Review Article

MALAT1 promotes migration and proliferation of colorectal cancer cells through targeted regulation of miR-205 expression

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Abstract: Objective: To understand the mechanism of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in colorectal cancer (CRC). Methods: qRT-PCR was applied to determine the expression of MALAT1 and microRNA-205 (miR-205) in CRC cells (DLD-1, SW480, HCT116, and SW620) and normal colon colonic epithelial cell (FHC). Stable MALAT1 inhibition vectors and miR-205 overexpression vectors were established and transfected into CRC cells (SW480 and HCT116), and then the cell counting kit-8 (CCK8), transwell assay, and flow cytometry were adopted to analyze the migration, proliferation, and apoptosis of the transfected cells, and the dual luciferase reporter assay was adopted to find out the correlation of MALAT1 with miR-205. Results: MALAT1 was up-regulated in the four kinds of purchased CRC cells, while the situation of miR-205 was opposite. Both inhibiting the expression of MALAT1 and up-regulating the expression of miR-205 could weaken the proliferation and migration abilities of SW480 and HCT116 cells and promote apoptosis of them. The dual luciferase reporter assay revealed targeted binding between MALAT1 and miR-205, and the rescue experiment revealed that inhibiting the miR-205 expression could prevent cell invasion, proliferation, and apoptosis caused by inhibiting MALAT1. Conclusion: MALAT1 could promote the development of CRC by inhibiting the expression of miR-205, and inhibition of MALAT1 is expected to be the treatment direction of CRC.

Keywords: MALAT1, miR-205, colorectal cancer, cell biological function

Introduction

Colorectal cancer (CRC) is a familiar cancer and the fourth major cause of cancer-related death in the world [1]. According to statistics, there were more than 1,000,000 CRC patients worldwide in 2018 and more than 550,000 people dead from CRC during the same period [2]. Although the diagnosis and treatment of CRC have improved to a certain extent, the 5-year survival rate of CRC patients is still unsatisfactory, and it is only 40-60% [3]. Therefore, it is currently a research hotspot to find out the pathogenesis of CRC and treatment targets for it.

Long non-coding RNA (LncRNA) is RNA longer than 200 nt [4]. Statistically, 58,648 IncRNAs have been found in human transcriptome [5]. Although IncRNA cannot directly encode proteins, it can affect gene expression at the transcriptional and post-transcriptional levels, thus influencing a variety of biological processes such as embryonic development, cell growth and tumorigenesis [6, 7]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a lncRNA that is highly conserved in species and widely expressed in humans [8]. It was reported that MALAT1 was abnormally expressed in cancers such as cervical cancer, gastric cancer, and ovarian cancer [9-11], and that it was involved in the pathogenesis of those diseases. A previous study showed that MALAT1 was up-regulated in CRC, and the up-regulation of it indicated a poor prognosis [12]. However, the relationship between MALAT1 and CRC remains unclear. We have found binding sites between microRNA-205 (miR-205) and MALAT1 through bioinformatics analysis. MiR-205 is located on chromosome 1, with a length of about 110 bp. MiR is a highly conserved endogenous short-chain non-coding RNA, which can participate in and regulate cell processes such as apoptosis, proliferation, and differentiation [13, 14], and it can serve as a tumor suppressor gene or oncogene in tumors.
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[15]. MiR-205 was found to be down-regulated in CRC [16], which indicated that it may be closely related to CRC.

According to the above, we suspected that MALAT1 promoted CRC cell progression by targeted regulation of miR-205 expression. Based on this hypothesis, we carried out the following research.

Materials and methods

Cell culture and transfection

CRC cell lines (DLD-1, SW480, HCT116, and SW620) and normal colon epithelial cell (FHC) from the ATCC Company of the United States were cultured in RPMI1640 (Gibco Company, the United States) with 1% penicillin-streptomycin (100X, Solarbio Company, the United States) and 10% fetal bovine serum (PBS), (Gibco Company, the United States) under 5% CO₂ at 37°C. The cells were transfected as follows: MALAT1 inhibitory plasmids (si-CASC19), miR-205 overexpression plasmids (miR-205-mimics), and blank controls (si-NC and miR-NC) were constructed. The constructed cell lines were transferred into a 24-well plate. After 48 hours, the cell lines were transfected with the cell plasmids using a Lipofectamine 2000 kit (Invitrogen Company, the United States) in strict accordance with the kit instructions.

qRT-PCR assay

The total RNA was sampled with a TRItol Kit (Invitrogen Company, the United States), and the concentration, purity, and integrity of the sample were verified using an ultraviolet spectrophotometer (Metash instrument Co., Ltd) and agarose gel electrophoresis. Reverse transcription was carried out using a TaqMan™ Reverse Transcription Reagents kit (Invitrogen Company, the United States) in strict accordance with the kit instructions. Amplification was carried out through SYBR_Premix ExTaq II (Takara Company, China) and ABI 7500 PCR instrument (Applied Biosystems, the United States) under the amplification system consisting of 20 μL of total volume having 10 μL of SYBR Premix Ex Taq II (2X), 2 μL of cDNA, 0.8 μL of upstream and downstream primer, and sterile purified water to adjust the volume. The amplification conditions were as follows: Pre-denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, and annealing and extension at 60°C for 30 s. Data in this study was analyzed using 2^ΔΔct [17].

Proliferation determination by CCK8 assay

CCK-8 (Beyotime Biotechnology, China) was employed to detect cell proliferation according to the kit instructions as follows: the cells were transfected for 24 h and were cultured in a 96-well plate at 2.5×10⁵ cells per well. A total of 10 μL of CCK-8 solution was added into each well at 24 h, 48 h, 72 h, and 96 h after culturing, respectively, and the plate was cultured at room temperature for 2 hours. Subsequently, the optical density of each well at 490 nm was detected using a microplate reader (Molecular Devices, the United States), and corresponding survival curves were drawn.

Cell migration assay

A Transwell kit (Gibco Company, the United States) was adopted for migration assay as follows: cells transfected for 24 h were collected, seeded into a 6-well plate at 5×10⁴ cells/well, and washed with PBS two times. The upper chamber and the lower chamber were given 200 μL of RPMI-1640 and 500 μL of RPMI-1640 containing 20% FBS, respectively. The plate was cultured for 48 h at 37°C, and the substrates and cells not passing through the membrane surface in the upper chamber were wiped off. The plate was cleaned with PBS three times, immobilized with paraformaldehyde for 10 min, and washed with double distilled water three times, and it was then stained with 0.5% crystal violet after being dried out. Finally, cell migration in the plate was analyzed using a microscope.

Cell apoptosis assay

The transfected cells were collected and digested with 0.25% trypsin, and then prepared into 1×10⁶ cells/mL suspension. The suspension was added with AnnexinV-FITC/PI (Shanghai Yeasen Biotechnology Co., Ltd., China), incubated at room temperature in the dark for 5 min, and finally detected using the FC500MCL flow cytometer system. The experiment was carried out triply.

Dual luciferase reporter assay

A Lipofectamine™ 2000 kit was applied to clone MALAT1 in pmirGLO dual-luciferase tar-
get expression vectors. The MALAT1-3'UTR wild type (Wt) and MALAT1-3'UTR mutant type (Mut) were constructed and transfected into the downstream of the luciferase reporter genes to sequence and identify the constructed plasmids. Correctly sequenced plasmids along with miR-205-mimics or miR-N were co-transfected into HEK293T cells. After 48 hours, a dual luciferase reporter gene detection kit (Promega Company, the United States) was used to detect the luciferase activity of the cells.

Statistical analysis

In this study, the collected data were analyzed statistically using SPSS 18.0, and visualized into required figures using GraphPad 7. Data distribution was analyzed using the Kolmogorov-Smirnov (K-S) test, and data in normal distribution were represented by the mean ± standard deviation (Mean±SD). Inter-group comparison was carried out with the independent t test, and multi-group comparison was carried out using the one-way ANOVA. Post hoc pairwise comparison was subject to the LSD-t test, and comparison in terms of expression at multiple time points was carried out through the repeated measures analysis of variance, and Bonferroni post hoc test was applied. P<0.05 suggested a significant difference.

Results

Expression of MALAT1 and miR-205 in CRC cells

qRT-PCR was applied to detect the expression of MALAT1 and miR-205 in CRC cell lines (DLD-1, SW480, HCT116, and SW620) and normal colon epithelial cell line (FHC). It was found that compared with FHC, the four kinds CRC cells showed significantly increased MALAT1 expression and significantly decreased miR-205 expression (both P<0.05). It was also found that MALAT1 was expressed highest in SW480 and HCT116 cells, so SW480 and HCT116 cells were used for subsequent cell experiments.

Effects of inhibiting MALAT1 on the biological function of CRC cells

For the purpose of determining the role of MALAT1 in CRC, si-MALAT1 and si-NC were transfected into SW480 and HCT116 cells, respectively, and the CCK-8 assay, transwell assay, and flow cytometry were employed to analyze the proliferation, migration, and apoptosis of transfected cells. The results revealed that transfection of si-MALAT1 could down-regulate MALAT1 in SW480 and HCT116 cells (P<0.05), and it could also suppress the proliferation and migration of the cells and promote apoptosis of them.

Effects of up-regulating miR-205 on the biological function of CRC cells

Previously, miR-205 was found to be down-regulated in the four purchased CRC cells, which suggested that miR-205 may be related to the progression of CRC. Therefore, we overexpressed miR-205 in SW480 and HCT116 cells and observed the influence on the biological function of the cells, in order to excavate the role of miR-205 in CRC. The results revealed that SW480 and HCT116 cells transfected with miR-205-mimics showed significantly increased
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miR-205 expression (P<0.05), and significantly suppressed proliferation and migration abilities, and intensified apoptosis. Figure 3.

Downstream target genes of MALAT1

In order to excavate the progression mechanism of MALAT1 in promoting CRC, we predicted and found that miR-205 could be used as the downstream target gene of MALAT1 through starBase2.0 website, and also found binding sites between them. We carried out a dual luciferase assay to verify their correlation, finding that transfection of miR-205-mimics could inhibit the luciferase activity of MALAT1-3'UTR Wt in HEK293T cells, but it posed no effect on that of MALAT1-3'UTR Mut. Subsequently, we employed qRT-PCR to detect the miR-205 expression in SW480 and HCT116 cells transfected with si-MALAT1, finding that the miR-205 expression decreased in them (P<0.05). Figure 4.

Effects of simultaneously inhibiting MALAT1 and miR-205 on the biological function of CRC cells

For the purpose of further investigating the correlation between MALAT1 and its downstream target gene, miR-205, we inhibited the expression levels of MALAT1 and miR-205 in SW480 and HCT116 cells at the same time, and then observed their biological function changes. It turned out that cells transfected with si-MALAT1+miR-205-inhibitor were not greatly different from those transfected with miR-NC in terms of cell proliferation, migration, and apoptosis, and they showed enhanced proliferation and migration abilities, and decreased apoptosis compared with those transfected with si-MALAT1. Figure 5.

Discussion

Because the diagnosis efficiency of early CRC is not high, most patients are already in the advanced stage at the time of diagnosis. Advanced CRC is characterized by high recurrence and easy metastasis, so the prognosis of CRC is very poor [18]. Proliferation, invasion, and metastasis of tumor cells are the key factors affecting the development and recurrence of tumor tissues [19]. MALAT1, mainly located in nuclear spots, contributes to the development of many tumors including CRC [20-22]. This study found that MALAT1 was up-regulated...
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MALAT1 promotes progression of colorectal cancer in 4 kinds of purchased CRC cells, and inhibition of it could inhibit cell proliferation and migration and increase apoptosis rate, which indicated that MALAT1 had potential to become a therapeutic target of CRC. However, the mechanism leading to the above results has not been fully clarified.

LncRNA acts on miRNA through sponge effects to regulate downstream targets of miRNA [23]. In order to understand the molecular mechanism of MALAT1 in acting as an oncogene in CRC, this study predicted downstream target genes of MALAT1 though the starBase2.0 website, and found targeted binding sites between MALAT1 and miR-205. MiR is a key regulator of gene expression, whose imbalance is related to the development and progression of various cancers [24]. MiR-205 has two sides in tumor occurrence and growth. On one hand, it can promote tumor growth. For example, miR-205 promotes tumor growth by silencing adenomatous polyposis coli (APC) in pancreatic cancer [25], and it can inhibit PTEN/SMAD4 in ovarian cancer, thus promoting the

Figure 3. Effects of up-regulating miR-205 on the biological function of CRC cells. A. SW480 and HCT116 cells showed increased miR-205 expression after being transfected with miR-205-mimics. B, C. SW480 and HCT116 cells showed suppressed proliferation ability after being transfected with miR-205-mimics. D. SW480 and HCT116 cells showed suppressed migration ability after being transfected with miR-205-mimics. E. SW480 and HCT116 cells showed enhanced cell apoptosis after being transfected with miR-205-mimics. F. Flow cytometry. Note: *indicates P<0.05.

Figure 4. Downstream target genes of MALAT1. A. There were binding sites between MALAT1 and miR-205 according to starbase 2.0 website prediction. B. Transfection of miR-205-mimics could lower the luciferase activity of MALAT1-3’UTR Wt in cells, but did not affect that of MALAT1-3’UTR Mut in cells. C. Transfection of si-MALAT1 could increase the expression of miR-205 in SW480 and HCT116 cells. Note: *indicates P<0.05.
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In addition, miR-205 can also play the role of an oncogene by regulating phosphatase and tensin homolog (PTEN) in lung cancer [27]. On the other hand, miR-205 can be a tumor suppressor in the development of triple negative breast cancer, liver cancer and prostate cancer [28-30]. This study found that miR-205 decreased in CRC cells, and up-regulating the expression of miR-205 could inhibit the cell migration and proliferation abilities and increase apoptosis, which indicated that miR-205 could curb the development of CRC. A previous report pointed out that miR-205 could be regulated by a variety of lncRNAs to affect the progress of diseases. For example, it can be inhibited by LncRNA GAS5 to prevent the growth of cervical cancer [31], and it can also be inhibited by LncRNA LINC00673 to promote the progression of liver cancer [32]. Some studies also revealed that miR-205 could be targeted and regulated by MALAT1 to promote the development of osteosarcoma and renal cell carcinoma [33, 34]. These studies show that LncRNA plays a key role in tumor regulation through miR-205. Our study found that the miR-205 expression in CRC cells increased after MALAT1 was inhibited, and the dual luciferase activity confirmed that MALAT1 and miR-205 can bind to each other in a targeted manner. Subsequently, we also found through rescue experiments that the simultaneous transfection of si-MALAT1+miR-205-inhibitor did not significantly change the proliferation, migration and apoptosis of CRC cells. Based on the above research results, we proved that MALAT1 could promote CRC development by inhibiting miR-205.

This paper has discussed the upstream regulation of miR-205 in CRC at the molecular level, and holds that MALAT1 promotes CRC cell growth by down-regulating miR-205, and MALAT1/miR-205 axis has a potential therapeutic value in CRC. Some studies concluded that miR-205 was involved in the cell life process by regulating PTEN [26, 27]. This study hypothesized that MALAT1 may regulate downstream PTEN-related pathways through miR-205 in CRC. This hypothesis will be investigated in future experiments. Moreover, this study has

![Figure 5. Effects of simultaneously inhibiting MALAT1 and miR-205 on the biological function of CRC cells. A, B. Cells transfected with si-MALAT1+miR-205-inhibitor were not greatly different from those transfected with miR-NC in terms of cell proliferation, and they showed enhanced proliferation compared with cells transfected with si-MALAT1. C. Cells transfected with si-MALAT1+miR-205-inhibitor were not greatly different from those transfected with miR-NC in terms of cell migration, and they showed enhanced migration compared with cells transfected with si-MALAT1. D. Cells transfected with si-MALAT1+miR-205-inhibitor were not greatly different from those transfected with miR-NC in terms of apoptosis rate, and showed decreased apoptosis rate compared with cells transfected with si-MALAT1. E. Flow cytometry. Note: *indicates P<0.05.](image-url)
only discussed the cytological influence of the MALAT1/miR-205 axis, and the regulatory network of the axis will be explored through in vivo experiments in mice in future studies.

To sum up, MALAT1 can promote the development of CRC by inhibiting the expression of miR-205, and inhibition of MALAT1 or up-regulation of miR-205 is expected to be the treatment direction of CRC.

Disclosure of conflict of interest

None.

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References


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[34] Li Q, Pan X, Wang X, Jiao X, Zheng J, Li Z and Huo Y. Long noncoding RNA MALAT1 promotes cell proliferation through suppressing miR-205 and promoting SMAD4 expression in osteosarcoma. Oncotarget 2017; 8: 106648-106660.