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Original Article

Mir-483 inhibits colon cancer cell proliferation and migration by targeting TRAF1

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Abstract MicroRNAs are important regulators during human growth and development. Emerging evidence indicates that microRNAs play important roles in colorectal cancer. The aim of this study is to reveal the biological function and direct target gene of miR-483 in colorectal cancer. The biological function of miR-483 on the proliferation and migration of colon cancer cells was then examined by Edu assay and transwell assay, respectively. Our findings revealed that miR-483 mimic could significantly inhibit cell proliferation and migration. The target gene of miR-483 was predicted by target scan software and identified by a dual fluorescence reporter system which showed that TRAF1 was a direct target gene of miR-483 in SW480 cell line. These data suggest that miR-483 is a colorectal cancer suppressor which could inhibit cell proliferation and migration, possibly via targeting TRAF1. The miR-483 could be a potential treatment target for colorectal cancer.
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Introduction

Colorectal cancer is one of the most common malignancies in the gastrointestinal tract [1]. The morbidity and mortality rate have increased year by year due to changes in lifestyle, diet, as well as living conditions [2]. Surgery and chemotherapy are the routine treatments of colorectal cancer. Colon cancer at early stage does not have obvious symptoms and can't be found; however, it has become a serious public health problem that threatens the health and lives of patients and becomes a social burden to patients with advanced colorectal cancer because of poor surgical results and drug resistance in chemotherapy [1,3]. Therefore, it is urgent to search new treatment for colorectal cancer and to explore its pathogenesis [4,5].

MicroRNAs (miRNA) are a class of non-coding RNAs with a length of about 21–25 nt, which inhibit or degrade the target gene by complementing the 3′ untranslated region (3′ UTR) of the mRNA of target gene. Many studies have shown that microRNAs are involved in the development and

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progression of multiple diseases by influencing the regulation of gene expression. It has been demonstrated that microRNAs have spatiotemporal expression specificity in different cells or tissues and the differentially expressed microRNAs can promote or inhibit the development of cancer. It is thought that differential expression of specific microRNAs in colon cancer cells may be involved in the occurrence and development of colon cancer and the metastasis of tumor cells [6—9]. So, it is necessary to explore the development process and new treatment for colon cancer by in-depth exploration of the function of microRNAs and their target genes. In this study, we studied the correlation between miR-483 and colon cancer and investigated the function of miR-483 in colon cancer. Additionally, we found that TRAF1 may be the target gene of miR-483.

Materials and methods

Cell culture and transfection

Human colon cancer cell lines SW480, HCT8 and normal colon epithelial NCM460 cells were purchased from Shanghai Institutes for Biological Sciences (Shanghai, China). Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). The cells were grown in a humidified incubator at 37 °C with 5% CO_2.

Based on the sequence of miR-483, the mimic, mimic control, inhibitor and inhibitor control were designed and chemically synthesized in GenePhama (GenePharma Co., Ltd, Shanghai, China). Transfections were performed using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) and cells were harvested 48 h after transfection for indicated study.

Real-time RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. cDNA synthesis was performed with the miRNA First-Strand cDNA Synthesis Kit (transgene, Beijing, China). The expression of miR-483 was measured by SYBR Green Master Mix (TOYOBO, Japan) using 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), U6 and GAPDH were used as an internal control for miRNA and mRNA respectively. The reverse transcription and quantitative PCR of miRNA were carried using special probes for miR-483 and U6 (RiboBio, Beijing, China). The specific primers for quantitative PCR of TRAF1 were: Forward,5′-GGAGGCCC AACTGCAATAA-3′ and reverse,5′-GTCAGCCGTGGGAACAATAA-3'. Relative miRNA and mRNA expression level was calculated by $2^{-\triangle\triangle ct}$ method.

Western blot analysis

Total protein was extracted using RIPA lysis buffer. After BCA quantification, the lysate was mixed with the protein loading buffer, heated at 100 °C for 10 min, separated by 10% SDS-PAGE electrophoresis followed by transfer to nitrocellulose filter membrane, and then the membrane was blocked in 5% skim milk at room temperature for 1 h, incubated with TRAF1 antibody (Abcam, Cambridge, MA USA, 1:1000) overnight at 4 °C, wash 3-5 X 10 min in TBS-T and incubate with an HRP-conjugated anti-mouse IgG secondary antibody (Jackson Immunoresearch Laboratories, Inc., West Grove, PA, USA, 1:10, 000) at 37 °C for 1 h. Signals were visualized by an ECL chemiluminescence detection kit (Millipore, Billerica, MA, USA) and semi-quantitated using ImageJ software. The protein loading was assessed by the expression of β-actin.

Cell proliferation and migration assays

The SW-480 cells were seeded in 24-well plates and incubated at 37 °C, in the presence of 5% CO_2.

For cell proliferation assay, after transfection, Edu (RiboBio, Beijing, China) was added to the culture medium to a concentration of 10 μmol/L and incubated in the incubator for 2 h. The cell was fixed with PBS containing 4% paraformaldehyde for 15 min at room temperature, and incubated for 30 min at room temperature with dyeing solution, DAPI buffer was added after twice washing, and the number of positive cells in each field of view was observed and recorded under fluorescence microscope.

For cell migration assay, a 1: 4 dilution of the artificial basement membrane Matrigel (BD Biosciences, Franklin, NJ, USA) was uniformly applied on the surface of the upper membrane of Transwell chamber which with a bottom 8 μm pore size polycarbonate microporous membrane. 600 μl of 1640 medium with 5% fetal bovine serum were added in the lower chamber, and 100 μl (2×10^5 cells/ml) were seeded in the upper chamber with 1% fetal bovine serum for 12 h. Then remove the Transwell chamber and carefully wipe the upper surface with the cotton ball. After staining by 1% crystal violet, cells in each field of view were observed and recorded under microscope. At least three fields in each group were counted.

Bioinformatics analysis

To investigate whether miR-483 targets TRAF1 or not, bioinformatics analysis was performed using TargetScan (www.targetscan.org). We searched the target gene of miR-483 directly on the TargetScan homepage and finally selected TRAF1 gene, miR-483 was shown to target sites 4262—4268 of the 3′-untranslated region (UTR) of TRAF1 mRNA (Fig. 3A).

Plasmid constructs

The TRAF1-3′UTR were amplified by PCR using human cDNA from 293 cells as template and was cloned into the pmirGLO vector (Promega, Madison, WI, USA) (TRAF1-pmirGLO). TRAF1-pmir-GLO-mutant had changed the putative binding site that can't combine with miR-483. The specific primers for TRAF1- pmir-GLO were: Forward, 5′-CTAGCTAG CCAGGCAATCCTCAGGCAACCCT-3′ and reverse, 5′-CGCGTCGAC GATGTGATCACAGCTCACTGCA-3'. The specific primers for TRAF1- pmir-GLO-mutant were: Forward,5′-

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