

Expression level of microRNA-195 in the serum of patients with gastric cancer and its relationship with the clinicopathological staging of the cancer

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Abstract. – **OBJECTIVE:** To study miR-195 (miR-195) expression in the serum and cancer tissue of patients with gastric cancer and to investigate the relationship between its expression and clinicopathological features of gastric cancer.

PATIENTS AND METHODS: Sixty-two patients with gastric cancer admitted to our institution were included in the study group, and 36 healthy persons undergoing health check-up at our institution served as control group. miR-195 expressions in the serum, gastric cancer tissue and corresponding paracancerous tissue in subjects of two groups were measured by using quantitative fluorescent real-time PCR (QF-RT-PCR), and the relationship between miR-195 and the clinicopathological features of the cancer was investigated.

RESULTS: miR-195 expression level in the serum of gastric cancer patients was significantly lower than that in the control group ($p < 0.05$). miR-195 expression in gastric cancer tissue was also significantly lower than that in corresponding paracancerous tissue ($p < 0.05$). The results of correlation analysis showed that low expression of miR-195 was negatively correlated with the infiltration depth, the extent of differentiation, the clinical staging and lymph node metastasis, all with statistical significance ($p < 0.05$), but not significantly correlated with tumor locations ($p > 0.05$).

CONCLUSIONS: Low expression of miR-195 in patients with gastric cancer may play a certain role in promoting the genesis and development of gastric cancer and it can function as a potential novel tumor marker for the early diagnosis and prognosis evaluation of gastric cancer.

Key Words:

microRNA-195, Gastric cancer, Staging.

Introduction

Gastric cancer, known as common cancer, is the fourth most prevalent malignancy following lung cancer worldwide and with high mortality according to statistics¹, and the second leading cause of cancer-related death globally². In China, gastric cancer remains one of the most common cancers. The early diagnosis of the cancer is difficult, with the disease often at an advanced stage with poor prognosis when detected. In addition, the morbidity and mortality of the disease have gradually increased in recent years, seriously threatening people's live and health. The pathogenesis and development of gastric cancer are a multi-factorial, multi-stage and multi-step process, which is resulted from the combined actions of genetic factors, environmental factors and biological factors. However, the detailed mechanism for gastric cancer pathogenesis is not fully understood.

In recent years, researchers have discovered a new class of small, non-coding and 22 nucleotide RNAs, i.e. microRNA (miRNA), which can induce mRNA degradation or suppress translation by binding to the 3'-untranslated region (UTR) of target mRNA. Numerous studies have suggested that miRNAs play a critical role in the regulation of the pathogenesis and development of cancer and their aberrant expression in tumor tissue is closely associated with the differentiation and metastasis of cancer³. Of all miRNAs, microRNA-195 (miR-195) is a new class of miRNA recently discovered. Certain studies have demonstrated aberrant miR-195 expressions in osteosarcoma, colorectal cancer and other tumors, involved in the regula-

tion of tumor cell apoptosis and tumorigenesis⁴⁻⁶. However, miR-195 in patients with gastric cancer and its relationship with the clinicopathology of these patients have not hitherto been reported. Therefore, the present study investigated, for the first time, the relationship between miR-195 and TNM staging of gastric cancer by assessing miR-195 expression in the serum and tissues of patients with gastric cancer, in an effort to provide relevant experimental evidence for the diagnosis, treatment and prognosis of gastric cancer.

Patients and Methods

Patients

Between November 2012 and August 2014, tumor samples and paracancerous tissues were collected from 62 patients who underwent surgery for gastric cancer in our institution. Of these patients, 29 were males and 33 females, with age range 52-77 years and mean age (65.15±6.51) years. Patients had received neither radiotherapy nor chemotherapy prior to surgery. The control group is made up of 36 people undergoing health check-up for the same period, including 15 males and 21 females of age range 64-78 years and mean age of (65.41±9.31) years.

Major Reagents

Trizol kit for RNA isolation from tissue and serum was purchased from Shanghai Invitrogen Biotechnology Co., Ltd., Shanghai, China. cDNA reverse transcription kit was purchased from Fermentas (Waltham, MA, USA). PCR kit was purchased from Sunshine Biotechnology Co., Ltd., Nanjing, China.

RNA Isolation and Reverse Transcription Reaction

Tumor tissues of gastric cancer and corresponding paracancerous tissues located at 2 cm-6 cm from the edge of the tumor were collected from 62 patients during surgery and examined. Additionally, 5 ml blood sample was individually collected from peripheral veins of each patient, complemented with an anticoagulant, EDTA (Shanghai Three Bio-Technology Co., Ltd., Shanghai, China), and centrifuged at 10,000 g for 10 min at 4°C within two hours from collection. The upper layer of serum was then isolated after centrifugation for further experiment.

A total of 100 mg tissue or 150 µl serum was collected and fully homogenized with 1ml Trizol

reagent. Total RNA was isolated following manufacturer's instruction and the resulting RNA was dissolved in 20 µl DEPC-treated water. Total RNA was quantified by using UV spectrophotometry, reverse transcribed with cDNA reverse transcription kit and stored at -20°C for further analysis. The PCR reaction conditions were initial denaturation at 95°C for 20s, followed by 50 cycles of 60°C for 20s and 70°C for 1s. All real-time PCR (RT-PCR) data was analyzed on ABI 7900 RT-PCR system and relative quantitative analysis was performed by using the $2^{-\Delta\Delta Ct}$ cycle method⁷. The sequences of primers for amplifying miR-195 were 5'-CGTAGCAGCACAGAAAT-3' and 5'-GTGCAGGGTCCGAGGT-3'[8]. U6 snRNA was used as the internal control to normalize miRNA expression. The sequences of primers for U6 were 5'-CTCGCTTCGGCAGCA CA-3' and 5'-AACGCTTCACGAATTTGCGT-3'.

Statistical Analysis

All data were analyzed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA) and graphs were generated using GraphPad Prism 5 software. Quantitative data were presented as mean ± standard deviation. Comparison between groups was performed using independent sample *t*-test. Spearman correlation analysis and Pearson correlation analysis were performed respectively. $p < 0.05$ was considered significantly different.

Results

The level of miR-195 mRNA decreased in serum of patients with gastric cancer

Compared to normal population, miR-195 mRNA expression in the serum of patients was significantly decreased (1.21±0.23 vs. 0.32±0.11, $t = 25.86$, $p < 0.05$), indicating that miR-195 may function as a tumor suppressor involved in inhibiting the progression of gastric cancer (Figure 1).

miR-195 mRNA Expression in Gastric Cancer Tissue and Corresponding Paracancerous Tissue

Compared with paracancerous tissue, miR-195 mRNA expression in gastric cancer tissue was significantly decreased (1.19±0.21 vs. 0.41±0.27, $t = 14.90$, $p < 0.05$), which further suggesting that low level of miR-195 in cancer tissue may be able to promote the development of gastric cancer (Figure 2).

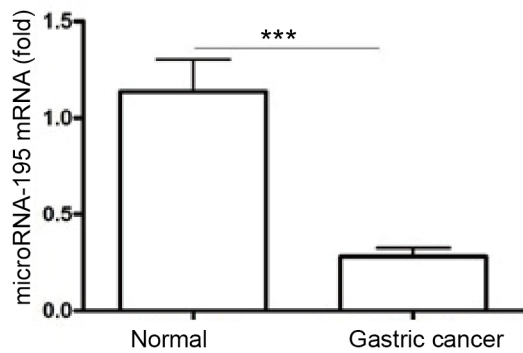


Figure 1. miR-195 mRNA expression in the serum of patients with gastric cancer. ***vs. normal group, $t = 25.86$, $p < 0.001$

Relationship Between miR-195 Expression Level in Cancer Tissue and the Clinicopathological Features of Gastric Cancer

The results showed that miRNA-195 mRNA expressions in cancer tissue of stage III and IV gastric cancers were significantly lower than those in stage I and II gastric cancer ($p < 0.05$). miR-195 mRNA expression level in well-differentiated tissue was significantly lower than that in poorly differentiated tissue ($p < 0.05$). miR-195 mRNA expression decreased as the extent of infiltration was aggravated ($p < 0.05$). Moreover,

miR-195 mRNA expression level in cancer with lymph node metastasis was significantly lower than that in cancer without metastasis ($p < 0.05$); however, no significant differences were observed in miR-195 mRNA expressions in different locations ($p > 0.05$) (Table I).

Correlation Between miR-195 Expression level in Tissue of gastric cancer and Clinicopathological Staging of Gastric Cancer

The results of Spearman correlation analysis demonstrated that miR-195 mRNA expression

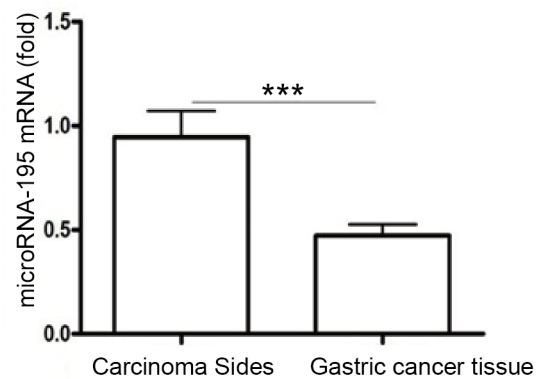


Figure 2. miR-195 mRNA expression level in gastric cancer tissue and corresponding paracancerous tissue. ***vs. paracancerous tissue, $t = 14.90$, $p < 0.001$

Table I. Relationship between microRNA-195 mRNA expression and the clinicopathological features of gastric cancer.

| Clinicopatholog-ical features | n | MicroRNA-195 mRNA expression | t/F [§] | p-value |
|-------------------------------|----|------------------------------|------------------|---------|
| Stage of cancer | | | | |
| Stage I and II | 30 | 0.47±0.31 | | |
| Stage III and IV | 32 | 0.32±0.22 | 2.21 | 0.031 |
| Tumor location | | | | |
| The gastric antrum | 18 | 0.41±0.31 | | |
| The gastric body | 20 | 0.38±0.28 | 0.39 | 0.649 |
| The gastric cardia | 24 | 0.45±0.24 | | |
| The extent of differentiation | | | | |
| Poorly differentiated | 38 | 0.48±0.29 | | |
| Well differentiated | 24 | 0.30±0.26 | 2.48 | 0.016 |
| The extent of infiltration | | | | |
| T2 | 15 | 0.49±0.21 | | |
| T3 | 28 | 0.40±0.25 | 4.72 | 0.017* |
| T4 | 19 | 0.31±0.26 | | |
| Lymph node metastasis | | | | |
| present | 28 | 0.32±0.21 | | |
| absent | 34 | 0.51±0.22 | 3.45 | 0.000 |

Note: §Comparison of tumor location and extent of infiltration among multiple groups were performed using F-test, and comparison between the rest two groups was performed using *t* test.

*Further pairwise comparisons using SNK method demonstrated statistical significance.

Table II. Relationship between microRNA-195 mRNA expression level and TNM staging of gastric cancer.

| TNM stage | No. | Serum of gastric cancer | Tissue of gastric cancer |
|-----------|-----|--------------------------|--------------------------|
| I | 13 | 0.51 ± 0.21 | 0.49 ± 0.23 |
| II | 17 | 0.46 ± 0.19 | 0.41 ± 0.46 |
| III | 14 | 0.35 ± 0.18 | 0.36 ± 0.43 |
| IV | 18 | 0.29 ± 0.18 [§] | 0.27 ± 0.55 [*] |

Note: VS TNM staging of gastric cancer, [§] $r=0.501, p<0.05$; ^{*} $r=0.554, p<0.05$

level in tissue of gastric cancer was negatively correlated with TNM staging of gastric cancer ($r = 0.501, p < 0.05$) (Table I), and miR-195 mRNA expression level in the serum was negatively correlated with TNM staging of gastric cancer ($r = 0.554, p < 0.05$) (Table II). These results indicated that the poorer the differentiation of gastric cancer, the lower the miR-195 expression (Table II).

Discussion

microRNA is a class of 19-25 nt single strand RNA, widely present in tissues, serum, plasma or other body fluids. These molecules are not protein-coding genes but play an important role in regulating mRNA of protein-coding genes⁹. In 2002, Calin et al¹⁰ reported, for the first time, that a significant correlation was observed between aberrant miRNA expression and presence of tumor. Since then, a growing number of studies have been focused on the effect of aberrant miRNA expression on tumor¹¹⁻¹³. A study¹⁴ has suggested that miRNA can affect tumor progression by regulating tumor suppressor genes or oncogenes. Currently, a large number of studies^{4,6} have showed that aberrant miR-195 expression was observed in various tumor tissues, involved in the regulation of tumor cell apoptosis and tumorigenesis. Zhao et al¹⁵ have assessed miR-195 expression level in the serum of 21 patients with breast cancer by using RT-PCR. The study showed that miR-195 expression was significantly lower than that of the normal population and suggested that miR-195 expression level can serve as a molecule for the specific diagnosis of breast cancer. In addition, the miR-195 level has been shown to be significantly suppressed in esophageal cancer tissue¹⁶. However, the miR-195 expression level in gastric cancer and its potential physiological function in gastric cancer have not been elucidated to date.

We showed that miR-195 expression level in cancer tissue was lower than that in the paracancer-

ous tissue of patients with gastric cancer, thereby suggesting miR-195 can play an important role in tumor suppression. Moreover, the present study also found out that miR-195 expression level in the serum of patients with gastric cancer was lower than that of the normal population, further confirming that miR-195 may function as a potential tumor suppressor in the genesis and development of gastric cancer.

We further analyzed the relationship between miR-195 expression in gastric cancer tissue and the clinicopathological features of cancer. The results demonstrated that miR-195 mRNA expressions in tissues of stage III and IV gastric cancer were significantly lower than those in stage I and II cancer tissue. miR-195 mRNA expression in well-differentiated gastric cancer was significantly lower than that of poorly differentiated cancer. Furthermore, we also observed that miR-195 mRNA expression decreased with the aggravation of the extent of infiltration, and miR-195 mRNA expression in cancer with lymph node metastasis was lower than that without metastasis. All of the above results indicate that low expression of miR-195 plays a certain role in promoting the genesis and development of gastric cancer and further suggest that miR-195 plays an important role in tumor suppression in gastric cancer.

Further correlation analysis showed that miR-195 expression in the cancer tissue and the serum of gastric cancer decreased with the increasing severity of gastric cancer based on TNM staging and a significantly negative correlation was observed between them. These results indicated that miR-195 might influence the malignancy of cancer cells in patients with gastric cancer, thereby suggesting that miR-195 might play potentially important roles in the diagnosis, staging and prognostic evaluation of gastric cancer. Deng et al¹⁷ found that administration of miR-195 mimics could significantly suppress the growth of gastric cancer cells, and thereby indicating that miR-195 is likely to be involved in tumor progression

by influencing the growth of gastric cancer cells. Studies¹⁸ also demonstrated that miR-195 can also inhibit the proliferation of human glioma cells by regulating the expression of cyclin D1 and cyclin E1, which suggested that miR-195 can be involved in the progression of gastric cancer by affecting cancer cell proliferation.

Conclusions

The low expression of miR-195 plays important roles in the pathogenesis and development of gastric cancer, possible by influencing the proliferation and growth of gastric cancer cells. In clinical practice, the detection of miR-195 plays a certain role in guiding the treatment and prognosis of patients with gastric cancer.

Conflict of Interest

The authors declare no conflict of interests.

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