

## Original Article

# Correlation between microRNA-421 expression level and prognosis of gastric cancer

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**Abstract:** The expression of microRNA-421 (miR-421) is significantly elevated in gastric carcinoma cells, thus may play an important role in tumor occurrence. This study thus aimed to further illustrate the correlation between miR-421 expression level and the progression and prognosis of gastric carcinoma. A total of 96 gastric carcinoma tissue samples were quantified for miR-421 expression level using quantitative PCR (qPCR) method. Kaplan-Meier survival curve was further deployed to analyze the postoperative survival of all patients. No significant correlation existed between miR-421 level and general information of patients such as age, sex, tumor size, location, invasion depth, TNM stage, differentiation stage and metastasis. However, miR-421 was significantly up-regulated in those tumors with lymph node metastasis ( $P < 0.05$ ), while those tumors with no lymph node metastasis had normal miR-421 level as those in control group ( $P > 0.05$ ). These results suggested the correlation between miR-421 up-regulation and lymph node metastasis. Those patients with high miR-421 expression had significantly shorter survival time compared to normal miR-421 patients (median: 37.34 months vs. 54.23 months,  $P < 0.01$ ), suggesting the correlation between miR-421 expression and prognosis of gastric cancer. MiR-421 level is correlated with lymph node metastasis and prognosis of gastric carcinoma, and is worth for further investigations.

**Keywords:** Gastric carcinoma, prognosis prediction, MicroRNA-421, correlation analysis

## Introduction

As the most common malignant tumor in gastrointestinal tract, gastric carcinoma has a high incidence in China. About 47% of gastric cancer cases all over the world occur in China, where more than 1 million newly diagnosed gastric cancers occur each year, causing about 260,000 deaths. Recent advancement of diagnosis and treatment of gastric carcinoma improved the treatment efficacy to some extents. However, unfavorable prognosis still exists as only 30% of patients survive after 5 years. Most patients, at their first diagnosis, already had lymph node metastasis or distal invasion, making the radical resection inaccessible. Therefore the early diagnosis of gastric cancer is of critical importance for timely treatment [1-3].

MicroRNA (miR) is a type of small single-stranded non-coding RNA molecule with ~22 nt length. It can specifically bind on the 3'-untrans-

lated region (3'UTR) of mRNA of target genes to inhibit the gene expression at translational level. It is now commonly accepted that miR play a critical role in both immune disease and tumor occurrence and metastasis. With deeper investigation of miR by oncologist, various tumor-related miR molecules have been discovered, benefiting the diagnosis and treatment of cancers [4, 5]. We have performed preliminary study using DNA microarray analysis to analyze gastric cancer related miR expressional spectrum, and found a total of 57 miRs with up-regulation in gastric carcinoma cells (14 of those molecules had more than 2-fold increase), and 42 miR molecules with down-regulation (12 of them had more than 2-fold decrease). Among all those miR molecules, miR-421 had the most significant elevated expression (8.25-fold increase). Therefore it is possible that miR-421 may exert modulation role in the pathogenesis of gastric cancer. Recent study also indicated the up-regulation of miR-421 as oncogene-like

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**Table 1.** MiR-421 level and clinical parameters of gastric cancer

Parameter	N	Percentage (%)	MiR-421 relative level	P value
<b>Sex</b>				
Male	74	77.08	1.47±0.16	0.32
Female	22	22.92	1.38±0.20	
<b>Age (years)</b>				
>60	46	47.92	1.43±0.21	0.75
≤60	50	52.08	1.46±0.15	
<b>Lauren type</b>				
Enteric adenocarcinoma	68	70.83	1.48±0.16	0.92
Diffused carcinoma	28	29.17	1.47±0.23	
<b>Differentiation stage</b>				
High	12	12.50	1.42±0.21	0.94
Moderate	46	47.92	1.45±0.17	
Low	38	39.58	1.48±0.25	
<b>UICC stage</b>				
I+II	38	39.58	1.43±0.17	0.55
III+IV	58	60.42	1.47±0.18	
<b>Invasive depth</b>				
T <sub>1</sub> +T <sub>2</sub>	54	56.25	1.45±0.16	0.69
T <sub>3</sub> +T <sub>4</sub>	42	43.75	1.47±0.24	
<b>Lymph node metastasis</b>				
No	22	22.92	1.35±0.16	0.01
Yes	74	77.21	1.92±0.21	
<b>Distal metastasis</b>				
No	70	73.83	1.42±0.20	0.30
Yes	26	26.05	1.46±0.18	
<b>Tumor location</b>				
Antrum	38	39.58	1.49±0.17	0.42
Body	6	6.25	1.28±0.13	
Cardia	26	27.08	1.46±0.23	
Whole stomach	26	27.08	1.41±0.15	
<b>Tumor size</b>				
≤5 cm	44	45.83	1.41±0.18	0.12
>5 cm	52	54.17	1.48±0.21	

role to facilitate tumor growth in gastric cancer cells [6]. Therefore, using miR-421 as the focus of our study, the correlation between miR-421 expression and pathogenesis, survival and prognosis of gastric cancer patients were further investigated, in an attempt to provide evidences for the prediction of target genes for cancer treatment.

### Materials and methods

#### Patient information

A total of 96 gastric cancer patients were recruited in this study between January 2012

and January 2015 in Department of Surgery in China-Japan Union Hospital. All patients were confirmed to have primary gastric carcinoma by pathological examination. No treatment such as radio-, chemo- and molecular target therapy has been received in all patients before the surgery. There were 74 males and 22 females in the patient group, with aging between 42 and 78 years old (average age = 61.34 years). Tumor samples were collected during the surgery, and were kept at -80°C. Pathological typing of tumor tissues including invasive grade, lymph node metastasis were performed according to international anti-cancer union standard and guideline of WHO [5, 7]. Post-operative follow ups were performed every month after discharge. This study has been pre-approved by the ethical committee of China-Japan Union Hospital and has obtained written consents from all participants.

#### RNA extraction and qPCR

Total RNA were extracted from tumor tissues using RNA extraction kit (Sigma, US) and tested for miR-421 relative expression level by TaqMan miRNA test kit (Santa Cruz, US). Using RNU6 as the internal reference, expression level of miR-421 was quantified by two-step assays. Firstly,

complementary DNA (cDNA) was synthesized by stem-loop primers, followed by in vitro reverse transcription (2 ng/μL RNA, 5 μL primers and 10 μL reaction buffer) under the following condition: 94°C 30 sec, 60°C 30 sec 72°C 60 sec and 72°C for 7 min. In a second step, TaqMan miRNA specific primers were mixed with 2X PCR TaqMan Universal mixtures, along with 5 μL cDNA in RNase-free water. The PCR parameters were: 40 cycles each containing 94°C denature for 30 sec, 60°C annealing for 30 sec and 72°C elongation for 60 sec, followed by 72°C elongation for 7 min in a fluorescent qPCR cyclers (Rotro-gene, Australia).

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**Table 2.** Correlation between miR-421 expression level and clinical parameters

Clinical index	OR (95% CI)	P value	Adjusted OR (95% CI) <sup>a</sup>	P value <sup>a</sup>
<b>Sex</b>				
Male	0.92 (0.25~3.17)	0.90	0.45 (0.09~2.01)	0.93
Female	1.00		1.00	
<b>Age (years)</b>				
>60	1.08 (0.45~3.03)	0.97	0.91 (0.25~3.19)	0.32
≤60	1.00		1.00	
<b>Invasive depth</b>				
T <sub>1</sub> +T <sub>2</sub>	1.00	0.08	1.00	0.11
T <sub>3</sub> +T <sub>4</sub>	2.92 (0.93~8.74)		3.08 (0.81~9.23)	
<b>Lymph node metastasis</b>				
No	1.00	0.04	1.00	0.03
Yes	3.51 (2.13~9.27)		5.16 (1.27~20.83)	
<b>Distal metastasis</b>				
No	1.00	0.65	1.00	0.80
Yes	1.48 (0.59~4.85)		0.73 (0.15~3.82)	
<b>Tumor location</b>				
Antrum	1.00	0.41	1.00	0.91
Other	1.79 (0.81~6.59)		1.16 (0.21~6.82)	

<sup>a</sup>Indicates the value of OR and P after adjustment.

Relative expression level of miR-421 was quantified by 2- $\Delta$ Ct method ( $\Delta$ Ct = CtmiR-421-CtRNU6).

### Statistical analysis

SPSS 21.0 software was used to process all collected data. Those samples with miR-421 level lower than average were classified in the low-expression group, while those with higher than average levels were recruited in the high-expression group. Stepwise regression was performed by analyzing the correlation between miR-421 level and disease indexes to screen out those parameters with statistical significance. Logistic regression analysis was deployed to evaluate the risk factor of pathological factors. Analysis of variance (ANOVA) was used to compare means between groups, while two-group-comparison was performed by student t-test. The comparison of ratios used chi-squared test. The postoperative survival of patients was analyzed by Kaplan-Meier survival curve, along with the correlation between miR-421 and survival rate. All statistical tests were performed in a two-tailed test scenario. Mann-Whitney U test was used to compare means between two groups. A statistical significance was defined when P<0.05.

## Results

### Correlation between miR-421 expression level and clinical parameters

The relative expression level of miR-421 in all 96 gastric cancer samples was 1.436 (95% CI: 0.323~2.534). Among all samples, there were 42 cases with lower-than-average level of miR-421, while the other 54 cases had higher expressions. No significant correlation existed between miR-421 and general information of patients such as age, sex, tumor size, location, invasion depth, TNM stage, differentiation and metastasis (P>0.05 in all cases,

**Table 1**). Those tumors with lymph node metastasis, however, had significantly elevated miR-421 relative level compared to those without lymph node metastasis (P<0.05, **Table 1**).

### Correlation between miR-421 and clinical features

Using Logistic regression analysis, we investigated the correlation between miR-421 and clinical factors of all cancer patients. It has been discovered that the elevated miR-421 increased the risk of lymph node metastasis (adjusted OR=5.16, 95% CI: 1.27~20.83, P=0.03, see **Table 2**).

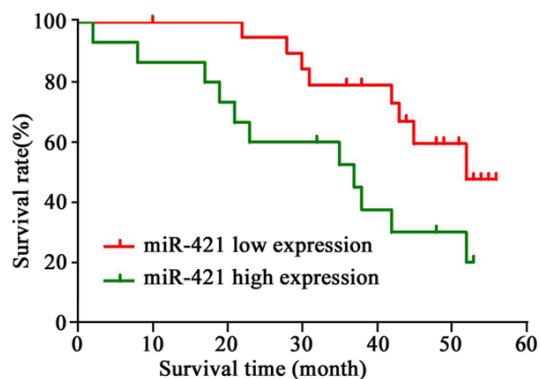
### Correlation between miR-421 level and disease prognosis

The median survival time for miR-421 high expression patients was 37.34 months, and was 54.23 months for those patients with miR-421 low expression, suggesting the correlation between miR-421 and prognosis of gastric cancer (**Figure 1**).

## Discussion

In clinical practice, most gastric cancer patients had on significant symptoms in the early stage of cancer, and have been in the late stage at

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**Figure 1.** Correlation between miR-421 level and survival rate.

the time of first diagnosis, causing unfavorable prognosis and lower survival rate [8]. MiR is a type of small molecules with significant biological functions via mediating target gene expression, and is one hotspot of current tumor studies about molecular mechanisms [9-12]. As one kind of novel non-coding single stranded RNA, miR is widely distributed in all eukaryotic cells. With the development of bioinformatics, miRNA has been revealed regarding its pluripotent functions in tumor proliferation, growth, apoptosis, development, metastasis and invasion. Moreover, the expressional spectrum of miR also determines the genotype of tumor, which plays a critical role in subtyping and prognosis prediction of tumors. The post-operative prognosis and survival time are also correlated with miRNA [13, 14].

We performed a high-throughput analysis, which showed the gastric cancer related miR-421. It is localized on human chromosome 1p34.23, and is one important member of miR-200 family. Past studies have revealed abnormal expression of miR-421 in various tumor tissues, along with differential modulatory functions [15-17]. Previous literatures showed the predictive value of miR-421 in the prognosis of colorectal, breast and cervical cancer, in addition to the role as molecular marker for late-stage prostate cancer [18, 19]. Over-expression of miR-421 may lead to the occurrence of endometrium carcinoma, as a result of inhibitory role on the growth of Ishikawa cells, in addition to the elevation of cisplatin toxicity on endometrium. The tumor mediatory function of miR was accomplished by its specific base-pairing with 3'-UTR of target gene mRNA, thus exerting a negative regulatory role for the fine-

tuned modulation of the whole expression spectrum [20]. The negative regulation of target genes can facilitate the occurrence and progression of tumors, while the inhibition of miR-overexpression can thus inhibit tumor proliferation and/or invasion, indicating the crucial role of miR in tumor regulation [21, 22]. This study showed no significant correlation between miR-421 relative expression and general information of patients such as age, sex, tumor size, location, invasion depth, TNM stage, differentiation and metastasis. However, lymph node metastatic tumors had elevated miR-421 expression, when compared to those without lymph node metastasis, suggesting the correlation between miR-421 and risk of lymph node metastasis, as high-expression individuals had elevated risk of lymph node invasion. In a further survival analysis, 42 cases of miR-421 low-expression patients had significantly longer survival time compared to miR-421 high-expression group, suggesting the correlation between miR-421 and gastric cancer prognosis.

In summary, this study demonstrated the correlation between miR-421 expression in gastric carcinoma cells and the prognosis of tumors, suggesting the involvement of miR-421 in regulating metastasis/invasion of gastric cancer. MiR-421 is thus one potential biological marker and drug target for early-stage gastric cancer, although comprehensive studies are still required for further illustration of molecular mechanism and biological prediction of miR-421 in gastric cancer in a large scale scenario.

### Disclosure of conflict of interest

None.

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