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Down-regulation of miR-1236-3p is correlated with clinical progression and unfavorable prognosis in gastric cancer

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Abstract. – OBJECTIVE: MiRNAs have been considered to participate in many processes of various cancers, including gastric cancer (GC). However, the significance of miRNAs in the progression prognosis of GC is largely unknown. The goal of this study was to determine the expression level of miR-1236-3p in GC and its clinical association.

PATIENTS AND METHODS: Clinical specimens from GC patients were obtained to quantify the expression level of miR-1236-3p using quantitative Real-time PCR. The correlation between the miR-1236-3p levels and the clinicopathological factors of the GC patients was analyzed. The association between miR-1236-3p expression and overall survival was estimated by the Kaplan-Meier method. The significance of different variables with respect to survival was analyzed using the univariate and multivariate Cox proportional hazards model.

RESULTS: We found that miR-1236-3p was significantly downregulated in GC tissues compared to non-tumor gastric tissues ($p < 0.01$). The levels of miR-1236-3p expression were associated significantly with clinical stage ($p = 0.002$), lymph node metastasis ($p = 0.000$) and distant metastasis ($p = 0.017$). Kaplan-Meier survival analysis showed that patients in the high miR-1236-3p expression group had better overall survival than those in the low miR-1236-3p expression group ($p = 0.0039$). Moreover, we confirmed that miR-1236-3p was an independent poor prognostic factor for GC patients through univariate and multivariate analysis.

CONCLUSIONS: Our findings provide evidence that miR-1236-3p expression is frequently decreased in GC tissues, and its low expression may be a significant prognostic factor for poor survival in GC patients.

Key Words:
miR-1236-3p, Gastric cancer, Prognosis.

Introduction

Gastric cancer (GC) is the fourth most common type of cancer causing ~800,000 mortalities each year and the leading cause of cancer-related death worldwide[1,2]. GC incidence rates vary wildly between men and women and across different countries[3]. The Asian countries account for the majority of GC cases, and almost 50% of these GC patients are diagnosed in China[4,5]. Although gastric endoscopy has contributed to this reduction by enabling early diagnosis GC, the prognosis of advanced gastric cancer still remains poor due to the recurrence, invasion and metastasis[6]. In order to improve the therapeutic outcome, it is urgent to explore novel potential molecule biomarkers for early diagnosis and accurate prognosis prediction. MicroRNAs (miRNAs) are small non-coding regulatory RNA molecules that which modulate gene expression at the post-transcriptional level by mainly binding to 3'-UTR of target messenger RNAs (mRNAs), which leading to mRNA translation inhibition or mRNA degradation[7]. Although miRNAs do not code proteins, more and more evidences indicate that they are involved in the regulation of various cellular processes, such as cell cycle, apoptosis, metabolism and metastasis[8,9]. More importantly, miRNAs are differentially expressed in various cells and

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tissues, underscoring their importance as biomarkers and therapeutic targets[10,11]. In addition, miRNA profiles also indicate that miRNAs can serve either as oncogenes or tumor suppressors in tumor progression[12,13]. Up to date, several miRNAs have been identified to be involved in the progression of GC, such as miR-31[14], miR-101[15] and miR-802[16]. MiR-1236-3p, located in chromosome 6p, is an intronic miRNA (102 bp in size). Previous studies have indicated that miR-1236-3p is a novel anti-oncogene with low expression in several cancers, including GC[17,18]. However, few studies have investigated the association between miR-1236-3p and GC development. Thus, the aim of this study was to evaluate whether miR-1236-3p was capable of acting as a prognostic biomarker for GC patients.

Patients and Methods

Human Tissues

Human GC and adjacent non-tumor tissues were obtained from 169 patients who underwent gastrectomy at the Yidu Central Hospital of Weifang, between June 2008 and December 2012. Tissue samples were quickly frozen in liquid nitrogen immediately after surgical removal and stored at -80°C. The samples had been clinically and histopathologically diagnosed according to the World Health Organization criteria between 2010 and 2012. All GC patients did not undergo radiotherapy or chemotherapy before their surgery. The clinicopathological features of 84 patients are summarized in Table I. Written informed consent was obtained from all patients prior to participation in the study. This study was reviewed and approved by the Institutional Review Board of the Yidu Central Hospital of Weifang.

RNA Extraction and Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA from GC tissues and normal gastric tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First strand cDNA synthesis was carried out using the miScript II RT Kit (Qiagen, Xuhui, Shanghai, Beijing) according to the manufacturer's protocol. Amplification and detection of miR-1236-3p were performed using a TaqMan Human MiRNA Assay Kit (Biosystems, Foster City, CA, USA) under ABI PRISM 7900 Sequence Detection System (Biosystems, Foster City, CA, USA). U6 was used to normalize miR-1236-3p expression. Relative quantification of miR-1236-3p expression was evaluated using the comparative cycle threshold (CT) method. Each sample was examined in triplicate. PCR primers for miR-1236-3p were synthesized by Bioasia (Hangzhou, Zhejiang, China). The specific primer sequences were as follows: miR-1236-3p forward, 5'-GGGCCTCTTCCCCTTGTCT-3' and reverse, 5'- TATGGTTGTTCTGCTCTCTGTCTC-3'; U6 forward, 5'- GTCCTGGCAGATATACACTA-AACAT-3' and reverse, 5'- CTCACGCTTGAAT-TCATGCGGCTT-3'.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 19.0 (IBM, Armonk, NY, USA). GraphPad 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) helped presenting these consequences. The differences between two groups were analyzed using the two-sided Student's *t*-test. Association between miR-1236-3p and clinicopathological parameters was analyzed by the x^2-test. The overall survival of patients with different expression of miR-1236-3p was estimated by the Kaplan-Meier analysis. Cox regression analysis was performed to analyze prognostic significance of each variable. A p-value < 0.05 was considered to indicate a statistically significant result.

Results

Expression of miR-1236-3p Was Down-Regulated in GC Patients

To explore the potential role of miR-1236-5p in progression of GC, we firstly examined miR-1236-3p expression level in 169 paired GC tissues and adjacent non-tumor tissues by qRT-PCR, and normalized to U6. The results were shown in Figure 1. We found that the expression of miR-1236-3p was significantly decreased in GC tissues compared with paired adjacent normal tissues ($p < 0.01$).

Association Between miR-1236-3p and Clinicopathological Parameters of GC Patients

In order to explore the clinical significance of miR-1236-3p expression, the association between miR-1236-3p expression and clinicopathological features in GC patients was further as-

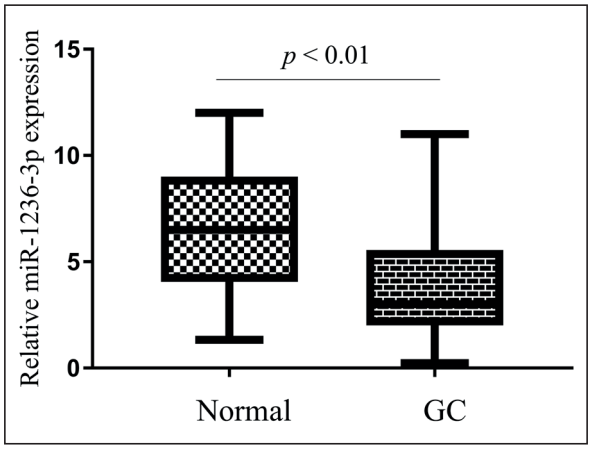

Figure 1. MiR-1236-3p expression was significantly downregulated in GC tissues than adjacent noncancerous tissues ($p < 0.01$).

sessed. As shown in Table I, our results showed that miR-1236-3p downregulation was correlated with clinical stage ($p = 0.002$), lymph node metastasis ($p = 0.000$) and distant metastasis ($p = 0.017$). However, there was no significant correlation between the expression level of miR-1236-3p and age, gender, perineural invasion, histological type or tumor depth of GC patients ($p > 0.05$).

Low miR-1236-3p Expression Levels Correlate With GC Patients' Poor Prognosis

Then, the prognostic value of miR-1236-3p expression was investigated using the Kaplan-Meier method and log-rank test. As shown in Figure 2, we found that the overall survival rate of GC patients with low miR-1236-3p expression was significantly lower than that of patients with high miR-1236-3p expression (log-rank test, $p = 0.0039$). Furthermore, the results of the univariate and multivariate Cox regression analyses for 5-year overall survival are shown in Table II. In the univariate analysis, clinical stage ($p = 0.005$), lymph node metastasis ($p = 0.001$), distant metastasis ($p = 0.021$) and miR-1236-3p expression ($p = 0.003$) were significant predictors for overall survival. Importantly, multivariate analysis demonstrated that miR-1236-3p (HR = 2.783; 95% CI, 1.025-4.246; $p < 0.009$) was a significant independent prognostic factor for overall survival in patients with GC (Table II).

Table I. Correlation between miR-1236-3p expression and different clinicopathological features in patients with GC.

Clinicopathological features	No. of cases	miR-1236-3p expression		p-value
		Low (n, %)	High (n, %)	
Gender				NS
Female	59	32 (54.2)	27 (45.8)	
Male	110	51 (46.4)	59 (53.6)	
Age (y)				NS
< 60	82	43 (52.4)	39 (47.6)	
≥ 60	87	40 (46)	47 (54)	
Perineural invasion				NS
Absence	121	62 (51.2)	59 (48.8)	
Presence	48	21 (43.8)	27 (56.2)	
Histological type				NS
Differentiated	98	48 (49)	50 (51)	
Undifferentiated	71	35 (49.3)	36 (50.7)	
Tumor depth				NS
T1-T2	109	48 (44)	61 (56)	
T3-T4	60	35 (58.3)	25 (41.7)	
Clinical stage				0.002
I-II	109	44 (41.3)	65 (58.7)	
III-IV	60	39 (65)	21 (35)	
Lymph node metastasis				0.000
No	102	39 (38.2)	63 (61.8)	
Yes	67	44 (65.7)	23 (34.3)	
Distant metastasis				0.017
No	103	43 (41.7)	60 (58.3)	
Yes	66	40 (60.6)	26 (39.4)	

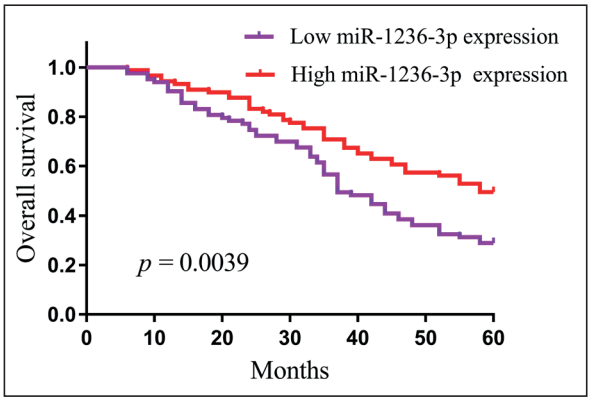

Figure 2. Kaplan-Meier analyses for the correlation between miR-1236-3p expression and survival. Log-rank test showed that patients with low miR-1236-3p expression had significantly poorer overall survival versus patients with high miR-1236-3p expression ($p = 0.0039$).

Discussion

GC is a major public health problem throughout the world. Over the past decades, in spite of observable advancement in surgical technique, chemotherapy and radiotherapy, the 5-year survival rate of GC patients remains unsatisfactory[19,20]. Considering that several studies have suggested substantial molecular markers for GC, it is essential to find new prognostic biomarkers for establishing targeted strategies to attenuate disease burden[21,22]. Researches have documented that miR-1236-3p plays essential roles in multiple cancers. For instance, Bian et al[23] found that miR-1236-3p expression was significantly down-regulated in lung adenocarcinoma. *In vitro* and *in vivo* experiments showed that overexpression of miR-1236-3p inhibited lung adenocarcinoma cells migration and invasion by targeting KLF8. Wang et al[24] reported that miR-1236-3p was significantly lowly expressed in ovarian carcinoma and its upregulation could suppress the cells migration and invasion abilities by targeting ZEB1. Zhang et al[25] found that miR-1236-3p served as a tumor suppressor in bladder tumors by modulating S-phase kinase-associated protein 2. Li et al[26] revealed that miR-1236 exerted its anti-cancer role in hepatocellular carcinoma by acting as a ceRNA of long noncoding RNA FAL1. Recently, An et al[18] reported that the expression levels of miR-1236-3p was significantly down-regulated in both GC tissues and cell lines. Further clinical information indicated that low miR-1236-3p expression was associated with lymph node metastasis, differentiation and TNM stage. In their gain-function assay, it was demonstrated that forced miR-1236-3p expression inhibited invasion and metastasis in gastric cancer by targeting MTA2. All these findings suggested that miR-1236-3p is a candidate tumor suppressor in the pathogenesis of cancers, including GC. However, whether miR-1236-3p is related to the prognosis of GC patients has not been reported. In this work, we also found that the expression levels of miR-1236-3p in human GC tissues were significantly decreased than in the paired normal tissues, suggesting that miR-1236-3p may be an anti-oncogenic miRNA in GC. Moreover, we found that miR-1236-3p expression was associated with clinical stage, lymph node metastasis and distant metastasis, indicating that miR-1236-3p might be involved in the carcinogenesis and metastasis of GC. Most importantly, for the first time, we showed that aberrant downregulation of miR-1236-3p was markedly correlated with shorter overall survival. In univariate and

Table II. Univariate and multivariate analyses of prognostic factors in GC patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender	1.323	0.774-1.832	0.322	–	–	–
Age (y)	1.556	0.813-2.131	0.135	–	–	–
Perineural invasion	1.677	0.563-2.556	0.188	–	–	–
Histological type	1.131	0.789-1.884	0.216	–	–	–
Tumor depth	2.213	0.677-2.889	0.089	–	–	–
Clinical stage	2.667	1.335-4.548	0.005	2.352	1.038-3.884	0.008
Lymph node metastasis	3.665	1.565-6.674	0.001	3.133	1.237-5.892	0.001
Distant metastasis	2.423	1.216-3.889	0.021	2.113	1.022-3.137	0.034
miR-1236-3p expression	3.137	1.213-5.129	0.003	2.783	1.025-4.246	0.009

multivariate analyses, a low level of expression of miR-1236-3p was associated with a bad prognosis for GC patients. Our findings suggested that miR-1236-3p is a potential unfavorable prognostic factor for GC. However, the precise molecular mechanisms behind the altered expression of miR-1236-3p in GC and its function are not very clear. However, further research is needed to solve this problem.

Conclusions

We showed that high expression of miR-1236-3p was correlated with poor prognosis of patients with GC and miR-1236-3p may be a predictive biomarker for GC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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