LINC00339 accelerates invasion and migration of colorectal cancer via mediating miRNA-30a-5p

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ABSTRACT

To analyze the biological function of LINC00339 in the progression of colorectal cancer (CRC). We aim to provide directions in the early-stage treatment of CRC. LINC00339 level in 60 paired CRC tissues and paracancerous tissues was examined by quantitative real-time polymerase chain reaction (qRT-PCR). The correlation between the LINC00339 level and clinical parameters was analyzed. Moreover, the LINC00339 level in CRC cell lines was determined as well. LINC00339 expression was changed in HCT-8 and HCT-116 cell lines by transfection of LINC00339 overexpression plasmid or anti-LINC00339. The regulatory effects of LINC00339 on the migratory and invasive abilities of CRC cells were evaluated through a series of functional experiments. Dual-luciferase reporter gene assay and rescue experiments were conducted to verify the interaction of LINC00339 and miRNA-30a-5p in mediating the progression of CRC. LINC00339 was upregulated in CRC tissues relative to paracancerous tissues. CRC patients with higher levels of LINC00339 had higher rates of lymph node metastasis and distant metastasis, and worse prognosis than those with lower levels. Knockdown of LINC00339 attenuated migratory and invasive abilities of HCT-116 cells. Overexpression of LINC00339 in HCT-8 obtained the opposite trends. In addition, we verified a negative correlation between LINC00339 and miRNA-30a-5p in CRC tissues. LINC00339 served as a ceRNA to absorb miRNA-30a-5p. Rescue experiments confirmed that miRNA-30a-5p knockdown reversed the regulatory effects of LINC00339 on the migratory and invasive abilities of CRC cells. LINC00339 was closely correlated to metastasis and poor prognosis of CRC. It accelerates CRC cells to migrate and invade via mediating miRNA-30a-5p.

Introduction

Colorectal cancer (CRC) is a high-risk digestive tract tumor throughout the world. Its mortality ranks third in Western countries (1-3). With the increased obesity rate, changing lifestyle and insufficient physical activity, the incidence of CRC gradually increasing in China. There are approximately 3.76 million new cases and 1.91 million death cases of CRC in the Chinese population (4,5). The pathogenesis of CRC involves both genetic and environmental factors (6,7). Although the screening strategies have advanced in recent years, there are still many CRC patients who are diagnosed in the advanced stage due to untimely screening and obscure symptoms7. Therefore, it is extremely important to clarify the pathogenesis of CRC to search for diagnostic and therapeutic targets (8,9).

Long non-coding RNAs (lncRNAs) are unable to encode proteins, which contain more than 200 nucleotides in length (10,11). LncRNA was originally thought to be a transcriptional noise or junk DNA that cannot influence the transcription and translation of proteins (12,13). Later, it was found that lncRNA exerts a vital role in biological progressions. LncRNA serves as a mediator in tumor development (14,15). LINC00339 is located on chromosome 9q34.3 and is 2176 bp in length, which is considered as a bidirectional lncRNA to direct the post-translational modification of snoRNA (16,17). LINC00339 has been reported to participate in the malignant behaviors of laryngeal squamous cell carcinoma, breast cancer, non-small cell lung cancer and glioma (18-21). This study focused on the potential role of LINC00339 in CRC and its mechanism.

MicroRNAs (miRNA) are widely expressed in animals, plants, and viruses. They bind to the 3' untranslated region (3'-UTR) of mRNAs catalyzed by RNA-induced silencing complexes (RISC), thus negatively regulating mRNAs by inhibiting translation or directly degrading them (22,23). MiRNA-30a-5p has been identified in many types of tumors (24). Nevertheless, its role in CRC is rarely reported. LncRNAs could act as ceRNAs to sponge miRNAs, and further mediate post-transcriptional regulation (10,13,15). Studies have shown that upregulation of LINC00339 accelerates the progression of non-small-cell lung cancer (NSCLC) by sponging miR-145 (10,13,15). LINC00339 promotes laryngeal carcinoma cells to proliferate and invade by sponging miR-145 (19). It is of great value to demonstrate the function of the lncRNA-miRNA-miRNA regulatory loop in tumor progression (15).

In this study, we screened the differentially expressed lncRNA and miRNA in the expression profile of CRC. LINC0039 was verified to promote malignant behaviors...
of CRC cells by sponging miRNA-30a-5p.

Materials and Methods

CRC samples

60 paired CRC tissues and paracancerous tissues were harvested from CRC patients undergoing radical operation. None of the patients were treated with anti-tumor therapy before surgery. Pathological staging was evaluated in accordance with the guidelines proposed by UICC (the Union for International Cancer Control). Patients and their families in this study have been fully informed. This study was approved by the Ethics Committee of our hospital.

Cell culture

CRC cell lines (HT29, HCT-8, HCT-116) and human-derived intestinal epithelial cell line (FHC) were provided by the American Type Culture Collection (ATCC) (Manassas, VA, USA). CRC cells were cultured in Dulbecco’s modified eagle medium (DMEM) (RPMI 1640 (Roswell Park Memorial Institute 1640) applied for HCT-116 cells) containing 10% FBS (Life Technologies, Gaithersburg, MD, USA) and maintained in a 37°C, 5% CO2 incubator. Until 80-90% of confluence, cell passage was conducted using 1×trypsin+EDTA (Ethylene Diamine Tetraacetic Acid).

Transfection

LINC00339 overexpression vector, NC, anti-LINC00339, anti-NC, miRNA-30a-5p mimics, miRNA-30a-5p inhibitor and miR-NC were provided by GenePharma (Shanghai, China). Cells seeded in the 6-well plates with 40% confluence were subjected to transfection. 48 hours later, transfected cells were harvested for functional experiments.

Cell proliferation assay

Cells were seeded in the 96-well plate with 5.0×10^4 cells per well. Viability was determined at the appointed time points (24 h, 48 h, 72 h and 96 h) using a cell counting kit-8 (CCK-8) kit (Dojindo Laboratories, Kumamoto, Japan). Absorbance at 490 nm was recorded for plotting the viability curve.

Transwell cell migration and invasion assays

Transfected cells for 48 h were digested and adjusted to 5.0×10^4/mL. 200 μL/well suspension was applied in the upper side of the Matrigel-coated Transwell chamber (Millicore, Billerica, MA, USA). On the bottom side, 700 μL of medium containing 20% FBS was applied. After 48 h of incubation, invasive cells were fixed in methanol for 15 min, dyed with 0.2% crystal violet for 20 min and counted using a microscope. Penetrating cells were counted in 5 randomly selected fields per sample. Transwell migration assay was conducted in the same procedures except for Matrigel pre-coating.

Quantitative real-time polymerase chain reaction (qRT-PCR)

We extracted total RNA from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and purified by DNase I treatment. Extracted RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using Primescript RT Reagent (TaKaRa, Otsu, Japan). The cDNA was amplified by real-time quantitative PCR using SYBR®Premix Ex Taq™ (TaKaRa, Otsu, Japan). Primer sequences were as follows: LINC00339, forward, 5′-GGTGAAGGTGGGCAATAC-3′; reverse, 5′-GCCCATCATTTACATGGA-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward, 5′-GGTGAAGGTGGGCAATAC-3′; reverse, 5′-CCATGTAGTTGGGCTACATGGA-3′; MiRNA-30a-5p, forward, 5′-TAGGGATCCTTTCATCCTTACCTTTTTCCCACA-3′; reverse, 5′-ATGCCGGAAACTAGAAGCTCGGTAGATAA-3′; U6, forward, 5′-CGTTTCGACGCACTATAC-3′; reverse, 5′-TTACGACATTTTGCGTGTCA-3′. Each sample was performed in triplicate, and analyzed by iQ5 2.0 (Bio-Rad, Hercules, CA, USA).

Dual-luciferase reporter gene assay

Based on the predicted binding sequences between miRNA-30a-5p and LINC00339, we constructed pmirGLO-LINC00339-wt, pmirGLO-LINC00339-mut and negative control pmirGLO. CRC cells were co-transfected with pmirGLO-LINC00339-wt/pmirGLO-LINC00339-mut/ pmirGLO and miRNA-30a-5p mimics/NC, respectively. After 48 h, cells were lysed and subjected to luciferase activity determination.

Statistical analysis

GraphPad Prism 5 V5.01 (La Jolla, CA, USA) was used for data analyses. Data were expressed as mean ± standard deviation. The student t-test was applied to analyze the intergroup differences. Differences among groups were analyzed by one-way analysis of variance (ANOVA), followed by a post-hoc test. The receiver operating characteristic (ROC) curve was introduced for evaluating the prognostic value of LINC00339. P<0.05 was considered statistically significant.

Results

High expression of LINC00339 in CRC

The expression pattern of LINC00339 in CRC was first determined by qRT-PCR. The data showed a higher abundance of LINC00339 in CRC tissues relative to paracancerous tissues (Figure 1A). We subsequently selected 16 pairs of CRC and paracancerous tissues for determining the mRNA level of LINC00339 and obtained identical results, suggesting the oncogenic role of LINC00339 in CRC (Figure 1B). Compared with the FHC cell line, LINC00339 showed a higher level in CRC cell lines (Figure 1C). ROC curve was introduced and revealed a certain prognostic value of LINC00339 in CRC (AUC=0.898, 95%C1=0.800-0.997, Figure 1D).

LINC00339 expression was correlated with pathological staging, lymph node metastasis, distant metastasis and overall survival of CRC patients

Based on the mRNA level of LINC00339, enrolled CRC patients were divided into a high-level group and a low-level group. Their clinical data were collected for correlation analyses. As Table 1 depicted, high-level LINC00339 was positively correlated to pathological staging, lymph node metastasis and distant metastasis of CRC patients. The age and gender of CRC patients had nothing to do with the LINC00339 level.
LINC00339 bound to miRNA-30a-5p

Based on the predicted binding sequences between miRNA-30a-5p and LINC00339, we constructed pmirGLO-LINC00339-wt, pmirGLO-LINC00339-mut and negative control pmirGLO for the subsequent dual-luciferase reporter gene assay. CRC cells were co-transfected with pmirGLO-LINC00339-wt/pmirGLO-LINC00339-mut/ pmirGLO and miRNA-30a-5p mimics/NC, respectively. Luciferase activity decline was pronounced in cells co-transfected with pmirGLO-LINC00339-wt and miRNA-30a-5p mimics rather than the other two groups (Figure 3A). It is confirmed that LINC00339 is directly

Knockdown of LINC00339 inhibited CRC cells to migrate and invade

To investigate the potential function of LINC00339 in CRC, we first constructed overexpression and knockdown lentivirus vectors of LINC00339. Transfection of LINC00339 overexpression vector markedly upregulated LINC00339 level in HCT-8 cells, and transfection of anti-LINC00339 downregulated its level in HCT-116 cells (Figure 2A). CCK-8 assay showed that HCT-8 cells overexpressing LINC00339 had higher proliferative ability relative to controls. Conversely, LINC00339 knockdown suppressed proliferative ability in HCT-116 cells (Figure 2B). Transwell assay indicated that the migratory and invasive abilities of CRC cells were elevated after LINC00339 overexpression, which was suppressed by knockdown of LINC00339 (Figure 2C).

Table 1. Association of LINC00339 expression with clinicopathologic characteristics of colorectal cancer.

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bound to miRNA-30a-5p.

We next detected miRNA-30a-5p level in 60 pairs of CRC and paracancerous tissues by qRT-PCR. Compared with paracancerous ones, miRNA-30a-5p was lowly expressed in CRC tissues (Figure 3B). Identically, it was downregulated in CRC cell lines (Figure 3C). Subsequently, we selected 16 cases of CRC tissues for determining LINC00339 and miRNA-30a-5p levels. A negative correlation between their levels was observed in CRC (r²=0.464, P<0.05, Figure 3D). Moreover, transfection of LINC00339 overexpression vector downregulated miRNA-30a-5p level in HCT-8 cells, and conversely, LINC00339 knockdown upregulated miRNA-30a-5p level in HCT-116 cells (Figure 3E).

LINC00339 modulated CRC progression via mediating miRNA-30a-5p

We speculated that LINC00339 may serve as a ceRNA to sponge miRNA-30a-5p, thus regulating the progression of CRC. Here, a series of rescue experiments were carried out. CRC cells were transfected with anti-NC+miR-NC, anti-LINC00339+miR-NC or anti-LINC00339+miRNA-30a-5p inhibitors, respectively. The downregulated LILINC00339 in the anti-LINC00339+miR-NC group was reversed by miRNA-30a-5p knockdown (Figure 4A). The viability curve revealed an inhibited proliferative rate in CRC cells transfected with anti-LINC00339+miR-NC, which was reversed in the anti-LINC00339+miRNA-30a-5p inhibitor group (Figure 4B). Similarly, suppressed migratory and invasive abilities of CRC cells due to LINC00339 knockdown were reversed by miRNA-30a-5p knockdown (Figure 4C).

Discussion

Although the molecular mechanism of CRC has achieved encouraging progress, patients with advanced CRC still suffer from a poor prognosis (1,7). Searching for the molecule switch that controls malignant performances of CRC and clarifying its metastatic mechanism have significant clinical values (4,5). Early diagnosis using biomarkers is an effective approach to reduce the mortality of CRC (7-9). In this study, we explored the possible mechanism by which LINC00339 acted as a ceRNA through sponging miRNA-30a-5p to mediate the progression of CRC.

Novel regulatory genes have been extensively discovered with the progress of molecular technology (15,22). LncRNAs exert complex regulations on cell proliferation, differentiation, apoptosis and epigenetic processes (10,12,25). LINC00339 is a novel gene that is closely involved in the progression of various tumors (18-21).
Here, we analyzed the differentially expressed lncRNA in CRC microarray (15). It was found that LINC00339 was remarkably upregulated in CRC. Through analyzing the clinical data of CRC patients, LINC00339 was found to be correlated with lymph node metastasis, distant metastasis and tumor stage of CRC. LINC00339 level was negatively correlated to the survival of CRC patients. In vitro experiments identified that LINC00339 promoted CRC cells to proliferate and metastasize.

The discovery of the ceRNA hypothesis makes lncRNA widely concerned as a component of the lncRNA-miRNA-mRNA axis (12,13,15). It is reported that lncRNA HULC promotes tumorigenesis and metastasis of hepatocellular carcinoma through mediating the miR-200a-3p/ ZEB1 axis (26). LncRNA RUSPI2 accelerates the tumorigenesis of cervical cancer by absorbing let-7a (27-29). In this paper, a negative correlation between LINC00339 and miRNA-30a-5p was observed. We, therefore, speculated whether LINC00339 could serve as a ceRNA in the regulation of CRC. Dual-luciferase reporter gene assay further proved their binding of LINC00339 to miRNA-30a-5p. We believed that miRNA-30a-5p was involved in LINC00339-mediated CRC progression.

MicroRNAs function as oncogenes or tumor-suppresser genes in various types of tumors (2,23). In this study, miRNA-30a-5p mediated cellular behaviors of CRC, thus influencing the occurrence and progression of CRC. MiRNA-30a-5p is directly bound to 3'UTR of LINC00339. We also revealed that expression changes in LINC00339 and miRNA-30a-5p were closely related to the progression of CRC. In conclusion, we believed that LINC00339 participated in the malignant behaviors of CRC by endogenously competing for miRNA-30a-5p. These findings deepened our understanding of lncRNA functions and the etiology and pathogenesis of CRC.

Conclusion

LINC00339 is highly expressed in CRC, which accelerates the migratory and invasive abilities of CRC cells via endogenously competing for miRNA-30a-5p.

Conflict of Interest

The authors declared no conflict of interest.

References


