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Original Article

Expression and clinical significance of miR-338 and miR-20a in serum of patients with gastric carcinoma

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Abstract: Objective: This research intended to explore the content and the role of miR-338 and miR-20a in the serum of patients with gastric carcinoma (GC). Methods: Sixty-seven patients with GC, diagnosed and treated for the first time in our hospital from February 2014 to October 2016 were selected as the observation group (OG), and 45 healthy people were selected as the control group (CG). miR-338 and miR-20a of the CG and the OG were tested using qRT-PCR, and the correlation between the two indexes was analyzed by Pearson test. The diagnostic value of miR-338 and miR-20a in GC was analyzed by receiver operating characteristic curve (ROC). The correlation of miR-338 and miR-20a with clinical data was compared, and the correlation of the two with the survival of patients was observed. The independent prognostic factors in patients with GC were analyzed by Cox regression. Results: miR-338 expression was low in GC patients' serum, while miR-20a was high in GC patients. The expression of the two indexes was negatively correlated (r=-0.609, P<0.001). The areas under the curve of miR-338 and miR-20a were 0.849 and 0.865 respectively. Low expression of miR-338 and high expression of miR-20a were correlated to large tumors, low differentiation degree, high possibility of lymph node metastasis, and late TNM stage of GC patients. Multivariate Cox results revealed that tumor size, lymph node metastasis, differentiation degree, TNM stage, miR-338 and miR-20a were independent prognostic factors. Conclusion: miR-338 and miR-20a are expected to be serological indicators for GC diagnosis and prognosis.

Keywords: miR-338, miR-20a, gastric carcinoma, expression, clinical significance

Introduction

GC is a common malignant tumor of the digestive tract, and the third cause of carcinoma death in the world [1]. In China, GC cases account for more than 40% of new cancer cases, with an annual death toll of about 350,000, accounting for 25% of all malignant tumor deaths [2, 3]. Patients with early stage GC usually undergo radical surgery and postoperative chemotherapy, and the 5-year survival is about 90%; but about 70% of patients miss the best treatment period due to delay of diagnosis, and the disease has developed into late stages [4]. Therefore, it is urgent to seek an efficient and valuable early diagnosis method in order to improve the prognosis of GC patients.

Endoscopy and biopsy are the gold standard of GC diagnosis, but they are invasive and have potential sampling errors. The current GC markers CA19-9 and CEA have low specificity and sensitivity [5, 6], so seeking a new and efficient diagnostic method is a current research hotspot. Studies have shown that the imbalance of miR has a correlation with the occurrence and growth of various carcinomas [7-9]. miR, a short-chain non-coding RNA, can regulate most gene transcription in the human body [10] and can also regulate the biological functions of carcinoma cells [11]. miR can exist stably in serum and plasma, and can be frozen and thawed many times because of its simple sampling [12, 13]. More and more studies have shown that there is a differential expression of miR in serum between GC patients and healthy people, so miR can be applied as a potential diagnostic index [14]. Studies have revealed that miR-338-3p can regulate the growth and metastasis of breast carcinoma [15], and miR-20a can be applied as a potential biomarker for diagnosing GC [16]. The clinical exploration of

miR is becoming more mature. However, there is a lack of studies on the diagnostic role of miR-338 and miR-20a in GC and the correlation of them with patients' survival. Therefore, this study tested miR-338 and miR-20a in GC patients and explored their clinical significance in GC.

Materials and methods

Clinical data

Sixty-seven patients with GC who were diagnosed and treated for the first time in Wuhan Fourth Hospital from February 2014 to October 2016 were selected as the observation group (OG), and 45 healthy people as the control group (CG). The laboratory indexes of healthy people were normal. Inclusion criteria: All patients were confirmed with GC by pathological diagnosis, and the diagnostic conditions were in line with the 2016 ESMO diagnostic guidelines [17]; the estimated survival time of patients was more than 3 months; patients had not received radiotherapy, chemotherapy and other anti-tumor treatment; patients had complete clinical data; patients could actively cooperate with hospital follow-up; patients and their families signed the informed consent. Exclusion criteria: Patients with communication barriers and liver and kidney dysfunction; patients who were pregnant or lactating; patients who quit halfway. This study conformed to the Medical Ethics Committee standards of our hospital.

qRT-PCR detection

miR-338 and miR-20a of the CG and the OG were tested. On the second day after the two groups were included, 5 ml fasting venous blood was collected, placed at room temperature for 30 min, centrifuged with a speed of 3000×g at 4°C for 10 min, and the supernatant was collected and stored at -80°C for testing. Total RNA was extracted using TRIzol kit, then reverse transcription was performed using TaqMan Reverse transcription kit (Invitrogen, USA), and cDNA was collected for PCR amplification. The amplification system was as follows: 1 μL cDNA, 0.4 μL upstream and downstream primers, 10 μl 2× TransStart® Green qPCR SuperMix UDG, 0.4 μl of Passive Reference Dye (50×) (optional), and finally Nuclease-free Water was added to supplement the volume to 20 μL. The amplification steps were as follows: 94°C for 10 min, 94°C for 5 s, and 60°C for 30 s, for a total of 40 cycles. Each sample was given 3 repeated wells. U6 was applied as the internal reference, and $2^{-\Delta\Delta Ct}$ was applied for data analysis.

Outcome measures

Main outcome measures: miR-338 and miR-20a of patients with GC was observed. ROC curve was applied to analyze the diagnostic value of miR-338 and miR-20a in GC. The relationship of miR-338 and miR-20a with clinical data was observed.

Secondary outcome measures: The relationship of the survival of patients with miR-338 and miR-20a was observed, and the independent prognostic factors of GC patients was analyzed by Cox regression.

Statistical analysis

SPSS 19.0 (Shanghai Yijun) was applied for statistical analysis, and GraphPad Prism 7 was used for illustrating the figures. The counting data was expressed by [n (%)], and the percentage between groups was compared by Chi-square, and the measurement data were represented by mean ± standard deviation (X ± SD). The difference was statistically significant when $P<0.05$.

Results

Comparison of general clinical data

By comparing the general clinical data of the CG and the OG, it was found that there was no statistical difference in age, sex, body mass index (BMI), smoking history, drinking history, residence and dietary preference between the OG and the CG, and as such the groups were comparable, as shown in **Table 1**.

miR-338 and miR-20a in serum of patients with GC

miR-338 and miR-20a of the CG and the OG were tested by qRT-PCR, which revealed that miR-338 in the OG (0.793±0.210) was evidently lower than that in the CG (1.021±0.086); while miR-20a in the OG (1.276±0.175) was evidently higher than that in the CG (1.026±0.082). Pearson correlation test revealed that miR-338 and miR-20a were negatively corre-

Table 1. Comparison of general clinical data between the OG and the CG

Factor	CG (n=45)	OG (n=67)	t/X^2	*P* value
Age (years)	55.4±8.1	57.2±7.4	1.215	0.227
Gender				
Male	21 (46.67)	38 (56.72)	1.091	0.296
Female	24 (53.33)	29 (43.28)		
BMI (kg/m^2)	21.64±1.76	22.03±2.08	1.033	0.304
History of smoking				
Yes	22 (48.89)	41 (61.19)	1.656	0.198
No	23 (51.11)	26 (38.81)		
History of drinking				
Yes	19 (42.22)	35 (52.24)	1.082	0.298
No	26 (57.76)	32 (47.76)		
Residence				
Urban	25 (47.27)	31 (46.27)	0.929	0.335
Rural	20 (52.73)	36 (53.73)		
Dietary preference				
Light	24 (53.33)	32 (52.24)	0.334	0.563
Greasy	21 (46.67)	35 (47.76)		
Tumor size				
<5 cm	0 (0.00)	44 (65.67)		
≥5 cm	0 (0.00)	23 (34.33)		
Lymph node metastasis				
Transferred	0 (0.00)	25 (37.31)		
Not transferred	0 (0.00)	42 (62.69)		
Degree of differentiation				
Poor differentiation	0 (0.00)	19 (28.36)		
Medium + high differentiation	0 (0.00)	48 (71.64)		
TNM staging				
I ± II	0 (0.00)	39 (58.21)		
III ± IV	0 (0.00)	28 (41.79)		

lated (r=-0.609, P<0.001), and the scatter diagram revealed that miR-338 decreased with the increase of miR-20a, as shown in **Figure 1**.

Diagnostic role of miR-338 and miR-20a in GC

miR-338 and miR-20a levels in the OG and the CG were collected to visualize the ROC curve. The results revealed that the area under miR-338 curve was 0.849, and the 95 CI% was 0.776-0.923. The area under miR-20a curve was 0.865, and the 95 CI% was 0.798~0.932, as shown in **Figure 2** and **Table 2**.

Correlation of miR-338 and miR-20a with clinical data

According to the median content of miR-338 and miR-20a in serum, the patients were grouped into high and low expression groups. The difference of general clinical data between the OG and the CG was analyzed and the results revealed that there was no evident difference in age, sex, BMI, smoking history, drinking history, residence and dietary preference, but there was a significant difference in tumor size, lymph node metastasis, degree of differentiation, and TNM stage (**Tables 3** and **4**).

Correlation of the survival of patients with miR-338 and miR-20a

According to the statistics of patient survival rates, 67 patients were followed up, and the total survival was 67.16%. The survival of the high miR-338 group was evidently higher than that of low miR-338 group, and there was a evident difference between the OG and the CG (P=0.029). The survival of the low miR-20a group was evidently higher than that of the high miR-20a group (P=0.003) (**Figure 3**).

Cox regression analysis

The clinical data of GC patients was obtained for Cox regression analysis. Univariate Cox results revealed that tumor size, lymph node metastasis, differentiation degree, TNM stage, miR-338 and miR-20a were prognostic factors of GC patients, while multivariate Cox results revealed that tumor size, lymph node metastasis, differentiation degree, TNM stage, miR-338 and miR-20a were independent prognostic factors, as shown in **Table 5**.

Discussion

GC is a very common disease in the world, with about one million new GC cases every year, and the prognosis is poor [18, 19]. Moreover, many patients suffer from inoperable diseases after

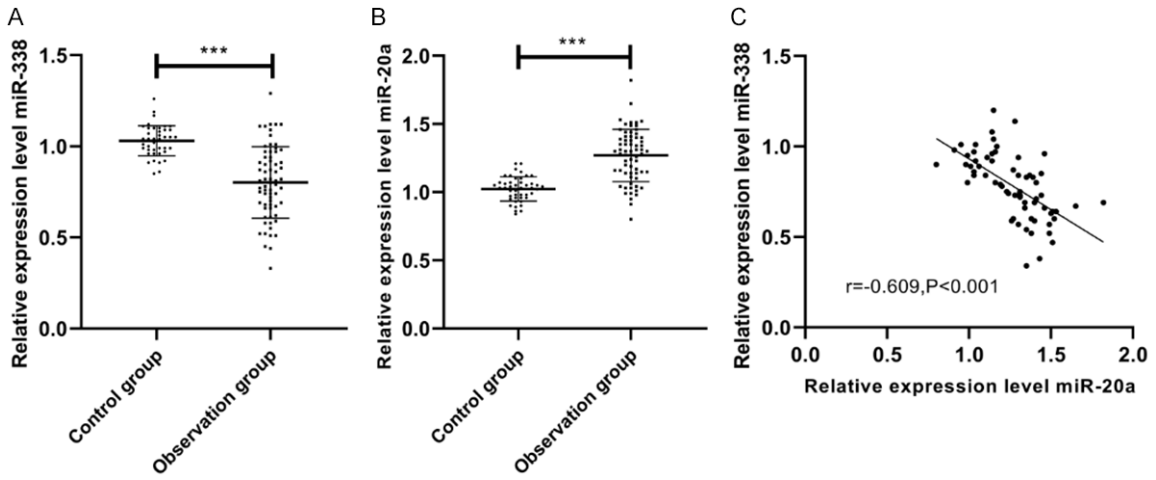

Figure 1. Expression of miR-338 and miR-20a in gastric carcinoma patients' serum. A. The relative expression level of miR-338 in the OG was evidently lower than that in the CG. B. The relative expression level of miR-20a in the OG was evidently higher than that in the CG. C. The expression of miR-338 in GC patients' serum decreased with the increase of miR-20a. *** indicates P<0.001.

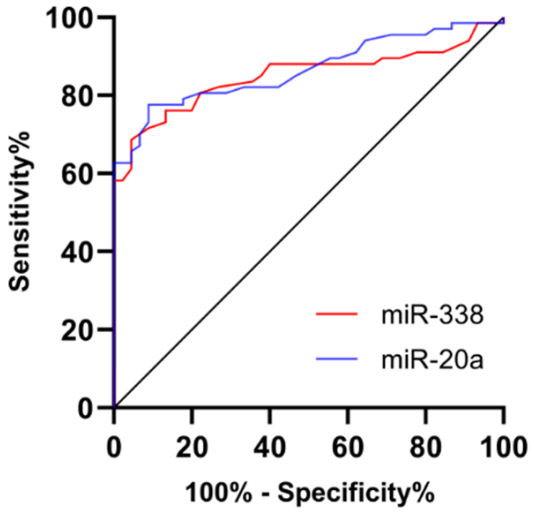

Figure 2. Diagnostic value of miR-338 and miR-20a in GC. The red line indicates the diagnostic value ROC curve of miR-338 in GC, and the area under the curve is 0.849. The blue line indicates the diagnostic value ROC curve of miR-20a in GC, and the area under the curve is 0.865.

diagnosis or relapse after radical gastrectomy, so it is of great significance to seek effective biomarkers for GC [20].

Molecular mechanisms are a new target for GC treatment and they may help with targeted therapy [21]. Many *in vitro* experimental studies have reported the regulatory role of miR in GC cells. Chen et al. [22] revealed that miR-338-3p in GC cells was evidently reduced, and can regulate cell proliferation, migration and invasion by regulating ADAM17, which may be a latent therapeutic target for GC. Xin et al. [23] reported that miR-20a was evidently enhanced in GC, and it can affect the apoptosis and autophagy of GC cells. Therefore, we speculated that miR-338 and miR-20a have extremely important clinical significance. At first, we compared miR-338 and miR-20a in GC patients and healthy people, and found that miR-338 was evidently reduced in GC patients' serum, while miR-20a was enhanced, suggesting that miR-338 and miR-20a were abnormal in GC, and might induce tumor occurrence and development, which may mean that they can be applied as biomarkers to distinguish GC from healthy people. Guo et al. [24] reported that miR-338 was down-regulated in GC patients and acted as a tumor suppressor gene. We speculated that miR-20a played a role in promoting oncogenes through its high expression. Pearson test analyzed the correlation of miR-338 with miR-20a, and found that the two indicators in GC were negatively correlated, suggesting that these two indicators might regulate the development of tumors induced by downstream target protein through antagonistic expression in GC. Then we analyzed the diagnostic value of miR-338 and miR-20a in GC by ROC curve, and found that the areas under the curve of miR-338 and miR-20a were 0.849 and 0.865, respectively, and both indicators had high specificity and good sensiti-

Table 2. ROC related parameters

Indicators	AUC	95 CI%	Specificity	Sensitivity	Youden index	Cut-off value
miR-338	0.849	0.776~0.923	95.56%	68.66%	64.21%	<0.905
miR-20a	0.865	0.798~0.932	91.11%	77.61%	68.72%	>1.130

Table 3. Relationship between miR-338 and clinical data of patients

Factor	High expression group (n=34)	Low expression group (n=33)	t/X^2	*P* value
Age (years)	56.3±7.5	58.2±7.1	1.215	0.227
Gender				
Male (n=38)	16 (47.06)	22 (66.67)	1.619	0.105
Female (n=29)	18 (52.94)	11 (33.33)		
BMI (kg/m^2)	21.58±1.81	22.11±2.13	1.099	0.276
History of smoking				
Yes (n=41)	18 (52.94)	20 (60.61)	0.401	0.527
None (n=26)	16 (47.06)	13 (39.39)		
History of drinking				
Yes (n=35)	17 (50.00)	21 (63.64)	1.268	0.260
No (n=32)	17 (50.00)	12 (36.36)		
Residence				
Urban (n=31)	21 (61.76)	17 (51.52)	0.717	0.397
Rural (n=36)	13 (38.24)	16 (48.48)		
Dietary preference				
Light (n=32)	18 (52.94)	20 (60.61)	0.401	0.527
Greasy (n=35)	16 (47.06)	13 (39.39)		
Tumor size				
<5 cm (n=44)	29 (85.29)	15 (45.45)	11.79	<0.001
≥5 cm (n=23)	5 (14.71)	18 (54.55)		
Lymph node metastasis				
Transferred (n=25)	6 (17.65)	19 (57.58)	11.41	<0.001
Not transferred (n=42)	31 (82.34)	42 (42.42)		
Degree of differentiation				
Poor differentiation (n=19)	4 (11.76)	15 (45.45)	9.356	0.002
Medium + high differentiation (n=48)	30 (88.24)	18 (54.55)		
TNM staging				
I ± II (n=39)	28 (82.35)	11 (33.33)	16.54	<0.001
III ± IV (n=28)	6 (17.65)	22 (66.67)		
miR-338	0.941±0.120	0.672±0.118	9.249	<0.001

vity, indicating that miR-338 and miR-20a have high diagnostic value for GC. This is similar to the research results of Jafarzadeh [25]. In view of the differential expression of miR-338 and miR-20a in GC, we divided the patients into high and low expression groups according to the median content of the two indexes, and analyzed the differences of the general clinical data between the OG and the CG. We found that there were statistical differences in tumor size, lymph node metastasis, differentiation degree and TNM stage. This revealed that the low miR-338 and high miR-20a were correlated with the large tumor, low degree of differentiation, and lymphatic metastasis. We can test the expression of these two indexes to seek the best treatment for patients to improve their prognosis.

In order to explore the relationship of miR-338 and miR-20a with the survival of GC, we followed up the GC patients for three years, and no

Table 4. Relationship between miR-20a and clinical data of patients

Factor	High expression group (n=34)	Low expression group (n=33)	t/X^2	*P* value
Age (years)	57.6±7.8	58.7±8.2	0.563	0.576
Gender				
Male (n=38)	19 (55.88)	19 (57.58)	0.020	0.889
Female (n=29)	24 (44.12)	29 (42.42)		
BMI (kg/m^2)	20.98±2.21	21.84±2.06	1.646	0.105
History of smoking				
Yes (n=41)	22 (64.71)	19 (57.58)	0.359	0.549
No (n=26)	16 (35.29)	13 (42.42)		
History of drinking				
Yes (n=35)	16 (47.06)	19 (57.58)	1.268	0.260
No (n=32)	18 (52.94)	14 (42.42)		
Residence				
Urban (n=31)	17 (50.00)	17 (42.42)	0.387	0.534
Rural (n=36)	13 (50.00)	16 (57.58)		
Dietary preference				
Light (n=32)	18 (41.18)	14 (54.55)	1.200	0.273
Greasy (n=35)	20 (58.82)	15 (45.45)		
Tumor size				
<5 cm (n=44)	8 (23.53)	30 (90.91)	30.97	<0.001
≥5 cm (n=23)	26 (76.47)	3 (9.09)		
Lymph node metastasis				
Transferred (n=25)	6 (17.65)	19 (57.58)	11.41	<0.001
Not transferred (n=42)	28 (82.35)	14 (42.42)		
Degree of differentiation				
Poor differentiation (n=19)	5 (14.71)	14 (42.42)	6.333	0.012
Medium + high differentiation (n=48)	29 (85.29)	19 (57.58)		
TNM staging				
I ± II (n=39)	10 (29.41)	29 (87.88)	23.53	<0.001
III ± IV (n=28)	24 (70.59)	4 (12.12)		
miR-20a	1.425±0.112	1.143±0.107	10.53	<0.001

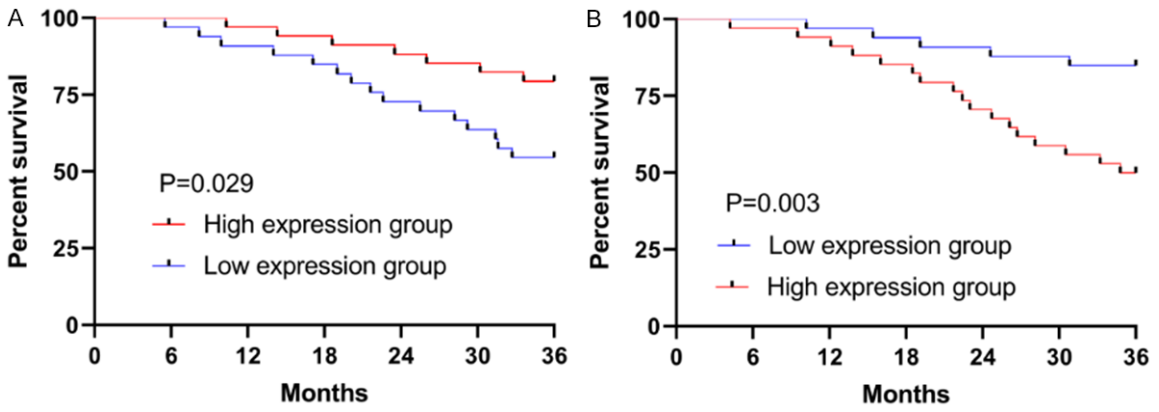

Figure 3. Correlation of patients' survival with miR-338 and miR-20a. A. The overall survival rate of miR-338 high expression group was evidently higher than that of low expression group, and there was a significant difference between the OG and the CG (P=0.029). B. The overall survival rate of miR-20a high expression group was evidently lower than that of low expression group, and there was a significant difference between the OG and the CG (P=0.003).

Table 5. Cox regression analysis

Factor	Univariate Cox			Multivariate Cox		
	Exp (B)	95 CI%	Sig.	Exp (B)	95 CI%	Sig.
Age	0.892	0.433~1.837	0.757			
Gender	0.631	0.769~1.172	0.631			
BMI	2.004	0.936~4.291	0.073			
History of smoking	0.441	0.169~1.153	0.095			
History of drinking	1.241	0.433~3.557	0.688			
Residence	0.781	0.379~1.607	0.501			
Dietary preference	1.279	0.668~2.654	0.527			
Lymph node metastasis	0.146	0.061~0.351	0.000	0.231	0.093~0.578	0.002
Degree of differentiation	0.254	0.127~0.509	0.000	0.327	0.156~0.685	0.003
TNM staging	5.304	2.745~10.247	0.000	3.195	1.636~6.236	0.001
miR-338	3.194	1.856~5.494	0.000	2.677	1.453~4.933	0.002
miR-20a	0.262	0.118~519	0.000	2.723	1.514~5.136	0.002

patients were lost to follow-up. Statistics revealed that the overall survival of GC patients was 67.16%. By visualizing the survival curves of the high and low levels of miR-338 and miR-20a, it was found that the survival of patients with high miR-338 was evidently better than that of patients with low miR-338, and the survival of low miR-20a was evidently better than that of high miR-20a; indicating that miR-338 and miR-20a may be expected to be potential prognostic indicators of GC patients. Therefore, we analyzed the clinical data of GC patients by Cox regression and found that tumor size, lymph node metastasis, differentiation degree, TNM stage, miR-338 and miR-20a were independent prognostic factors. Liu et al. [26] revealed that the decrease of miR-338-3p in GC tissue had a correlation with the low degree of tumor differentiation and lymph node infiltration, suggesting that patients had poor prognosis and low overall survival rate. miR-338-3p can be applied as a biomarker for predicting the sensitivity of radiotherapy and chemotherapy [27]. Yang et al. [16] reported that serum miR-20a had a correlation with GC tumor stage, differentiation degree and lymph node metastasis, and can be used as a molecular marker for GC diagnosis, evaluation of treatment effect and prognosis, and monitoring recurrence of GC patients. Wang et al. [28] found that the tumor volume decreased with the decrease of miR-20a in a GC mouse model. Combined with our research, it was revealed that monitoring miR-338 and miR-20a in the serum of patients with GC played an important role in the diagnosis, treatment and prognosis of GC, and these two indicators are expected to be potential serological indicators of GC.

There are still some shortcomings in this study. Our results revealed that miR-338 had a correlation with miR-20a in GC patients, but we have not made a combined diagnosis of the two indicators, and we do not know the value of the two indicators in predicting the survival of patients. We will supplement these questions in the future.

To sum up, miR-338 and miR-20a are expected to be serological indicators for GC diagnosis and prognosis.

Disclosure of conflict of interest

None.

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