

Study of miR-143 expression in stomach cancer

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Abstract. The present study was planned to investigate miR-143 expression during stomach cancer. The study explored the relationship between miR-143 expression and clinicopathological characteristics including proliferation, migration and apoptosis of stomach cancer cells. Sixty-three samples from each of stomach cancer tissue and surrounding tissue were obtained. Total RNA was extracted. The expression levels of miR-143 from stomach cancer tissue as well as from surrounding tissue were measured by semi-quantitative PCR. The effects of miR-143 overexpression on the migration of stomach cancer cells were examined by Transwell assay. The effects of miR-143 overexpression on the apoptosis of stomach cancer cells were examined by flow cytometer. The expression level of miR-143 was significantly decreased in stomach cancer tissues in comparison to surrounding tissues ($P<0.01$). Moreover, the expression of miR-143 related well with the tumor size, TNM stage, lymphatic metastasis and relapse ($P<0.01$). On the other hand, stomach cancer cell line with overexpression of miR-143, showed significant decline in proliferation rate and migration rate comparison to control cells ($P<0.01$). However, it showed significant higher in apoptosis rate ($P<0.01$). The present study concluded that expression of miR-143 is low during stomach cancer. Further, higher expression levels of miR-143 have the ability to decline proliferation and migration of stomach cancer cells. In this manner, the expression level of miR-143 could be used as an important factor to determine the severity of stomach cancer.

Introduction

The pathogenesis of stomach cancer is related to several factors including bad dietary habits (1,2). The advances in medical technology have resulted in significant rise in the rate of detection and treatment of stomach cancer. However, despite so many advances the cure rate is still poor especially in the cases of elderly patients (3,4). The growing knowledge and interest in the field of molecular biology has resulted in the study of microRNA (miRNA) as the spotlight of medicine and life science. A large number of evidence demonstrated that miRNA is closely associated with the pathogenesis and development of multiple cancers (5,6). Zhang *et al* found that low expression of miR-143 significantly increased the incidence of pancreatic cancer. Thus, the drugs that blocked the expression of miR-143 could significantly decrease the survival of patients (7). Another study revealed that the expression level of miR-143 could affect the pathogenesis and development of breast cancer (8). However, study focusing on the relationship of miR-143 expression and stomach cancer has not been reported. Therefore, in the present study we measured the expression of miR-143 in stomach cancer tissues and explored the relationship between miR-143 and stomach cancer. We describe the relationship between the pathogenesis and development of stomach cancer and the expression level of miR-143. The study provided new aspect for the better management and diagnosis of stomach cancer.

Materials and methods

This study included the stomach cancer patients admitted to the Department of Oncology, Xiangyang Central Hospital (Xiangyang, China) from February 2010 to February 2014. All patients underwent excision of the lesion of stomach cancer. The collected samples were then subjected to pathological examination for stomach cancer confirmation. A total of 63 patients (52-79 years) were confirmed with stomach cancer, in which, 35 were males and 28 were females. Other patients with chronic wasting diseases were excluded. The surrounding tissue was 3 cm away from the stomach cancer lesion. Complete clinical and pathological information was preserved for these patients. Sixty-three healthy individuals confirmed with physical examination were included as study controls. All patients had one-year follow-up records and adequate treatment options. The stomach cancer tissues from all patients were stored in liquid nitrogen. The study was approved by the

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Ethics Committee of Xiangyang Central Hospital (Xiangyang, China) and written informed consents were signed by the patients and/or guardians.

MGC-803 cell line was procured from Kunming Cell Bank, Chinese Academy of Sciences. All chemicals required for the experimentation were procured either from Sigma-Aldrich (St. Louis, MO, USA) or Millipore (Billerica, MA, USA). Trizol kit and reverse transcription polymerase chain reaction kit were obtained from Invitrogen; Thermo Fisher Scientific, Inc., (Waltham, MA, USA). Inverted fluorescence microscope and flow cytometer were from Thermo Fisher Scientific, Inc. PCR amplifier and UV imaging system were procured from Biometra GmbH, Göttingen, Germany.

The expression of miR-143 in the stomach cancer tissues and surrounding tissues. Total RNA was extracted from the tissues with a TRIzol kit. The integrity of the extracted RNA was confirmed by agarose gel electrophoresis. cDNA was obtained with a reverse transcription polymerase chain reaction kit. The expression level of miR-143 in tissues was measured by semi-quantitative PCR with GAPDH as the internal reference. The primers synthesized by Tiangen Biotechnology Co., Ltd. (Beijing, China) are shown in Table I. The PCR products were separated by agarose gel electrophoresis followed by examination under UV imaging system.

The construction of a cell line with overexpressing miR-143. The growing cells (6×10^4) were cultured in a 6-well plate and incubated with both Lipofectamine™ 2000 and miR-143d complex. After 48 h, the RNA was extracted and reverse transcribed into cDNA. The expression level of miR-143 was measured by PCR. Successful transfection enabled high expression level of miR-143. The cells of successful transfection were selected and assigned into overexpression group and control group. The cells of successful transfection were cultured in the incubator with 5% CO₂ at 37°C for further use.

The effects of overexpression of miR-143 on stomach cancer cells

Effects on the proliferation of stomach cancer cells. The effects of overexpression of miR-143 on the growth of stomach cancer cells were determined by MTT assay: The cells (3×10^4 /ml) were cultured in a 96-well plate for 48 h and then incubated with MTT 5 mg/ml for 4 h. The optical density read at 570 nm by a microplate reader (9).

Effects on the migration of stomach cancer cells. The effects of overexpression of miR-143 on the migration of stomach cancer cells were determined by Transwell assay: The starved cells (5×10^5 /ml) were added into a Transwell chamber. After staining and fixation, the cells that crossed the chamber were counted under a microscope (Leica, Solms, Germany) (10).

Effects on apoptosis of stomach cancer cells. The effects of overexpression of miR-143 on apoptosis of stomach cancer cells were determined by flow cytometer: The cells (5×10^5 /ml) were prepared into single cell suspension and cultured in a cell culture plate for 24 h. The cell suspension was spun and the supernatant was discarded. Then the cells were incubated with

Table I. PCR primers.

Primer sequences	
miR-143	F: 5'-GTGCGTGGAGAGTGTGAAGCACTG-3' R: 5'-GTGTGGACGTGTCGGCAACTC-3'
GAPDH	F: 5'-CAGGGCTGCTTTTAACTCTGGTAA-3' R: 5'-GGGTGGAATAATCTTGAACATGT-3'

F, forward; R, reverse.

Table II. The relationship between the expression level of miR-143 and clinicopathological features.

Items	No.	miR-143	P-value
Age (years)			0.0697
≤60	27	2.73±1.33	
>60	36	2.69±1.45	
Tumor size			0.0326
≤5 cm	39	6.98±1.37	
>5 cm	24	5.28±1.21	
TNM stage			0.0075
I-II	43	6.29±1.38	
III-IV	20	3.21±2.06	
Lymphatic metastasis			0.0082
Yes	15	2.06±1.52	
No	48	5.88±1.86	
Relapse			0.029
Yes	28	3.76±1.87	
No	35	4.28±1.59	

Annexin V: 1 ml at room temperature for 10 min and stained with PI in the dark at 4°C for 30 min. The cells were measured by a flow cytometer.

Statistical analysis. The data were represented by mean ± SD. Statistical analysis was performed by SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Measurement data were analyzed by t-test and analyzed by Chi-square test. Further, survival analysis was performed by Kaplan-Meier with log-rank test. $P \leq 0.05$ was considered to indicate a statistically significant difference.

Results

The expression of miR-143 in stomach cancer patients. Sixty-three samples from each of stomach cancer tissue and surrounding tissue were obtained. The expression levels of miR-143 (Fig. 1) were significantly lower in stomach cancer tissue than surrounding tissue ($P < 0.01$).

miR-143 expression and clinicopathological features. The relative expression levels of miR-143 in stomach cancer

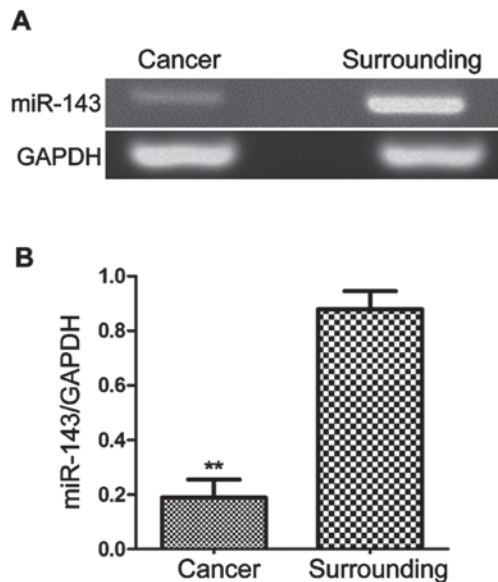


Figure 1. The expression levels of miR-143 in stomach cancer tissue and surrounding tissue. (A) Agarose gel electrophoresis; (B) the relative expression levels of miR-143. The relative expression levels of miR-143 were significantly lower in stomach cancer tissue than surrounding tissue (** $P<0.01$).

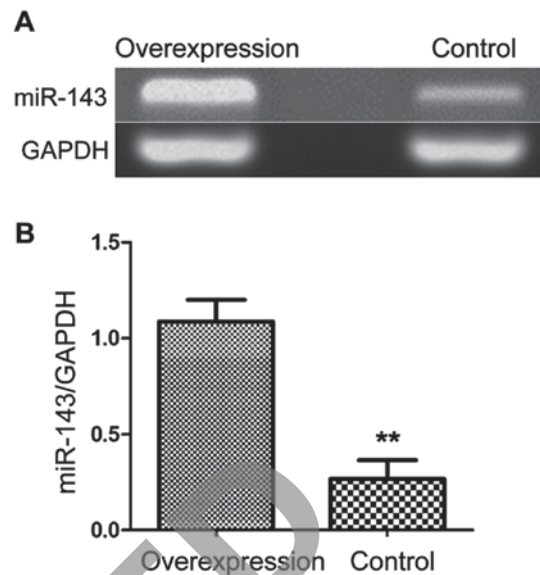


Figure 3. (A) Agarose gel electrophoresis showed the expression of over-expressed miR-143 in MGC-803 cells. (B) The histogram showed that the expression level of miR-143 in MGC-803 cells was significantly higher than control cells (** $P<0.01$).

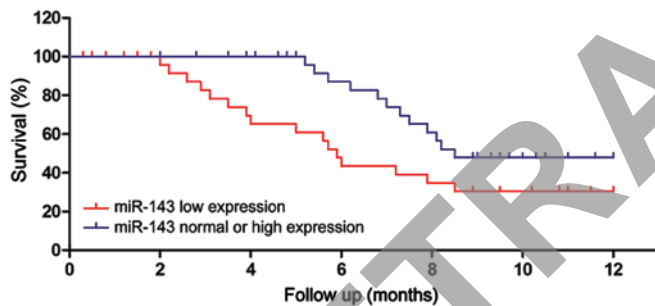


Figure 2. The relationship between the expression level of miR-143 and the survival of patients.

tissue and surrounding tissue were analyzed with respect to age, tumor size and clinical stages of the stomach cancer patients. As shown in Table II, the relative expression levels of miR-143 in stomach cancer tissue were not related to the age of patients ($P=0.0697$), yet related to the tumor size, TNM stage, lymphatic metastasis and relapse ($P<0.05$).

The patients were assigned into high expression group, normal expression group and low expression group according to the relative expression levels of miR-143. Statistical analysis of the survival showed that the survival of the patients in low expression group was significantly shorter than normal expression group or high expression group ($P<0.01$). The survival curves are shown in Fig. 2. The survival of the patients in low expression group was significantly shorter than normal expression group or high expression group ($P<0.01$).

The construction of MGC-803 cell line with overexpression of miR-143. Stomach cancer cell MGC-803 were transfected with Lipofectamine 2000 and miR-143d complex to upregulate the expression of miR-143. The RNA was extracted and reverse

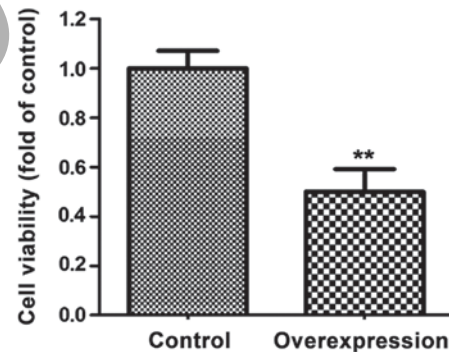


Figure 4. Cell proliferation measured by MTT assay. The effects of over-expression of miR-143 on cell proliferation. The cellular number was significantly lower in the cells with overexpression of miR-143 than control cells (** $P<0.01$).

transcribed into cDNA for measurement of the expression level. As shown in Fig. 3, the expression level of miR-143 was significantly higher in MGC-803 cells than control cells ($P<0.01$). This indicated successful construction of MGC-803 cell line with overexpression of miR-143.

The effects of miR-143 overexpression on cell proliferation. The number of MGC-803 cells were significantly lower in MGC-803 cells than control cells ($P<0.01$) (Fig. 4).

The effects of miR-143 overexpression on cell migration. The migration of the cells with overexpression of miR-143 was significantly less than control cells ($P<0.01$) (Fig. 5).

The effects of overexpression of miR-143 on cell apoptosis. When miR-143 was overexpressed, the apoptosis rate of MGC-803 cells was 29.8% and was significantly higher than control cells ($P<0.01$) (Fig. 6).

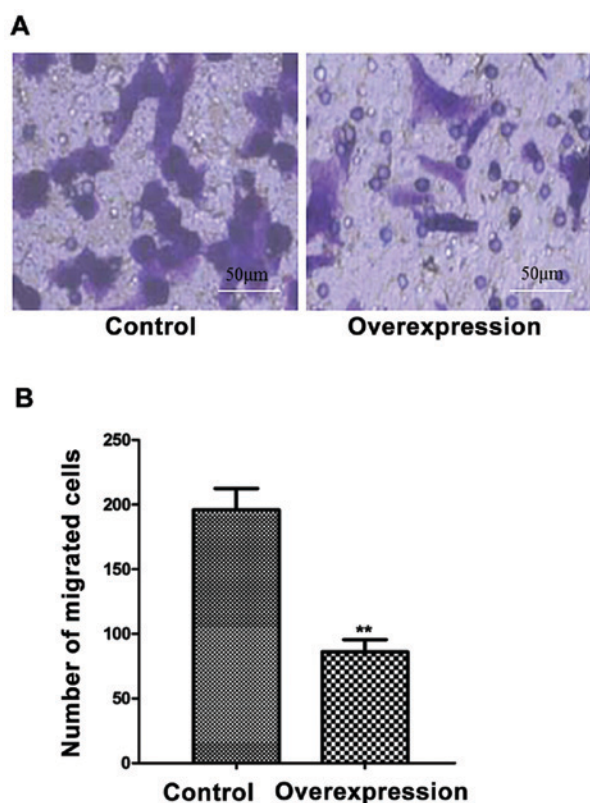


Figure 5. Cell migration capacity measured by Transwell assay. (A) The cells under microscopy (bar, 50 μm). (B) Histogram. The migration capacity of the cells with overexpression of miR-143 was significantly decreased compared to control cells (** $P < 0.01$).

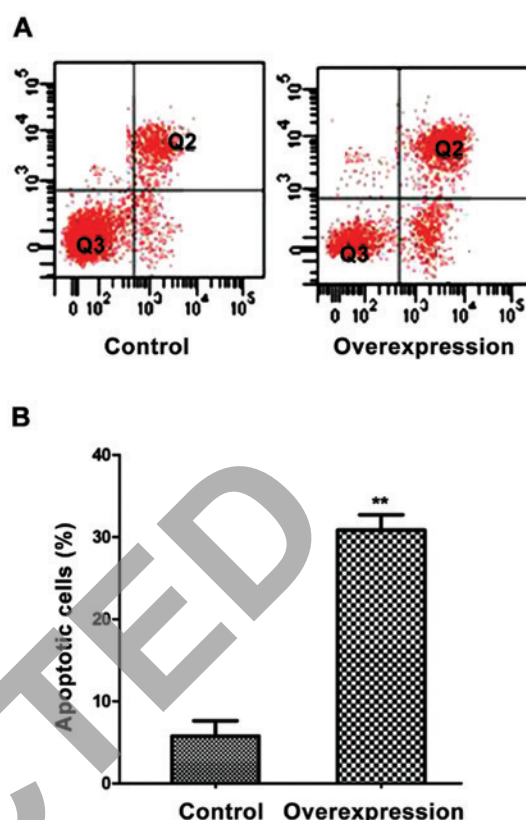


Figure 6. Cell apoptosis measured by Flow cytometry. (A) The histogram of cell apoptosis generated by Flow cytometry software. (B) Apoptosis in control cells and cells with overexpression of miR-143. The apoptosis rate in the cells with overexpression of miR-143 was significantly higher than control cells (** $P < 0.01$).

Discussion

The miRNAs regulate genetic transcription and the expression levels of proteins after transcription (9). Moreover, miRNA could affect the transcription and translation in tumor cells, leading to abnormal metabolism (10,11). miRNA higher expression result in abnormal proliferation and migration of tumor cells (12). Extensive studies on miRNAs revealed that the expression levels of miR-143 in many tumor cells were significantly lower than normal tissues (13,14).

The present study aimed to explore miR-143 expression during stomach cancer. The expression level of miR-143 in surrounding tissue was significantly higher than stomach cancer tissue. This observation was consistent in both the pancreatic cancer and colon cancer (15,16). In the present study, the analysis of miR-143 expression showed it was not related to the age of patients, yet related to clinical stage, metastasis and relapse. This indicated that the expression level of miR-143 was closely related to the pathogenesis and development of stomach cancer. The regulation of miR-143 expression could be a potential strategy in the treatment of stomach cancer. This study determined the changes in cell proliferation, migration and apoptosis with overexpression of miR-143 by MTT assay, Transwell assay and flow cytometry. When miR-143 was overexpressed, the proliferation as well as migration of MGC-803 cells was significantly decreased. On the other hand, the apoptosis rate was significantly increased. This confirmed that miR-143 might regulate the development

of stomach cancer through modulation of the proliferation and migration of stomach cancer cells. Moreover, it could be suggested that miR-143 induces cell apoptosis to promote the death of stomach cancer cells.

The patient clinicopathological data combined with the expression level of miR-143 indicated that the small tumors that hardly migrated showed good prognosis and few relapse when miR-143 was overexpressed. The results are in agreement with the results of the cellular assay. Both clinical data and cell assay confirmed that miR-143 is closely related to the proliferation and migration of stomach cancer cells. Yan *et al* also confirmed that in tumor cells with high miR-143 expression, and the capacity of distant metastasis was significantly lower than that of low or normal expression (17). Similarly, another study reported that miR-143 could affect the proliferation and migration of tumor cells in animal sarcoma model (18). On the other hand, when miR-143 was knocked out, the proliferation and migration of tumor cells was significantly increased. Further, Wu *et al* reported that miR-143 was closely related to the pathogenesis of stomach cancer and the possibility of stomach cancer was significantly increased in the patients with low expression of miR-143 (19).

The present study concludes that miR-143 expression is low in stomach cancer tissues. Thus, miR-143 could be used as the diagnostic gene of stomach cancer and novel therapy of stomach cancer could be developed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SJH and ZL wrote the manuscript and performed PCR. YFZ constructed cell line. QFL contributed to MTT assay. ZYL helped with transwell assay. WRF detected apoptosis of cell. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xiangyang Central Hospital (Xiangyang, China) and informed consents were signed by the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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