RESEARCH ARTICLE

Effect of miR27a on Proliferation and Invasion in Colonic Cancer Cells

Yang Gao*, Bao-Dong Li, Yong-Gang Liu

Abstract

The aim of this study was to detect the expression of miR196a, miR146a, miR27a and miR200a in patients with colon cancer, and investigate the effect of miR27a expression on proliferation and invasion in colonic cancer cells. RT-PCR was employed to detect the expression levels in colon cancers. Then, colon cancer cells were cultured and transfected with 100 nM of miR27a mimics (80 nmol/L) or 80 nM miR27a inhibitors (80 nmol/L) in 24-well plates. Proliferation and invasion of colonic cancer cells were then determined by CCK-8 and Transwell assays, respectively. Our data showed miR27a to be high-expressed in patients with colon cancer. In addition, proliferation and invasion in the miR27a mimic group were significantly higher than in the control group and negative group ($P<0.05$), while, proliferation and invasion in the miR27a inhibitor group were obviously lowered ($P<0.05$). In conclusion, high expression of miR27a may play an important role in enhancing proliferation and invasion of colon cancer cells.

Keywords: Colon cancer cells - miR27a - proliferation - invasion

Introduction

MicroRNAs (miRNAs) are a class of small non-coding single-stranded RNA molecules that participate in control of gene expression by repression protein translation or by destabilizing target mRNA via cleavage or deadenylation (Gargalionis et al., 2013; Samimi et al., 2013). Up to now, over 700 miRNAs have been identified (Li et al., 2009). Many miRNAs such as let-7, miR15a, miR 21, miR 34a and miR 155 have been found to be associated with different kinds of cancer including lung, pancreas and colon cancers (Li et al., 2011; Okayama et al., 2012; Schultz et al., 2012; Rothschild, 2013). Any changes in the expression level of specific miRNAs are believed to be involved in cancer progression and can be prognostically indicative for human cancers (Bullock et al., 2013; Kahlert et al., 2013).

Colon cancer is one of the most common causes of cancer death in China (Yan et al., 2012). As the present report, expression of miR143 and miR145 have been found in colon cancer cells (Schepeler et al., 2008; Zhang et al., 2010). An increasing number of in vitro studies have demonstrated an important role for some miRNAs such as miR196a, miR146a, miR27a and miR200a in regulating tumor growth, metastasis and chemotherapy resistance. However, little is known about the relationship between the expression of these miRNAs in human colon cancer (Kong et al., 2011). Therefore, the aim of this study was to detect the expression of miR196a, miR146a, miR27a and miR200a in patients with colon cancer, and further investigate the role of miR27a expression on colon cancer cells proliferation and invasion.

Materials and Methods

Patients

After having obtained informed consent, colon tissue samples were collected from 33 healthy volunteers, 26 patients with colitis and 42 patients with colon cancer. The use of tissues for this study that we collected from clinic were divided into three groups: Normal group, Colitis group and Colon cancer group. Each group in vitro experiments was equal or larger than 6, and each experiment was repeated for 3 times. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Henan Cancer Hospital. Written informed consent was obtained from all participants.

RT-PCR

Total RNA was extracted with miRNeasy Mini Kit (Qiagen, Hilden, Germany), and cDNA was prepared with ReverAidTM First Strand Cdna Synthesis Kit (Thermo Scientific, Rockford, IL, USA), and then RT-PCR was performed using the FastvStart Universal SYBR Green Master Kit (Roche, Basel, Swiss) following the manufacturer's protocol.

Cell culture

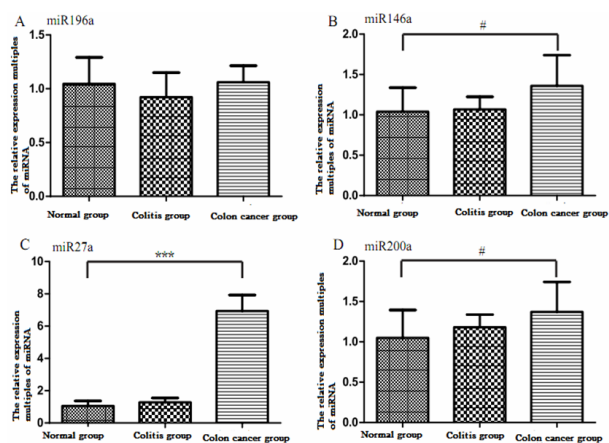The LoVo is a cell line that is established from human

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Figure 1. Expression Levels of miR196a, miR146a, miR27a and miR200a in Colon Cancer Tissues

Figure 2. Expression Levels of miR27a in Colon Cancer Cells after Transfection

colon cancer. LoVo cell line was maintained in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% FCS (Gibco, Grand Island, NY, USA) and 50 mg/ml penicillin/ streptomycin(Gibco, Grand Island, NY, USA) in a humidified incubator containing 5% CO_2 at 37 °C. Cells were fed three times a week and passaged every 7 days. LoVo was purchased from Saimo Company, Xuzhou, China.

Transfection

For increasing the transfection efficiency, we carried out an assay to analyse the transfection concentration. 24 h before transfection LoVo cells were seeded per well in 24 well plate and allowed to grow overnight. The cells were then transfected with five different concentration of Transfection Control (Cy3) (40 nM, 60 nM, 80nM, 100 nM and 120 nM, respectively) using lipofectamine 2000 (Invitrogen, Carlsbad, USA) according to the manufacture's protocol. 24 h after transfection miR27a expression level would be detected by PCR.

After determining the optimization concentration, we further performed another transfection assay. LoVo cells were divided in 5 groups: Control group that was without any treament, Liposome group that was transfected by lipofectamine 2000 only, Negative (RiBoBio Inc., Guangzhou, China) group, miR27a mimics (RiBoBio Inc., Guangzhou, China) group and miR27a inhibitors (RiBoBio Inc., Guangzhou, China) group. 24 h before transfection LoVo cells were seeded per well in 6 well plate and allowed to grow overnight. The cells were then transfected with Negative or 100 nM of miR27a mimics (80 nmol/L) or 80 nM miR27a inhibitors (80 nmol/L) using lipofectamine 2000 according to the manufacture's protocol. 6 h after transfection, medium was replaced with fresh RPMI 1640 containing 10% FCS. 24~48 h after culturing, transfected cells were collected for further analysis. This assay was repeated for 3 times.

Proliferation assay

Proliferation assay was carried out in triplication experiments using cell counting kit-8 (CCK-8). 4×10^3 LoVo cells were seeded per well in 96 well plate and allowed to grow overnight. Then, 24 h, 48h and 72h, respectively after transfection LoVo cells were treated with
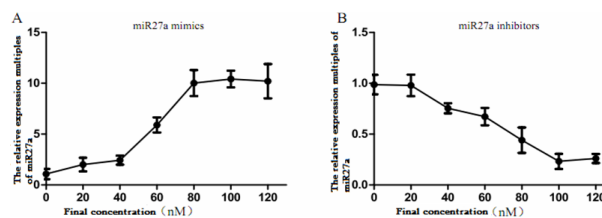
10 µL CCK-8 for 2h at 37 °C. Thereafter, the value of 450 nm was measured by ELISA. This assay was repeated for 3 times. CCK-8 was purchased from BiYuntian Company, Shanghai, China.

Invasion assay

Invasion assay was carried out in triplication experiments using Transwell chambers (Millipore, Boston, MA, USA). 24 h after transfection, the cells were centrifuged and washed by PBS for 1~2 times and then suspended with L-15 containing 0.1%BSA. Then, 1×10^5/ mL cells were then performed with the standard Transwell protocol according to the manufacturer's instructions. Moreover, selected cells would be observed by inverted microscope. And finally, the number of the cells would be counted. This assay was repeated for 3 times.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). All values were expressed as mean± SD. $P<0.05$ was considered as statistically significant.

Results

Expression of miR196a, miR146a, miR27a and miR200a in colon cancer tissues

No statistically significant was found between the expression levels of miR196a, miR146a and miR200a in Normal, Colitis and Colon cancer groups ($P>0.05$) (Figure 1A, B, C, D). As shown in Figure 1C, miR27a expression in Colon cancer group was much higher than Normal and Colitis groups ($P<0.05$), while, there was no difference of miR27a expression between Normal group and Colitis group ($P>0.05$).

Analysis of transfection concentration

In miR27a mimics group, miR27a expression was gradually increased when transfection concentration of miR27a mimics raised, reaching the highest at 80nM and then getting into the plateau if keep on raising (Figure 2A). However, the miR27a level of miR27a inhibitors group was gradually decreased with the raise of miR27a inhibitors concentration, reaching the lowest at 100nM and then would be equability if keep on raising (Figure 2B).

Effect of miR27a on proliferation in colonic cancer cells

As shown in Figure 3, proliferation rate in miR27a mimics group was significantly higher than Negative group ($P<0.05$), whereas, the proliferation rate of miR27a inhibitors group was obviously lower compared to that of
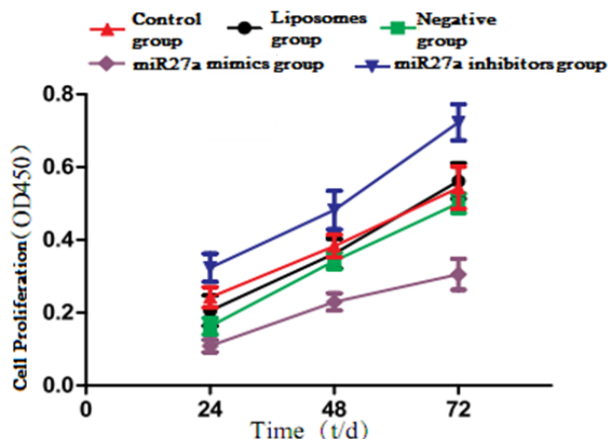
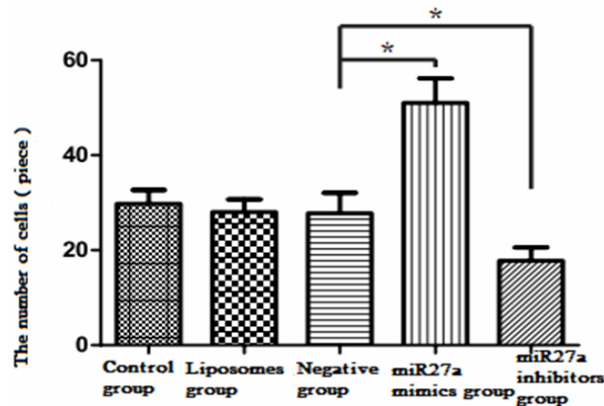**Figure 3. Effect of miR27a on Proliferation in Colonic Cancer Cells**

Figure 4. Effect of miR27a on Invasion Colonic Cancer Cells

Negative group (*P*<0.05). Besides, there was no difference of proliferation rate in Control, Liposome and Negative groups (*P*>0.05) (Figure 3).

Effect of miR27a on invasion colonic cancer cells

As shown in Figure 4, the invasion in miR27a mimics group was much stronger than that of those in Negative group (*P*<0.05), but the invasion in miR27a inhibitors group were significantly poorer than Negative group (*P*<0.05). In addition, there was no difference of invasion rate in Control, Liposome and Negative groups (*P*>0.05).

Discussion

MicroRNAs are tiny regulatory molecules that have important role in various biological processes such as proliferation, cell death, differentiation and metabolism (Filipowicz et al., 2008; Mohammed et al., 2009; Zhao et al., 2012; Fendler et al., 2013; Lucas et al., 2013). miR27a is located at chromosome 19 and has been shown to be expressed in breast cancer, gastric adenocarcinoma and cervical cancer (Mertens-Talcott et al., 2007; Wang et al., 2008; Liu et al., 2009). It has been identified as an oncogenic miRNA, and its important role in cancer development has been demonstrated in a few studies. miR27a had been reported to regulate cell growth and division in a dose-dependent manner (Liu et al., 2009; Lerner et al., 2011), and it might mediate the drug resistance of esophageal cancer cells (Zhang et al., 2010)

and ovarian cancer cells (Li et al., 2010). But reports about the expression of miR27a in human colon cancer were few.

In this study, we firstly used a RT-PCR approach to detect the miR27a level in human colon cancer. Our result has demonstrated that miR27a level of Colon cancer group was much higher than that of Normal and Colitis groups (*P*<0.05). A recent study suggested that miR27a may have a high expression level in gastric cancer (Liu et al., 2013). This is similar with the result made in our paper.

With the purpose of determining the transfection efficiency, we carried out an assay to analysis the transfection concentration. Figure 2 showed that there was a significant correlation between expression level of miR27a and concentrations of miR27a mimics and miR27a inhibitors. We finally obtained that the optimization transfection concentration of miR27a mimics was 80nM, and miR27a inhibitors was 100 nM.

Proliferation assay was performed and results showed that the proliferation rate in miR27a mimics group were significantly higher than Negative group (*P*<0.05) .The result indicated that miR27a may enhance colon cancer cell proliferation strongly. On the other hand, we further utilized miR27a inhibitors for repression of LoVo cells growth, proliferation and invasion. The results of Transwell assay suggested that miR27a may also reinforce the invasion of colon cancer cells obviously. Tumor invasion and metastasis contribute to the great majority of cancer deaths. Our efforts towards the diminution of the disease should include developing novel biomarkers to use in screening for patients with a high risk of metastasis.

In conclusion, our data for the first time indicated that up-regulation of miR27a may play an important role in enhancing the proliferation and invasion of colon cancer cells. Meanwhile, further investigation would be necessary for identification of the exact mechanism through which miR27a influence the colon cancer cells proliferation and invasion.

Acknowledgements

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