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Original Research

Serum miR-885-5p can be used as a marker for efficacy prediction and prognosis of advanced liver cancer

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Abstract: This study set out to observe the value of miR-885-5p in the prognosis evaluation of liver cancer patients after transcatheter arterial chemoembolization. Sixty liver cancer patients treated in Zhuji City People's Hospital between January 2019 and September 2019 were enrolled. The patients were categorized into survival (SG) and death groups (DG) according to prognosis. Sixty-five healthy individuals were included as a control. The expression of miR-885-5p and miR-21were measured by the qRT-PCR and predictive value of diagnostic efficiency was determined by the ROC curve. Multivariate Cox regression analysis was performed to explore independent risk factors. Results showed that levels of miR-885-5p in the serum of liver cancer patients were obviously lower than that of the control group; however, levels of miR-21 in the serum of liver cancer patients were higher than that of healthy individuals. Patients with miR-885-5p low expression and miR-21 high expression had poor differentiation, stages III+IV, and their incidence of lymphatic invasion and distal metastasis was higher ($P<0.05$). AUC values of miR-885-5p and miR-21 single diagnosis were both > 0.8. The relative level of miR-885-5p in the serum of the SG was dramatically higher than that of the DG, and its relative level of miR-21 in serum was significantly decreased compared with DG. ROC curves in diagnosing the prognosis of liver cancer patients were drawn, AUC values of serum miR-885-5p and miR-21 in diagnosing their prognosis were both > 0.8. Cox regression analysis was carried out on independent factors affecting their prognosis. The results revealed that the TNM stage, lymphatic invasion, distal metastasis and miR-885-5p were independent prognostic factors affecting their 5-year survival. It was concluded that miR-885-5p exhibited the potential to be a serum biomarker to evaluate the efficacy of advanced liver cancer.

Key words: miR-885-5p; miR-21; Hepatocarcinogenesis; The short-term prognosis.

Introduction

Liver cancer is a major chronic malignant tumor. During the past two decades, its morbidity has not only been increasing but also showing a trend of rejuvenation, which seriously threatens people's life and health (1-2). Due to the limited choice of conventional biochemical testing methods for predicting the efficacy of advanced liver cancer, it cannot reflect their efficacy after treatment timely. Recurrence or metastasis of advanced liver cancer after treatment is an important reason for their high mortality. Recently, people have a deeper understanding of the pathogenesis of liver cancer, and studies have found that good biomarkers help diagnose and evaluate it (3-5). MicroRNA (miRNA) is a kind of endogenous non-coding single-stranded RNA (6-7). Current research shows that tumor-related miRNAs in blood circulation are closely related to EMT and metastasis of lesions of tumors in the body (8-10). At the moment, more and more studies show that serum miRNA is better for improving the prognosis of patients with advanced liver cancer (11-12).

At present, in the study of liver cancer-related miRNA, it has been confirmed that the miR-21 expression level is related to the invasion potential in liver cancer cells (13). Silencing miR-21 expression can inhibit their invasion and metastasis (14-16). However, the characteristics of the two miRNAs in liver cancer are still unclear. Therefore, we conducted prospective clinical studies to observe the miR-885-5p and miR-21 expression in serum of those with liver cancer, analyze their clinical value and short-term prognosis analysis, so as to provide a new theoretical basis for the diagnosis and treatment of liver cancer prognosis in molecular biology.

Materials and Methods

Patients

60 patients with advanced liver cancer treated in Zhuji City People's Hospital between January 2019 and September 2019 were selected as group A (37 male and 23 female patients); they were (60.10±1.00) years old on average. Sixty-five healthy people were enrolled as a control group (B, 40 male and 25 female patients); they were (60.00±1.00) years old on average. Exclusion and inclusion criteria: All liver cancer patients included met the diagnostic guidelines of the Liver Cancer Expert Committee (17); liver and kidney function is normal and there are no other malignant tumors. All those who received chemotherapy, immunotherapy and radiotherapy before the operation were excluded. This study was approved by the Ethics Committee of Zhuji City People's Hospital. Patients and their families were

Table 1. Primers used in this study.

Target	Upstream sequence	Downstream sequence
miR-885-5p	5'-UCC AUU ACA CUA CCC UGC CUC U-3'	5'-AGG CAG GGU AGU GUA AUG GAU U-3'
miR-21	5'-ACA CTC CAG CTG GGT AGC TTA TCA GAC TGA-3'	5'-CTC AAC TGG TGT CGT CCA GCC ATG TTA TGA GTT GCA TCA ACA TC -3'
U6	5'-GAG CCA AGG CTA TGA CA-3'	5'-GCC TAC TGC AGA ACG GCA AC-3'

informed in advance before the study was carried out, and they signed informed consent forms.

Reagents

Trizol reagent, Real time-PCR reagent and reverse transcription kit were purchased from Invitrogen (USA), the enzyme label analyzer and real-time quantitative PCR instrument were ordered from BioRad company (USA), the primers targeting miR-885-5p, miR-21 and U6 were ordered from Invitrogen and shown in Table 1.

Detection of miR-885-5p and miR-21

Five mL of elbow venous blood in the subjects was taken and added into a vacuum blood collection vessel. Subsequently, samples were subjected to centrifugation at 3,500 rpm for 10 minutes. Sera were collected and stored in an EP tube for later use and placed in a low-temperature refrigerator at 80 ℃. Real-time PCR technology was carried out to measure the miR-885-5p and miR-21 expression in serum of group A and group B. The sera RNA was isolated using Trizol according to the manufacturer's instructions. The cDNA was then synthesized by minScript reverse transcription kit according to the manufacturer's instructions. The reaction system contains 1 μl of M-MLV, 1 μl of Oligo (dT), 0.5 μl of RNA inhibitor, 1 μl of dNTPs and non-RNase water up to 20 μl, following incubation at 55℃ for 1 hour and 75℃ for 5 s. Subsequently, Real time-PCR was performed with the synthesized cDNA as a template with reaction system as follows: 2.5 μl of 10×PCR buffer, 1 μl of dNTPs, 1 μl of forward and reverse primer each, 0.25 μl of Taq DNA polymerase and non-RNase water supplemented to 50 μl. Under below conditions: denaturation at 95℃, 15 min, denaturation at 95℃, 15 s, annealing at 60℃, 30 s, a total of 35 cycles, and a final extension at 72℃ for 15 min. The Real-time PCR efficacy was determined by the melting curve and amplification curve. The relative expression of each target was calculated by the $2^{-\Delta\Delta Ct}$ method with the U6 as a reference gene.

Statistical methods

SPSS 10.0 software system was performed for statistical analysis. The counting data were expressed by the number of cases/percentages [n(%)], and the χ^2 test was used for comparison between the two groups. The measurement data were expressed by mean number (X±sd), and comparison between groups was conducted by t-test or F test. ROC was used to draw the diagnostic value, K-M survival curve was used to draw the 5-year survival condition of patients, Log-rank test was used to analyze, multivariate Cox regression was used to investigate the independent risk factors affecting their prognosis, and one-way analysis of variance was used for comparison among multiple groups, expressed by F. A p-value lower than 0.05 was considered as statistical difference.

Results

General clinical data of patients

No significant differences in age, gender, drinking,

Table 2. Clinical general characteristics.

Group	Group A (n=60)	Group B (n=65)	t/F/X²	P
Age (years)	60.10±1.00	60.00±1.00	0.559	0.578
Gender			0.000	0.988
Male	37 (61.67)	40 (61.54)		
Female	23 (38.33)	25 (38.46)		
BMI (kg/m²)	19.71±1.04	19.68±1.11	0.156	0.877
Education background			0.310	0.577
Bachelor degree or above	32 (54.24)	32 (49.23)		
Below bachelor degree	27 (45.76)	33 (50.77)		
Smoking			2.031	0.154
Yes	49 (81.67)	46 (70.77)		
No	11 (18.33)	19 (29.23)		
Drinking			0.193	0.661
Yes	50 (83.33)	56 (86.15)		
No	10 (16.67)	9 (13.85)		
Differentiation			-	-
Poorly differentiated	34 (56.67)	-		
Moderately+Highly differentiated	26 (43.33)	-		
TNM stage			-	-
Stage II	33 (55.00)	-		
Stages III+IV	27 (45.00)	-		
Lymphatic invasion			-	-
Yes	35 (58.33)	-		
No	25 (41.67)	-		

Table 3. Diagnostic value of two miRNAs in serum for liver cancer before treatment.

Indicators	miR-885-5p	miR-21
AUC	0.8949	0.9008
95%CI	0.8561-0.9336	0.8481-0.9535
Std. Error	0.0198	0.0268
Cut-off value	0.335	0.525
Sensitivity(%)	85.48	84.29
Specificity(%)	75.63	82.50

Table 4. Relationship between miR-885-5p and pathological data of liver cancer.

Parameter		miR-885-5p expression		x^2 value	P-value
		Low expression	High expression		
Gender					
	Male (n=37)	19 (63.33)	18 (60.00)	0.071	0.791
	Female (n=23)	11 (36.67)	12 (40.00)		
Age					
	≥ 60 years old (n=40)	20 (66.67)	20 (66.67)	0.000	1.000
	< 60 years old (n=20)	10 (33.33)	10 (33.33)		
Lesion location					
	Right/left lobe of liver (n=35)	16 (53.33)	19 (63.33)	0.617	0.432
	Others (n=25)	14 (46.67)	11 (36.67)		
Differentiation					
	Poor differentiation (n=34)	24 (80.00)	10 (33.33)	13.300	<0.001
	Moderate+high differentiation (n=26)	6 (20.00)	20 (66.67)		
TNM stage					
	Stage II (n=33)	11 (36.67)	22 (73.33)	8.148	0.004
	Stage III+IV (n=27)	19 (63.33)	8 (26.67)		
Lymphatic invasion					
	Yes (n=35)	22 (73.33)	13 (43.33)	5.554	0.018
	No (n=25)	8 (26.67)	17 (56.67)		
Vascular invasion					
	Yes (n=33)	16 (53.33)	17 (56.67)	0.067	0.795
	No (n=27)	14 (46.67)	13 (43.33)		
Distant metastasis					
	Yes (n=27)	20 (66.67)	7 (23.33)	11.38	<0.001
	No (n=33)	10 (33.33)	23 (76.67)		

educational background and clinical general characteristics between group A and group B were observed (P>0.05, Table 2).

Comparison of miR-885-5p and miR-21 between two groups before TACE

As shown in Figure 1 and Table 3, the miR-885-5p expression levels in two groups were (0.30±0.10) and (0.5±0.10) respectively, and the miR-21 expression levels in group A and group B were (0.6±0.10) and (0.40±0.10) respectively. Compared with the two groups, the miR-885-5p expression in the serum of the patient group was markedly lower compared to that of the health group (P<0.001), and miR-21 levels in the serum of patient group was markedly higher than that of health group (P<0.001).

Analysis of miR-885-5p and miR-21 expression levels and clinicopathological characteristics in liver cancer patients

As shown in Tables 4 and 5, according to the median miR-885-5p, liver cancer patients were divided into high and low expression groups. Analysis of the differences between the pathological data of the two groups showed that low expression patients had poor differentiation, stages III+IV, and higher incidence of lymphatic invasion and distal metastasis (P<0.05). Patients with liver cancer were divided into high and low expression groups according to the median value of miR-21. Analysis of the differences between the pathological data of both groups showed that patients with high expression had low differentiation, stages III+IV, and higher

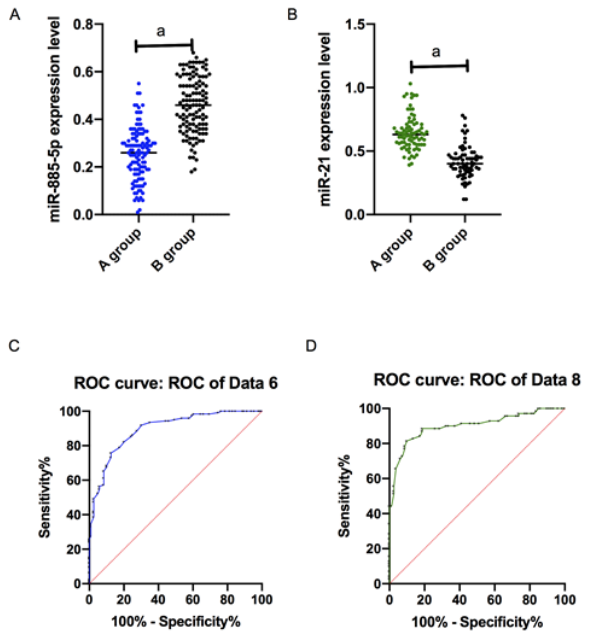

Figure 1. Comparison of relative expression results of serum miR-885-5p and miR-21 between groups A and B. A: The relative expression of serum miR-885-5p in group A was remarkably higher than that in group B (P<0.001). B: The relative expression of serum miR-21 in group A was remarkably lower than that in group B (P<0.001). Note: a means P<0.001. C: In the diagnosis of liver cancer patients, the sensitivity, specificity and AUC values of miR-885-5p single diagnosis were 85.48%, 75.63% and 0.8949 respectively. D: In the diagnosis of liver cancer patients, the sensitivity, specificity and AUC values of miR-21 single diagnosis were 84.29%, 82.50% and 0.9008 respectively.

Table 5. Expression levels of miR-21 and clinicopathological characteristics of liver cancer patients.

Parameter		miR-21 expression		x^2 value	P value
		Low expression	High expression		
Gender				0.635	0.426
	Male (n=37)	17 (56.67)	20 (66.67)		
	Female (n=23)	13 (43.33)	10 (33.33)		
Age				1.200	0.273
	≥ 60 years old (n=40)	18 (60.00)	22 (73.33)		
	< 60 years old (n=20)	12 (40.00)	8 (26.67)		
Lesion location				0.068	0.793
	Right/left lobe of liver (n=35)	17 (56.67)	18 (60.00)		
	Others (n=25)	13 (43.33)	12 (40.00)		
Differentiation				9.774	0.002
	Poor differentiation (n=34)	11 (36.67)	23 (76.67)		
	Moderate+high differentiation (n=26)	19 (63.33)	7 (23.33)		
TNM stage				15.150	<0.001
	Stage II (n=33)	24 (80.00)	9 (30.00)		
	Stages III+IV (n=27)	6 (20.00)	21 (70.00)		
Lymphatic invasion				6.452	0.011
	Yes (n=35)	13 (43.33)	24 (75.00)		
	No (n=25)	17(56.67)	8 (25.00)		
Vascular invasion				0.067	0.795
	Yes (n=33)	16 (53.33)	17 (56.67)		
	No (n=27)	14 (46.67)	13 (43.33)		
Distant metastasis				8.148	0.004
	Yes (n=27)	8 (26.67)	19 (63.33)		
	No (n=33)	22 (73.33)	11 (36.67)		

incidence of lymphatic invasion and distal metastasis (P<0.05).

Association between miR-885-5p, miR-21 and survival of patients with liver cancer

As shown in Figure 2, all the patients were followed up for 5 years, and their 5-year survival rate was 10%. miR-885-5p high expression was dramatically increased the rates than that of those with low expression. However, high miR-21 expression was dramatically decreased the rates than that of those with low expression (P<0.05).

ROC analysis of miR-885-5p and miR-21 in predicting prognosis of liver cancer patients

As shown in Table 6 and Figure 3, according to a 5-year follow-up, we divided group A into a survival group (SG) (6 cases) and death group (DG) (54 cases) according to whether liver cancer patients were alive or not. The relative expression levels of miR-885-5p and miR-21 in the serum of the SG were (0.5±0.10) and (0.46±0.10) respectively, and those in the DG were (0.35±0.10) and (0.65±0.10) respectively. The relative expression of serum miR-885-5p in the SG was markedly higher than that in the DG, and the relative expression of serum miR-21 in the SG was markedly lower than that in the DG (P<0.001). ROC curve of serum miR-885-5p and miR-21 in diagnosing the prognosis of liver cancer patients was drawn. AUC value of serum miR-885-5p in diagnosing their prognosis was 0.8117, the sensitivity was 92.59%, the specificity was 66.67%, and the cut-off value was 0.465. AUC value of serum miR-21 in diagnosing their prognosis was 0.8410, sensitivity was 88.89%, specificity was 83.33%, and cut-off value was 0.535.

Cox regression analysis of 5-year survival of patients with advanced liver cancer

As shown in Table 7, the cox regression analysis was carried out on patients with liver cancer, and independent factors affecting their prognosis were observed. The results signified that the TNM stage, lymphatic invasion, distal metastasis and miR-885-5p were independent prognostic factors affecting their 5-year survival.

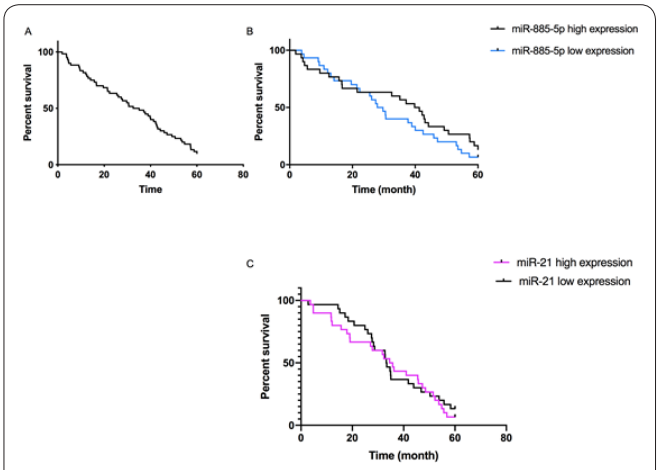

Figure 2. Relationship between miR-885-5p, miR-21 and 5-year survival of liver cancer patients. A: Overall survival rate of liver cancer patients. B: the 5-year survival rate of patients with miR-21 high expression group was lower than that of those with low expression group (P<0.05). C: the 5-year survival rate of patients with miR-885-5p high expression was higher than that of those with low expression (P<0.05).

Discussion

The factors affecting the development of liver cancer are complex; miRNAs, as oncogenes or tumor suppressor genes, have a great influence on regulating tumor signal pathways. Clinically, more sensitive and accurate efficacy prediction and prognostic markers for advanced liver cancer have been continuously sought (18-19). Currently, traditional diagnostic methods still have some disadvantages in the prognosis of liver cancer, so it is particularly important to explore biomarkers

Table 6. Diagnostic value of serum miR-885-5p and miR-21 in the prognosis of liver cancer patients before treatment.

Indicators	miR-885-5p	miR-21
AUC	0.8117	0.8410
95%CI	0.6273-0.9962	0.5964-1.000
Std. Error	0.0941	0.1248
Cut-off value	0.465	0.535
Sensitivity (%)	92.59	88.89
Specificity (%)	66.67	83.33

Table 7. Univariate and multivariate Cox regression analysis of 5-year survival prognosis in liver cancer patients.

Factor	5-year univariate Cox		5-year multivariate Cox	
	P value	HR (95CI%)	P value	HR (95CI%)
Gender	0.746	0.819 (0.257-3.463)	-	-
Age	0.529	1.132 (0.493-4.475)	-	-
Lesion location	0.793	0.688 (0.204-1.915)	-	-
Vascular invasion	0.444	1.126 (0.755-5.135)	-	-
Differentiation	0.032	0.277 (0.038-0.832)	0.520	0.725 (0.146-3.831)
TNM stage	0.000	3.784 (1.642-6.767)	0.000	8.204 (4.206-23.004)
Lymphatic invasion	0.002	0.168 (0.018-0.489)	0.001	0.266 (0.100-0.688)
Distant metastasis	0.001	0.261 (0.023-0.682)	0.000	0.085 (0.021-0.973)
miR-885-5p	0.023	0.544 (0.225-0.891)	0.022	2.361 (1.265-8.257)
miR-21	0.008	0.331 (0.260-0.815)	0.010	2.482 (1.254-8.902)

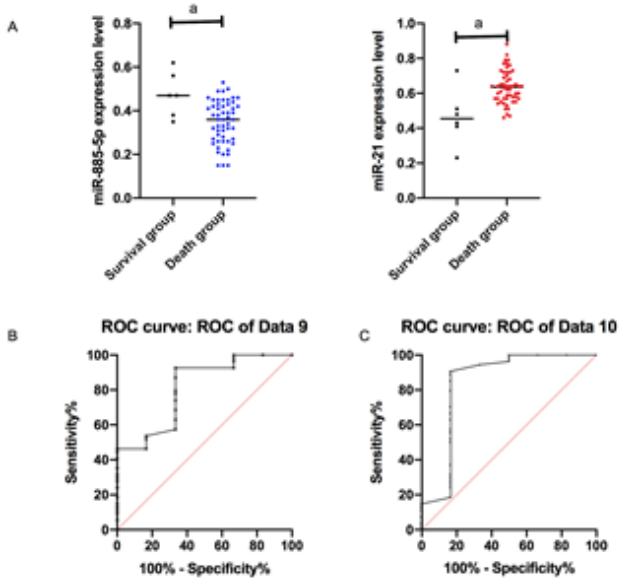

Figure 2. ROC curve of serum miR-885-5p and miR-21 in diagnosing prognosis of liver cancer patients. A: Relative expression levels of miR-885-5p and miR-21 in the serum of the two groups (a indicates P<0.001). B: AUC value of serum miR-885-5p for diagnosing their prognosis was more than 0.8. C: AUC value of serum miR-21 for diagnosing their prognosis was greater than 0.8.

relevant to its diagnosis and prognosis (20).

miRNAs are a class of endogenous non-coding single-stranded RNA with the function of promoting cancer or inhibiting cancer (21-22). Relevant research results on regulating the development and change of cancer in liver cancer reveal that miR-885-5p, as a new tumor influencing factor, has been controversial about its expression difference in liver diseases. Others believe that miR-885-5p is remarkably down-regulated in liver cancer and inhibits its metastasis by inhibiting Wnt /β-catenin signaling pathway (23). Nevertheless, miR-21 has been proved to be highly expressed in digestive tract diseases such as liver cancer (24). Nowadays, more scientific reports on the level changes of two miRNAs are lacked to predict the efficacy of advanced liver cancer. In the current work, the expression differences of miR-885-5p and miR-21 in the liver cancer patient and health individual serum were analyzed first and found that miR-885-5p in the serum of those with liver cancer was down-regulated while miR-21 was overexpressed. However, it was found that miR-885-5p and miR-21 expression were not tied to gender, age and lesion location of liver cancer, but were related to vascular invasion, differentiation, TNM stage, lymphatic invasion and distal metastasis. In recent years, researchers have focused on the biological treatment of miRNAs. At present, studies have shown that miR-21 is up-regulated by the same miRNA in individuals infected with chronic hepatitis B or hepatic cellular cancer, and the overexpression of serum miR-21 is more obvious with the aggravation of TNM stage (25-26). Based on the ROC study data, we believe that serum miR-885-5p and miR-21 are all used to assess the severity of the disease and to monitor the later health. Ultimately, we will analyze the serum miR-885-5p and miR-21 expression according to the different prognosis of liver cancer patients. It is found that the serum miR-885-5p of the surviving patients is higher than that of the dead patients, while the relative expression of serum miR-21 is obviously lower than that of the dead patients. At the end of the research, we analyzed the 5-year prognosis factors of liver cancer patients. Through analysis, the TNM stage, lymphatic invasion, distal metastasis, miR-885-5p and miR-21 are independent prognostic factors of them. Previous related studies have found that the TNM stage, lymphatic invasion and distal metastasis are independent prognostic factors affecting the prognosis of liver cancer patients (27). But, it is the first report that miR-21 and miR-885-5p can be independent prognostic factors for them. Through this study, we have preliminarily determined the function of two miRNAs in liver cancer, which are expected to become its potential diagnostic and prognostic indicators. Both miR-885-5p and miR-21 play a vital role in the clinical diagnosis and prognosis of liver cancer.

This study confirmed the prognostic analysis importance of two miRNAs in liver cancer, but there are still some limitations. If there is no more specific analysis

of the regulatory effect of miR-885-5p and miR-21 expression changes on related leukocytes in liver cancer patients, there is no further explanation of their biological functions. Furthermore, the clinical routine inflammatory factors of miR-885-5p, miR-21 and liver cancer are not analyzed, which have a certain influence on the improvement of research design. Hence, we will refer to the latest research in real-time in the later period and add corresponding research schemes to make up for design defects so as to continuously improve the research. The study of gene expression has been used in many studies as an important marker. These markers have been used for many traits, including as markers for diseases such as cancer (28-39).

In a word, miR-885-5p is up-regulated in liver cancer. Conversely, miR-21 is increased in the serum of liver cancer patients. miR-885-5p and miR-21 could be involved in its cancer occurrence and development. Therefore, these miRNAs have the potential to be developed as indicators to evaluate liver cancer metastasis and predict the cancer prognosis evaluation.

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