EFFECT OF MIR-138 EXPRESSION ON CLINICOPATHOLOGICAL PARAMETERS IN PATIENTS WITH GASTRIC CANCER

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ABSTRACT

Objective: *In this study, the clinical significance of miR-138 was explored by analyzing the effect of miR-138 expression on clinical pathological parameters of gastric cancer patients.*

Methods: *42 patients with confirmed gastric cancer were selected. The expression of miR-138 in tumor tissues and matched adjacent normal tissues was detected by real-time fluorescence quantitative PCR (RT-PCR). The correlation between the expression level of miR-138 and the basic clinicopathological parameters of gastric cancer patients was analyzed.*

Results: *The expression level of miR-138 in the tumor tissues of 42 patients with gastric cancer was significantly lower than that in adjacent normal tissues (P<0.05). The expression of miR-138 was remarkably correlated with TNM stage and with or without lymph node metastasis in patients with gastric cancer (P<0.05). That is, the lower the expression level of miR-138, the later the tumor stage tended to be, and patients with lymph node metastasis had lower miR-138 levels.*

Conclusion: *The expression of miR-138 was down-regulated in gastric cancer, and its expression was closely related to tumor stage and with or without lymph node metastasis in patients with gastric cancer, which indicates that miR-138 has the potential to be a tumor marker in the process of diagnosis, curative effect evaluation and prognosis judgment of gastric cancer.*

Keywords: *miR-138, gastric cancer, tumor markers.*

DOI: 10.19193/0393-6384_2019_6_550

Received *November 30, 2018;* ***Accepted*** *February 20, 2019*

Introduction

Gastric cancer is one of the most common malignant tumors of digestive system. The incidence and mortality of gastric cancer rank fourth and third among all malignant tumors in the world[(1)]. With the continuous improvement of medical technology, the survival of patients with gastric cancer has been improved to a certain extent compared with the past, but the overall 5-year survival rate is still low[(2)]. The pathogenesis of gastric cancer is hidden, early gastric cancer usually has no typical symptoms, and there is a lack of effective early screening methods in clinic, so that most patients with gastric cancer are in the middle and advanced stage when they go to the hospital. This kind of patients can not only fail to carry out radical resection, but also have tumor cell metastasis, which severely reduces the therapeutic effect, leading to poor prognosis and also has a great impact on patients' quality of life[(3)]. At present, many studies on early diagnosis of gastric cancer are under way, but the markers of gastric cancer that can be applied in clinic are still very limited[(4-5)]. Therefore, it is very important to find reliable molecular markers which can be used in early diagnosis, therapeutic target and prognosis of gastric cancer to improve the survival cycle of patients with gastric cancer.

MiRNAs, as a small molecule RNA, which widely exists in a wide range of human tissues, has a regulatory effect on a variety of human genes[(6)]. MiR-138 is a kind of miRNA, with tumor regulatory function discovered in recent years, which has been found to be down-regulated in non-small cell lung cancer, hepatocellular carcinoma, esophageal squa-

mous cell carcinoma, renal cell carcinoma and other malignant tumors. It can also inhibits the progress of tumors and plays a similar function as tumor suppressor gene. It has been reported that miR-138 is down-regulated in cervical cancer cells and over-expression of miR-138 can inhibit the proliferation and migration ability of cervical cancer cells Hela. Further analysis showed that miR-138 could specifically inhibit the expression of hTERT in Hela cells[7]. He et al. detected the expression levels of miR-138 in serum of patients with non-small cell lung cancer (NSCLC). It was found that the expression level of miR-138 in patients with NSCLC was significantly lower than that in healthy people, and the expression level was closely related to the occurrence of lymph node metastasis[8].

Li et al. found that the expression of miR-138 in serum of patients with clear cell carcinoma of ovary was down-regulated, and the miR-138 expression was significantly increased after surgical treatment compared with the preoperative level. In addition, it was found that the expression of miR-138 in peripheral blood of patients with gastric cancer was significantly lower than that of healthy controls, and its expression level was significantly correlated with tumor metastasis and tumor stage[9]. More and more attention has been paid to the regulatory role of miR-138 in tumor, but the mechanism of the effect of miR-138 in the gastric cancer is still short of systematic research.

The purpose of this study was to investigate the relationship between miR-138 and the occurrence and development of gastric cancer. 42 patients with gastric cancer admitted to our hospital was selected, the expression level of miR-138 in the tumor tissues was detected, the relationship between miR-138 expression and the clinicopathological parameters of patients with gastric cancer was analyzed, and its clinical value was preliminarily discussed.

Methods

Clinical sample

A total of 42 gastric cancer patients diagnosed in xx hospital from June 2018 to June 2019 were selected, and the tissue samples of the gastric cancer and adjacent normal tissues were collected (the distance from the edge of cancer focus tissue was greater than 5cm).

Prior to sample collection, all patients signed informed consent and obtained permission from Ethics committee of our hospital. All patients were diagnosed as gastric cancer by pathology department before operation, and had not received radiotherapy and chemotherapy. The obtained tissue samples were immediately rinsed with normal saline solution and stored in liquid nitrogen.

Detection of miR-138 expression in tissues by RT-PCR

Total RNA was extracted by Trizol method and the purity and concentration of RNA were determined. Reverse transcription and PCR reactions were carried out strictly in accordance with the instructions of TAKARA company (Japan). Three complex holes were set up in each group, and GAPDH was used as internal parameter for relative quantification, which was detected by real-time quantitative fluorescence PCR instrument (ABI Company, US). GAPDH-F, 5'-CGGAAAGCTCGGTATCGTAT-3'; GAPDH-R, 5'-AGCCTTTGGACGTAGAAGAC-3'; miR-138-F, 5'-ACACTCCAATGCTACATGAACATGAGA-3'; miR-138-R, 5'-TGGTGTGATGCATCGAGTCG-3'.

The RT-PCR reaction conditions were as follows: 94 oC for 3 min, 94 oC for 20 s, 60 oC for 40 s, with 40 cycles. The relative expression levels of miR-138 was calculated by $2^{-\triangle Ct}$ method. $\triangle Ct=\triangle Ct_{miR-138}-\triangle Ct_{GAPDH}$.

Statistical analysis

SPSS21.0 software was used to analyze the data, and Graphpad Prism5.0 software was used for graph analysis. All the data are represented as mean ± standard deviation ($\bar{x}\pm s$). The quantitative data were processed by single factor variance analysis or t test, and χ^2 test was used to process the qualitative data. $P<0.05$ indicates that the difference between groups has statistical significance.

Results

Down-regulation of miR-138 expression in gastric cancer tissue

The expression of miR-138 in 42 cases of gastric cancer was detected by RT-PCR assay. As shown in figure 1, the results showed that the relative expression levels of miR-138 in gastric cancer tissues was (2.375±1.766), which was significantly lower than that in adjacent normal tissues (7.221±2.482), and the difference was statistically significant ($P<0.05$). It was indicated that the expression of miR-138 in gastric cancer was down-regulated.

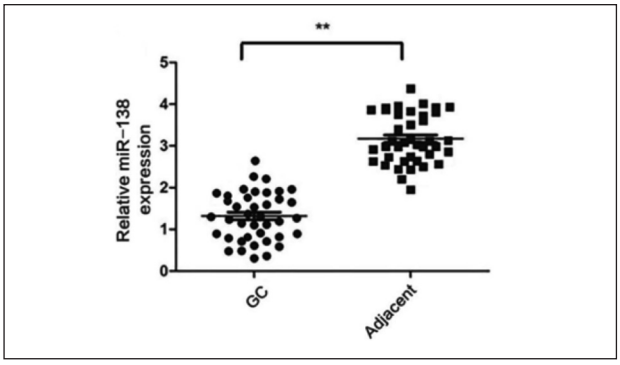

Figure 1: Expression of miR-138 in gastric cancer and paracancerous tissues. **indicates that the difference has statistical significance (P<0.05).

Correlation analysis between the expression of miR-138 and clinicopathological parameters in patients with gastric cancer

According to the median miR-138 expression, gastric cancer patients were divided into high expression group (n = 20) and low expression group (n = 22). The correlation between miR-138 expression and clinical parameters of gastric cancer patients was analyzed. The results showed that the expression of miR-138 in gastric cancer tissues was significantly correlated with TNM stage and with or without lymph node metastasis (P<0.05), but not with age, sex, tumor diameter and differentiation (P>0.05). Patients with lymph node metastasis and higher stages tended to have lower levels of miR-138 expression, as shown in Table 1. It is suggested that miR-138 is involved in the occurrence of gastric cancer and is related to the severity of gastric cancer progression.

Items	miR-138 expression		F value	P value
	Low expression (n=22)	High expression (n=20)		
Sex			1.905	0.722
Male	12	12		
Female	10	8		
Age			2.512	0.496
<50	6	7		
≥50	16	13		
Tumor diameter (cm)			1.923	0.161
<5	5	12		
≥5	17	8		
Differentiation degree				
High differentiation	11	9		
Moderate differentiation	5	4	3.022	0.211
Low differentiation	6	7	2.155	0.752
TNM stage			2.082	0.000
T1+T2	9	16		
T3+T4	13	4		
With or without of vascular invasion			2.664	0.000
With	15	8		
Without	7	12		

Table. 1: The relationship between miR-138 expression level and clinicopathological parameters of gastric cancer patients.

Discussion

The tumor cell can release the intracellular miRNA out of the cell through the way of the exosome body. Exocrine bodies exist in peripheral blood or urine, and miRNA has been confirmed to exist stably in serum or plasma. This provides a theoretical basis for the detection of miRNAs as a serum or plasma marker for tumors (10). Compared with biopsy with gastroscopy, the detection of miRNAs in peripheral blood is convenient and easy to carry out, and has the advantages of no innovation and reproducibility. It is more suitable for wide-scale clinical promotion, and has the potential to become a marker of gastric cancer detection. It can be used for the early diagnosis and recurrence monitoring of gastric cancer.

Chemotherapy is a very important treatment for patients with advanced gastric cancer or patients with metastasis. However, in clinic, the frequency of chemotherapy resistance of gastric cancer is high, which is also an important reason for the failure of gastric cancer treatment. The regulatory role of miRNAs in the occurrence and development of gastric cancer has made some progress. The researchers also pay more attention to the study of the mechanism of gastric cancer with miRNAs as the target, and provide a new theoretical reference for improving the curative effect of the treatment of gastric cancer. Previous studies have shown that miR-138 plays a more important role as tumor inhibitor gene in malignant tumors and plays a regulatory role in many downstream target genes[11-13]. At the same time, the expression of miR-138 is also regulated by several upstream genes. In addition, miR-138 can also affect the activation of multiple signaling pathways[14-16]. Ma et al. found that the expression of miR-138 was down-regulated in gallbladder carcinoma. Over-expression of miR-138 could inhibit the proliferation activity and promote apoptosis of gallbladder cancer cells, and its possible mechanism was to target the expression of Bag-1 gene[17].

Yu et al. reported that miR-138 can target FOXC1 expression to exert its inhibitory effect on the proliferation and migration ability of pancreatic cancer cells[18]. MiR-138 can not only play the role of tumor suppressor gene through down-regulation of expression, but also play a similar role as tumor-promoting gene. In the glioma cells, the expression level of miR-138 is significantly higher than that of the normal cells, and the high expression of the miR-138 can promote the migration and the invasion ability of the brain glioma, and also inhibit the apoptosis

of the glioma cells[19]. Therefore, in different tumor tissues, miR-138 can play both the function of tumor suppressor gene and cancer-promoting genes by up-regulating the expression.

In this study, the level of miR-138 in the tumor tissues and adjacent normal tissues of 42 patients with gastric cancer was detected using RT-PCR assay. The results showed that the expression of miR-138 in gastric cancer tissues was down-regulated, indicating that miR-138 was involved in the carcinogenesis of gastric cancer. In order to explore the clinical significance of differential expression of miR-138, complete clinical information of patients with gastric cancer was collected, and the correlation between the expression level of miR-138 and the clinicopathological parameters of patients was analyzed. The results indicated that there was a significant correlation between miR-138 and the TNM stage and with or without lymph node metastasis in the patients with gastric cancer, indicating that the expression of miR-138 affects the degree of tumor progression in patients with gastric cancer.

Through this study, it was confirmed that there is a differential expression of miR-138 in gastric cancer tissues, which is closely related to the clinicopathological parameters and prognosis of patients, that is, miR-138 is involved in the occurrence of gastric cancer and affects the severity of gastric cancer progress.

This indicates that miR-138 can be a potential marker in the process of diagnosis, efficacy evaluation and prognosis of gastric cancer. Gastric cancer is a complex process involving polygenic regulation. It is necessary to understand the mechanism of miR-138 affecting the occurrence and development of gastric cancer in order to better apply it to clinical practice. In the future, the expression level of miR-138 in gastric cancer cell line and the effect of its differential expression on proliferation, apoptosis, migration and invasion of gastric cancer cells can be studied at the cell level, and the downstream target of miR-138 can be further discussed.

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