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Reduced miR-490-3p expression is associated with poor prognosis of *Helicobacter pylori* induced gastric cancer

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Abstract. – OBJECTIVE: *Helicobacter pylori* (HP) infection has been demonstrated to be a risk factor accounting for the initiation and development of gastric cancer (GC). The aim of the present study was to investigate the clinical significance of miR-490-3p in HP associated GC.

PATIENTS AND METHODS: We measured the expression level of miR-490-3p in human GC tissues by quantitative Real-time PCR (qPCR). Then the association between miR-490-3p and clinical features of GC was further investigated.

RESULTS: Our results showed that miR-490-3p levels exhibited a progressive downregulation in gastritis, intestinal metaplasia, HP negative GC and HP positive GC. In addition, miR-490-3p expression was significantly correlated with various clinicopathological parameters such as lymph node metastasis and clinical stage in HP-positive GC. Moreover, GC patients with lower miR-490-3p had a shorter 5 years overall/disease free survival time in the HP positive cohort. Finally, multivariate analysis showed that low miR-490-3p was an independent risk factor for HP associated GC.

CONCLUSIONS: miR-490-3p is downregulated in HP-positive GC and associated with poor clinical outcome, indicating that miR-490-3p is a promising prognostic biomarker for HP positive GC.

Key Words:
Biomarker, Gastric cancer, *Helicobacter pylori*, miR-490-3p, Prognosis.

Introduction

Gastric cancer (GC) is one of the most common malignant diseases and the second cause of cancer-related mortality around the world[1]. Surgical resection, chemotherapy, and radiotherapy are the standard treatment options for GC. Despite great advance has been made in the diagnosis and therapy, the incidence and mortality of GC remain high[2]. Tumor-node-metastasis (TNM) staging system is the most widely used indicator for predicating the prognosis of GC[3]. However, it is not sufficiently sensitive and specific. Therefore, it is important to explore novel prognostic biomarker for GC.

MicroRNAs (miRNAs) are a family of short non-protein-coding RNAs which negatively regulate gene expression at the posttranscriptional level[4]. Various bioinformatic analyses have demonstrated that there are hundreds of downstream targets for each miRNA, indicating miRNAs affects almost, if not all, genetic pathways[5]. Aberrant expression of miRNAs has been implicated in the pathogenesis of a number of human diseases such as cancer, cardiovascular diseases, and diabetes mellitus[6-9]. Upregulation of miR-186 suppressed the proliferation, migration, and invasion of gastric cancer cells, and contrary findings were observed when miR-186 was downregulated. In addition, reduced miR-186 expression was possibly associated with larger tumor size and advanced clinical stage. Moreover, Twist1 was identified as a direct downstream target of miR-186, indicating miR-186 played a tumor suppressive role in GC[10]. Yanaka et al[11] reported that miR-544a could promote the epithelial-mesenchymal transition of gastric cancer cells by activating the Wnt signaling pathways, suggesting that miR-544a acted as an oncogene in GC.

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Helicobacter pylori (HP) infection plays a central role in the initiation and progression of GC. It can effectively promote the carcinogenesis by either activating onco-miRNAs or epigenetic silencing of tumor suppressor miRNAs[12,13]. Deregulated miR-490-3p expression has been reported in many types of cancer[14-16]. However, its role in GC is poorly understood. Thus, the goal of the current study was to determine the potential clinical significance of miR-490-3p in GC.

Patients and Methods

Study Population

Patients with gastritis, intestinal metaplasia, GC were prospectively recruited from General Hospital of Daqing Oil Field. The study design was approved by the Ethics Committee of the General Hospital of Daqing Oil Field and written informed consent was obtained from all individual participants. All the patients with intestinal metaplasia or GC were pathologically confirmed. GC cases were classified according to the 7th edition American Joint Committee on Cancer (AJCC) staging system. Follow-up data for all GC patients were acquired. Overall survival was defined as the time from initial diagnosis to the date of the last follow-up or death. Disease-free survival (DFS) was calculated from the date of diagnosis to the first evidence of recurrence or metastasis. A summary of the baseline characteristics of GC cases was presented in Table I.

Quantitative Real-Time PCR

Total RNA was extracted from tissue samples using Trizol (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. Reverse-transcription was carried out with the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed in triplicate for each sample using SYBR Green (Applied Biosystems) and the 7500 Real-Time PCR System (Applied Biosystems) with the following conditions: 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 60s. Relative miRNA levels were calculated using the comparative Ct and U6 was used as the internal control.

Statistical Analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, CA, USA). $p<0.05$ was considered statistically significant. The expression level of miR-490-3p was evaluated using nonparametric Kruskal-Wallis test for multiple groups. Associations between clinicopathological parameters and miR-490-3p expression were evaluated using a Chi-squared test. Survival curves were evaluated by the Kaplan-Meier method and analyzed using the log-rank test. The Cox proportional hazard regression analysis was used to identify the prognostic value of miR-490-3p in GC.

Results

miR-490-3p was Significantly Downregulated in HP Positive GC

We compared miR-490-3p levels in gastritis, intestinal metaplasia, HP-negative GC and HP-positive GC. Our real-time PCR showed that miR-490-3p was progressively reduced from gastritis, intestinal metaplasia, HP-negative GC and HP-positive GC ($^{**}p<0.01$; $^{***}p<0.001$). However, no significant difference was found between in-

Table I. The correlation between miR-490-3p expression level and the clinicopathological parameters in HP positive GC.

		miR-490-3p expression		
Parameters	**Cases**	**Low**	**High**	***p***
Age				0.858
<60	37	18	19	
≥60	45	21	24	
Gender				0.492
Male	58	29	29	
Female	24	10	14	
Location				0.991
Middle and proximal third	40	19	21	
Distal third	42	20	22	
Tumor size				0.173
<5	38	15	23	
≥5	44	24	20	
TNM Stage				<0.001
I/II	31	7	24	
III/IV	51	32	19	
Lymph node metastasis				0.038
No	26	8	18	
Yes	56	31	25	
Distant metastasis				0.621
No	69	32	37	
Yes	13	7	6	
Differentiation				0.003
Well/moderate	33	9	24	
Poor	49	30	19	

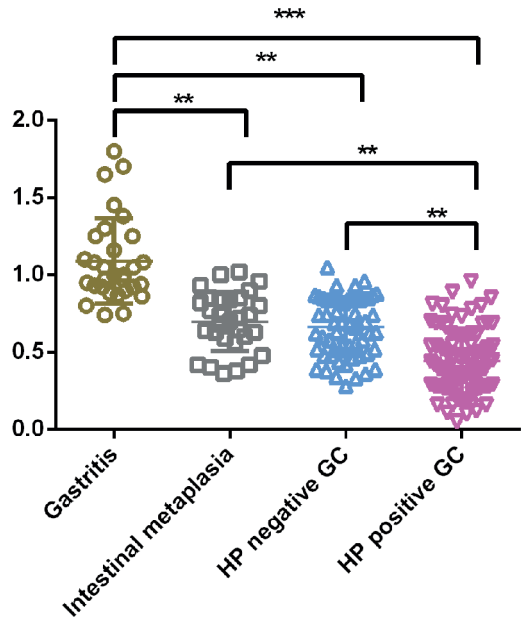

Figure 1. The expression level of miR-490-3p in GC.

testinal metaplasia and HP-negative GC (p>0.05) (Figure 1).

The Association Between miR-490-3p Levels and Clinicopathological Parameters of GC

For HP positive GC, low miR-490-3p levels were positively associated with lymph node metastasis (p=0.038), advanced TNM stage (p<0.001) and high histological grade (p=0.003). However, it was not associated with age, gender, location, tumor size and distant metastasis. For HP negative GC, low miR-490-3p levels were only positively correlated with advanced clinical stage (p=0.047) (Tables I-II).

The Prognostic Value of miR-490-3p in GC

For HP positive GC, the survival analysis demonstrated that GC patients in the low miR-490-3p group had a significantly shorter overall (p<0.001) and disease free (p=0.004) survival time than the patients in the high miR-490-3p (Figure 2A). However, no significant difference was found about the overall and disease-free survival time between HP-negative GC patients in the high and low miR-490-3p group (Figure 2B).

Our multivariate analysis demonstrated that miR-490-3p was an independent prognostic risk factor for both overall (HR=3.18, 95% CI=1.38-5.73, p=0.015) and disease-free survival (HR=3.67, 95% CI=1.53-6.72, p=0.012) in GC patients with HP infection (Table III).

Discussion

In the present study, our data showed that miR-490-3p was progressively reduced from normal controls, gastritis, intestinal metaplasia and GC. Also, miR-490-3p was significantly downregulated in HP-positive GC when compared to HP negative GC. miR-490-3p levels were associated with various clinicopathological parameters including TNM stage, lymph node metastasis, and differentiation. Moreover, patients with lower miR-490-3p expression suffered more unfavorable overall and disease-free survival in the positive HP infection cohort. miR-490-3p was also an independent prognostic factor for HP positive GC, indicating miR-490-3p was significantly associated with HP infection and its downregulation could promote the initiation and development of GC. We conjectured that HP was able to epigenetically silent the expression of miR-490-3p, leading to the carcinogenesis of GC. However, the

Table II. The correlation between miR-490-3p expression level and the clinicopathological parameters in HP negative GC.

		miR-490-3p expression		
Parameters	**Cases**	**Low**	**High**	***p***
Age				0.813
<60	26	13	13	
≥60	32	17	15	
Gender				0.649
Male	43	23	20	
Female	15	7	8	
Location				0.301
Middle and proximal third	27	12	15	
Distal third	31	18	13	
Tumor size				0.293
<5	29	13	16	
≥5	29	17	12	
TNM stage				0.047
I/II	14	4	10	
III/IV	44	26	18	
Lymph node metastasis				0.647
No	17	8	9	
Yes	41	22	19	
Distant metastasis				0.699
No	53	27	26	
Yes	5	3	2	
Differentiation				0.306
Well/moderate	25	11	14	
Poor	33	19	14	

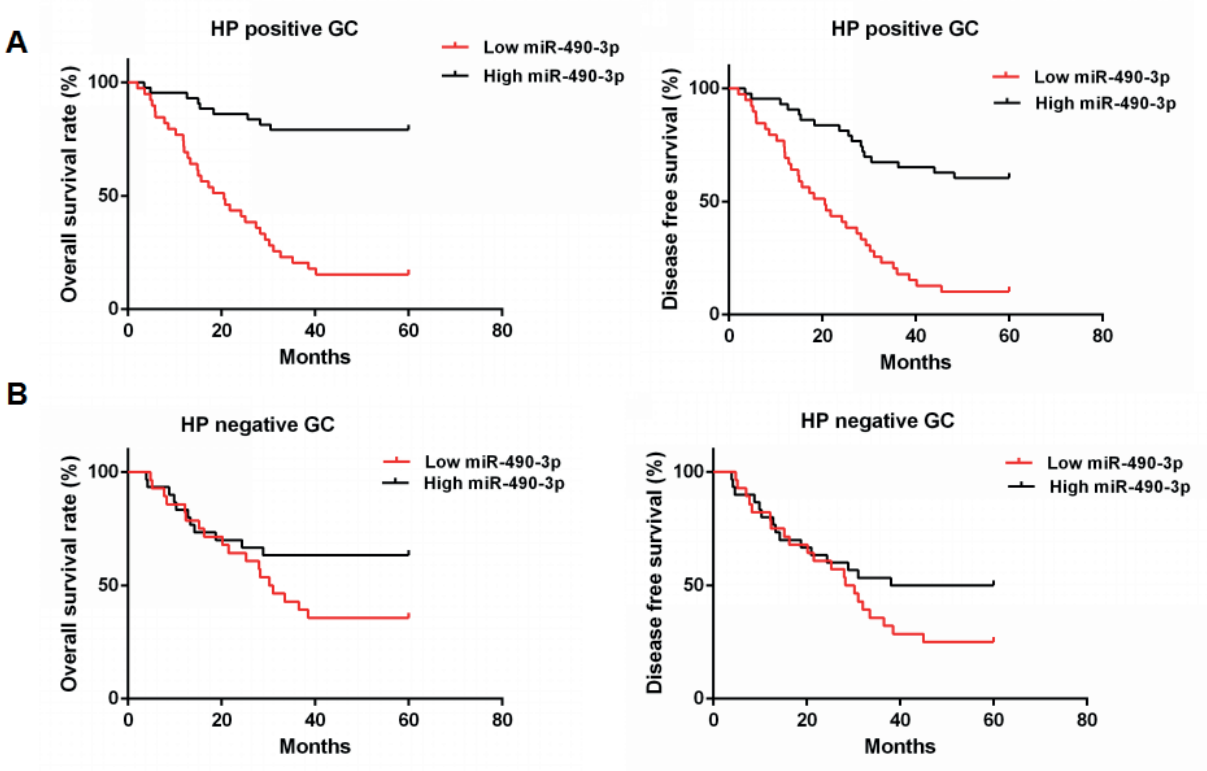

Figure 2. The association between overall/disease free survival and miR-490-3p levels.

concrete molecular mechanisms needed further investigation. Similar to our results, miR-490-3p was reported to be downregulated in GC tissues and its regulatory region was hypermethylated. In addition, SMARCD1 was proved to a downstream target of miR-490-3p[17]. This study further corroborated our findings and speculation.

miR-490-3p acts as a tumor suppressor gene in many types of cancers. miR-490-3p expression was decreased in ovarian carcinoma and significantly associated with clinical stage and differentiation. In addition, ectopic expression of miR-490-3p reduced the proliferation, migration and invasion capacity of ovarian cancer cells, while promoted their apoptosis. CDK1 was demonstrated to be a downstream target of miR-490-3p[18]. Zheng et al[19] reported that miR-490-3p promoter was hypermethylated in colorectal cancer (CRC) tissues. Furthermore, overexpression of miR-490-3p suppressed the oncogenic behaviors of CRC cells both *in vitro* and *in vivo,* and *vice versa*. Similarly, miR-490-3p was also found to inhibit tumorigenesis and progression in breast cancer by targeting RhoA directly, indicating miR-490-3p functioned as a tumor suppressor in breast cancer[20].

However, miR-490-3p is also able to promote cancer development. The expression level of miR-490-3p was significantly increased in hepatocellular carcinoma (HCC) tissues and cell lines. Upregulation of miR-490-3p promoted the proliferation, migration and invasion capability of HCC cells as well as induced EMT, while miR-490-3p downregulation led to opposite results. ERGIC3 was a direct downstream target of miR-490-3p[21]. miR-490-3p was also associated with drug resistance. Enhanced miR-490-3p could promote drug-resistance of human ovarian cancer cells, which was not consistent with

Table III. Multivariate analysis of clinicopathological factors for overall/disease free survival of GC patients.

Variable	HR	95% CI	*p*
Overall survival			
Differentiation	2.54	1.22-3.81	0.029
Lymph node metastasis	1.89	1.02-2.52	0.048
TNM stage	4.82	1.63-8.26	0.004
miR-490-3p	3.18	1.38-5.73	0.015
Disease free survival			
Differentiation	2.42	1.31-4.56	0.035
Lymph node metastasis	2.06	1.24-3.92	0.043
TNM stage	5.51	1.84-10.32	0.001
miR-490-3p	3.67	1.53-6.72	0.012

previous findings[18,22]. Further studies should carry out to elucidate the role of miR-490-3p in other types of cancers. We believed that the molecular functions of miR-490-3p are very diverse and its concrete activities are closely correlated with tumor microenvironment.

Conclusions

We showed that miR-490-3p levels were remarkably downregulated in HP associated GC tissues. Low miR-490-3p was significantly correlated with unfavorable clinical outcome in GC patients with HP infection, indicating miR-490-3p was a promising prognostic biomarker for this malignancy.

Conflict of interest

The authors state that they have no conflicts of interest.

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