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ORIGINAL ARTICLE

The expression and prognostic value of miR-195-5p in patients with advanced gastric cancer after chemotherapy

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Summary

Purpose: *To explore miR-195-5p expression and prognostic value in advanced gastric cancer (AGC) patients after chemotherapy.*

Methods: *114 patients in total with AGC admitted to our hospital undergoing chemotherapy created the gastric cancer group, and 100 simultaneous healthy subjects undergoing physical examination created the normal group. MiR-195-5p level in both groups was detected.*

Results: *miR-195-5p level was evidently lower in the gastric cancer group, and miR-195-5p could be used in diagnosing gastric cancer patients. miR-195-5p was significantly associated with clinical stage and grade of differentiation. It was found that carcinoembryonic antigen (CEA) and carbohydrate antigen 19.9 (CA19.9) were significantly negatively associated with miR-195-5p level before treatment in patients with AGC. Subjects were divided into effective group and ineffective group based on the therapeutic effect, mir-195-5p level in the ineffective group was notably lower than that in the effective group, and the serum miR-195-5p level was available for efficacy prediction. Patients were separated to high expression group and low expression group based on the median of the mir-195-5p expression. High expression group had remarkably better survival in comparison with the low expression group, and miR-195-5P expression of dead patients was lower than that of the surviving patients.*

Conclusion: *Serum miR-195-5p is decreased in AGC patients, and can be effectively utilized as a biomarker for the diagnosis and prognosis of patients with gastric cancer.*

Key words: *miR-195-5p, advanced gastric cancer, chemotherapy, prognostic analysis*

Introduction

As one of the fatal malignancies worldwide, gastric cancer ranks fourth in the incidence of malignant tumors, and second in mortality [1,2]. The occurrence of the disease varies greatly regionally, with more than 50% of new cases occurring in developing countries, and East Asia is the main high-risk area [3]. According to statistics, the annual incidence of gastric cancer in China accounts for about 42.6% of the world, and the mortality rate accounts for about 45% [4]. Since early gastric cancer patients have no obvious symptoms, statistics show that most people diagnosed with advanced gastric cancer (AGC) cannot be operated on or relapse after undergoing surgery [5]. Previous relevant literature reports that patients diagnosed with AGC have a higher mortality rate, with a 5-year survival rate of only about 20% [6]. Due to the limitation of patients with advanced disease in the treatment process, systemic chemotherapy is still the main method for the treatment of patients with AGC [5].

MicroRNA (miR) is a new biomarker for gastric cancer, which has been widely used in recent years [7]. MiR is an endogenous non-coding protein RNA gene (approximately 18-24 nucleotides in length) that is vital in many diseases, which mediates and

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regulates the production of proteins by interacting with mRNA [8]. MiR can play a role in a variety of cancers, including the diagnosis, severity judgment and prognosis prediction of gastric cancer [9,10]. For example, Li et al [11] detected the expression of serum miR-17-92 and found that miR-17-92 could be used for early diagnosis of gastric cancer and was a potential biomarker of gastric cancer. MiR-195-5p can target various cancer-related genes and participate in the regulation of the biological behavior of a variety of diseases [12]. Elevated plasma miR-195-5p level is reported to be associated with soluble vascular endothelial growth factor receptor 1 (sFlt-1) level in preeclampsia, suggesting that miR-195-5p can be used as a biomarker in preeclampsia patients. However, there has been no study on the clinical value of serum miR-195-5p in AGC patients [13].

Herein, miR-195-5p level and prognostic value in AGC patients after chemotherapy were investigated by determining of miR-195-5p in serum of AGC patients.

Methods

General data

Totally 114 patients with AGC were included in the gastric group, with 71 males and 43 females and a mean age of 61.34±5.76 years. Simultaneously, 100 cases of healthy subjects undergoing physical examination in our hospital were included in the normal group, WITH 58 males and 42 females AND A MEAN age of 61.86±6.09 years. This study has been approved by the ethics committee. The patients and their families have been informed in advance, and informed consent form has been signed. Inclusion criteria: Patients diagnosed with AGC by histology. Patients with good compliance. Patients with complete clinical data. Patients accompanied by family members when admitted. Patients without other malignant tumors. Patients without autoimmune diseases. Subjects with similar educational levels. Exclusion criteria were as follows: Subjects with history of mental disease; subjects dropped out of the study or lost to follow up; subjects not willing to participate in this study; subjects with severe organ diseases (heart, kidney, liver, lung) after examination.

Therapies

Patients with AGC were treated with chemotherapy, and subjected to abdominal CT and ultrasound. On the first day, 130 mg/m^2 oxaliplatin (Jiangsu Hengrui Medicine Co., Ltd., SFDA approval number: 20050962) was given intravenously to patients for 2 h, and 200 mg/m^2 folinic acid (Jiangsu Hengrui Medicine Co., Ltd., SFDA approval number: H20000584) was intravenously infused to patients for 2 h. Then, 450 mg/m^2 of 5-fluorouracil (Tianjin KingYork Group Co., Ltd., SFDA approval number: H12020959) was given intravenously for 22 h. This was repeated every 3 weeks for a total of 2 cycles.

Detection methods

Five ml fasting elbow venous blood was collected from gastric cancer patients before and after treatment in the morning and in normal subjects during physical examination. Centrifugation of blood was performed at 1006.2xg for 10 min at 4°C to acquire the upper serum and then placed in a refrigerator at -80°C for later use. Extraction of the total RNA was conducted in the light of the instructions of Trizol kit (Invitrogen, CA, USA). Purity and concentration were determined by UV spectrophotometer (Youpu General Technology Co., Ltd., Beijing, China). RNA samples were reverse-transcribed with reference to the manual of the cDNA reverse transcription kit (Takara, Dalian, China). PCR experiments were conducted on an ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with SYBR Green PCR Master Mixes (Thermo Fisher Scientific, MA, USA). PCR reaction conditions: 95°C for 30 s, 95°C for 5 s, 60°C for 34 s, with a total of 40 cycles. Guangzhou Ruibo Biotechnology Co., Ltd. (China) was responsible for design and synthesis of primer sequences. U6 was taken as the internal reference, and the specific primer sequences are shown in Table 1. miR-195-5p content was calculated using 2 method. All determinations were repeated three times.

Efficacy evaluation

The efficacy evaluation according to the criteria of solid tumor RECIST1.1 [14] were: Complete response (CR) : all lesions disappeared after examination, and no lesions appeared for at least 2 weeks. Partial response (PR) : no new lesions were found in patients after examination, and the sum of the maximum lengths and diameters of the tumor were significantly reduced, with reduction ≥30%. Progressive disease (PD) : new lesions appeared in patients, or the sum of the maximum lengths and diameters of the tumor increased ≥20% after detection. Stable disease (SD) : between PR and PD. Effective group = CR+PR, ineffective group = PD+SD.

Standards of observation

miR-195-5p level in serum was determined to analyze its diagnostic value of patients with AGC. Serum

Table 1. Sequences

	Upstream	*Downstream*
miR-195-5p	5'-GGGGTAGCAGCACAGAAAT-3'	5'-TCCAGTGCGTGTCGTGGA-3'
U6	5'-TGCGGGTGCTCGCTTCGCAGC-3'	5'-CCAGTGCAGGGTCCGAGGT-3'

carbohydrate antigen 19.9 (CA19.9) and carcinoembryonic antigen (CEA) in the gastric cancer group were detected by automated chemiluminescence immunoassay analyzer (Roche, Switzerland, model: cobas e411), and the relevant reagents were products of Roche. The specific operation was conducted in strict accordance with the suggestion of the manufacturer. The correlation of miR-195-5p with tumor markers CA19.9 and CEA was analyzed, as well as the efficacy and prognostic value of miR-195-5p in AGC treatment.

Statistics

Statistical analyses were performed with SPSS 20.0 (IBM Corp, Armonk, NY, USA). GraphPad Prism 7 (GraphPad software Co., Ltd., San Diego) was utilized for image rendering of data. Enumeration data were represented by [n (%)]. Inter-group comparison was done by chi-square test. Measurement data were presented in the form of mean±SD. T-test was applied for comparison between groups, and one-way analysis of variance (ANOVA) was adopted for comparison in multiple groups. LSD-t test was utilized for postmortem analysis. Analysis of the correlation between the two groups of variables was performed by Pearson's method. Kaplan-Meier method was applied for drawing survival curves, and Log-rank test was utilized for survival analysis. Receiver operating characteristic (ROC) curve was adopted, and the area under the curve (AUC) was counted to evaluate the diagnostic and predictive value of miR-195-5p in AGC. A statistically significant difference was set at $p<0.05$).

Results

General data

General clinical data of two groups of subjects were collected and shown in Table 2. There was no considerable difference in gender, age, body mass index (BMI), average height, smoking and drinking between the gastric cancer group and the normal group ($p>0.05$), indicating comparability.

miR-195-5p expression in AGC

Determination of mir-195-5p in serum showed that its level was evidently lower in AGC patients

Table 2. Comparison of general data

	Gastric cancer group (n=114) n (%)	*Normal group (n=100) n (%)*	x^2/t	*p*
Gender			0.408	0.523
Male	71 (62.28)	58 (58.00)		
Female	43 (37.72)	42 (42.00)		
Average age, years	61.34±5.76	61.86±6.09	0.642	0.522
≥60	75 (65.79)	62 (62.00)	0.332	0.564
<60	39 (34.21)	38 (38.00)		
Average BMI (kg/m^2)	24.67±5.33	24.87±4.87	0.285	0.776
Average height (cm)	168.33±8.09	168.42±8.43	0.080	0.937
Smoking or not			0.139	0.906
Yes	27 (23.68)	23 (23.00)		
No	87 (76.32)	77 (77.00)		
Drinking			0.833	0.362
Yes	48 (42.11)	36 (36.00)		
No	66 (57.89)	64 (64.00)		

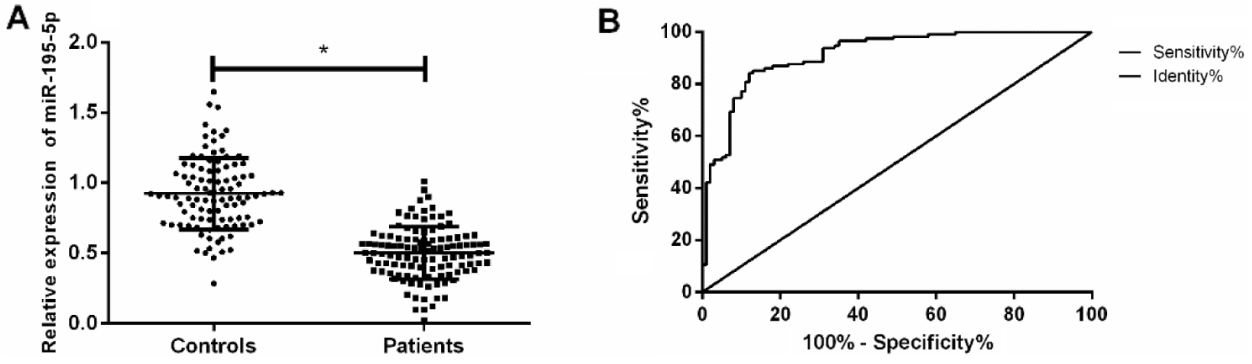

Figure 1. miR-195-5p expression in AGC. **A:** the serum miR-195-5p level was significantly lower in the gastric cancer group (*$p<0.05$). **B:** the AUC of miR-195-5p in the diagnosis of gastric cancer was 0.9148, the sensitivity was 84.21%, and the specificity was 88.00%.

Table 3. Relationship between miR-195-5p and pathological characteristics of AGC patients (mean±SD)

	n	*miR-195-5p*	*t/F*	*p*
Gender			0.278	0.781
Male	71	0.51±0.20		
Female	43	0.50±0.16		
Age, years			1.138	0.258
≥60	75	0.48±0.14		
<60	39	0.51±0.12		
Clinical stage			5.312	<0.001
III	44	0.60±0.18		
IV	70	0.44±0.14		
Tumor size (cm)			1.395	0.166
<6	53	0.52±0.13		
≥6	61	0.48±0.17		
Grade of differentiation			5.340	<0.001
High+middle differentiation	50	0.59±0.18		
Poor differentiation	64	0.43±0.14		
Depth of invasion			0.890	0.376
T3	55	0.53±0.19		
T4	59	0.50±0.17		
Tumor location			0.042	0.960
Cardia, gastric fundus	23	0.51±0.11		
Gastric body	51	0.51±0.21		
Antrum, pylorus	40	0.52±0.15		

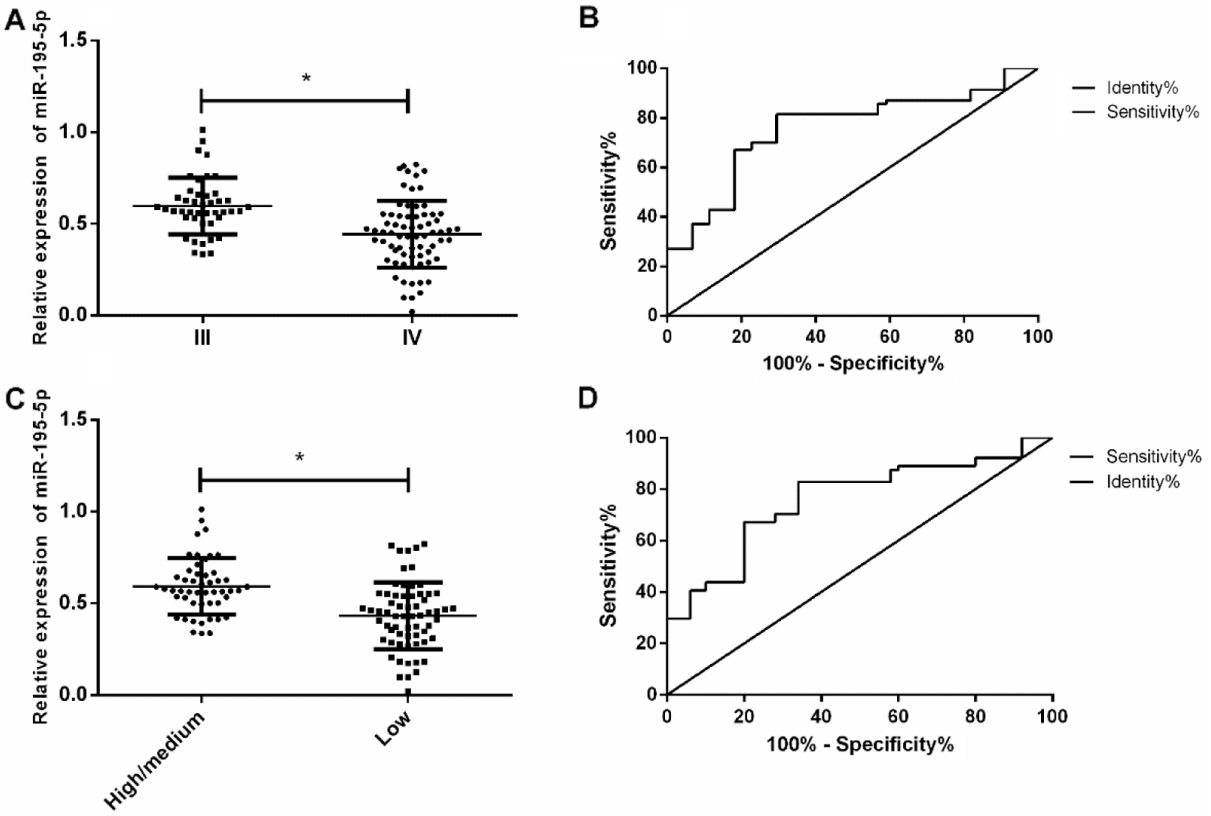

Figure 2. Relationship between miR-195-5p and pathological characteristics. **A:** serum miR-195-5p in patients with clinical stage III was higher than that in patients with stage IV (*p<0.05). **B:** serum miR-195-5p could be used to diagnose the clinical stage of AGC, with an AUC of 0.7565. **C:** serum miR-195-5p in patients with high and moderate differentiation was higher than that in patients with low differentiation (*p<0.05). **D:** serum miR-195-5p could be used to diagnose the differentiation of patients with AGC, with an AUC of 0.7588.

(p<0.05) (Figure 1). It was also observed that miR-195-5p could be used in the diagnosing gastric cancer patients, with AUC of 0.9148, sensitivity of 84.21% and specificity of 88.00%.

Relationship between miR-195-5p and pathological characteristics of AGC patients

The relationship between miR-195-5p and pathological characteristics is shown in Table 3.

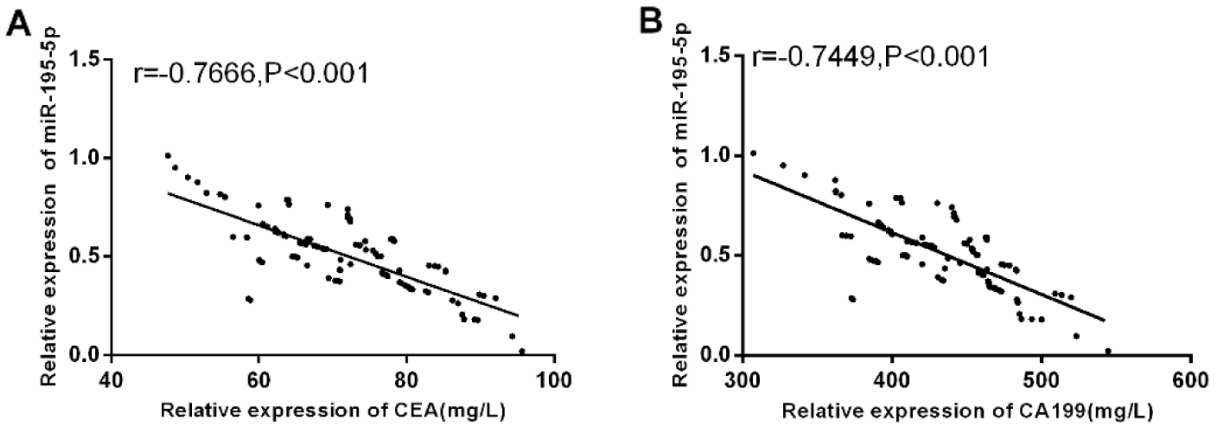

Figure 3. Correlation of miR-195-5p with CEA and CA199 levels in AGC patients. **A:** miR-195-5p was inversely correlated with CEA in AGC patients (r=-0.7666, p<0.001). **B:** miR-195-5p was inversely correlated with CA199 in AGC patients (r=-0.7449, p<0.001).

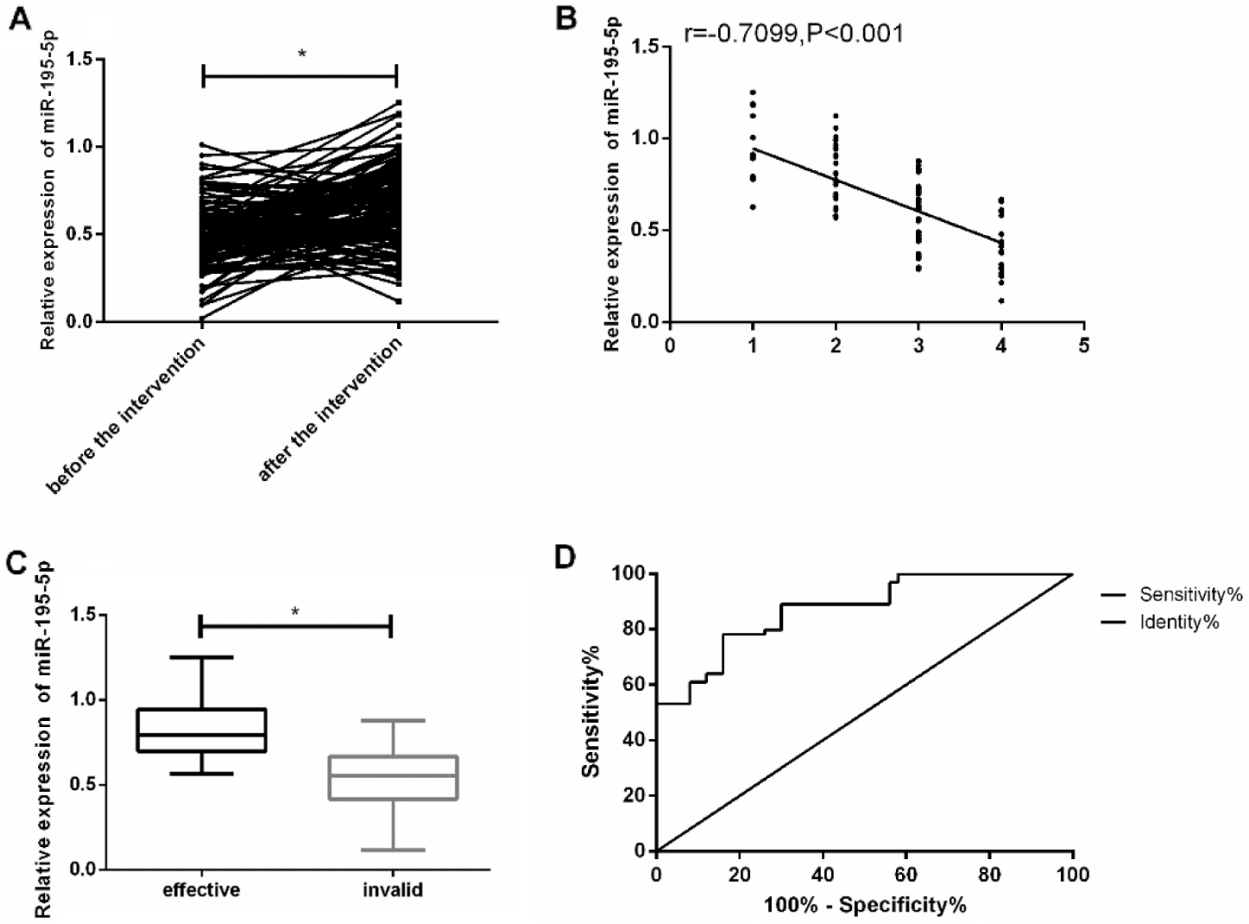

Figure 4. miR-195-5p expression in AGC patients before and after treatment. **A:** miR-195-5p was notably elevated in AGC patients after chemotherapy (p<0.05). **B:** miR-195-5p level after treatment was inversely correlated with the therapeutic effect (r=-0.7099, p<0.001). **C:** serum miR-195-5p level in the ineffective group was notably lower than that in the effective group (*p<0.05). **D:** miR-195-5p level after treatment could be used to predict the efficacy, with an AUC of 0.8734, a sensitivity of 78.13%, and a specificity of 84.00%. 1 represents CR, 2 represents PR, 3 represents SD and 4 represents PD.

miR-195-5p was notably associated with clinical stage and grade of differentiation (p<0.05). According to Figure 2, miR-195-5p could be used for the diagnosis of clinical stage and differentiation grade, and its expression level gradually decreased with the aggravation of the disease and the decrease of differentiation grade (p<0.05).

Correlation of miR-195-5p with CEA and CA199 levels in AGC patients

CEA and CA19.9 expression in the serum of patients with AGC are shown in Figure 3. Pearson's correlation analysis revealed a remarkable negative correlation of CEA and CA199 with miR-195-5p levels before treatment in patients with AGC (r=-0.7666, -0.7449, p<0.001).

miR-195-5p expression AGC patients before and after treatment

After chemotherapy, there were 15 patients with CR, 35 patients with PR, 39 patients with SD and 25 patients with PD. After treatment, serum miR-195-5p level was significantly increased (p<0.05), and was inversely correlated with therapeutic effect (r=-0.7099, p<0.001). Subsequently, patients were separated to effective group and ineffective group on the basis of therapeutic effect, and serum miR-195-5p level was notably lower in the ineffective group than the effective group (p<0.05). Moreover, the serum miR-195-5p level after treatment could be used for chemotherapy efficacy prediction with an AUC of 0.8734, a sensitivity of 78.13%, and a specificity of 84.00%. More details are shown in Figure 4.

Relationship between miR-195-5p and prognosis of AGC patients

After chemotherapy, 37 patients with AGC survived and 77 died, and the 3-year survival rate was 32.45%. All patients were divided into high expression group and low expression group on the basis of miR-195-5P median expression. High expression group had remarkably better survival than the low expression group, and miR-195-5P of dead patients was notably lower than that of surviving patients (p<0.05). The AUC of miR-195-5p level after treatment in predicting the survival of AGC was 0.7473, the sensitivity was 53.25%, and the specificity was 89.19%. More details are shown in Figure 5.

Discussion

Gastric cancer is one of the most frequent malignancies of digestive system [15]. Currently, surgical treatment is the main method in treating this disease, which is also the only way to cure it.

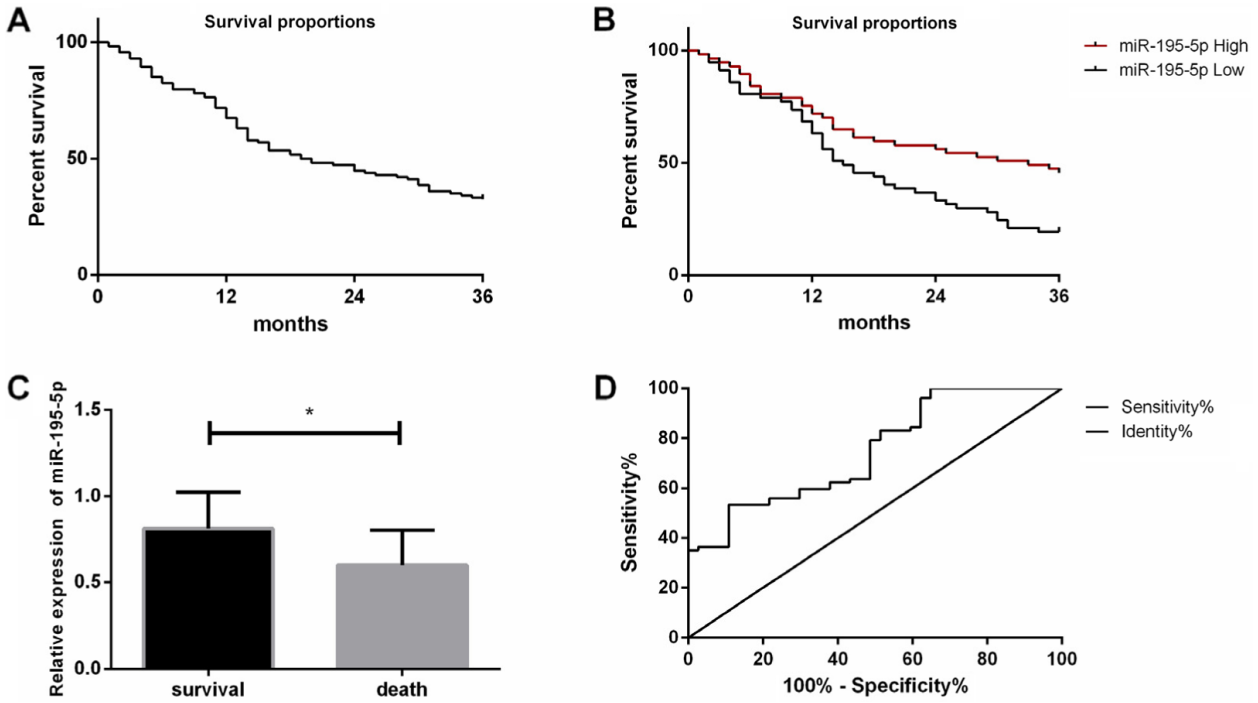

Figure 5. Relationship between miR-195-5p and prognosis of AGC patients. **A:** after chemotherapy, 37 patients with AGC survived and 77 died, with the 3-year survival rate of 32.45%. **B:** the survival of the high expression group was notably better than that of the low expression group (p<0.05). **C:** miR-195-5p expression in the dead patients was notably lower than that in the surviving patients (*p<0.05). **D:** the AUC of miR-195-5p level after treatment in predicting the survival of AGC was 0.7473, the sensitivity was 53.25% and the specificity was 89.19%.

With the development of medical technology, the patient survival has been improved in those diagnosed with early gastric cancer, with the 5-year survival rate of up to 95% [16]. However, due to the low diagnostic rate of early gastric cancer, most of patients are in advanced stage when they are diagnosed, and they have missed the best time for surgical treatment. At this time, chemotherapy is often used. Due to drug resistance and other conditions in the treatment, however, the treatment effect is seriously affected, leading to poor prognosis [17,18]. Therefore, it is of great importance to find biomarkers that can predict the treatment and prognosis of chemotherapy, and it is conducive to the adjustment of treatment of patients with AGC.

Currently, abnormal miRs expression has been found in numerous cancers, and miRs play a role of suppressing/promoting tumor development [19]. miR-195-5p is a member of the miR-15 family that locates on chromosome 17p13.1 [20]. Its abnormal expression has been confirmed in many human malignant tumors, such as breast cancer [21] and prostate cancer [22], and plays a tumor suppressive role. Through cell experiments, latest research has found that miR-195-5p is related to differentiation grade and multidrug resistance of gastric cancer cells. miR-195-5p up-regulation negatively regulates the protein level of zinc finger protein 139 (ZNF139) and promotes gastric cancer cell chemosensitivity via affecting p-glycoprotein (P-gp), b-cell lymphoma-2 (Bcl-2) and multidrug resistance-associated protein 1 (MRP1) expression [23]. This study investigated miR-195-5p regulatory mechanism on the sensitivity of drug-resistant cells in gastric cancer, but it has not reported whether miR-195-5p can be utilized for the prediction of chemotherapy efficacy in AGC patients.

In recent years, studies on peripheral blood miRs have become a hot topic. It is reported that miR changes can be detected in whole blood, plasma or serum of gastric cancer, and the detection of serum samples has the advantages of being non-invasive and convenient. Therefore, changes in serum miR can effectively reflect disease type and severity [24-26]. Here, miR-195-5p expression in serum of AGC patients was detected, and the level was lower in AGC patients than in normal subjects. Sueta et al [27] indicated that miR-195-5p was evidently reduced in breast cancer, suggesting that miR-195-5p might play a part in tumor inhibition. Besides, through the ROC curve, it was found that miR-195-5p had a high diagnostic value in patients with AGC. Previous studies have reported the diagnostic efficacy of various miRs in gastric cancer [28]. In combination with this study, we concluded that miR-195-5p could be used as a biomarker for gastric cancer. By analyzing the relationship between miR-195-5p and clinical characteristics of AGC, it was found that miR-195-5p had no significant correlation with gender, age, tumor size, invasion depth or tumor location of patients with AGC. miR-195-5p was notably associated with clinical stage and differentiation grade, and had the value of differential diagnosis for pathological parameters of clinical stage and differentiation grade, indicating that it could be used as a marker in evaluating gastric cancer. Moreover, miR-195-5p decreased gradually with the aggravation of the disease and the reduction of the grade of differentiation. Kong et al [29] elaborated that high level of miR-25 was evidently related to depth of invasion, lymph node metastasis and disease stage in gastric cancer patients. However, only patients with AGC were selected in this study, and due to differences in miR, there was no considerable association between miR-195-5p, depth of invasion and tumor location of AGC.

CEA and CA19.9 are commonly used biomarkers for malignant tumors and can be used for gastric cancer diagnosis, treatment and recurrence evaluation. Serum CEA and CA19.9 levels can accurately predict treatment efficacy for gastric cancer patients [30,31]. Moreover, CEA and CA19.9 levels gradually increased with disease severity [30]. Herein, the level of tumor markers was examined in gastric cancer patients on admission, and CEA and CA19.9 were evidently negatively correlated with miR-195-5p level before treatment. It was speculated that miR-195-5p might have the same biological efficacy as tumor markers. After chemotherapy, miR-195-5p level in AGC patients was notably higher than that before treatment. Previous studies have revealed that miR can be used for the assessment of therapeutic effect of neoadjuvant chemotherapy in gastric cancer patients [32]. In addition, previous literature has suggested that miR may be an effective predictor for palliative care of gastric cancer patients, but the types of miRs studied are limited [33]. This research revealed that serum mir-195-5p level after treatment was associated with the effect of chemotherapy in patients with AGC. Moreover, the serum miR-195-5p level after treatment could be used for efficacy prediction, with an AUC of 0.8734, a sensitivity of 78.13%, and a specificity of 84.00%. After 3 years of follow-up, patients were separated to a high expression group and a low expression group with reference to miR-195-5p expression. The survival was considerably better in the high expression group when compared with the low expression group, and the miR-195-5p in dead patients was evidently lower than that in the surviving patients. The miR-

195-5p level after treatment was used to predict the survival of AGC. It could be seen that serum miR-195-5p level had significant application value in gastric cancer diagnosis, staging and prognosis assessment, and can provide a favorable reference for patient's clinical diagnosis and treatment.

This study explored the expression and significance of serum miR-195-5p in AGC patients from multiple aspects. However, there are still some limitations. The subjects included in this study are only patients with AGC. Samples and types can be expanded in future studies to further study the occurrence, development and mechanism of miR-195-5p in gastric cancer patients.

In conclusion, serum miR-195-5p is notably decreased in patients with AGC, and can be effectively utilized as a biomarker for the assessment of diagnosis and prognosis of gastric cancer patients.

Conflict of interests

The authors declare no conflict of interests.

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