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Correlation between microRNA-766 expression in patients with advanced gastric cancer and the efficacy of platinum-containing chemotherapy

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Abstract. – OBJECTIVE: We aimed at observing the correlation between microRNA-766 expression and the efficacy of platinum-containing chemotherapy in patients with stage IV gastric cancer (GCa).

PATIENTS AND METHODS: Tissue specimens were obtained from 100 patients with stage IV GCa who received platinum-based chemotherapy, and microRNA-766 expression in these samples was examined by quantitative real-time polymerase chain reaction (qPCR) analysis. Survival analysis was carried out through Kaplan-Meier test. The influencing factors of survival were assessed through COX univariate and multivariate regression.

RESULTS: GCa tissues contained significant lower expression of microRNA-766 than adjacent tissues. The degree of tumor differentiation and peritoneal metastasis were confirmed to have great relevance to microRNA-766 level. Patients with high microRNA-766 expression have better chemotherapy efficacy and longer progression-free survival.

CONCLUSIONS: Our study shows for the first time that the highly expressed microRNA-766 in tumor tissues of patients with stage IV GCa predicts better platinum-containing chemotherapy efficacy and prognosis.

Key Words:
GCa, MicroRNA-766, Chemotherapy, Prognosis.

Introduction

GCa is the fourth most common malignancy in the world and the second most deadly. More than 70% of new GCa cases and deaths occur in developing countries, including China[1]. What's worse, more than 85% of cases are diagnosed as advanced accounting for its untypical early symptoms[2]. Currently, for patients with advanced GCa, chemotherapy has been the main treatment method, among which platinum is the most crucial and basic first-line drug for GCa chemotherapy[3]. Sadly, only a fraction of patients may receive effective chemotherapy due to drug resistance. Literature has reported that alternation of DNA damage repair ability is the main reason for platinum resistance, and nucleotide excision repair (NER) plays a pivotal role[4,5].

MiRNAs are a group of non-coding RNAs, consisting of 17-22 nucleotides, which promote or inhibit translation by binding to the 3'-untranslated regions (3'-UTR) of the mRNA[6]. MiRNAs can be engaged in tumor formation, growth, and metastasis and serve as potential molecular markers for tumor diagnosis and prognosis prediction[7-9]. Each miRNA can regulate different genes through different molecular pathways[10]. Therefore, it is important to understand the molecular mechanisms by which miRNAs inhibit tumors.

For advanced GCa, two- or three-drug combination chemotherapy containing platinum preferred. At present, the main chemotherapy plans are as followed: cisplatin combined with 5-fluorouracil, cisplatin combined with capecitabine, oxaliplatin combined with 5-fluorouracil, oxaliplatin combined with capecitabine, etc. However, the effective rate is only 29-48% due to primary or secondary drug resistance[11-13]. Patients accepted ineffective treatments only suffer the toxic side effects without clinical benefits. Hence, molecular markers that can predict the curative effect of patients are urgently needed to guide the treatment plan so as to avoid ineffective and excessive treatment.

Abnormal miRNAs expression results in multiple tumor drug resistance[16-19]. Of note, the inhibition effect of microRNA-200c on SGC7901/DDP

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cell proliferation was relevant to the induction of expression of calcinetin E, protein tyrosine phosphatase gene protein, BAX protein, inhibition of activation of Akt pathway and down-regulation of Bcl-2 protein expression[14]. However, the correlation between microRNA-766 and GCa remains elusive. Therefore, in this study, we analyzed whether microRNA-766 could predict the efficacy and prognosis of platinum-containing chemotherapy for GCa patients through the measurement of microRNA-766 in the tissues of GCa patients and the study of its correlation with the efficacy of platinum, so as to guide the individualized treatment of GCa patients.

Patients and Methods

Clinical Data

The score of the Eastern Cooperative Oncology Group (ECOG) was 0-1 for 60 cases and 2 for 40 cases. Inclusion criteria are as follows: (1) tumor tissues specimens were pathologically confirmed as GCa; (2) stage IV patients were selected based on 2010 AJCC/TNM stage; (3) patients have not received chemoradiotherapy before or have received but more than 6 months since the last chemotherapy; (4) patients above the age of 18; ECOG score 0-2 points (5) the long diameter of metastatic organ lesions was greater than 10 mm and the short diameter of lymph nodes was greater than 15 mm as measured by CT or MRI. Patients with any of the following items shall not be included in this study: (1) patients who have developed or currently suffer from other malignant tumors within 5 years; (2) have accepted systemic anti-tumor therapy, including cytotoxic therapy, signal transduction inhibitors, immunotherapy and radiotherapy before treatment; (3) patients who have seizures and need treatment; patients having a history of psychotropic substance abuse and cannot be cured or have mental disorders; (4) pregnant or lactating patients. All subjects must sign informed consent to receive 2 cycles of platinum-based chemotherapy with follow-up. This investigation was approved by the Ethics Committee of Shanxi Cancer Hospital.

Chemotherapy Regimens

Platinum (cisplatin 15 mg/m^2 d1 -- 5 or oxaliplatin 85 mg/m^2 d1) combined with 5 fluorouracil (capecitabine 2000 mg/m^2 d1 -- 14, teggio 80 mg/m^2 d1 -- 14, 5 fluorouracil 400 mg/m^2 iv d1 1200 mg/m^2 for 24 hours d1-2) combined with or without taxol (paclitaxel 135 mg/m^2 d1 or docetaxel 75 mg/m^2 d1). The regimen repeats every 3 weeks (FOLFOX repeats every 2 weeks).

Curative Effect Evaluation

The efficacy was assessed according to RECIST 1.1 standard[15]. The specific situation is as follows:

(1) assessment of target lesions: complete response (CR): the short diameter of all pathological lymph nodes must be reduced to less than 10 mm and the target lesions disappeared. Partial response (PR): total maximum diameter of the target lesion was reduced by ≥30% from the baseline level. Stable disease (SD): the sum of the maximum diameters of the lesions decreased but did not reach PR, or increased but did not reach PD. Progressive disease (PD): the sum of the maximum diameters of the lesions increased by ≥20% from the baseline level and the absolute value of the target lesions increased by more than 5 mm. (2) Evaluation of non-target lesions: CR: tumor marker levels returned to normal and lesions disappeared. Non-CR/non-PD: persistent presence of one or more non-target lesions and/or persistently higher than normal levels of tumor markers. PD: the appearance of new lesions and/or clear progression of non-target lesions. (3) The overall efficacy evaluation was shown in Table I. Efficacy evaluation was conducted after 2 cycles of treatment. The same patient was evaluated by the same imaging method before and after che-

Table I. Evaluation of overall efficacy.

Target lesion	Non-target lesions	New lesion	Overall efficacy
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes/No	PD
Any	PD	Yes/No	PD
Any	Any	Yes	PD

motherapy, and all CT or MRI results were evaluated by the same examiner.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Detection

Total RNAs were extracted using the miRNeasy Mini Kit (Qiagen). Subsequently, 5 µg RNA was diluted in DEPC water for 20 times, and the optical density at 260 nm and 280 nm was respectively recorded. RNA samples with OD_{260}/OD_{280} of 1.7-2.1 were considered to be highly purified and qualified for qRT-PCR. Using the reversely transcribed cDNAs as templates, qRT-PCR was conducted at 95°C for 10 min, followed by 40 cycles at 95°C for 10 s, 60°C for 20 s and 72°C for 34 s. Primer sequences were as follows: microRNA-766: 5'-TCGAGTACTTGAGATGGAGTTTT-3' (forward) and 5'-GGCCGCGTTGCAGTGAGCCGAG-3' (reverse); U6: 5'-GCTTCGGCAGCACATATACTAAAAT-3' (forward) and 5'-CGCTTCACGAATTTGCGTGTCAT-3' (reverse). The threshold was selected manually at the lowest level where each logarithmic amplification curve was rose in parallel to obtain the Ct value of each sample. Relative level was calculated by $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct=[Ct_{(target\ gene)}-Ct_{(U6)}]_{target\ gene}-[Ct_{(target\ gene)}-Ct_{(U6)}]_{U6}$. Each experiment was conducted in triplicate.

Postoperative Follow-Up

All postoperative patients were followed up for 5 years, 1 every 3 months in the first year, and 1 every 6 months thereafter.

Statistical Analysis

All data were processed by Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp., Armonk, NY, USA). Measurement data were presented as $\bar{x} \pm$ SD (standard deviation). Differences between two groups were analyzed using the Student's t-test. p less than 0.05 was statistically significant. *$p<0.05$.

Results

Expression of MicroRNA-766 in Patients with Advanced GCa

QRT-PCR analysis detected a significant reduction in microRNA-766 expression in GCa tissue samples collected from 100 cases (Figure 1), indicating that microRNA-766 may have an inhibitory effect on GCa progression.

Relationship Between MicroRNA-766 Level and Clinicopathological Characteristics of Patients with Advanced GCa

Statistical analysis revealed that in cancer tissues of GCa patients in stage IV, microRNA-766 showed no link to clinical indicators such as age, gender, ECOG score, tumor size, and metastasis incidence ($p>0.05$). However, microRNA-766 expression was downregulated in moderately and highly differentiated cancer tissues and cases with peritoneal metastasis ($p<0.05$) (Table II). The above observations indicate that microRNA-766 level is relevant to the degree of cancer differentiation and the presence or absence of peritoneal metastasis.

Expression of MicroRNA-766 in Tumor Tissues of Patients with Advanced Gastric Cancer in Chemotherapy Effective Group and Ineffective Group

After two cycles of platinum-containing chemotherapy, the evaluation was based on the RESICT 1.1 standard, with 59 PR, 23 SD, and 18 PD. Patients with a CR evaluation of CR and PR were classified into the chemotherapy-effective group, with a total of 59 patients. PD was classified as a chemotherapy ineffective group with a total of 18 persons. The mean ± SD of microRNA-766 expression in tumor tissue samples of patients in the chemotherapy-effective group was 2.35 ± 0.702 and was 0.89 ± 0.461 those in the

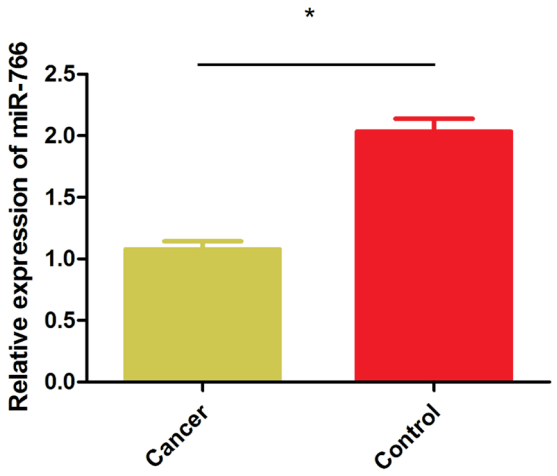

Figure 1. Relative expression of microRNA-766 in cancerous tissue and adjacent tissues. The expression of microRNA-766 in cancer tissues was significantly lower than that in adjacent tissues (*$p<0.05$).

Table II. Correlation analysis between miRNA-766 expression and clinicopathological characteristics.

Variables	n	miRNA -766	t	p
Age				
<60	47	1.87±0.64	0.238	0.812
≥60	53	1.84±0.62		
Sex				
Male	62	2.12±0.77	1.174	0.243
Female	38	2.31±0.81		
ECOG score				
0-1	60	1.78±0.56	1.364	0.176
2	40	1.95±0.68		
Tumor size				
<5	66	2.05±1.02	0.864	0.39
≥5	34	1.87±0.92		
Differentiation				
Medium/High	55	1.76±0.88	3.035	0.003
Low	45	2.37±1.13		
Liver metastasis				
Yes	49	1.69±0.82	1.247	0.215
No	51	1.92±1.01		
Peritoneal metastasis				
Yes	54	1.87±0.53	2.622	0.01
No	46	2.23±0.83		

chemotherapy-ineffective group (Figure 2). Taken together, these results indicate that the high expression of microRNA-766 contributes to the efficacy of platinum-based chemotherapy in GCa.

Association Between MicroRNA-766 Level and PFS/OS in Tumor Tissues of Patients with Advanced GCa

After 5 years of follow-up, patients were divided into microRNA-766 high expression and low expression group, based on the average level of microRNA-766 in tumor tissues. The Kaplan-Meier curve suggested that patients with highly expressed microRNA-766 had longer PFS (HR = 7.12, p=0.0076) (Figure 3A) and longer OS (HR = 5.005, p=0.0253) (Figure 3B). The above results suggest that high microRNA-766 expression predicts a better prognosis of patients with advanced GCa.

Univariate and Multivariate Cox Regression Analysis of Influencing Factors Affecting PFS and OS of Patients with Advanced GCa

Cox proportional hazard model was applied for univariate analysis of PFS/OS influencing factors in patients with GCa. It is suggested that peritoneal metastasis, poor efficacy and low microRNA-766 levels in tumor tissues are risk factors for progression-free survival in GCa patients in stage IV (Table III). The significant single factor was further analyzed by multivariate analysis. Table IV shows that the statistically significant multivariate independent variables were the efficacy of chemotherapy and the level of microRNA-766 in tumor tissues (p<0.01).

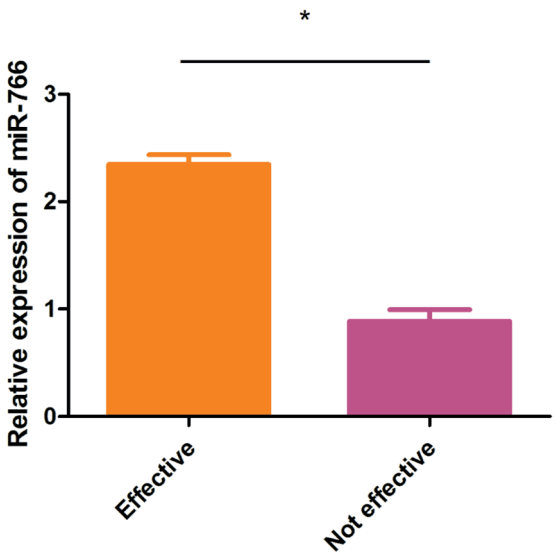

Figure 2. MicroRNA-766 level in cancer tissues from the effective and ineffective groups of GCa patients. The relative expression of microRNA-766 in the effective group was higher than that in the ineffective group (*p<0.05).

Table III. Univariate Cox regression analysis of PFS and OS influencing factors in patients with advanced GCa.

	PFS			OS		
Variables	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age						
<60	1			1		
≥60	0.847	0.528-1.864	0.36	0.763	0.511-1.783	0.42
Sex						
Male	1			1		
Female	1.426	0.953-1.978	0.24	1.297	0.867-1.868	0.26
ECOG score						
0-1	1			1		
2	1.825	0.726-4.757	0.52	1.802	0.921-2.872	0.73
Tumor size						
<5	1			1		
≥5	1.048	0.792-1.528	0.62	1.323	0.871-2.765	0.54
Differentiation						
Medium/High	1			1		
Low	1.772	0.933-2.247	0.18	1.565	0.653-3.013	0.32
Liver metastasis						
Yes	1			1		
No	1.829	0.845-2.855	0.35	1.662	0.761-2.883	0.32
Peritoneal metastasis						
Yes	1			1		
No	1.559	1.258-2.931	0.015	2.013	1.562-4.881	0.028
Chemotherapy						
Two drugs	1			1		
Three drugs	0.856	0.542-3.220	0.37	1.302	0.882-2.995	0.45
Curative effect						
PD	1			1		
CR+PR	0.076	0.021-0.371	<0.001	0.392	0.192-0.73	0.006
PD	1			1		
SD	0.327	0.138-0.765	0.002	0.531	0.431-0.882	0.013
MiR-766						
Low level	1			1		
High level	0.526	0.321-0.720	0.006	0.543	0.377-0.871	<0.001

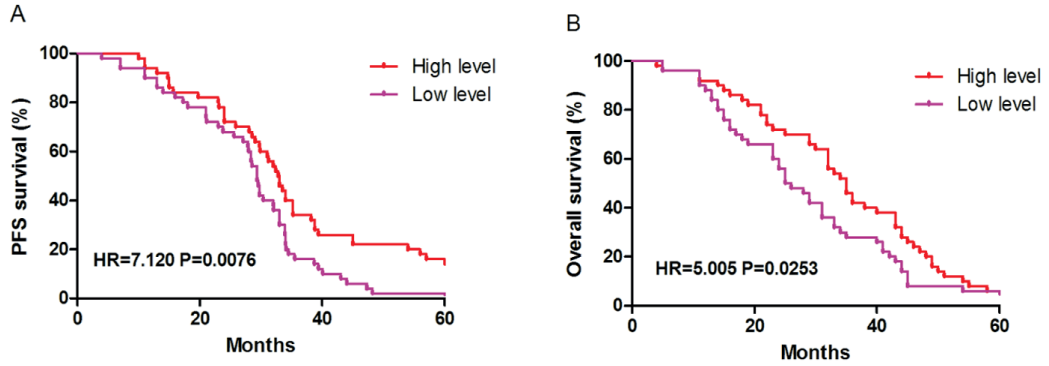

Figure 3. Relationship between microRNA-766 expression level and PFS/OS in tumor tissue of GCa patients. **A,** Kaplan-Meier curve suggested that patients with high expression in microRNA-766 had longer PFS in the lower expression group ($p<0.01$). **B,** Kaplan-Meier curve suggested that patients with high expression in microRNA-766 had longer OS in the lower expression group ($p<0.01$).

Table IV. Multivariate Cox regression analysis of factors affecting PFS and OS in patients with advanced GCa.

	PFS			OS		
Variables	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
PD	1			1		
CR+PR	0.027	0.021-0.141	<0.001	0.142	0.094-0.431	0.007
PD	1			1		
SD	0.391	0.214-0.684	0.023	0.432	0.392-0.732	0.011
miR-766						
Low level	1			1		
High level	0.652	0.415-0.832	0.037	0.559	0.356-0.872	0.003

Discussion

Although a large number of studies have been devoted to finding biomarkers that can predict the efficacy and prognosis of GCa[16], it is still a huge challenge to find clinically useful biomarkers due to the complexity of GCa[17]. MiRNAs regulate the expression of target genes by binding to the 3-' UTR of target genes to regulate the translation and degradation of mRNA[18]. Nearly 50% of miRNAs are located at tumor-associated loci or fragile sites[19]. Increasing evidence suggests that miRNAs are associated with the diagnosis, prognosis and chemotherapy resistance of GCa[20,21].

MiRNAs can serve as indicators for cisplatin resistance in GCa. So, microRNA-106a in gastric cancer resistant cells SGC7901/DDP was overexpressed as compared with the parent cell SGC7901[22], while inhibition of microRNA-106a can enhance cisplatin induced apoptosis and reverse cisplatin resistance; overexpressed microRNA-181b up-regulated Bcl-2 and enhanced the sensitivity of SGC7901/DDP to cisplatin[23]. MiRNA-27a targets the cystine/glutamate exchanger SLC7A11 and promotes cisplatin resistance by regulating GSH biosynthesis[24]. In addition, microRNA-21 has been confirmed to enhance tumor cell resistance to cisplatin by activating the Akt signaling pathway, and inhibition of the Akt signaling pathway by phosphatidylinositol 3-kinase inhibitors can reverse the impact of microRNA-21 on prolonging cell survival[25]. Downregulation of microRNA-27a can reduce P-gp and improve the expression of p21 to reverse the sensitivity of GCa cells to cisplatin[26]. In this study, we found that microRNA-766 in GCa tissues was markedly down-regulated in comparison to adjacent tissues, and the expression of microRNA-766 can predict the degree of tumor differentiation and peritoneal metastasis in patients. Moreover, the high expression of microRNA-766 increased the sensitivity of patients to platinum-containing chemotherapy and improved therapy efficacy.

MiRNAs can be used as prognostic indicators of GCa[27]. Tan et al[28] found that microRNA-185 inhibited tumor metastasis, suggesting that it can predict prognosis. Other authors have shown that the combined diagnosis of seven miRNAs helps predict PFS and OS of patients[29,30]. Previously overexpressed microRNA-21 was associated with differential tumor differentiation, lymph node metastasis, and TNM staging, suggesting the prognostic value of microRNA-21[31,32]. In the present study, GCa patients in stage IV with higher expression of microRNA-766 had a longer PFS and OS and a better prognosis. In addition, Cox univariate and multivariate regression analysis also showed that high expression of microRNA-766 could improve PFS and OS in patients, suggesting the potential value of microRNA-766 as a prognostic indicator.

In this paper we found that microRNA-766 regulated the sensitivity of radiotherapy and chemotherapy. MicroRNA-766 is very likely to become a drug target for gastric cancer, laying a new theoretical foundation for the diagnosis and treatment of gastric cancer.

Conclusions

In short, we found that GCa patients in stage IV with high expression of microRNA-766 receive a better platinum-based chemotherapy efficacy and longer PFS and OS. MicroRNA-766 can serve as an index to predict the efficacy and prognosis of platinum-containing chemotherapy for stage IV GCa.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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