RESEARCH ARTICLE

Upregulation of MicroRNA 181c Expression in Gastric Cancer Tissues and Plasma

Mei-Hua Cui*, Xiao-Lin Hou, Xiao-Yan Lei, Fang-Hong Mu, Gui-Bin Yang, Lin Yue, Yi Fu, Guo-Xing Yi

Abstract

**Objective:** To test the microRNA-181c (miR-181c) expression in tissues and plasma of gastric cancer (GC) cases, analyze any correlations, and explore the possibility of miR-181c as a potential molecular marker for GC diagnosis. **Materials and Methods:** Relative miR-181c expression levels in cancers and plasma from 30 GC patients was tested using reverse transcription–real-time fluorescent quantitation PCR and compared to that in samples from 30 gastric ulcer and 30 chronic gastritis patients. **Results:** The miR-181c expression level in the GC tissues was significantly higher than that in the gastric ulcer and chronic gastritis tissues \( (P = 0.000) \), as was the miR-181c expression level in the GC plasma \( (P = 0.000) \). We determined that miR-181c expression in GC plasma was positively correlated to its expression in the GC tissues \( (P = 0.000) \). **Conclusions:** The expression of miR-181c is upregulated in GC tissues and plasma, and the miR-181c expression level in GC plasma is positively correlated to that in the corresponding cancer tissues. Plasma miR-181c is possibly a new serological marker for GC diagnosis.

**Keywords:** MicroRNA - 181c (miR-181c) - gastric cancer - plasma - cancer tissue

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Introduction

MicroRNAs (miRNAs) are tiny, non–protein-coding RNA molecules composed of 19-26 nucleotides that regulate the level of posttranscriptional gene expression and are associated with a variety of tumors when expressed abnormally (Jay et al., 2007). Recently, there have been concerns regarding the correlation of miRNA-181a (miR-181c) to tumors. Studies have shown that abnormal miR-181c expression is closely related to glioma, squamous cell carcinoma of the tongue, breast cancer, and other tumors (Wong et al., 2008; Lowery et al., 2009; Slaby et al., 2010). Scholars have studied the correlation of miR-181c to gastric cancer (GC) at cellular level and found that the miR-181c expression varies according to the GC cell line: it is downregulated in the KATO-III, MKN45, MKN74, AGS cell lines, and so on, and upregulated in the HSC58, MKN7, and GCIY cell lines, and the like (Hashimoto et al., 2010). However, whether miR-181c expression in the tissue and plasma samples from GC patients is increased or decreased currently remains unclear.

GC is one of the most common malignant tumors in China, ranked first in terms of morbidity and mortality rates. Early diagnosis and treatment can significantly improve the GC cure rate and prognosis. Nevertheless, effective methods for the early diagnosis of GC are lacking. Recent studies have found that tumor-related miRNA can also be detected in the peripheral blood of patients with cancer (Mitchell et al., 2008). This means that it is possible to use miRNA as a novel, noninvasive molecular marker for cancer diagnosis. We tested the expression of miR-181c in the tissue and plasma of patients with GC, analyzed the correlation between them, and evaluated the possibility of using miR-181c as a potential serological molecular marker for GC diagnosis.

Materials and Methods

**Subjects**

Thirty patients each with GC, gastric ulcer, and chronic gastritis as confirmed at our hospital from June 2010 to March 2011 based on gastrointestinal symptoms were included. All the patients were examined and their diagnosis confirmed by gastroscopy and biopsy pathology, and we excluded those with other malignant tumor histories. The age range of the 24 men and 6 women with gastric adenocarcinoma was 37-83 years; the mean age was 68.5 ± 10.4 years. The 21 men and 9 women with gastric ulcer were 34-78 years old; the mean age was 63.7 ± 12.6 years. The 20 men and 10 women with chronic gastritis were 34-83 years old; the mean age was 62.4 ± 12.2 years. The sexes and ages in the three groups were not significantly different \( (P > 0.05) \). The biopsy sites examined with gastroscopy were cancer tissue, gastric
ulcer tissues, and the antral mucosa in the GC, gastric ulcer, and chronic gastritis patients, respectively. The biopsy lesion tissues were underwent routine pathological examination when all the patients were examined by gastroscopy, two pieces of the appropriate biopsy tissues were additionally obtained with clippers, and 3 mL venous blood was obtained before or after the gastroscopy examination. The Medical Ethics Committee of Aerospace Center Hospital ratified the study program and all patients signed informed consent forms. The study conformed to The Code of Ethics of the World Medical Association (Declaration of Helsinki) printed in the British Medical Journal (18 July 1964).

Reagents
We used TRI Reagent BD (mrcgene; Cincinnati, OH), Total RNA Isolation Kit (Ambion; Austin, TX), reverse transcription (RT) kit (Qiagen; Hilden, Germany), and a SYBR Green Real-Time PCR Master Mix (Takara; Dalian, China). All the other chemicals used were of analytical reagent grade.

Samples
The GC, gastric ulcer, and chronic gastritis mucosa tissue samples were harvested during the gastroscopy, quickly frozen in liquid nitrogen, and stored at -80°C. Three milliliters of peripheral blood was collected before and after the gastroscopy, and the plasma was harvested after centrifugation and stored at -80°C.

RNA isolation and real-time RT–PCR
For the real-time RT–PCR, RNAs were extracted from homogenized tissues or plasma using TRIzol according to the manufacturer’s instruction. Genomic DNA contaminants were removed using DNA-free DNase Treatment and Removal (Ambion). The RNA purity was assessed by spectrophotometry (A260/A280 > 1.8). Single-stranded cDNAs were generated using the RT kit according to the manufacturer’s directions. The real-time quantitative PCR experiments were performed with an ABI Prism 9700 Sequence Detection System (Applied Biosystems; Foster City, CA) using the SYBR Green PCR Master Mix according to the manufacturer’s protocol. The primer sequences were as follows: hsa-miR-181c sense: 5′-AACATTCAACCTGTCGGTGAGT-3′; internal control U6 sense: 5′-CAAGGATGACACGCAAAATTCC-3′. All anti-sense primers were universal primers provided in the kit. Each dilution was amplified with triplicate PCR and plotted as the mean values. Fluorescence measurements were made in every cycle. The following cycling conditions were used: 95°C for 30 s, and 40 cycles of 95°C for 5 s and 60°C for 34 s. The fold-change in the relative gene expression to that of the control was determined by the standard 2-ΔΔCt method.

Statistical analysis
The experimental data were analyzed using SPSS13.0 statistical software and expressed as mean ± standard deviation. The difference in the relative levels of miR-181c expression in the GC, gastric ulcer, and chronic gastritis tissues and plasma were compared using one-way ANOVA. The correlation of miR-181c expression in the GC tissues and plasma was defined using bivariate correlation analysis and linear regression analysis. The test level at α = 0.05, P < 0.05 was considered significantly different.

Results
Difference of miR-181c expression in GC, gastric ulcer, and chronic gastritis tissues
The relative levels of miR-181c expression in the GC, gastric ulcer, and chronic gastritis tissues were 2.37 ± 1.11, 1.29 ± 0.59, and 1.16 ± 0.55, respectively. The level of miR-181c expression in the GC tissues was significantly higher than that in the gastric ulcer and chronic gastritis tissues (P = 0.000), whilst the expression levels in the gastric ulcer and chronic gastritis tissues were not significantly different (P = 0.536, Figure 1).

Difference of miR-181c expression in GC, gastric ulcer, and chronic gastritis plasma
The relative levels of miR-181c expression in the GC, gastric ulcer, and chronic gastritis plasma were 3.27 ± 1.30, 1.31 ± 0.62, and 1.12 ± 0.45, respectively. The level of miR-181c expression in the GC plasma was significantly higher than that in the gastric ulcer and chronic gastritis plasma (P = 0.000), whilst the levels of miR-181c expression in the gastric ulcer and chronic gastritis plasma were not significantly different (P = 0.404, Figure 1).

Relation of relative levels of miR-181c expression in the GC plasma and tissues
After they were defined using bivariate correlation...
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regression and correlation analysis approaches revealed that the relative levels of miR-181c expression in the GC plasma and tissue were positively correlated. This suggested that if there were a higher level of expression in GC tissues, the level of miR-181c expressed in the plasma would increase. Plasma specimens are obtained more easily than tissue specimens are. This suggests that plasma miR-181c has the potential to be a new serum marker for the diagnosis and follow-up of GC.

It is still unclear why miR-181c expression in the peripheral blood plasma of patients with GC is upregulated. Current studies have conjectured that the increased plasma miRNA level is due to the release of overexpressed miRNA from tumor tissues into the peripheral blood. There are two theories regarding the release mechanism: the injury theory and the super-microvesicle theory. The former states that miRNA is released into the peripheral blood during the proliferation and dissolving of cancer cells, while the latter states that the miRNA is transmitted intercellularly through super-microvesicles and is released extracellularly and to the peripheral blood when the super-microvesicles shed off the cell membrane during transmission (Ji et al., 2009; Brase et al., 2010). Additional studies have found that many tumor cells present in the microenvironment of GC, such as macrophages, myeloid cells, dendritic cells, and T cells, and so on, can release a secretory product (exosomes) functionally. The secretory product transmits the miRNA to other cells or the circulating blood (Valadi et al., 2007). Based on the above hypotheses, it is believed that the increased level of miR-181c expression in GC plasma may be due to the release of overexpressed miR-181c from cancer tissue into the peripheral blood somehow, or that miR-181c is upregulated in some tumor-associated cells and transmits the miRNA to the peripheral blood through the secretory product.

To conclude, the upregulated miR-181c expression in the plasma of patients with GC was positively correlated with its level of expression in GC tissues; plasma miR-181c has the potential to be a new, noninvasive serological marker for GC diagnosis. Further studies must be performed to explore its clinical value, for example, by increasing the sample size to identify the diagnosis thresholds and analyzing GC patients hierarchically according to tumor stage to identify the plasma miR-181c values in early GC diagnosis and to prospectively understand the function of plasma miR-181c on the follow-up and prognostic evaluation after GC.

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