.18%, respectively. More details are shown in Fig. 5 and Table VI.

Discussion

CC is not only the most common malignant tumor in digestive tract organs, but also one of the most common malignancies throughout the body (20). In addition, the incidence of the disease has been on the increase in recent years and is getting younger, which does harm to the human body increasingly day by day (21). At present, the pathogenesis of CC is not clear. It has been suggested that the occurrence of CC is related to external factors such as diet, smoking, obesity, diabetes and drinking, as well as internal factors such as cell and gene changes (22). Therefore, there is always a lack of specific tumor markers as

early diagnostic criteria for CC in clinical practice. With the application of microRNAs being regarded as early screening indicators for tumors, however, microRNAs have gradually become a major research focus in China and abroad, and it is crucial that potential markers of CC in clinical practice be identified. This study explored the clinical significance of miR-135a and MMP-13 for CC, which is of great significance for the diagnosis and treatment of CC in the future.

The results showed that miR-135a had a low expression in CCC patients, while MMP-13 was significantly increased in CCC patients, suggesting that miR-135a and MMP-13 may be involved in the occurrence and development of CC. Previous studies have confirmed miR-135a was decreased in breast cancer and MMP-13 was increased in lung cancer (23,24), which also support the results of this study. miR-135a is a recently discovered miRNA. Xu and Wen (25) proposed that miR-135a participates in the progress of acute myeloid leukemia by regulating HOXA10. Zhou *et al* (26) indicated that miR-135a affects non-small-cell lung cancer through the pathway of IGF-1/P13K/Akt. Studies have suggested that miR-135a can bind to MTSS1, and its synthetic substances can effectively inhibit the proliferation and invasion ability of tumor cells (27), and MTSS1 has been proven to be significantly reduced in CC (28). Thus, we speculated that miR-135a might also bind to MTSS1 in CC and played a role of tumor suppressor gene. Cheng *et al* (29) proved that miR-135a targeting FAK pathway could inhibit tumor metastasis and angiogenesis, which may be one of the pathways in which it plays a role in CC. However, the basic experiment was not conducted in this experiment, and the mechanism of miR-135a could not be determined. MMPs is a kind of proteolytic enzyme dependent

on calcium ion and zinc ion, which is composed of 10 exons and 9 introns. MMPs can promote the peripheral development of tumor cells by enhancing the intercellular adhesion and can participate in the immune process of tumor cells by stimulating some potential biological activities (30). MMP-13 belongs to collagenase type I, which has been confirmed to not only degrade interstitial collagen, but also degrade extracellular matrix molecules, and regulate the process of invasion and metastasis of tumor (31). Currently, MMP-13 has been proved to be abnormally expressed in both osteosarcoma and gastric cancer (32,33), and its mechanism of action in CC is speculated to be associated with its ability in promoting angiogenesis. By analyzing the diagnostic value of miR-135a and MMP-13 for CC, we found that the combined detection of the two had a good prediction effect for the occurrence of CC, indicating that the two could be used as a screening indicator for CC in future clinical practice, so as to improve the early detection rate of CC. Compared with traditional imaging methods, miR-135a combined with MMP-13 has the advantage of convenient detection and intuitive detection results, without relying on the clinician's previous judgment experience to analyze the image results. Moreover, the preservation time of peripheral blood samples is longer, which is conducive to clinical review at any time. Compared with traditional tumor markers, the detection advantage of miR-135a combined with MMP-13 is that it has a higher degree of speciality, which can assist clinicians to make early judgments on tumor types and implement relevant interventions.

We further analyzed the correlation of miR-135a and MMP-13 with traditional cancer marker CEA, and found that miR-135a was negatively correlated with CEA, while MMP-13 was positively correlated with CEA, which further confirmed the application value of miR-135a and MMP-13 in tumor diagnosis in the future. In addition, according to its relationship with the clinical pathological features, we found that miR-135a and MMP-13 were bound to T stage, N stage, clinical stage, tumor type, organization type, lymphatic metastasis, differentiation degree of CC. However, due to the limited experimental conditions, we did not analyze the diagnostic value of miR-135a and MMP-13 in different pathological features in more detail. A more in-depth analysis and discussion is required in future research. The results of this study also confirmed that miR-135a and MMP-13 are related to the tumor progression of CC, which is a potential therapeutic target of CC. It is hoped that relevant researchers can conduct experimental verification and analysis against our conjecture. Through the follow-up of the patient's prognosis, we found that miR-135a and MMP-13 are closely related to the prognosis of patients, and the expression of the two has a good predictive value for the prognosis and death of patients, suggesting that monitoring of miR-135a and MMP-13 in patients clinically can help clinicians to judge the conditions of recovery and prognosis of patients in the future. Currently, the incidence of CC is on the rise, as is the threat of death from the disease. By analyzing miR-135a and MMP-13 in CC, this study confirmed that both the two had great value in evaluating the prognosis of patients. According to the results of this study, the lower miR-135a was, the higher MMP-13 was, and the higher the prognosis and death risk of patients was. Therefore, we speculated that miR-135a and MMP-13 may also be potential

therapeutic targets of CC, and effective treatment may be achieved in the future through targeted regulation of the two. Thus, more experimental analysis is needed to confirm this point, which is the main direction of our follow-up research.

This study aimed to explore the situation of miR-135a and MMP-13 in CCC patients. However, due to the limited experimental conditions, there are still some deficiencies, such as the study period was short and the impact of miR-135a and MMP-13 on the long-term prognosis of CCC patients could not be determined. In addition, this experiment lacked the support of *in vitro* experiments, and the mechanism of miR-135a and MMP-13 affecting CC could not be fully clarified. von Felden *et al* (34) suggested in the study that miR-135a is highly expressed in hepatic cancer; the differences between this study and our results might be attributed to the specific expression of miR-135a in different tumor diseases and the different biological effects. Further experiments will be conducted to verify our view, as well as a more comprehensive and complete analysis on the application of miR-135a and MMP-13 in CC to obtain the optimal experimental results. In addition, in this study, we did not analyze the diagnosis of miR-135a and MMP-13 CC at different pathological stages, which may lead to certain errors in the early screening of CC. This study preliminarily confirmed the clinical significance of miR-135a and MMP-13 in CC, and more in-depth and detailed experimental analysis is needed to improve our results.

In conclusion, miR-135a is lowly expressed in CC and MMP-13 is increased in CC. The combined detection of the two has a good diagnostic effect on the occurrence of CC, and is closely related to the prognosis of CCC patients, which may be an excellent potential indicator for the diagnosis and treatment of CC in the future.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XZ, DY and PX conceived and designed the study, and drafted the manuscript. XZ, DY, XD and PX collected, analyzed and interpreted the experimental data. PX revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Sheng Li Oil Field Central Hospital. Signed written informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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