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ORIGINAL RESEARCH

Serum miR-191 and miR-425 as Diagnostic and Prognostic Markers of Advanced Gastric Cancer Can Predict the Sensitivity of FOLFOX Chemotherapy Regimen

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Purpose: miR-191 and miR-425 have been proved to be highly expressed in gastric carcinoma (GC). However, little research has been done on their clinical value in serum of patients with advanced GC. In addition, it is not clear whether they can be used as markers for the response and prognosis of GC patients treated with oxaliplatin combined with 5-fluorouracil and FOLFOX chemotherapy.

Patients and Methods: A total of 230 patients with advanced GC admitted to our hospital were selected as the study objects, all of whom received FOLFOX chemotherapy regimen. Another 100 cases of healthy subjects were included. QRT-PCR was employed to detect the serum expression of miR-191 and miR-425 in patients.

Results: Compared with the healthy subjects, the serum expressions of miR-191 and miR-425 in GC patients were significantly upregulated, which were correlated with differentiation degree and TNM staging, respectively. According to the ROC curve, the AUC of miR-191 and miR-425 for GC diagnosis was 0.937 and 0.901, respectively, while the AUC for differentiation degree diagnosis was 0.854 and 0.822, and that for TNM staging diagnosis was 0.860 and 0.829, respectively. The predictive AUC of miR-191 and miR-425 for chemosensitivity was 0.868 and 0.835, respectively, with a combined predictive AUC of 0.935. Low differentiation degree, high TNM staging, high miR-191 and high miR-425 expressions were independent risk factors for chemotherapy insensitivity. Differentiation degree, TNM staging, chemotherapy effect, miR-191 and miR-425 were independent influencing factors for the prognosis of GC patients.

Conclusion: Up-regulated expression of miR-191 and miR-425 in the serum of patients with advanced GC are effective biomarkers for the diagnosis, chemotherapy and prognosis evaluation of GC.

Keywords: miR-191, miR-425, FOLFOX, chemotherapy, prognosis, GC

Introduction

Gastric carcinoma (GC) ranks the fourth place in the incidence rate of all malignant tumors, just followed by lung cancer, colon cancer and breast cancer, whose mortality rate ranks the second among all cancers.[1] The incidence and mortality of GC vary geographically. About half of the cases occur in East Asia, among which China accounts for about 42.6% of the world's new cases and 45% of all

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GC-related deaths every year.[2] Surgical resection can be performed in patients with early or locally advanced GC, with the 5-year survival rate of 90%.[3] However, as the disease presents no obvious clinical symptoms and characteristics at the early stage, a majority of patients only to be found in the advances stages when diagnosed, resulting in poor diagnostic rate, with an overall 5-year survival rate of less than 30%.[4] Chemotherapy becomes the prioritized surgical approach for advanced GC patients, but only serve the purpose of palliative treatment as GC is too difficult to cure.[5] Clinically, oxaliplatin combined with 5-fluorouracil and folinic acid (FOLFOX) is an effective chemotherapy regimen for patients with advanced GC.[6] However, GC is a kind of malignant tumor with strong heterogeneity, whose primary or acquired drug resistance prevent chemotherapy from completely destroying the tumor cells, while chemotherapy insensitivity is a common cause of tumor recurrence and metastasis.[7] Therefore, the evaluation of the chemotherapy effect and survival rate of patients with advanced GC can help optimize the treatment strategy.

MiRNA is a class of endogenous non-coding small RNA, which can directly bind to the mRNA 3′ non-coding region of the target gene, thus directly degrading the mRNA or inhibiting the translation process.[8] MiRNA can alter the occurrence and development of various malignant tumors by affecting their biological functions, including proliferation, migration, and invasion.[9] It also plays a role in the diagnosis, severity judgment and prognosis of various malignant tumors including GC.[10,11] MiR-191 and miR-425 are abnormally expressed in various cancers, such as lung cancer, liver cancer, GC, etc.[12,13] MiR-191, a part of miR-191/miR-425 clusters, is upregulated in the blood of patients with various malignant tumors, which can be used as a non-invasive biomarker for tumor diagnosis and prognosis.[14] Studies in recent years have shown that the overexpression of miR-191/miR-425 clusters in breast cancer cells can lead to changes in gene expression profiling, thereby fundamentally changing the occurrence and progression of breast cancer.[15] In addition, Vaira[16] revealed that miR-425-3p could predict the response of hepatocellular carcinoma to sorafenib treatment. However, the role of blood miR-191 and miR-425 in the diagnosis, FOLFOX chemotherapy, and prognosis of GC patients remains poorly understood.

The expression of serum miR-191 and miR-425 of GC patients was detected by qRT-PCR to explore their clinical value in GC patients and their relationship with chemotherapy response and prognosis.

Materials and Methods

General Information

A total of 230 patients with advanced GC admitted to Affiliated Cancer Hospital of Zhengzhou University from February 2011 to May 2014 were enrolled, including 156 males and 74 females, aged 36–78 years. The inclusion criteria were as follows: GC patients diagnosed histologically or pathologically without any previous radiotherapy or chemotherapy prior to this study, whose Eastern Cooperative Oncology Group (ECOG) score[17] was no more than 2 points, and received at least two cycles of chemotherapy with an estimated survival time of no less than 12 weeks. The exclusion criteria were as follows: Patients with other malignant tumors. Patients with central nervous system metastasis. Patients with mental disorders who cannot cooperate in this study. Patients withdraw from the experiment or lost to follow-up. Another 100 healthy subjects from the same period were selected, including 60 males and 40 females, aged 35–76 years. The research program was approved by the Medical Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University and the experiment was carried out in accordance with the Helsinki Declaration. Written informed consent forms were obtained from all patients in this study.

Chemotherapy

All GC patients received FOLFOX chemotherapy regimen.[18] On the first day, oxaliplatin (130mg/m^2) was given intravenously for 2 hrs, followed by leucovorin (200mg/m^2) for 2 hrs, and then 5-fluorouracil (450mg/m^2) for 22 hrs. Chemotherapy was repeated every 3 weeks, with 21 days as a cycle, and a total of 2 cycles were performed. In comply with the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) of United States,[19] the dosage of 5-fluorouracil was reduced by 15% in the event of grade 3–4 diarrhea, stomatitis or dermatitis, and the dosage of oxaliplatin was decreased by 15% in the case of persistent paresthesia and functional impairment during the chemotherapy cycle.

Curative Effect Evaluation

According to RECIST1.1 solid tumor efficacy evaluation criteria,[20] the diagnosis and corresponding clinical symptoms were as follows: Complete response (CR): The target lesion disappeared completely and remained for at least 2 weeks. Partial response (PR): A reduction of at least 30% in the sum of the maximum length and diameter of baseline lesions, without the occurrence of new lesions. Progressive disease (PD): The sum of the maximum length and diameter of baseline lesions increases by at least 20% or new lesions appear. Stable disease (SD): The sum of the maximum length and diameter of baseline lesions decreased but did not reach PR, or increased but did not reach PD, falling between PR and PD.

QRT-PCR Detection

An amount of 5mL of peripheral venous blood samples were taken from all study subjects and placed in vacuum blood collection vessels. The samples were then centrifuged at 1500g for 10min at 4°C, and 1.0mL of the obtained serum was collected and stored at -80°C for later use. Next, the total RNA was extracted using MagMAX mir Vana isolation kit (Shanghai Even bridge biotechnology Co., Ltd., A27828), whose RNA concentration and purity were detected by NanoDrop 1000 ultramicrospectrophotometer (NanoDrop, Wilmington, DE, USA), while RNA integrity identified by agarose gel electrophoresis. The RNA samples were retrotranscribed according to the instructions of TaqMan MicroRNA reverse transcription kit (Shanghai Even bridge biotechnology Co., Ltd., 4366596). PCR experiments were performed in triplicate using TaqMan Universal PCR Master Mix (Shanghai Runwell Technology Co., Ltd., AB-4324018) of ABI 7300 real-time fluorescence quantitative PCR system (Applied Biosystems, Foster City, CA, USA, 4318157). The amplification conditions of qPCR were as follows: 94°C: 5min, 94°C: 30s, 55°C: 30s, 72°C: 30s, totaling 40 cycles. Finally, the cyclic threshold (Ct) value was calculated by SDS 2.0.1 software, and the data were analyzed by $2^{-\Delta\Delta ct}$. The primer sequence was designed and synthesized by Guangzhou Ruibo Biotechnology Co., Ltd.

Primer sequence of MiR-191

Forward: 5′-AAGGAATCTTTCTGCACTCAAGCAT-3′,

Reverse: 5′-ATGCTTGAGTGCAGAGATTCCCTT-3′.

Primer sequence of MiR-425:

Forward: 5′-ACACTCCAGCTGGGAATGACACGATCACTCC-3,

Reverse: 5′-TGGTGTCGTGGAGTCG-3′.

Primer sequence of U6:

Forward: 5′-CTCGCTTCGGCAGCACA-3′,

Reverse: 5′-ACGCTTCACGAATTTGCGT-3′.

Statistical Methods

The counting data were expressed by case/percentage n(%) and a chi-square test was adopted for comparison of counting data between groups. Data with normal distribution were presented as mean ± standard deviation (Meas±SD). A *t*-test was adopted for inter-group comparison, and a paired *t* test was employed for comparison between groups before and after chemotherapy. Receiver operating characteristic curve, also known as ROC, and area under the curve (AUC) were employed to evaluate the diagnostic value of miR-191 and miR-425 in GC. With miR-191 and miR-425 as independent variables, logistic regression model was established to fit the ROC curve of joint detection according to the probability value. Spearman rank correlation coefficient was applied for correlation analysis. Logistic single-factor and multiple-factor regression analysis was used to analyze the risk factors of the efficacy of radiotherapy and chemotherapy in GC patients. Kaplan-Meier survival curve was drawn to calculate the survival rate, and Log rank test was used for survival analysis. Multivariate Cox regression analysis was adopted to analyze the risk factors affecting the prognosis of GC patients. $P<0.05$ was considered to be statistically different. SPSS 22.0 software (Company, Chicago, Illinois, USA) was employed for statistical analysis.

Results

Clinical Value of Serum miR-191 and miR-425 in GC

QRT-PCR showed that the serum miR-191 and miR-425 in GC patients were significantly up-regulated compared with those in healthy subjects ($P<0.001$). Further observation of the relationship between the clinical characteristics and the expressions of miR-191 and miR-425 indicated that miR-191 and miR-425 were related to the degree of differentiation and TNM staging, respectively ($P<0.05$). In addition, ROC curve analysis revealed that the AUC of serum miR-191 and miR-425 for GC diagnosis was 0.937 and 0.901, respectively, the AUC of serum miR-191 and miR-425 for differentiation degree diagnosis was 0.854 and 0.822, and the AUC of

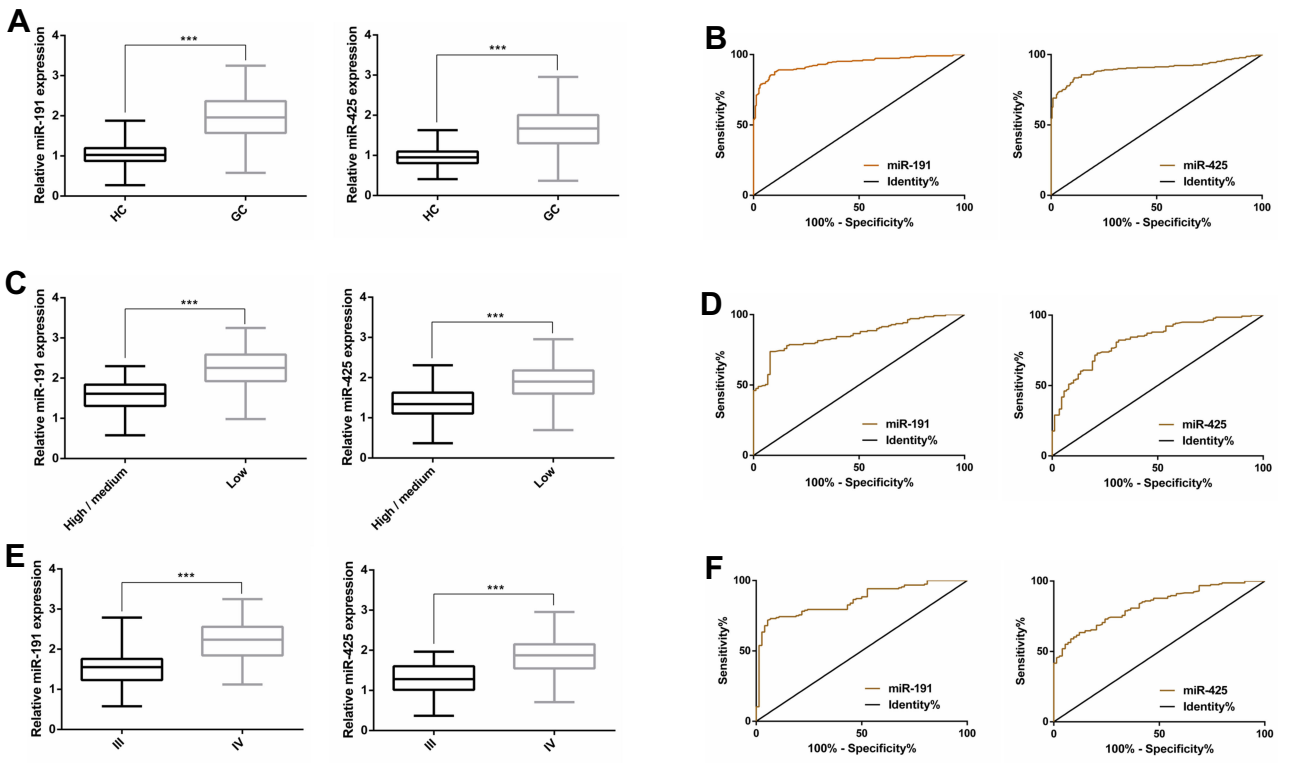

Figure 1 Clinical value of serum miR-191 and miR-425 in GC. (**A**) The expression of miR-191and miR-425in serum of GC patients; (**B**) the ROC curves of miR-191and miR-425for GC diagnosis; (**C**) the expression of miR-191and miR-425 in differentiation degree; (**D**) the ROC curves of miR-191and miR-425for differentiation degree diagnosis; (**E**) the expression of miR-191 and miR-425 in TNM staging; (**F**) the ROC curves of miR-191 and miR-425 for TNM staging diagnosis.
Note: ***P<0.001.

serum miR-191 and miR-425 for TNM staging diagnosis was 0.860 and 0.829, respectively (Figure 1, Tables 1 and 2).

Relationship Between Serum miR-191, miR-425 and Chemotherapy Sensitivity of GC Patients

Of the 230 GC patients who received chemotherapy, the cases/(percentage) and corresponding diagnosis were as follows: 7/(3.05%): CR, 80/(34.78%): PR, 86/(37.39%): SD, and 57/(24.78%): PD. No clinical symptoms of sepsis or inflammatory diseases were observed during chemotherapy. Besides, qRT-PCR was employed to detect the expression of miR-191 and miR-425 in serum of GC patients before and after chemotherapy. It was found that both miR-191 and miR-425 were significantly down-regulated after chemotherapy (P<0.001). Based on the treatment effect, CR was set as 1, PR as 2, SD as 3, and PD as 4. Spearman correlation coefficient showed that miR-191 and miR-425 were both positively correlated with the chemotherapy effect (r_{miR-19}=0.686, P<0.001, $r_{miR-425}$=0.661, P<0.001). Serum miR-191 and miR-425

Table 1 ROC Parameters

Parameters	AUC	95%CI	Cut-Off	Specificity (%)	Sensitivity (%)
miR-191					
GC	0.937	0.914–0.960	1.352	90.00	87.83
Differentiation degree	0.854	0.806–0.902	1.994	92.13	73.76
TNM staging	0.860	0.812–0.908	1.964	94.59	71.79
miR-425					
GC	0.901	0.869–0.932	1.200	89.13	83.48
Differentiation degree	0.822	0.768–0.876	1.652	78.65	73.05
TNM staging	0.829	0.777–0.880	1.720	87.84	63.46

Table 2 Relationship Between miR-191, miR-425 and Clinicopathological Parameters of GC Patients (Meas±SD)

Clinicopathological Parameters	n	miR-191	t/F	P	miR-425	t/F	P
Gender			0.264	0.792		0.298	0.766
Male	156	1.980±0.563			1.675±0.498		
Female	74	1.959±0.545			1.654±0.487		
Age (years)			1.654	0.100		1.057	0.292
<60	138	1.924±0.578			1.640±0.541		
≥60	92	2.047±0.516			1.710±0.412		
Drinking			0.730	0.466		0.734	0.464
No	128	1.997±0.568			1.689±0.502		
Yes	102	1.943±0.541			1.641±0.484		
ECOG performance status			1.340	0.182		1.563	0.119
0–1	148	1.937±0.534			1.630±0.481		
2	82	2.039±0.591			1.736±0.512		
Differentiation degree			10.980	<0.001		9.651	<0.001
High+medium differentiation	89	1.563±0.385			1.315±0.394		
Low differentiation	141	2.232±0.488			1.890±0.415		
TNM staging			10.660	<0.001		9.462	<0.001
III	74	1.509±0.388			1.288±0.384		
IV	156	2.194±0.483			1.848±0.435		
Tumor size (cm)			1.493	0.137		1.258	0.210
<6	140	1.929±0.575			1.692±0.509		
≥6	90	2.041±0.520			1.631±0.469		
Tumor site			0.222	0.801		0.850	0.429
Cardia, gastric fundus	30	1.931±0.627			1.623±0.528		
Corpus ventriculi	105	1.961±0.559			1.714±0.500		
Gastric antrum, pylorus	95	2.000±0.534			1.631±0.476		
CEA (ng/mL)			1.030	0.304		1.323	0.187
<5	155	1.947±0.567			1.638±0.528		
≥5	75	2.027±0.531			1.730±0.410		
CA199 (kU/L)			1.522	0.130		0.995	0.321
<37	143	1.930±0.565			1.643±0.525		
≥37	87	2.045±0.537			1.709±0.437		

were significantly different between chemotherapy-sensitive and insensitive patients (P<0.001). In addition, the ROC curve indicated that the AUC of predicting chemosensitivity of miR-191 and miR-425 were 0.868 and 0.835, respectively, while that of miR-191/425 combined was 0.935 (Figure 2, Table 3).

Relationship Between the Clinicopathological Parameters and the Sensitivity to Chemotherapy in GC Patients

According to the therapeutic effect of patients, CR and PR were defined as chemotherapy-sensitive (n=87), while SD and PD were defined as chemotherapy-insensitive (n=143). The correlation between between the clinicopathological parameters, miR-191 and miR-425 and the sensitivity to chemotherapy in GC patients was analyzed. Univariate analysis results showed that age, differentiation degree, TNM staging, tumor size, miR-191, and miR-425 were correlated with the sensitivity to chemotherapy (P<0.05). The median values of miR-191 (1.958) and miR-425 (1.667) were set as segmentation points, and the binary Logistic regression equation was employed to carry out multivariate logistic regression analysis of the factors with differences. The results demonstrated that the

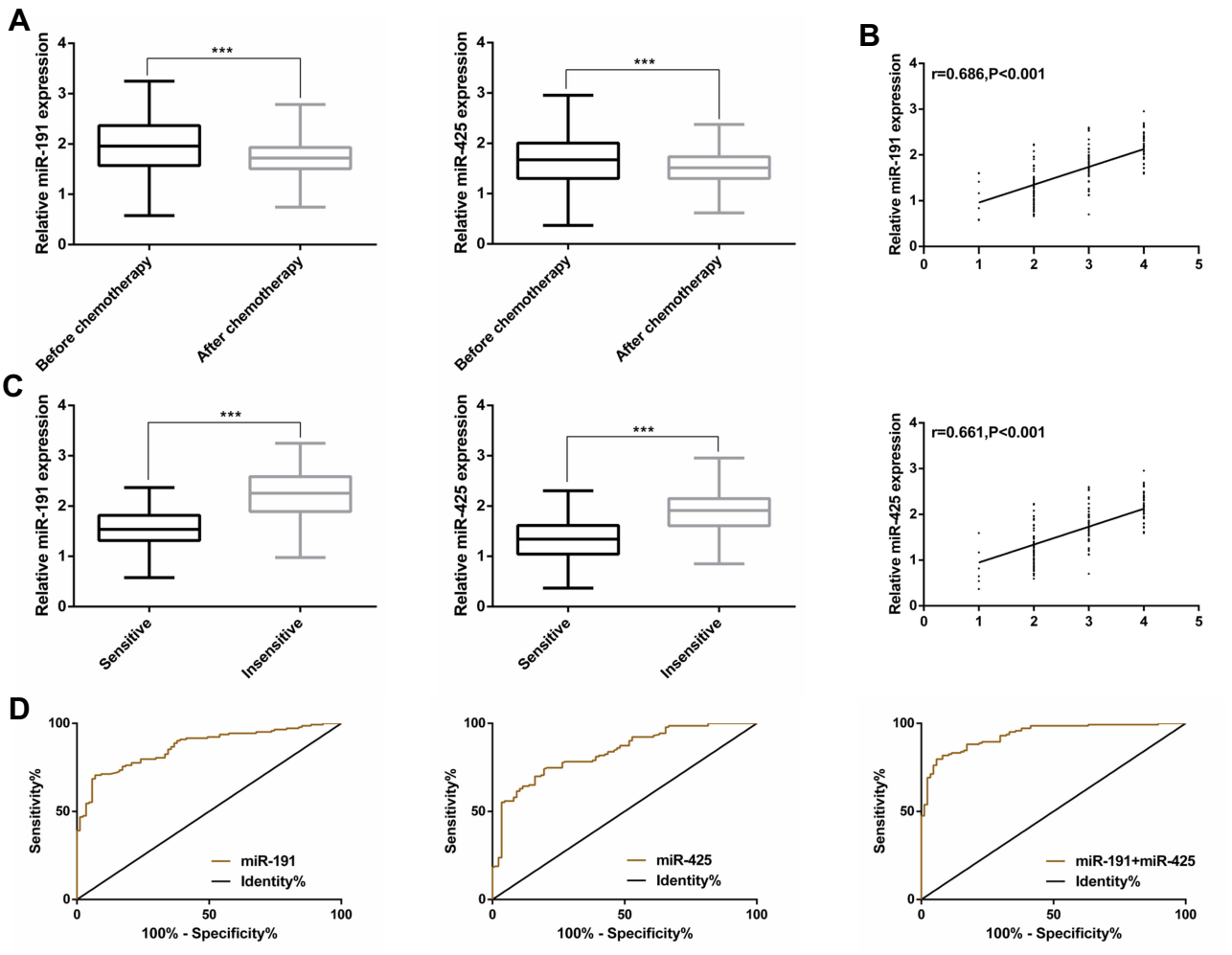

Figure 2 Relationship between serum miR-191, miR-425 and chemotherapy sensitivity of GC patients. (**A**) Expression of serum miR-191 and miR-425 before and after chemotherapy of GC patients; (**B**) MiR-191 and miR-425 were positively correlated with chemotherapy effect; (**C**) expression of serum miR-191 and miR-425 in chemotherapy-sensitive and insensitive patients. (**D**) The ROC curve of miR-191 miR-425 and miR-191/425 combined for predicting the sensitivity to chemotherapy.
Note: ***P<0.001.

differentiation degree, TNM staging, miR-191 and miR-425 were independent risk factors for chemotherapy sensitivity in GC patients (P<0.05). (Tables 4–6)

Relationship Between Clinicopathological Parameters, miR-191 and miR-425 and Prognosis of GC Patients

The median follow-up time of 230 GC patients was 12.9 months (ranging from 3.0 to 50.0 months), with a median survival time was 13.0 months. Univariate analysis demonstrated that age, differentiation degree, TNM staging, chemotherapy effect, miR-191, and miR-425 were associated with median survival time (P<0.05). The median survival time of patients with miR-191 <1.958 was significantly longer than that of patients with miR-191 ≥1.958 (P<0.01), while that of patients with miR-425 <1.667 was also significantly longer than that of patients with miR-425 ≥1.667

Table 3 ROC Parameters

Parameters	AUC	95%CI	Cut-Off	Specificity (%)	Sensitivity (%)
miR-191	0.868	0.823–0.913	2.029	93.10	70.63
miR-425	0.835	0.784–0.887	1.644	80.46	74.13
miR-191+miR-425	0.935	0.904–0.965	0.729	94.25	79.72

Table 4 Relationship Between the Clinicopathological Parameters, miR-191 and miR-425 and the Sensitivity to Chemotherapy in GC Patients [n(%)]

Factors	n	Sensitive (n=87)	Insensitive (n=143)	χ^2	P
Gender				0.336	0.562
Male	156	61 (70.11)	95 (66.43)		
Female	74	26 (29.89)	48 (33.57)		
Age (years)				4.687	0.030
<60	138	60 (68.97)	78 (54.55)		
≥60	92	27 (31.03)	65 (45.45)		
Drinking				3.246	0.072
No	128	55 (63.22)	73 (51.05)		
Yes	102	32 (36.78)	70 (48.95)		
ECOG performance status				2.029	0.154
0–1	148	61 (70.11)	87 (60.84)		
2	82	26 (29.89)	56 (39.16)		
Differentiation degree				8.324	0.004
High+medium differentiation	89	44 (50.57)	45 (31.47)		
Low differentiation	141	43 (49.43)	98 (68.53)		
TNM staging				12.220	<0.001
III	74	40 (45.98)	34 (23.78)		
IV	156	47 (54.02)	109 (76.22)		
Tumor size (cm)				6.348	0.012
<6	140	62 (71.26)	78 (54.55)		
≥6	90	25 (28.74)	65 (45.45)		
Tumor site				1.913	0.384
Cardia, gastric fundus	30	13 (14.94)	17 (11.89)		
Corpus ventriculi	105	43 (49.43)	62 (43.36)		
Gastric antrum, pylorus	95	31 (35.63)	64 (44.76)		
CEA (ng/mL)				0.224	0.636
<5	155	57 (65.52)	98 (68.53)		
≥5	75	30 (34.48)	45 (31.47)		
CA199 (kU/L)				0.00	0.980
<37	143	54 (62.07)	89 (62.24)		
≥37	87	33 (37.93)	54 (37.76)		
miR-191				11.550	<0.001
<1.958	115	56 (64.37)	59 (41.26)		
≥1.958	115	31 (35.63)	84 (58.74)		
miR-425				8.153	0.004
<1.667	115	54 (62.07)	61 (42.66)		
≥1.667	115	33 (37.93)	82 (57.34)		

(P<0.01). Further multivariate Cox regression analysis demonstrated that differentiation degree, TNM staging, chemotherapy effect, miR-191, and miR-425 were independent prognostic factors for GC patients (P<0.05). (Figure 3, Tables 7 and 8).

Discussion

GC patients are usually in an advanced stage when diagnosed, at which time platinum compounds are the main chemotherapy regimen in clinical practice.[21] However, some patients present primary or secondary drug

Table 5 Logistic Regression Analysis Assignment

Factors	Variables	Assignments
Age (years)	X1	<60=1, ≥60=2
Differentiation degree	X2	High+medium differentiation=1, low differentiation=2
TNM staging	X3	III=1, IV=2
Tumor size (cm)	X4	<6=1, ≥6=2
miR-191	X5	<1.958=1, ≥1.958=2
miR-425	X6	<1.667=1, ≥1.667=2

Table 6 Multivariate Logistic Regression Analysis

Factors	β	S.E	Wals	OR (95% CI)	P
Age (years)	0.180	0.286	0.399	1.198 (0.684–2.097)	0.527
Differentiation degree	1.450	0.681	4.534	4.265 (1.122–16.209)	0.033
TNM staging	2.121	0.786	7.283	8.339 (1787–38.911)	0.007
Tumor size (cm)	1.159	0.630	3.380	3.185 (0.996–10.955)	0.066
miR-191	1.851	0.768	5.809	6.369 (1.413–28.702)	0.016
miR-425	1.509	0.591	6.515	4.521 (1.419–14.398)	0.011

resistance in chemotherapy application, leading to poor therapeutic effect and seriously affecting prognosis.[22] Therefore, the differentiation of patients with poor prognosis after chemotherapy can help optimize the treatment for patients with advanced GC.

Many studies have confirmed that abnormal miRNA expression in blood is closely related to the severity and prognosis of various malignant tumors.[23,24] MiR-191 and miR-425 are believed to be highly expressed in GC.[25] As reported by Shi,[26] the up-regulated expression of miR-191 in GC cells and tissues can promote the growth of GC cells by inhibiting n-deacetylase/n-sulfone-based transferase 1 (NDST1). It has also been reported that the up-regulated expression of miR-425 in human GC cells can promote invasion and metastasis.[27] Therefore, it can be concluded that miR-191 and miR-425 play an essential role in the occurrence and development of GC. However, previous studies focus on GC tissues or cells, in which tumor tissues need to be acquired by means of surgical resection or puncture, which is relatively traumatic, while serum miR detection stands out for its small trauma.[28] In the present study, the expression levels of miR-191 and miR-425 in serum of GC patients were significantly up-regulated compared with normal people, and further ROC curve was drawn to find that the AUC of miR-191 and miR-425 in diagnosing GCwas 0.937 and 0.901, respectively, with good diagnostic value. Previous studies have shown that the expression of miR-191/425 clusters in GC tissues and serum increased significantly, and the AUC values of serum miR-191 and miR-425 for GC diagnosis were 0.849 and 0.548, respectively.[29] This may be due to the fact that only advanced GC patients were included in this study, resulting in differences in diagnostic efficacy. In addition, by analyzing the relationship between the two and the clinical pathological parameters of GC patients, it was found that miR-191 and miR-425 were related to the differentiation degree and TNM staging, respectively, and had differential diagnostic value for these pathological parameters, which indicated that miR-191 and miR-425 could be used as markers for the evaluation of GC.

FOLFOX is supposed to be an effective palliative treatment for advanced GC patients.[30] It is well established that markers can be used to predict the efficacy of GC chemotherapy. For example, in the study of Oh,[31] vascular endothelial growth factor (VEGF) gene polymorphism can distinguish the response rate of FOLFOX chemotherapy in advanced GC patients (22.2% vs 32.3%), and the increased expression is a prognostic factor affecting progression-free survival. In addition, it has been observed that serum miR-19a is significantly up-regulated in serum during the drug-resistant phase of colorectal cancer, which can be used as a predictive marker of drug resistance in FOLFOX chemotherapy regimen.[32] In this study, the expressions of miR-191 and miR-425 in serum of GC patients after chemotherapy were detected, and it was found both expressions were significantly down-regulated after chemotherapy, which indicated that the FOLFOX chemotherapy could inhibit the expression of both; however, the mechanism remains unclear. Next, the relationship between miR-191, miR-425 and the chemotherapy efficacy was analyzed, and the results exhibited that serum miR-191 and miR-425 were positively correlated with the chemotherapy efficacy, respectively, before chemotherapy. Moreover, ROC curve revealed that the AUC of predicting chemosensitivity of miR-191 and miR-425 were 0.868 and 0.835, respectively, while that of combined prediction of miR-191 and miR-425 was 0.935, suggesting that the combined detection of the two has a high predictive value for chemosensitivity. What is more, logistic regression analysis revealed that patients with low differentiation, TNM staging, high miR-191 and miR-425 expression in GC were at increased risk of chemotherapy insensitivity. Previous studies have supported that miR-191 can be acted as a candidate target gene for the treatment of hepatocellular carcinoma.[33] According to Zhang,[34] miR-425 can regulate the chemical resistance of

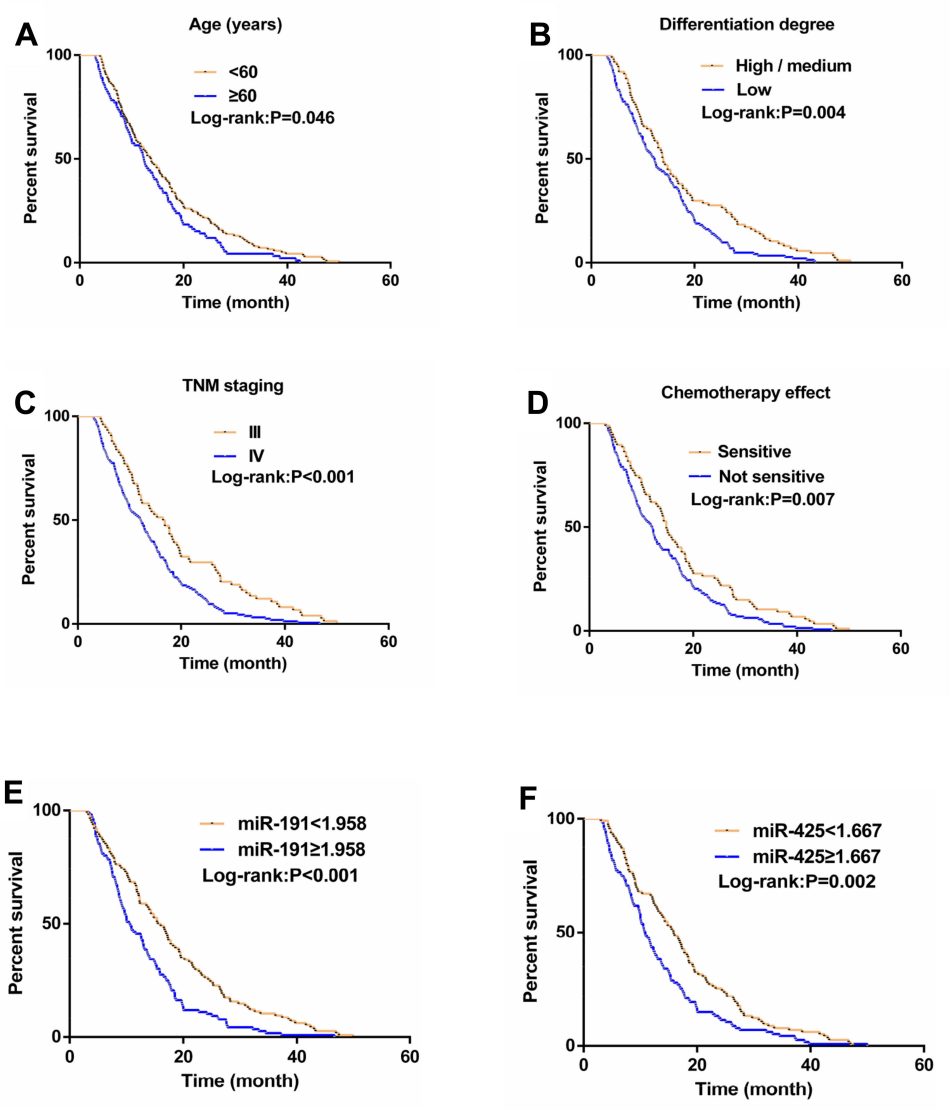

Figure 3 Relationship between clinicopathological parameters, miR-191 and miR-425 and prognosis in GC patients. The overall survival curve was plotted according to age (**A**), differentiation (**B**), TNM staging (**C**), chemotherapy effect (**D**), miR-191 (**E**), and miR-425 (**F**).

colorectal cancer cells by regulating programmed cell death 10 (PDCD10). Combined with this study, miR-191 and miR-425 may play a role in the chemotherapy of various tumors, but the drug resistance mechanism of both in GC chemotherapy remains a subject of investigation. Further observation of the relationship between miR-191, miR-425 and the prognosis of GC patients demonstrated that the median survival time of low miR-191 (<1.958) and miR-425 (<1.667) was significantly prolonged, and the differentiation degree, TNM staging, chemotherapy effect, miR-191 and miR-425 were independent prognostic factors for GC patients. Although previous studies have confirmed that differentiation degree and TNM staging can affect the prognosis of GC patients after chemotherapy,[35,36] here it is the first time that micR-191 and miR-425 are verified to be the influencing factors of chemotherapy and prognosis in GC patients.

Taken together, this study confirmed that miR-191 and miR-425 were upregulated in serum of patients with advanced GC, which are expected to be effective biomarkers for GC diagnosis, chemotherapy and prognosis evaluation. However, there are still shortcomings in the present study. To begin with, in vitro experiments are absent, and we failed to observed drug resistance mechanism of miR-191 and miR-425 in GC cells. And secondly, miR-191 and miR-425 need to be combined

Table 7 Univariate Analysis of Prognostic Factors in Patients with GC

Factors	n	Median Survival Time (Month)	χ^2	P
Gender			0.182	0.670
Male	156	12.6		
Female	74	14.0		
Age (years)			3.988	0.046
<60	138	13.8		
≥60	92	12.4		
Drinking			1.424	0.233
No	128	13.1		
Yes	102	12.9		
ECOG performance status			2.052	0.152
0–1	148	13.0		
2	82	12.8		
Differentiation degree			8.169	0.004
High+medium differentiation	89	14.0		
Low differentiation	141	12.3		
TNM staging			13.690	<0.001
III	74	16.9		
IV	156	12.3		
Tumor size (cm)			0.926	0.336
<6	140	13.7		
≥6	90	11.4		
Tumor site			3.966	0.138
Cardia, gastric fundus	30	12.9		
Corpus ventriculi	105	13.0		
Gastric antrum, pylorus	95	12.6		
CEA (ng/mL)			2.839	0.092
<5	155	14.0		
≥5	75	10.9		
CA199 (kU/L)			1.004	0.316
<37	143	14.1		
≥37	87	12.3		
Chemotherapy effect			7.201	0.007
Sensitive	87	14.8		
Insensitivity	143	12.1		
miR-191			15.450	<0.001
<1.958	115	15.8		
≥1.958	115	10.7		
miR-425			9.253	0.002
<1.667	115	15.9		
≥1.667	115	10.9		

Table 8 Multivariate Cox Regression Analysis of Prognosis in Patients with GC

Factors	HR (95% CI)	P
Age (years)	1.483 (0.781–2.768)	0.224
Differentiation degree	2.142 (1.161–3.957)	0.015
TNM staging	3.786 (2.324–6.167)	<0.001
Chemotherapeutic effect	2.138 (1.167–3.982)	0.017
miR-191	3.517 (1.978–6.267)	<0.001
miR-425	2.367 (1.175–4.683)	0.013

with traditional biomarkers of gastric cancer such as carbohydrate antigen 724 and pepsinogen in the clinical practice of pancreatic cancer. Nevertheless, these deficiencies will be addressed in follow-up studies.

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Disclosure

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