

Original Article

miR-29c expression predicts poor prognosis in patients with gastric cancer and its role in ROCK-I expression and cell apoptosis

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Abstract: Objective: To explore the expression of miR-29c in gastric cancer cells and the effect on the RhoA/ROCK pathway. Method: MiR-29c, RhoA and ROCK-1 protein expression in cancer tissues and para-carcinoma tissues from 114 gastric cancer patients were detected and their relationships with prognosis were explored. MiR-29c-mimic/miR blank vector (miR-control) was successfully transferred to human gastric cancer cell line SGC-7901 and their effects on ROCK-1 cell migration and apoptosis were detected. Untreated SGC-7901 cells were set as a blank control group. Pearson analysis was used to analyze the correlation between miR-29c and ROCK-I protein expression. Results: MiR-29c was down-regulated and RhoA and ROCK-1 proteins were upregulated in gastric cancer tissues over those in para-carcinoma tissues ($P < 0.001$). The low expression of miR-29c and the high expression of ROCK-1 protein was related to a low 5-year survival (both $P < 0.05$). The relative expression level of miR-29c in the miR-29c-mimic was higher than miR-control and the control group ($P < 0.05$). The relative expression levels of RhoA protein and ROCK-I protein, as well as cell penetrating numbers in the miR-29c-mimic were significantly lower than those in the miR-control and blank control groups ($P < 0.05$). The apoptosis rate of miR-29c-mimic were significantly higher than those in the miR-control and blank control groups ($P < 0.05$). Pearson correlation analysis results showed that miR-29c was negatively correlated with the expression level of ROCK-I protein ($r = -0.500$, $P = 0.025$). Conclusion: MiR-29c plays a similar role to an anti-oncogene and might be involved in the regulation of migration and apoptosis of gastric cancer cells through RhoA/ROCK signaling.

Keywords: miR-29c, gastric cancer, RhoA/ROCK, apoptosis

Introduction

Gastric cancer accounts for 6.8% of all new cancer cases around the world, and the prognosis is not optimistic. Only 5% of patients with gastric cancer survive for more than five years after diagnosis [1, 2]. Although many tumor-associated molecules have been found in the development and mechanisms of gastric cancer, the pathogenesis is still not fully explored. It is generally believed that the gradual change of multiple molecules causes gastric cancer [3, 4]. Therefore, it is meaningful to find molecular markers that can be used as prognostic factors and effective treatments for gastric cancer patients.

MicroRNAs can regulate gene expression by inhibiting mRNA translation. More evidence sho-

ws that microRNAs play an important role in proliferation, development and apoptosis of cancer cells [5, 6]. MiR-29c may have anti-cancer effects on gastric cancer cells, and its decreased expression may promote the growth of cancer cells by down-regulating the expression of RCC 2 [7]. Other studies have reported that the expression level of miR-29c in gastric cancer tissues was lower than that in para-carcinoma tissues, and it was closely related to neoplasm staging [8]. However, studies on the relationship between prognosis of gastric cancer and miR-29c have rarely been reported. In other reports of digestive tract tumors, miR-29c showed high application value in esophageal cancer screening [9]. MiR-29c is also associated with shorter of overall survival of pancreatic cancer patients. Up regulation of miR-29c can inhibit the migration and invasion of pancreatic

cancer cells [10]. These studies prove that miR-29c plays an important role in the occurrence and development of tumors. However, they did not explore the prognostic value of miR-29c in gastric cancer and its potential anti-cancer mechanisms.

RhoA/ROCK is a gastric cancer related pathway, and inhibition of its expression can promote the apoptosis of gastric cancer cells [11]. Studies have reported that miR-148a can inhibit ROCK 1, so that migration and invasion of gastric cancer cells can be inhibited [12]. The RhoA/ROCK pathway is also regulated by miR-26b and participates in the invasion and migration of lung cancer cells [13]. These studies suggest that the effects of the RhoA/ROCK pathway are regulated by microRNAs. However, there are few reports about the relationship between miR-29c and the RhoA/ROCK pathway.

Therefore, in this study, the expression of miR-29c and the RhoA/ROCK pathway in gastric cancer cells was detected. The effect of miR-29c on the proliferation and migration of gastric cancer cells and its mechanisms were analyzed. The relationship between miR-29c and RhoA/ROCK pathway was explored.

Materials and methods

Study subjects

On hundred fourteen patients with gastric cancer from April 2012 to February 2013 were selected and their cancer tissues and corresponding para-carcinoma tissues were obtained during surgery; the ages of patients were 55-75 years old. Inclusion criteria: All patients were diagnosed with gastric cancer in our hospital; all cases were primary tumors; all cases were stage II-III; there was no distal lymph node metastasis; all patients have complete medical records with 5-year follow-up data. Exclusion criteria: patients with other benign tumors or malignant tumors; patients with prior history of tumor; patients with malignant pleural effusion; patients with serious diseases such as cerebrovascular disease, liver and kidney dysfunctions; patients with severe infections such as sepsis; pregnant women or lactating women, were excluded. This study was approved by the Hospital Ethics Association. Patients and their families were all consulted by telephone or letter, and they all signed informed consent.

Human gastric cancer cell line SGC-7901 cells were purchased from Shanghai Aolu Biological Technology Co., Ltd., [item number XB-2497]. The culture medium was DMEM containing 10% of fetal bovine serum (fetal bovine serum was purchased from Beifang Zhijiefangyuan Technology Co., Ltd., [item number 10099141]; DMEM medium was purchased from Xiamen Yanke Biological Technology Co., Ltd., [item number 10567014]). The culture conditions were 37°C, 5% CO₂, and the humidity was 90%.

Cell passage

After the resuscitation of SGC-7901 cells, the cells were digested with 0.25% trypsin when the cell adherence density reached 90%. The cells were observed under a microscope. When cells achieved around-shape, and the intercellular space was enlarged, and some cells detached from the bottle wall, then the cells were transplanted into DMEM medium and cultured in an incubator. The condition of incubator was 37°C and 5% CO₂. Cells were cultivated to the third generation and collected as stand-by application.

Construction and transfection of miR-29c expression vector

MiR-29c overexpression vector (miR-29c-mimic) and miR blank vector (miR-control) were designed and synthesized by Thermo Fisher Scientific. Untreated SGC-7901 cells were set as the blank control group. Twenty-four hours before transfection, SGC-7901 cells were digested by trypsin. When cells were fused to about 80%, the transfection of expression vector was performed according to the instruction of the transfection kit. The specific operation steps can be referred to in the instructions of the kit. The culture medium was incubated in incubator under 37°C and 5% CO₂ for 48 hours, and it was replaced for every 6 hours. Forty-eight hours later, qRT-PCR was used to detect the transfection results and the levels of cell migration and apoptosis. Lipofectamine TM2000 transfection kits were purchased from Chengdu Dongsheng Kechuang Technology Co., Ltd., [item number 11668019].

qRT-PCR

Fifty mg of cancer tissues or para-carcinoma tissues were homogenized. One ml of TRIzol

Table 1. Primer sequence

	Upstream	Downstream
miR-29c	5'-GATGATGACGACAAAGCTAGCGACATTTGCTCTTGAGTCTG-3'	5'-GCGGCCGCTCAGTTATCTAGAGACCACTTCTCATTGCCATA-3'
U6	5'-CGGAGTCAACGGATTGGTGTGAT-3'	5'-AGCCTTCTCCATGGTGGTGAAGAC-3'

lysate was added to the tissues to extract the total RNA. The concentration of SGC-7901 cell suspension was adjusted to 1×10^7 /ml. Suspension and TRIzol lysate were added with the ratio of 3:1, to extract total RNA. After extraction, RNA integrity was analyzed by 1.5% agarose gel electrophoresis. The purity of the extracted RNA was detected by trace nucleic acid analyzer. If A260/A280 value was 1.8 to 2.1, it met the experimental requirements. After RNA extraction, reverse transcription reaction were performed. PCR amplification was carried out after the first strand of cDNA was synthesized. The PCR amplification system was as follows: 2 μ L of cDNA template, 1 μ L of cDNA dilution, 5 μ L of 2*SYBR Green mixture, 1 μ L of upstream primers and 1 μ L of downstream primers; double distilled water added to 10 μ L; degradation for 30 s under 95°C; annealing for 30 s under 60°C; extension for 30 s under 75°C; repeated for a total of 40 cycles. After the experiment, the dissolution curve was analyzed, and U6 was used as the internal reference. All samples were repeated in 3 replicates, and $2^{-\Delta\Delta CT}$ was used to analyze the results. TRIzol™ Reagent was purchased from Chengdu Dongsheng Kechuang Technology Co., Ltd., [item number 15596026]. SYBER GREEN real-time fluorescence quantitative PCR kits were purchased from Nanjing Kebai Biotechnology Co., Ltd., [item number 4310251]. The primer sequence was designed and synthesized by HePeng (Shanghai) Biotechnology Co. Co., Ltd. (Table 1).

Tunel experiment

The concentration of SGC-7901 cells after 48-hours of chemotherapy was adjusted to 5×10^7 /ml. 4% of neutral formaldehyde was used to fix for 10 minutes at room temperature. Cells were washed two times with PBS, and the excess liquid was removed, for 5 minutes each time. PBS containing 2% of H₂O₂ was applied for 5 minutes at room temperature. The excess liquid was removed, and the cells were washed with PBS 2 times, 5 minutes each time. Cell staining was performed according to the instructions of Tunel kit. Image analysis software (Image-pro-

Plus5.0) was used to count the number of Tunel positive cells under five 40*10 field of view, and the apoptosis rate was calculated. This was repeated 3 times. Tunel kits were purchased from Xiamen Huijia Biological Technology Co., Ltd., [item number 4815-30-K].

Transwell matrigel invasion assay

Matrix glue was paved in Transwell chambers in advance. SGC-7901 cells were made into 1×10^5 /mL/mL of single arranged cell suspensions, and 100 μ L of cell suspension was inoculated in the Transwell chambers. In the upper chamber, RPMI1640 culture medium + 10% of calf serum + cell suspension were added, and the total volume was 100 μ L; in lower chamber, RPMI1640 culture medium + 20% of calf serum + cell suspension were added, and the total volume was 500 μ L; all suspension was cultured for 48 hours under 37°C and 5% CO₂; after staining, the number of passaged cells was detected; there were a total of 6 chambers, and each chamber was counted for 6 views. The 3 parallel experiments were carried out at the same time. Transwell chambers and related reagents were purchased from Shanghai Yuanzi Biotechnology Co., Ltd.

Western blot

The repeated freeze-thaw method was used to extract proteins from cancer tissues and SGC-7901 cells. The protein was separated by polyacrylamide gel electrophoresis. The initial voltage was 90 V, and then the voltage was increased to 120 V, to move the samples to suitable positions on the separation gel. After the electrophoresis, a transfer membrane was carried out. The condition was 100 V constant pressure for 100 minutes, and then it was sealed for 60 minutes at 37°C. Then the transfer membrane was placed and sealed in 5% of skim milk, and immune reactions were carried out. The membrane was incubated with primary antibodies at 4°C for one night. On the next day, the membrane was washed with PBS 3 times, and 5 minutes each time. Membranes were incubated with second antibody at room

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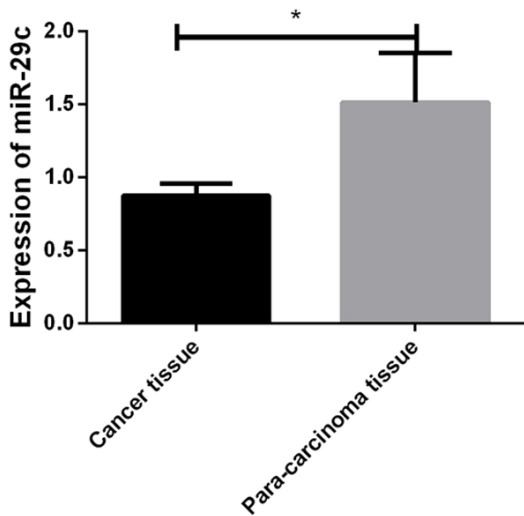


Figure 1. The expression level of miR-29c in gastric cancer patients' tissues, * $P < 0.05$.

temperature for one hour. ECL luminescent reagent was used to develop the film. Quantity One software was used to analyze the strip. β -actin was set as the internal reference protein. The relative expression level of protein = strip gray value/Internal reference grayscale value. Western blot detection kits were purchased from Shanghai Youyu Biotechnology Co., Ltd., [item number was JC-445]. RhoA antibody (batch number: RS-1180R; manufactured by Abcam Company, UK), ROCK-I antibody (batch number: RS-1166R, manufactured by Abcam Company, UK) and sheep anti-mouse IgG second antibody were purchased from Shanghai Xinyu Biotechnology Co., Ltd., [item number was xyKS016].

Statistical analysis

SPSS 19.0 (Asia Analytics Formerly SPSS China) was used to carry out statistical analysis. The variables data were expressed as %, and χ^2 test was used for comparison of rates. The attributes data were expressed as Mean \pm standard deviation (mean \pm sd). Independent t test was used for comparison between two groups, and one-way analysis of variance with post hoc LSD t test was used for comparison among groups and for comparison at different time points in same group.

Pearson analysis was used to analyze the correlation between miR-29c and ROCK protein. The Kaplan-Meier survival curve was used to analyze the relationship of miR-29c and ROCK protein, and the survival time of patients. There

was a statistically significant difference when $P < 0.05$.

Results

Expression level of miR-29c

The relative expression level of miR-29c in gastric cancer tissues and para-carcinoma tissues was (0.873 ± 0.082) and (1.515 ± 0.338) , respectively, with significant a difference ($P < 0.05$). The expression level of miR-141 in gastric cancer tissues was significantly lower than that in the para-carcinoma tissues ($P < 0.001$) (**Figure 1**).

Expression level of RhoA/ROCK

Western blot detection showed that the relative expression levels of RhoA protein and ROCK-I protein in gastric cancer tissues were respectively (1.253 ± 0.103) and (1.275 ± 0.134) . The relative expression levels of RhoA protein and ROCK-I in para-carcinoma tissues were respectively (0.362 ± 0.012) and (0.483 ± 0.014) . The expression level of RhoA protein and ROCK-I protein in gastric cancer tissues was significantly higher than para-carcinoma tissues ($P < 0.001$) (**Table 2** and **Figure 2**).

Relationship of miR-29c and ROCK-I protein and survival time of patients

According to the relative expression level of miR-29c and ROCK-I protein in gastric cancer tissues, the median expression level of miR-29c was 1.537. The patients were divided into high expression group (>1.537) and low expression group (≤ 1.537) according to the median expression value. The median expression level of ROCK-I protein was 1.369. The patients were divided into high expression group (>1.369) and low expression group (≤ 1.369) according to the median expression value. Kaplan-Meier survival analysis showed that the 5-year survival rate of miR-29c high expression group was 85.96% (49 cases), and the 5-year survival rate of miR-29c low expression group was 68.42% (39 cases). The 5-year survival rate of miR-29c low expression group was lower than the miR-29c high expression group ($P = 0.038$). The 5-year survival rate of ROCK-I protein high expression group and low expression group was 64.91% (37 cases) and 82.46% (47 cases), respectively. The 5-year survival rate of ROCK-I protein low expression group was higher than

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Table 2. The expression level of RhoA/ROCK in gastric cancer patients' tissues

	Cancer Tissues	Para-carcinoma Tissues	t	P
RhoA protein	1.253±0.103	0.362±0.012	91.741	<0.001
ROCK-I Protein	1.275±0.134	0.483±0.014	62.745	<0.001

miR-control and blank control group ($P = 0.644$), indicating that transfection was successful (**Figure 4**).

Expression of RhoA/ROCK in gastric cancer SGC-7901 cells after transfection

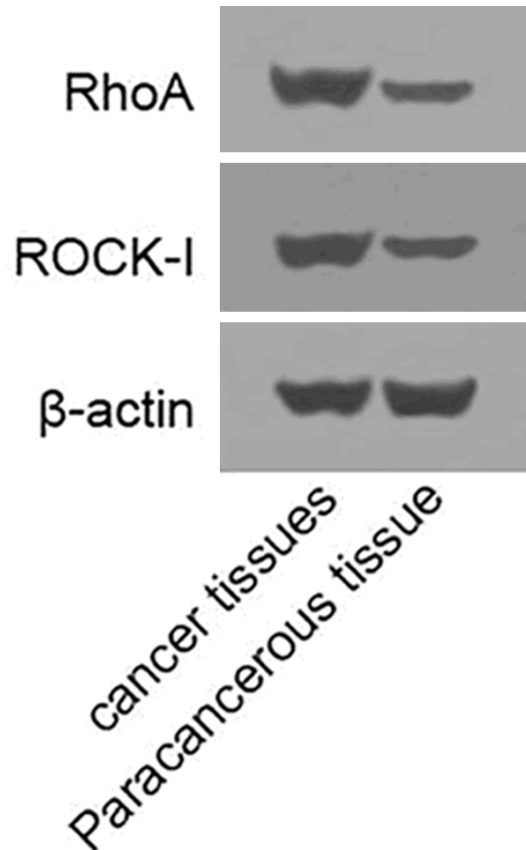


Figure 2. Western blot results of RhoA protein, ROCK-I protein

the ROCK-I protein high expression group ($P = 0.023$) (**Figure 3**).

Analysis of transfection results of gastric cancer SGC-7901 cells

QRT-PCR detection results showed that after transfection, the relative expression level of miR-29c in miR-29c-mimic, miR-control and blank control is (2.015 ± 0.762), (1.015 ± 0.454), and (1.145 ± 0.613), respectively. Significant differences were observed among the three groups ($P = 0.002$). The relative expression level of miR-29c-mimic was higher than miR-control ($P = 0.002$) and the control group ($P = 0.004$), and there was no difference between

Western blot detection results showed that after transfection, the relative expression levels of RhoA protein and ROCK-I protein in miR-29c-mimic, miR-control and blank control was (1.051 ± 0.141) and (1.864 ± 0.158), (1.365 ± 0.141) and (1.295 ± 0.177), and (1.442 ± 0.125) and (1.227 ± 0.138), respectively. There was a significant difference between the results, and the relative expression level of RhoA protein and ROCK-I protein in miR-29c-mimic was significantly lower than the miR-control and blank control ($P < 0.001$), and there was no significant difference between miR-control and blank control (**Table 3** and **Figure 5**).

Apoptosis level of gastric cancer SGC-7901 cells after transfection

The results of TUNEL cell apoptosis assay showed that the apoptosis rate of miR-29c-mimic, miR-control and blank control gastric cancer SGC-7901 cells was ($11.32 \pm 1.07\%$), ($24.14 \pm 1.55\%$) and ($25.75 \pm 1.37\%$), respectively. The apoptosis rate of miR-29c-mimic was lower than miR-control and blank control ($P < 0.001$), and there was no significant difference between miR-control and blank control (**Figure 6**).

Cell migration ability of gastric cancer SGC-7901 cells after transfection

The results of TUNEL cell apoptosis assay showed that the cell penetrating number of miR-29c-mimic, miR-control and blank control gastric cancer SGC-7901 cells was (19.58 ± 6.34), (45.32 ± 9.47) and (47.72 ± 8.65), respectively. The cell penetrating number of miR-29c-mimic was significantly lower than miR-control and blank control ($P < 0.001$), and there was no significant difference between miR-control and blank control (**Figure 7**).

Correlation analysis between miR-29c and ROCK protein

Pearson correlation analysis showed that miR-29c was negatively correlated with the expression level of ROCK-I protein ($r = -0.500$, $P = 0.025$) (**Figure 8**).

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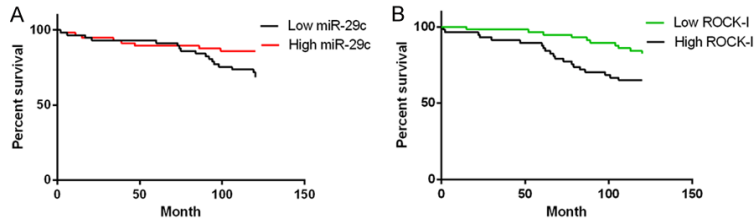


Figure 3. The relationship between miR-29c, ROCK-I protein and survival time of gastric cancer patients. A. The relationship between miR-29c and survival time of gastric cancer patients. B. The relationship between ROCK-I protein and survival time of gastric cancer patients.

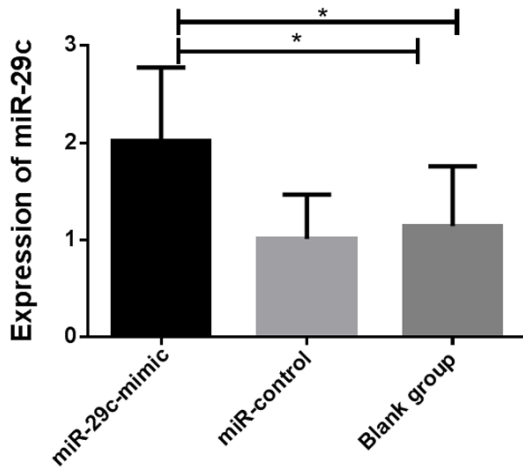


Figure 4. The expression level of miR-29c in SGC-7901 cells after transfection. *P<0.05.

Discussion

Gastric cancer is one of the most common malignant tumors, and the incidence of gastric cancer has been increasing in recent years. With the development of medical technology, there are more and more treatments for gastric cancer, but the therapeutic effect is still unsatisfactory [14, 15]. Molecular targeting therapy is a new treatment method in recent years. At present, the etiology and pathogenesis of gastric cancer have not been fully clear. More and more studies have reported that miR-29c and RhoA/ROCK can regulate the biological behavior of cells in tumors [16, 17]. However, there are few reports on the effect of miR-29c and RhoA/ROCK in the prognosis of tumors, and the relationship between them has not been reported yet. This study analyzed the effects and their relationship, to provide experimental basis for gastric cancer molecular therapy.

In this study, 114 patients with gastric cancer were selected. The expression levels of miR-

29c, RhoA protein and ROCK-I protein in cancer tissues and para-carcinoma tissues were detected. The results of qRT-PCR detection showed that the expression level of miR-29c in gastric cancer tissues was significantly lower than para-carcinoma tissues, and the expression levels of RhoA protein and ROCK-I protein were higher than para-carcinoma tissues. The prognosis was lower

for patients with low expression of miR-29c and high expression of ROCK-I protein. A study of gastric cancer and microRNAs microarrays [18] also reported that the expression of miR-29c increased in gastric cancer tissues, and the expression of miR-29c was related to the degree of differentiation of gastric cancer. The expression level of miR-29c in highly differentiated gastric cancer tissues was significantly higher than undifferentiated gastric cancer tissues.

The selective cox-2 inhibitor celecoxib can improve the combination of C/EBP α and promoter, so that miR-29c is activated, and apoptosis is induced. In a report [19] the expression of miR-29c was low in head and neck squamous cell carcinoma tissues, and it was related to the relapse-free survival time of patients. MiR-29c is also an independent risk factor of poor prognosis for patients with liver cancer [20, 21]. These studies have demonstrated the oncogene inhibitory effect of miR-29c in cancers. There are few reports about ROCK-I protein and prognostic survival time of patients with gastric cancer. Some studies reported that the 5-year survival rate of patients with colorectal cancer was negatively correlated with the expression level of ROCK-I [22]. Some studies [23] reported that expression level of ROCK-I protein was associated with overall prognosis and survival of patients. About 46.6% of patients with poor prognosis showed high expression of ROCK-I. These studies confirmed the impact of miR-29c and ROCK-I proteins on the prognosis of patients with gastric cancer. Although there are few reports on these two factors and prognosis, with deepening of the research, there will be more and more evidence to prove the predictive value of miR-29c and ROCK-I proteins for the prognosis of gastric cancer patients.

We then analyzed the effects of miR-29c on some biological behaviors of gastric cancer

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Table 3. Expression of RhoA/ROCK in gastric cancer SGC-7901 cells after transfection

	miR-29c-mimic	miR-control	Blank control	F	P
RhoA protein	1.051±0.141	1.365±0.141	1.442±0.128	6.971	0.027
ROCK-I Protein	0.864±0.158	1.295±0.177	1.227±0.138	7.987	0.020

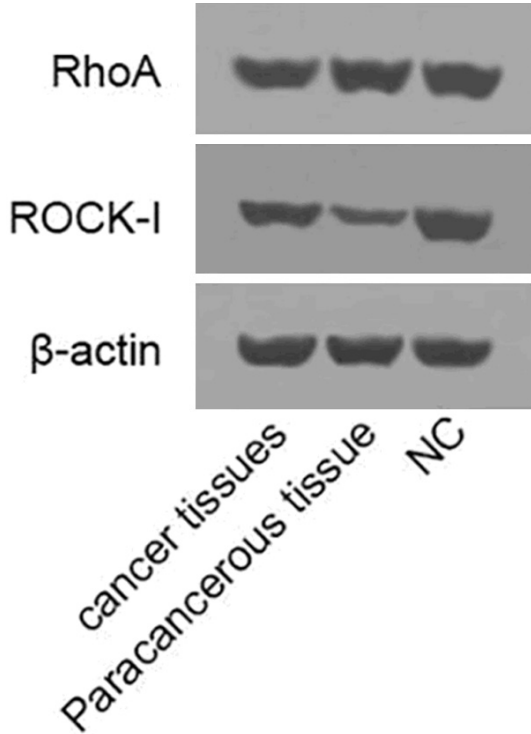


Figure 5. Western blot analysis of RhoA protein, ROCK-I protein results.

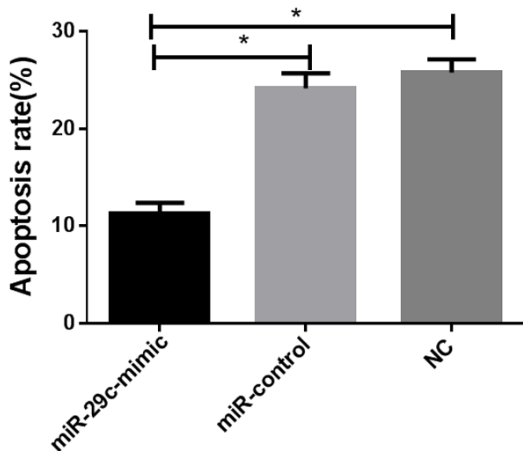


Figure 6. Apoptosis level analysis of SGC-7901 cells after transfection. *P<0.05.

cells. First, we constructed the miR-29c overexpression vector. Then blank vector was taken

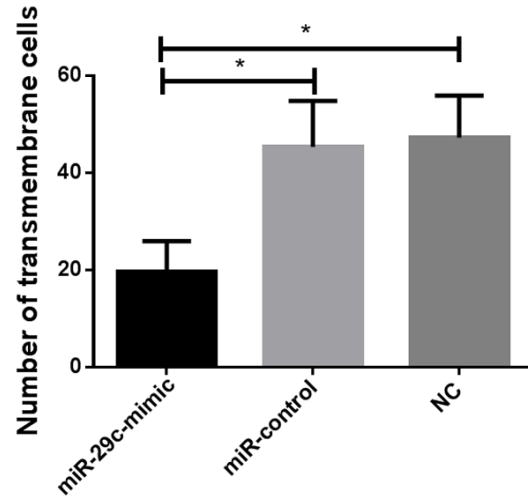


Figure 7. Migration capability analysis of SGC-7901 cells after transfection. *P<0.05.

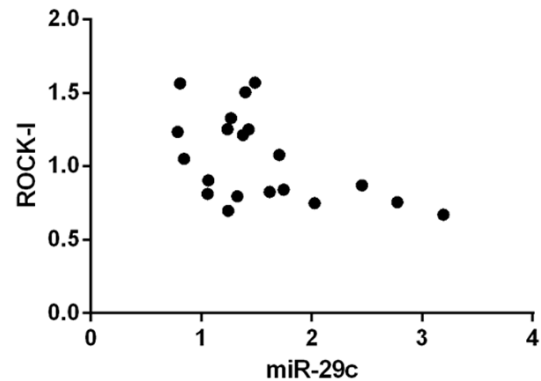


Figure 8. Analysis of the correlation between miR-29c and ROCK protein. MiR-29c was negatively correlated with expression level of ROCK-I protein ($r = -0.500$, $P = 0.025$).

as control, and gastric cancer SGC-7901 cells were transfected. The results of qRT-PCR showed that after transfection, the expression level of miR-29c increased significantly, indicating the transfection was successful. Then the migration ability and apoptotic level of gastric cancer cells in miR-29c-mimic and miR-control were detected. The migration ability of gastric cancer cells with high expression of miR-29c decreased, while the apoptotic rate increased.

It indicated that miR-29c has anti-tumor effects in gastric cancer. We also found that for SGC-7901 cells with high expression level, the expression levels of RhoA protein and ROCK-I protein in SGC-7901 cells decreased. Pearson correlation analysis showed that the expression level of ROCK-I protein was positively correlated with the expression level of miR-29c, indicating that miR-29c had regulatory effects on the RhoA/ROCK signaling pathway. There are few reports about the relationship between miR-29c and the RhoA/ROCK signaling pathway. We will verify this in a series of future studies and provide experimental basis. A study [24] reported that MiR-29c targeting inhibits downstream target gene DNA methyltransferase 3A. It has been additionally [24] reported that overexpression targeted regulation of nuclear autoantigen sperm protein of miR-29c can inhibit cell proliferation, and it also can promote cell apoptosis and blockade of G1/G0 cell cycle. These reports confirmed the validity and reliability of some of our research, while our study also suggested another mechanism of miR-29c in anti-tumor effects.

There are some shortcomings in this study. The research subjects are gastric cancer cell models. Although it can provide the growth conditions of gastric cancer cells, it cannot simulate the complex tumor microenvironment *in vivo*. Therefore, our results need to be verified by clinical experiments. This study only verified the effect of miR-29c on the RhoA/ROCK signaling pathway, but it is unknown whether miR-29c has effects on other signaling pathways. This study did not predict the target gene of miR-29c, and we will supplement our results by experiments in the future.

In conclusion, the expression of miR-29c in gastric cancer plays a role similar to an anti-oncogene. The 5-year survival rate was lower for gastric cancer patients with high expression of RhoA/ROCK and low expression of miR-29c or ROCK-I. MiR-29c may be involved in the regulation of migration and apoptosis of gastric cancer cells through the RhoA/ROCK signaling pathway.

Disclosure of conflict of interest

None.

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References

- [1] Li Z, Lei H, Luo M, Wang Y, Dong L, Ma Y, Liu C, Song W, Wang F, Zhang J, Shen J and Yu J. DNA methylation downregulated mir-10b acts as a tumor suppressor in gastric cancer. *Gastric Cancer* 2015; 18: 43-54.
- [2] Shen L, Li J, Xu J, Pan H, Dai G, Qin S, Wang L, Wang J, Yang Z, Shu Y, Xu R, Chen L, Liu Y, Yu S, Bu L and Piao Y. Bevacizumab plus capecitabine and cisplatin in Chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: randomized, double-blind, phase III study (AVATAR study). *Gastric Cancer* 2015; 18: 168-176.
- [3] van der Post RS, van Dieren J, Grelack A, Hoogerbrugge N, van der Kolk LE, Snaebjornsson P, Lansdorp-Vogelaar I, van Krieken JH, Bisseling TM and Cats A. Outcomes of screening gastroscopy in first-degree relatives of patients fulfilling hereditary diffuse gastric cancer criteria. *Gastrointest Endosc* 2018; 87: 397-404, e2.
- [4] Van Cutsem E, Bang YJ, Feng-Yi F, Xu JM, Lee KW, Jiao SC, Chong JL, Lopez-Sanchez RI, Price T, Gladkov O, Stoss O, Hill J, Ng V, Lehle M, Thomas M, Kiermaier A and Ruschoff J. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. *Gastric Cancer* 2015; 18: 476-484.
- [5] Reddy KB. MicroRNA (miRNA) in cancer. *Cancer Cell Int* 2015; 15: 38.
- [6] Jiang B, Wei Y, Xu J, Yuan R and Xiang Y. Coupling hybridization chain reaction with DNAzyme recycling for enzyme-free and dual amplified sensitive fluorescent detection of methyltransferase activity. *Anal Chim Acta* 2017; 949: 83-88.
- [7] Matsuo M, Nakada C, Tsukamoto Y, Noguchi T, Uchida T, Hijiya N, Matsuura K and Moriyama M. MiR-29c is downregulated in gastric carcinomas and regulates cell proliferation by targeting RCC2. *Mol Cancer* 2013; 12: 15.
- [8] Liu L, Ye JX, Qin YZ, Chen QH and Ge LY. Evaluation of miR-29c, miR-124, miR-135a and miR-148a in predicting lymph node metastasis and tumor stage of gastric cancer. *Int J Clin Exp Med* 2015; 8: 22227-22236.
- [9] Xu H, Yao Y, Meng F, Qian X, Jiang X, Li X, Gao Z and Gao L. Predictive value of serum miR-10b, miR-29c, and miR-205 as promising biomarkers in esophageal squamous cell carcinoma screening. *Medicine (Baltimore)* 2015; 94: e1558.

Role of miR-29c in gastric cancer

- [10] Jiang J, Yu C, Chen M, Zhang H, Tian S and Sun C. Reduction of miR-29c enhances pancreatic cancer cell migration and stem cell-like phenotype. *Oncotarget* 2015; 6: 2767-2778.
- [11] Xu XT, Song QB, Yao Y, Ruan P and Tao ZZ. Inhibition of RhoA/ROCK signaling pathway promotes the apoptosis of gastric cancer cells. *Hepatogastroenterology* 2012; 59: 2523-2526.
- [12] Zheng B, Liang L, Wang C, Huang S, Cao X, Zha R, Liu L, Jia D, Tian Q, Wu J, Ye Y, Wang Q, Long Z, Zhou Y, Du C, He X and Shi Y. MicroRNA-148a suppresses tumor cell invasion and metastasis by downregulating ROCK1 in gastric cancer. *Clin Cancer Res* 2011; 17: 7574-7583.
- [13] Gao Y and Yang F. [MiR-26b regulates invasion and migration of lung cancer cells through targeting hENT1 depending on RhoA/ROCK-1 pathway]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2017; 42: 755-761.
- [14] Shimada H, Noie T, Ohashi M, Oba K and Takahashi Y. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. *Gastric Cancer* 2014; 17: 26-33.
- [15] Kim JW, Nam KH, Ahn SH, Park DJ, Kim HH, Kim SH, Chang H, Lee JO, Kim YJ, Lee HS, Kim JH, Bang SM, Lee JS and Lee KW. Prognostic implications of immunosuppressive protein expression in tumors as well as immune cell infiltration within the tumor microenvironment in gastric cancer. *Gastric Cancer* 2016; 19: 42-52.
- [16] Zhao X, Li J, Huang S, Wan X, Luo H and Wu D. MiRNA-29c regulates cell growth and invasion by targeting CDK6 in bladder cancer. *Am J Transl Res* 2015; 7: 1382-1389.
- [17] Sun K, Duan X, Cai H, Liu X, Yang Y, Li M, Zhang X and Wang J. Curcumin inhibits LPA-induced invasion by attenuating RhoA/ROCK/MMPs pathway in MCF7 breast cancer cells. *Clin Exp Med* 2016; 16: 37-47.
- [18] Saito Y, Suzuki H, Imaeda H, Matsuzaki J, Hirata K, Tsugawa H, Hibino S, Kanai Y, Saito H and Hibi T. The tumor suppressor microRNA-29c is downregulated and restored by celecoxib in human gastric cancer cells. *Int J Cancer* 2013; 132: 1751-1760.
- [19] Hudcova K, Raudenska M, Gumulec J, Binkova H, Horakova Z, Kostrica R, Babula P, Adam V and Masarik M. Expression profiles of miR-29c, miR-200b and miR-375 in tumour and tumour-adjacent tissues of head and neck cancers. *Tumour Biol* 2016; 37: 12627-12633.
- [20] Dong CW, Wang YX, Du FT, Ding W and Hu SY. Low miR-29c expression is a prognostic marker in hepatocellular carcinoma. *Genet Mol Res* 2016; 15.
- [21] Li J, Bharadwaj SS, Guzman G, Vishnubhotla R and Glover SC. ROCK 1 has more accurate prognostic value than MET in predicting patient survival in colorectal cancer. *Anticancer Res* 2015; 35: 3267-3273.
- [22] Byrd TT, Fousek K, Pignata A, Szot C, Samaha H, Seaman S, Dobrolecki L, Salsman VS, Oo HZ, Bielamowicz K, Landi D, Rainusso N, Hicks J, Powell S, Baker ML, Wels WS, Koch J, Sorensen PH, Deneen B, Ellis MJ, Lewis MT, Hegde M, Fletcher BS, St Croix B and Ahmed N. TEM8/ANTXR1-Specific CAR T cells as a targeted therapy for triple-negative breast cancer. *Cancer Res* 2018; 78: 489-500.
- [23] Cui H, Wang L, Gong P, Zhao C, Zhang S, Zhang K, Zhou R, Zhao Z and Fan H. Deregulation between miR-29b/c and DNMT3A is associated with epigenetic silencing of the CDH1 gene, affecting cell migration and invasion in gastric cancer. *PLoS One* 2015; 10: e0123926.
- [24] Yu B, Chen X, Li J, Gu Q, Zhu Z, Li C, Su L and Liu B. microRNA-29c inhibits cell proliferation by targeting NASP in human gastric cancer. *BMC Cancer* 2017; 17: 109.