

## Original Article

# Expression and clinical study of gastric carcinoma hsa-miR-204 in tissues of gastric adenocarcinoma patients

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Received December 4, 2019; Accepted January 3, 2020; Epub June 15, 2020; Published June 30, 2020

**Abstract:** Objective: This study aimed to analyze the expression and clinical significance of gastric carcinoma hsa-miR-204 in tissues of gastric adenocarcinoma patients. Methods: 106 gastric adenocarcinoma patients who received operative treatment in our hospital from January 2015 to January 2018 were selected as the objects of this study, and the gastric adenocarcinoma tissues were collected during the operation. At the same time, normal gastric tissues of 3 cm adjacent to the cancer (pathology showed no cancer cells) were collected as control. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the expression of miR-204 in cell lines, normal gastric specimen and gastric adenocarcinoma specimen. The correlation between relative expression of miR-204 and pathological parameters in gastric adenocarcinoma tissues was analyzed. miR-204 was transfected into human gastric cancer cell lines, and the proliferation and apoptosis of human gastric cancer cell lines in the transfected and untransfected groups were compared. Results: (1) The relative expression of miR-204 in gastric adenocarcinoma specimen was  $1.95 \pm 0.75$ , which was much lower than that of  $4.02 \pm 1.28$  in normal gastric specimen ( $P < 0.05$ ). (2) The expression levels of miR-204 in patients with moderate and good differentiation were higher than those in patients with poor differentiation ( $P < 0.05$ ); the expression level of miR-204 in patients with lymph node metastasis was lower than that in patients without lymph node metastasis ( $P < 0.05$ ); and the expression level of miR-204 in patients at stages I-II of TNM staging was higher than that in patients at stages III-IV ( $P < 0.05$ ). (3) The apoptosis rates of BGC823 and SGC7901 in the untransfected group were  $8.29 \pm 0.22\%$  and  $4.05 \pm 0.25\%$ , which were much lower than those of  $9.15 \pm 0.25\%$  and  $6.52 \pm 0.32\%$  in transfected group ( $P < 0.05$ ). (4) On the third day, the absorbances of BGC823 and SGC7901 were respectively  $2.49 \pm 0.09$  OD490 and  $3.18 \pm 0.06$  OD490 in untransfected group, which were higher than those in transfected group ( $P < 0.05$ ). Conclusion: During the occurrence and development of gastric adenocarcinoma, miR-204 may play an important role as a cancer suppressor gene and can be regarded as a new molecular marker to provide vital foundation for prognosis and treatment of gastric adenocarcinoma patients.

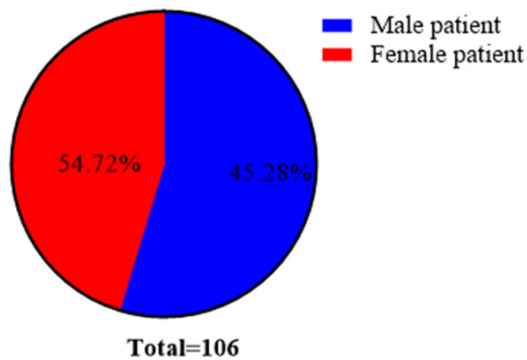
**Keywords:** Gastric carcinoma, hsa-miR-204, gastric adenocarcinoma, tissue, expression, cell apoptosis, cell proliferation

## Introduction

Gastric carcinoma is one of the malignant tumors with high clinical morbidity and mortality. Its specific pathogenesis is not completely known now, but it is generally believed that the pathogenesis is closely related to gene, heredity, precancerous lesions, helicobacter pylori infection, life style, dietary habit, and region, etc. [1, 2]. Gastric adenocarcinoma is a common type of gastric carcinoma, accounting for 95% of malignant gastric tumors. It is called adenocarcinoma because it is caused by the

malignant transformation of gastric gland cells [3].

Micro RNA (miRNA), as a RNA molecule extensively existing in eukaryotes, can mediate the formation of RNA-induced silencing complex in cytoplasm and promote the degradation of messenger RNA in cytoplasm. Besides, it can regulate the expression of downstream genes after transcription [4]. Studies have shown that miRNA expression is closely related to the occurrence and development of various clinical malignant tumors [5]. miRNA-204, mainly locat-



**Figure 1.** Analysis on gender ratio of patients. Among 106 patients involved in this study, the males accounted for 54.72% and the females accounted for 45.28% ( $P>0.05$ ).

ed on human chromosome 9, usually has abnormal expression in patients with gastric carcinoma, breast cancer, hepatic carcinoma, lung cancer, rectal carcinoma, etc. So it is speculated that miRNA-204 may play a vital role during the occurrence and development of tumors [6]. However, there is no unified conclusion in clinical practice to define the regulating effect of miRNA-204 in the process of tumor occurrence and development.

This study aimed to analyze the regulating effect of miRNA-204 on the expression of gastric adenocarcinoma tissues and its influence on the apoptosis of gastric carcinoma cells; analyze the relative expression of miRNA-204 in cell lines, normal gastric specimen and gastric adenocarcinoma specimen; discuss the correlation between relative expression of miR-204 and pathological parameters in gastric adenocarcinoma specimen; and analyze the influence of miRNA-204 on proliferation and apoptosis of gastric carcinoma cells in gastric adenocarcinoma tissues.

## Materials and methods

### Materials

106 gastric adenocarcinoma patients who received operative treatment in our hospital from January 2015 to January 2018 were selected as the objects of this study. (1) Inclusion criteria: Written informed consent was provided by patients and their family members. Patients were diagnosed with gastric adenocarcinoma according to histopathology. The estimated sur-

vival time was more than 3 months. Patients did not receive chemoradiotherapy in the past. (2) Exclusion criteria: Patients who had the relapse of gastric adenocarcinoma; patients with incomplete medical records; patients who suffered from other malignant tumors. This study was approved by medical ethics committee of our hospital. Patients aged 51-78 years old, with the average age of  $62.58\pm 5.88$ . There were 58 male patients and 48 female patients (**Figure 1**); 44 patients with lymph node metastasis and 62 patients without lymph node metastasis; 56 patients with poor differentiation and 50 patients with moderate and good differentiation; 72 patients in stage I-II and 34 patients in stage III-IV according to TNM staging; and 79 patients with transfection and 27 patients without transfection. The excised specimen was stored in liquid nitrogen at first and then in the refrigerator at  $-80^{\circ}\text{C}$ .

## Methods

### Cell culture

Shanghai Institute of Cell Biology provided immortalized gastric epithelial cell line (GES-1) and gastric carcinoma cell lines (SGC7901 and BGC823). RPMI-1640 culture medium (produced by Wisent Biotechnology (China) Co., Ltd.) was taken as cell culture fluid, which was placed in the  $\text{CO}_2$  constant temperature incubator produced by ThermoFisher for culture. The temperature was controlled at about  $37^{\circ}\text{C}$  and the cell culture fluid was changed every 2 days.

### Cell transfection

miRNA-204 was added into RNase-free water to prepare the solution, with the concentration of  $20\ \mu\text{mol/L}$ . SGC7901 and BGC823 cells in log phase were collected and the culture medium was used for cell culture upon the completion of trypsinization. The inoculation was performed in a 6-well plate. Then, the Lipofectamine 2000 transfection reagent was added, which was followed by dilution and still standing of 5 min in sequence. The mixed liquor was added into cell culture plate, with the dripping amount of  $500\ \mu\text{L}$  in each well. After 4-6 h, the mixed liquor was changed. After transfection,  $4\ \mu\text{L}$  of 5-Fu solution of was added, which was followed by the still standing of 24 h to digest cells.

**Table 1.** Analysis on relative expression of miR-204 in cell lines, normal gastric specimen and gastric adenocarcinoma specimen ( $\bar{x} \pm sd$ )

Group	Relative expression of miR-204
Gastric adenocarcinoma specimen	1.95±0.75
Normal gastric specimen	4.02±1.28
Cell line BGC823	1.88±0.25
Cell line SGC7901	1.18±0.15
Cell line GES-1	5.29±0.68
F	15.268
P	0.002

*Reverse transcription-polymerase chain reaction (RT-PCR) method*

The total RNA was extracted from cell lines, normal gastric specimen and gastric adenocarcinoma specimen through Trizol reagent and was transcribed into cDNA. The forward primer and reverse primer of miR-204 were 5'-AACCGAUCCCCGUCUGAGAUUG-3' and 5'-CCGGAUCAAGAUUAGUUCGGUU-3' respectively. The internal reference was  $\beta$ -actin and its forward primer and reverse primer were 5'-AGCGGGTC-TGACG-TAAAGCGA-3' and 5'-GTGGACGGGAGAGGAC-TGG-3' respectively. The reaction condition of PCR was set as predegeneration of 10 s at 95°C for 50 cycles: 95°C for 10 s, 65°C for 10 s and extension of 60°C for 8 s. The amplification curve was plotted at the same time. Upon the completion of reaction, the cycle thresholds of all reactions were obtained. At last,  $2^{-\Delta\Delta ct}$  method was used to quantify the relative expression of miRNA-204.

*Cell apoptosis assay*

The cell concentration was adjusted and controlled at  $1 \times 10^6$ /mL. The centrifugation was performed at the speed of 1,000 r/min for 10 min and the supernatant was discarded. 1 mL of PBS buffer solution was added and shaken mildly, and then the centrifugation was performed at the speed of 1,000 r/min for 10 min, and the supernatant was discarded. Cell apoptosis was assayed through Annexin V-FITC/PI double staining method. All the operations were carried out in strict accordance with kit instructions and all kits were provided by Shanghai Qcbio Science & Technologies Co., Ltd.

*Cell proliferation assay*

The methyl thiazolyl tetrazolium assay method was used to detect the proliferation of human gastric carcinoma cell lines untransfected and transfected with miR-204. After digestion, the adherent cells were beaten evenly in each group and then were transferred into the 96-well plate. 20  $\mu$ L of the reaction solution was dripped into each well and cultivated in the CO<sub>2</sub> constant temperature incubator for 2 h of culture. The culture temperature was controlled at 37°C. The absorbance of each group was assayed at the position where the wave length was 490 nm.

*Observation targets*

(1) To analyze the relative expression of miR-204 in cell lines, normal gastric specimen and gastric adenocarcinoma specimen. (2) To analyze the correlation between relative expression of miR-204 and pathological parameters in gastric adenocarcinoma specimen. (3) To analyze the influence of miR-204 on cell apoptosis. (4) To analyze the influence of miR-204 on cell proliferation.

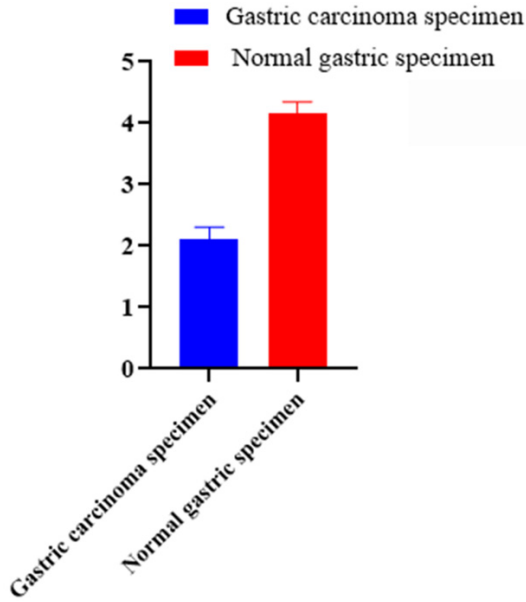
*Statistical analysis*

SPSS22.0 was used for statistical analysis; the measurement data were expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD); the independent-samples *t* test was used for the data in conformity with normal distribution; Mann-Whitney U test was used for the data not in conformity with normal distribution; the paired-samples *t* test was used for comparison before and after experiment in group; the enumeration data were expressed as [n (%)]; and the  $\chi^2$  test was used for comparison of enumeration data between groups. Thereinto,  $P < 0.05$  was considered statistically significant.

**Results**

*Relative expression of miR-204 in cell lines, normal gastric specimen and gastric adenocarcinoma specimen*

The relative expression of miR-204 was 1.95±0.75 in gastric adenocarcinoma specimen, which was much lower than that of 4.02±1.28 in normal gastric specimen, indicating statistical difference ( $P < 0.05$ ) (Table 1 and Figure 2). The

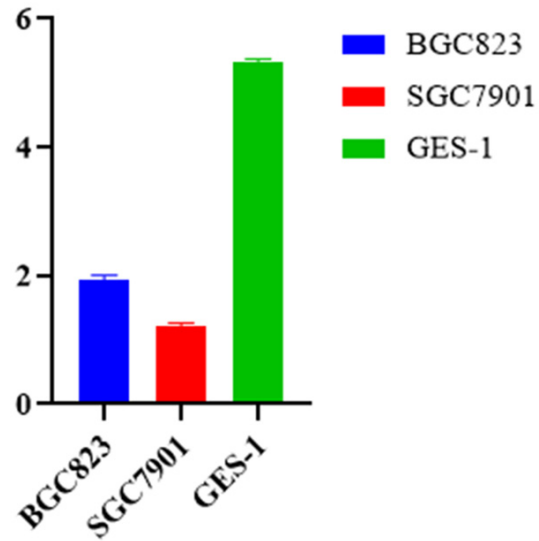


**Figure 2.** Relative expression of miR-204 in normal gastric specimen and gastric adenocarcinoma specimen. The relative expression of miR-204 in gastric adenocarcinoma specimen was lower than that in normal gastric specimen ( $P<0.05$ ).

relative expressions of miR-204 were  $1.88\pm 0.25$  and  $1.18\pm 0.15$  respectively in gastric carcinoma cell line SGC7901 and BGC823, which were lower than that of  $5.29\pm 0.68$  in immortalized gastric epithelial cell line GES-1, indicating statistical difference ( $P<0.05$ ) (Table 1 and Figure 3).

*Analysis on the correlation between relative expression of miR-204 and pathological parameters in gastric adenocarcinoma specimen*

There was no statistical difference in the expression level of miR-204 between males and females ( $P>0.05$ ), and between patients aged  $<60$  years old and those aged  $\geq 60$  years old ( $P>0.05$ ). The expression levels of miR-204 in patients with moderate and good differentiation were higher than those in patients with poor differentiation, which indicated statistical difference ( $P<0.05$ ); the expression level of miR-204 in patients with lymph node metastasis was lower than that in patients without lymph node metastasis, which showed statistical difference ( $P<0.05$ ); and the expression level of miR-204 in patients at stages I-II was higher than that in patients at stages III-IV according to TNM staging, which indicated statistical difference ( $P<0.05$ ) (Table 2).



**Figure 3.** Relative expression of miR-204 in cell line BGC823, cell line SGC7901 and cell line GES-1. The relative expression of miR-204 in gastric carcinoma cell line SGC7901 was lower than that in immortalized gastric epithelial cell line GES-1 ( $P<0.05$ ); and the relative expression of miR-204 in gastric carcinoma cell line SGC7901 was lower than that in immortalized gastric epithelial cell line GES-1 ( $P<0.05$ ).

*Analysis on the influence of miR-204 on cell apoptosis*

The apoptosis rates of BGC823 and SGC7901 were  $8.29\pm 0.22\%$  and  $4.05\pm 0.25\%$  respectively in untransfected group, which were much lower than  $9.15\pm 0.25\%$  and  $6.52\pm 0.32\%$  in transfected group, indicating statistical difference ( $P<0.05$ ) (Table 3 and Figure 4).

*Analysis on the influence of miR-204 on cell proliferation*

There was no statistical difference in absorbance of BGC823 and SGC7901 between transfected group and untransfected group on the first day ( $P>0.05$ ). On the third day, the absorbances of BGC823 and SGC7901 was  $(2.49\pm 0.09)$  OD490 and  $(3.18\pm 0.06)$  OD490 respectively in untransfected group, which were higher than those in transfected group, showing statistical difference ( $P<0.05$ ) (Table 4).

**Discussion**

Gastric carcinoma is a malignant tumor of the digestive tract, ranking only second to lung cancer and hepatic carcinoma in terms of incidence. Its pathogenesis is various and closely

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**Table 2.** Analysis on the correlation between relative expression of miR-204 and pathological parameters in gastric adenocarcinoma specimen ( $\bar{x} \pm sd$ )

Pathological parameters	Feature	Number of cases	Expression level of miR-204	t	P
Gender	Male	58	2.05±0.71	0.915	0.363
	Female	48	1.92±0.75		
Age	<60 years old	40	2.02±0.61	1.548	0.125
	≥.1 years old	66	1.82±0.72		
Degree of differentiation	Moderate and good differentiation	50	2.19±0.71	5.291	0.024
	Poor differentiation	56	1.71±0.62		
Lymph node metastasis	Yes	62	1.72±0.75	3.14	0.002
	No	44	2.18±0.78		
TNM staging	Stage I-II	72	2.22±0.85	3.954	0.000
	Stage III-IV	34	1.72±0.23		

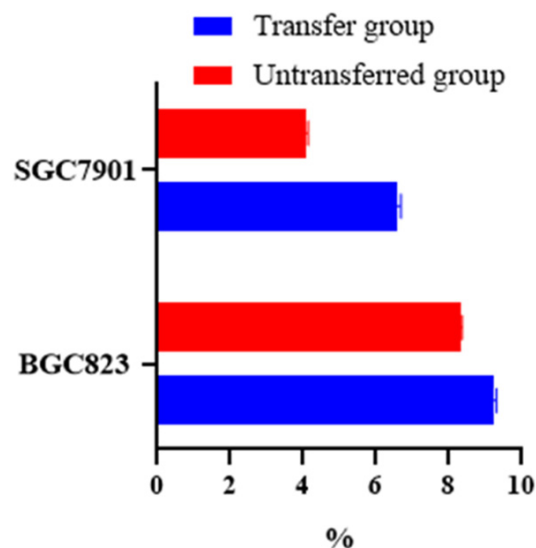
**Table 3.** Analysis on the apoptosis of human gastric carcinoma cell lines in untransfected group and transfected group ( $\bar{x} \pm sd$ , %)

Group	BGC823	SGC7901
Transfected group	9.15±0.25	6.52±0.32
Untransfected group	8.29±0.22	4.05±0.25
t	26.588	62.624
P	0.000	0.000

related to gene, heredity, precancerous lesions, helicobacter pylori infection, etc. [7, 8]. In order to better prevent and treat gastric carcinoma, it is very important to strengthen the research on the mechanisms of its occurrence and development. At present, there is no unified conclusion about the specific pathogenesis of gastric carcinoma.

miRNA is small non-coding RNA, with the length between 21 and 25 nucleotide. It can achieve the specific recognition on 3-UTR (3'-untranslated region) of mRNA, promote the translation inhibition and degradation of targeted mRNA and thus realize the useful effect of negative regulation of gene expression [9, 10]. Furthermore, miRNA also plays a crucial role in processes of growth, development, proliferation, differentiation, metabolism and apoptosis [11].

In recent years, many clinical studies have shown that miRNA plays an important role in the occurrence and development of tumors, which mainly acts as cancer suppressor gene or cancer gene. Most scholars have paid much attention to targeted miRNA therapy [12, 13]. Chandrasekaran [14] and other scholars found



**Figure 4.** Analysis on the apoptosis of human gastric carcinoma cell lines in untransfected group and transfected group. The apoptosis rates of BGC823 and SGC7901 in untransfected group were lower than those in transfected group ( $P < 0.05$ ).

that miR-182 had high expression in patients with ovarian cancer, cervical cancer and prostatic cancer. miR-182 acted as a cancer gene to promote the metastasis, infiltration, proliferation and formation of drug resistance of tumor cells. The expression level of miR-130α reduced obviously in tumor tissues of prostatic cancer, leukemia and glioma, etc., and it acted as a cancer suppressor gene to inhibit the metastasis and proliferation of tumors [15, 16]. Some studies have found that miR-204 had obviously different effects in different tumors [17]. miR-204 had high expression in tumor tissues of



**Table 4.** Analysis on the absorbance of human gastric carcinoma cell lines in untransfected group and transfected group ( $\bar{x} \pm sd$ , OD490)

Group	BGC823		SGC7901	
	1 d	3 d	1 d	3 d
Transfected group	1.91±0.12	2.32±0.02	2.61±0.02	2.91±0.02
Untransfected group	1.93±0.13	2.49±0.09	2.62±0.11	3.18±0.06
t	1.579	18.984	0.921	43.953
P	0.116	0.000	0.358	0.000

renal carcinoma, acute lymphoblastic leukemia and prostatic cancer, etc., which acted as a cancer gene to promote the metastasis and proliferation of tumors [18, 19]. However, the expression level of miR-204 reduced obviously in colorectal cancer, endometrial cancer and nasopharyngeal carcinoma, which acted as cancer suppressor gene to inhibit the growth and formation of tumors [20, 21].

In order to investigate the correlation between miR-204 and the occurrence and development of gastric adenocarcinoma, the expression level of miR-204 in gastric adenocarcinoma tissues was assayed and compared with that in normal gastric tissues in this study. The relative expression quantity of miR-204 in gastric adenocarcinoma specimen was lower than that in normal gastric specimen, and the relative expressions of miR-204 in gastric carcinoma cell line SGC7901 and BGC823 were lower than that in immortalized gastric epithelial cell line ( $P<0.05$ ), which implied that the expression level of miR-204 is reduced obviously in both gastric carcinoma tissues and gastric carcinoma cell lines. By analyzing the correlation between miR-204 expression and pathological parameters such as TNM staging, degree of differentiation, situation of lymph node metastasis, gender, age, etc. in gastric adenocarcinoma patients, it was found that the expression level of miR-204 in patients with moderate and good differentiation was higher than that in patients with poor differentiation; the expression level of miR-204 in patients with lymph node metastasis was lower than that in patients without lymph node metastasis; and the expression level of miR-204 in patients at stages I-II was higher than that in patients at stages III-IV according to TNM staging ( $P<0.05$ ). It was further speculated that miR-204 primarily acted as a cancer suppressor gene in gastric adenocarcinoma. In order to verify the above hypoth-

esis, the cell transfection experiment *in vitro* was performed in this study. The results indicated that the apoptosis rates of BGC823 and SGC7901 in untransfected group were lower than those in transfected group after the gastric carcinoma cell line BGC823 and SGC7901 were transfected with miR-204, and the absorbance of BGC823 and SGC7901 of untransfected

group was higher than that of transfected group on the third day ( $P<0.05$ ), which implied that the proliferation ability of gastric carcinoma cell lines reduced obviously after they were transfected with miR-204, so they could promote cell apoptosis effectively. The combined action of various pathways promoted miR-204 to regulate cell proliferation and apoptosis [22]. Kang [23] and other scholars believed that miR-204 could promote the down-regulation of SOX4 gene. And SOX4 gene mainly had high expression in various malignant tumors and thus could regulate the proliferation, migration and apoptosis of tumor cells. So this may be an important pathway for miR-204 to affect the development of tumors [23]. Yan [24] and other scholars found that miR-204 expression was down-regulated obviously in hepatic carcinoma group and was negatively correlated with histone deacetylase 1 protein expression and Bcl-2 protein expression. It was speculated that miR-204 may promote the apoptosis of hepatic carcinoma cells and inhibit the proliferation by inhibiting histone deacetylase 1 protein expression and Bcl-2 protein expression.

In conclusion, during the occurrence and development of gastric adenocarcinoma, miR-204 may play an important role as a cancer suppressor gene and can be regarded as a new molecular marker to provide vital foundation for prognosis and treatment of gastric adenocarcinoma patients. Besides, the targeted miR-204 therapy is very likely to become a potential new treatment method for gastric adenocarcinoma.

However, the study has some limitations. The results of this study were not representative enough due to fewer objects of study; therefore, much attention shall be paid to this aspect in future studies. Meanwhile, it is necessary to further research the type of signaling pathway or molecular mechanism through which miR-

204 affects the metastasis and proliferation of gastric adenocarcinoma cells.

### Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

### Disclosure of conflict of interest

None.

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