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Decreased long noncoding RNA ADIPOQ promoted cell proliferation and metastasis *via* miR-219c-3p/TP53 pathway in colorectal carcinoma

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Abstract. – OBJECTIVE: To investigate the expression of Long non-coding RNA ADIPOQ and its facilitating effects on proliferation and invasion of colorectal cancer by modulating the expression of TP53 via sponging with miR-219c-3p.

PATIENTS AND METHODS: qRT-PCR was performed to detect the expressions of ADIPOQ and TP53 in human colorectal cancer tissues and cells. CCK-8 assay was performed to evaluate the Caco-2 cells proliferation and transwell assay was performed to evaluate the Caco-2 cells migration. The relationship between ADIPOQ and miR-219c-3p was detected by statistical analysis. Target prediction and Luciferase activity assay were conducted to investigate the binding site and interaction between ADIPOQ and miR-219c-3p. Further, we cloned the mice TP53 3′-UTR into the Luciferase reporter vector and constructed miR-219c-3p binding mutants to verify the inhibited regulation of miR-219c-3p to the TP53 expression.

RESULTS: The results suggested that the expression of ADIPOQ and TP53 was downregulated in human colorectal cancer tissues and Caco-2 cells. qRT-PCR and CCK-8 assay showed that ADIPOQ expression is correlated with the proliferation of colorectal cancer cells. Transwell assay showed that ADIPOQ regulated the migration ability of colorectal cancer cells. The bioinformatics prediction and Luciferase assay demonstrated that ADIPOQ serves as ceRNA for miR-219c-3p to further regulate the expression of TP53.

CONCLUSIONS: For the first time, we found that lncRNA-ADIPOQ was downregulated in human colorectal cancer cells, which could facilitate tumor proliferation, migration and invasion as a ceRNA by sponging with miR-219c-3p.

Key Words:
ADIPOQ, MiR-219c-3p/TP53, Colorectal carcinoma.

Introduction

Colorectal carcinoma (CRC) is a type of malignant neoplasm in the human digestive tract with a high morbidity rate[1]. Its incidence and mortality are ranked third and fourth highest, respectively, of all malignant tumors[2,3]. Therefore, it is regarded as a serious threat to human health. Because of economic development and changes in people's lifestyle, the incidence of CRC is much higher than before[4]. In recent years, the rising morbidity rate of CRC has slowed down[5]. However, because of its subclinical manifestations and its characteristic poor prognosis, CRC has a substantial negative impact on human health[6]. Therefore, CRC is still one of the most serious human diseases.

Research into the mechanisms underlying tumorigenesis in CRC has primarily focused on protein-coding genes[7]. However, some studies[8-10] have revealed that non-coding RNAs (ncRNAs) are involved in regulating a wide range of physiologic functions, including cell proliferation, migration, apoptosis and tumor development. NcRNAs are comprised of microRNAs (miRNAs), tRNAs, siRNAs, snoRNAs and long non-coding RNAs (lncRNAs)[11]. MiRNAs have attracted a fair amount of attention over the past few decades[12]. They can induce mRNA degradation or translational inhibition and hence can mediate post-transcriptional gene silencing[13,14]. Specifically, approximately 30% of human genes are estimated to be regulated by miRNAs and their dysregulation is associated with several types of cancer[15,16]. In addition, it has been shown that miRNAs can influence cell cycle progression,

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cell proliferation, cell invasion and apoptosis in cancer by binding with their mRNA targets[17]. In addition to miRNAs, much evidence has demonstrated that lncRNAs, defined as a type of RNA with a length of > 200 nucleotides, can regulate physiological and pathological processes by interacting with DNA, RNA and protein molecules[18]. The lncRNAs are generally deregulated in a wide variety of diseases, including Alzheimer's disease, heart disease and cancer[19-21]. To date, a few lncRNAs have been reported to be related to the progression of human cancer[22,23]. Xu et al[24] demonstrated that the long noncoding RNA SNHG1 regulates colorectal cancer cell growth through interactions with EZH2 and miR-154-5p.

LncRNA ADIPOQ is located on chromosome 12F1 and its expression is upregulated in several normal tissues such as colon, stomach and liver[25]. Tan et al[26] found that ADIPOQ was specifically downregulated in colorectal cancer patients. However, the roles and functions of ADIPOQ in colorectal cancer are poorly understood so far. In this study, we aimed to explore the biological roles of ADIPOQ in colorectal cancer, as well as to illustrate the molecular mechanisms. We first detected the ADIPOQ levels in colorectal cancer tissues and Caco-2 cell line. Then, the proliferation, migration and invasion abilities of Caco-2 cells were measured after transfection of lentiviral ADIPOQ. Finally, we assessed the regulatory relationship between ADIPOQ and miR-219c-3p, miR-219c-3p and TP53, respectively, and found that ADIPOQ could bind with miR-219c-3p to further regulate the expression of TP53. Our study uncovered a critical role of lncRNA-ADIPOQ in colorectal cancer progression, which serves as ceRNA by modulating the inhibitory effect of miR-219c-3p on TP53.

Patients and Methods

Patients and Tumor Samples

In this work, 10 pairs of colorectal cancer tissue samples and adjacent normal tissues were collected from surgically treated colorectal cancer cases and then stored at -80°C. Patient information was included in Table I. No significant differences were found in the 10 pairs of samples in terms of diagnostic indicators and prognostic factors. This investigation was approved by the Ethics Committee of our Hospital. Patients and their families had been fully informed that their specimens would be used for scientific research, and all participating patients had signed informed consent.

Table I. Demographic data.

	Age < 45 years	Age > 45 years
Patients numbers	7	8
Sex ratio (M/F)	3/4	4/4
BMI (kg/m^2) ± SD	22.9 ± 3.6	23.2 ± 2.9

All the patients were selected randomly.

Cell Culture

Human colorectal cancer cells Caco-2 cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) supplied with 10% FBS (Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin (Gibco, Rockville, MD, USA) and incubated at 37°C in an atmosphere of 5% CO_2.

Construction of Lentivirus and Cell Transfection

Lentiviral Lnc ADIPOQ and lnc ADIPOQ shRNA were synthesized and constructed by Shanghai GenePharma Co., Ltd (Shanghai, China). For miRNA analysis, the miR-219c-3p mimic, miR-219c-3p inhibitor and the negative control were constructed by Shanghai GenePharma Co., Ltd (Shanghai, China). To knock down TP53, si-TP53 plasma and negative control plasma were constructed by Shanghai GenePharma Co., Ltd (Shanghai, China). For transfection, 1×10^4 cells were seeded in 6-well plates and cultured with RANKL (100 ng/mL) and M-CSF (100 ng/mL). Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA) and Opti-MEM® I reduced serum medium were used for transfection. For analysis of Lnc-ADIPOQ, cells were transfected with Lnc-ADIPOQ shRNA (referred as to sh) and negative control shRNA (referred as to nc), respectively. For analysis of miR-219c-3p, cells were transfected with miR-219c-3p inhibitor, and control cells were transfected with empty vector respectively. The cells without transfection were used as the control (referred as to control). After incubated for 30 min, cultures were replaced with DMEM containing 10% FBS. Then, at indicated time point after transfection, cells were harvested for further study.

Transwell Assay

To test the migration ability of Caco-2 cells, transwell plates with a pore size of 8 μm (Millipore Inc., Billerica, MA, USA) were used to conduct transwell assay. Caco-2 cells were treated differently, and the lower chamber was added with DMEM supplemented with 20% FBS. The upper side of the membrane was wiped with a cotton swab to remove the cells that did not migrate, and the cell numbers in five random fields were counted in each sample.

RNA Extraction and qRT-PCR

After taking out the culture plates, the cells were washed with phosphate-buffered saline (PBS). After treatment, total RNA of cells was extracted by using TRIzol reagent (Life Technologies, Waltham, MA, USA) according to the manufacturer's instructions. Samples were stored at room temperature for 30 min. The reverse transcription of cDNA was performed with a PrimeScript$^{\text{TM}}$ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. For qRT-PCR, PCR primers were synthesized by GenePharma (Shanghai Gene Pharma, Shanghai, China) and sequences were listed in Table II. SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) was used to detect the expression.

CCK-8 Assay

The CCK-8 kit (Dojindo Molecular Technologies, Kumamoto, Japan) was used to measure the cells proliferation according to the manufacturers' instructions. In brief, 5×10^3 cells were seeded in 96-well plates uniformly. After treated with regulated medium, the medium was removed, and cells were washed with PBS solution for 3 times. Then, CCK-8 dilution was added to the 96-well plates and incubated at 37°C in an atmosphere of 5% CO_2 for 2 hours. After incubation, the plates were taken out, and cell proliferation was measured using multi-detection microplate reader. The absorbance (OD) value at 490 nm of each well was detected.

Luciferase Assay

After transfection for 48 h, the luciferase activities were measured using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) according to the manufacturer's protocol. *Renilla* Luciferase activities were normalized to the firefly Luciferase activities and the data were expressed as the fold change relative to the corresponding control groups, which were defined as 1.0.

Statistical Analysis

Unless otherwise indicated, all data are processed by Statistical Product and Service Solutions (SPSS) 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Each assay was applied at least three independent experiments or replicates. All data were presented as mean ± SD. Student's *t*-test, one-way analysis of variance (ANOVA) and multiple comparison between the groups was performed by using SNK method, in which $*p < 0.05$, $**p < 0.01$ represented as the difference significance.

Results

ADIPOQ Was Downregulated in Human Colorectal Cancer Tissues and Cells

We first used the previously described gene expression data of 15 patients with colorectal cancer tissues to screen the lncRNAs that specifically low expressed in colorectal cancer tissues. Cluster heatmap showed the differential expressed lncRNAs between colorectal cancer tissues and adjacent normal tissues (Figure 1A). By bioinformatics screening, we identified four lncRNAs including Fntb, Lgi1, AK052226 and ADIPOQ

Table II. Primer sequences for qRT-PCR.

Genes	Forward	Reverse	Tm (°C)
ADIPOQ	5'-CAGCCCGACACAAGCATTCC-3'	5'-CATCCATTTTCAGGATGCGGA-3'	60
miR-219-3p	5'-GGTTATAGCCCTAGAGAGACA-3'	5'-ACACACCGGAAGACATTTACA-3'	61
TNFSF11	5'-GCTCCTCTTAGGGGCCACT-3'	5'-GAGACGGGACCCTTAGGCCAT-3'	62
TNFSF13	5'-AAGTCTCAATTCCTCCCAGG-3'	5'-AGATGCTTGTCTGGGTATATGT-3'	62
TP53	5'-CACTCCCACCCTGAGATTTGT-3'	5'-CATCGTCTGCACGGTTCATGCG-3'	60
GAPDH	5'-TGGATTTGGACGCATTGGTC-3'	5'-TTTGCACTGGTACGTGTTGAT-3'	62

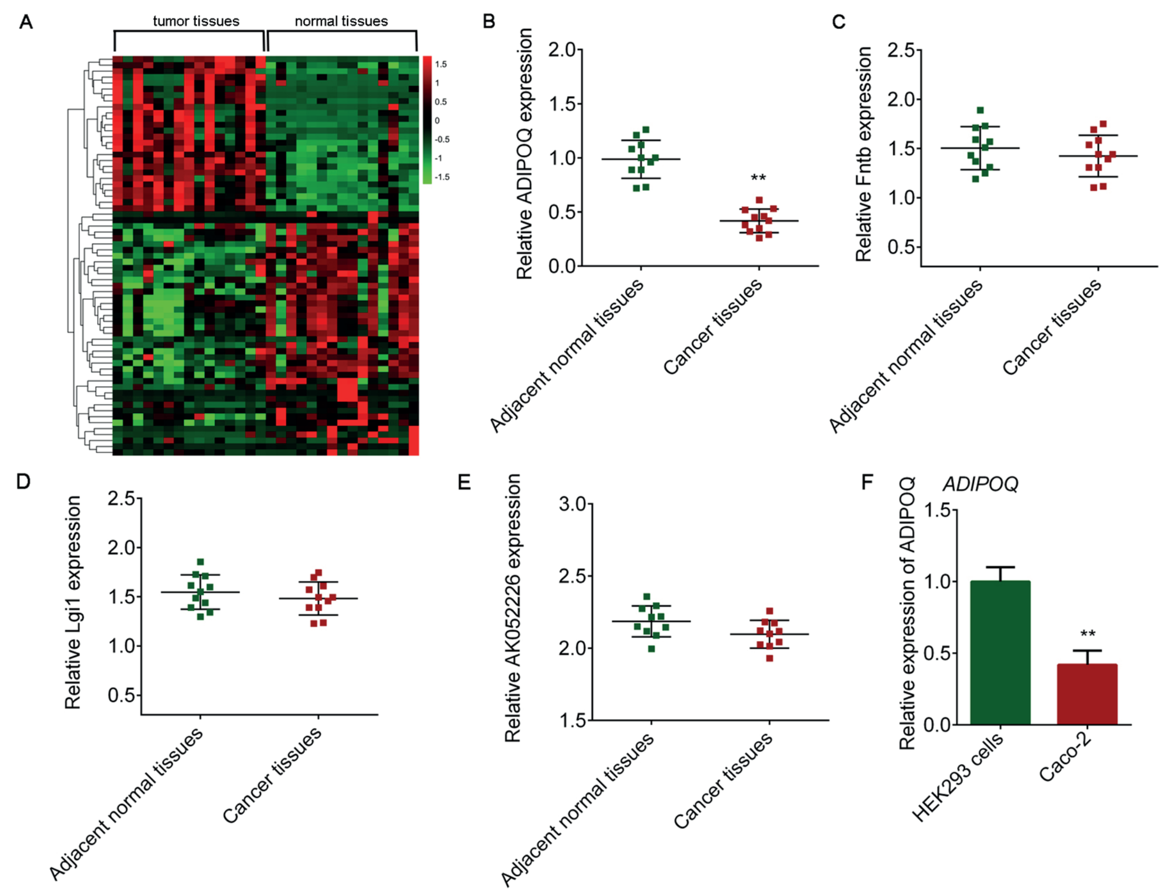

Figure 1. ADIPOQ was downregulated in human colorectal cancer tissues and cells. **A**, Heatmap of differentiated expressed LncRNA in 15 human colorectal carcinoma and adjacent non-cancerous normal tissues. **B**, Relative mRNA expression levels of ADIPOQ in colorectal cancer tissues and adjacent normal tissues. **C-E**, Relative mRNA expression levels of Fntb, Lgi1 and AK052226 in colorectal cancer tissues and adjacent normal tissues. **F**, Relative mRNA expression levels of ADIPOQ in human colorectal cancer cell line Caco-2 and HEK293 cells. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

that might downregulate in colorectal cancer tissues. We then performed qRT-PCR to detect those gene expressions in our clinical samples (n=10). The results showed that ADIPOQ was significantly reduced in colorectal cancer tissues (Figure 1B), while no differences had been found in other three lncRNAs (Figure 1C-E). To further interpret the biological functionality of ADIPOQ in colorectal cancer, we performed qRT-PCR analysis to detect ADIPOQ expression in human colorectal cancer cell lines Caco-2. The results suggested that ADIPOQ expression was also significantly downregulated in Caco-2 cells compared with human epithelia cells HEK293 cells ($p < 0.05$) (Figure 1F).

Upregulation of ADIPOQ Inhibited the Proliferation of Human Colorectal Cancer Cells

To explore the functions of ADIPOQ in colorectal cancer progression, ADIPOQ overexpressing lentiviral (Lnc-ADIPOQ) was constructed and transfected into Caco-2 cells. Besides, small interfering RNA for ADIPOQ (si-ADIPOQ) was also synthesized and transfected into Caco-2 cells. The expression of ADIPOQ was subsequently detected by qRT-PCR. The results showed that the expression of ADIPOQ in the Lnc-ADIPOQ group was significantly enhanced compared with the vectorl group ($p < 0.05$), while the expression levels of ADIPOQ were reduced in the si-ADIPOQ group compared with the negative control vector2 group ($p < 0.05$) (Figure 2A, 2B). To verify the role of ADIPOQ on cell proliferation, CCK8 assay was performed on Caco-2 cells after regulation of ADIPOQ expression. The results revealed that overexpression of ADIPOQ significantly reduced colorectal cancer cells proliferation compared with the control group, whereas inhibition of ADIPOQ expression remarkably increased the cell proliferation number at 3 days (Figure 2C, 2D). qRT-PCR analysis showed that the expression of tumor proliferation

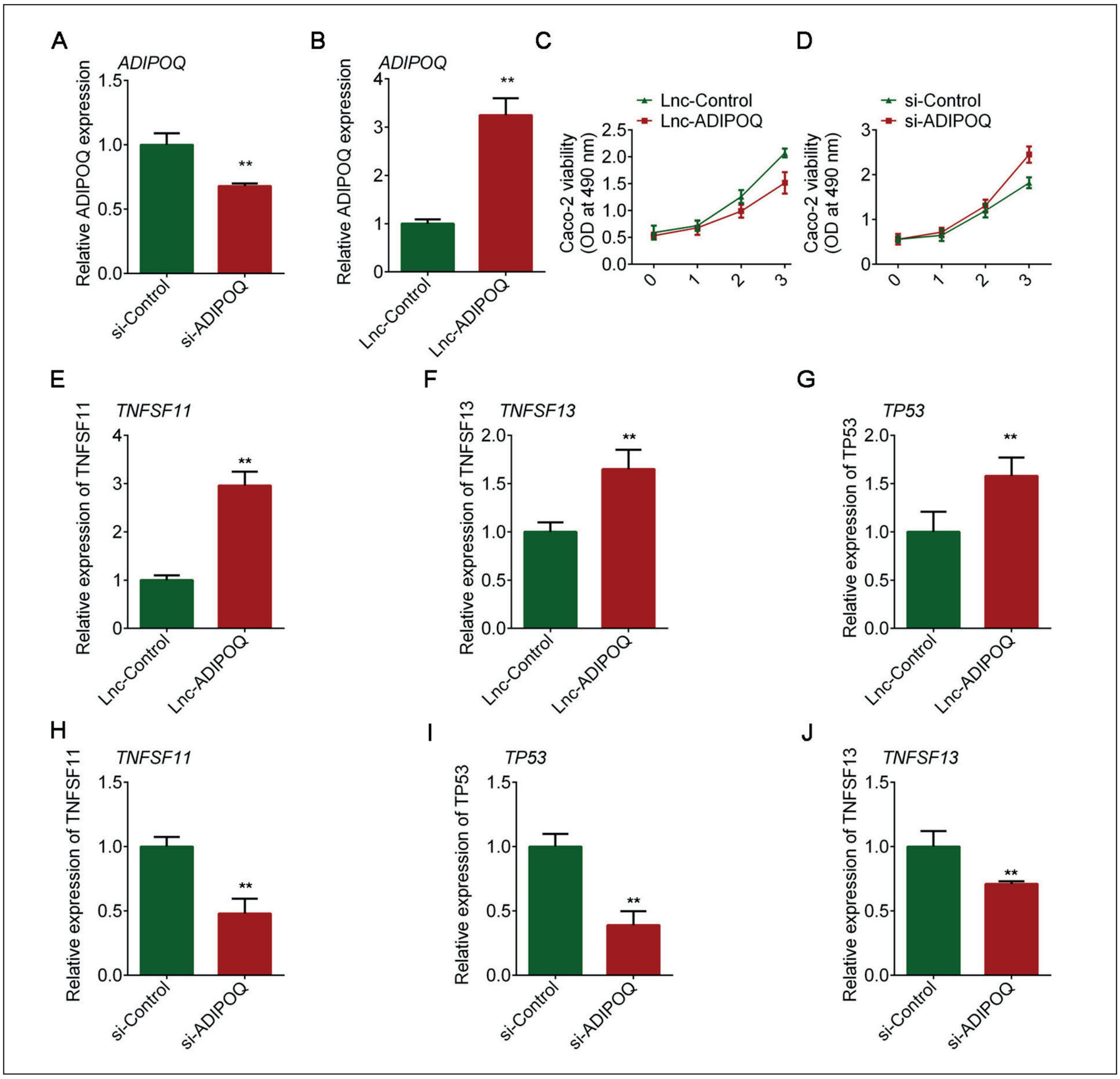

Figure 2. Upregulation of ADIPOQ inhibited the proliferation of human colorectal cancer cells. **A**, Relative mRNA expression levels of ADIPOQ in Caco-2 cells transfected with si-Control and si-ADIPOQ. **B**, Relative mRNA expression levels of ADIPOQ in Caco-2 cells transfected with ADIPOQ overexpressing lentiviral (Lnc-ADIPOQ) and Lnc-Control. **C**, Absorption at 490 nm of Caco-2 cells treated with Lnc-ADIPOQ and Lnc-Control detected by CCK-8 assay at 1 d, 2 d and 3 d. **D**, Absorption at 490 nm of Caco-2 cells treated with si-ADIPOQ and si-Control detected by CCK-8 assay at 1 d, 2 d and 3 d. **E-G**, Relative mRNA expression levels of TNFSF11, TNFSF13 and TP53 in Caco-2 cells transfected with ADIPOQ overexpressing lentiviral (Lnc-ADIPOQ) and Lnc-Control. **H-J**, Relative mRNA expression levels of TNFSF11, TNFSF13 and TP53 in Caco-2 cells transfected with si-Control and si-ADIPOQ. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

suppressor genes including TNFSF11, TNFSF13 and TP53 were upregulated after overexpression of ADIPOQ (Figure 2E-2G), and it was reversed after ADIPOQ inhibition (Figure 2H-2J). These results suggested that changing the expression of ADIPOQ could transform the proliferation of colorectal cancer cells.

The Migration Ability of Colorectal Cancer Cells Was Significantly Inhibited After Upregulating the ADIPOQ Expression

To further explore whether ADIPOQ affects the migration and invasion of tumor cells, we performed transwell assay to detect the migration ability after the expression of ADIPOQ was increased in Caco-2 cells. The results revealed that after ADIPOQ upregulation, the number of Caco-2 cells that transferred through transwell chambers was significantly reduced in response to fetal bovine serum compared with control group (Figure 3A). Besides, after inhibition of ADIPOQ expression, the number of Caco-2 cells transferred through transwell chambers was significantly increased (Figure 3B). These data suggested that ADIPOQ alteration regulated the migration ability of human colorectal cancer cells; upregulated ADIPOQ can effectively inhibit the migration and invasion of colorectal cancer cells, making ADIPOQ a potential target for tumor therapy.

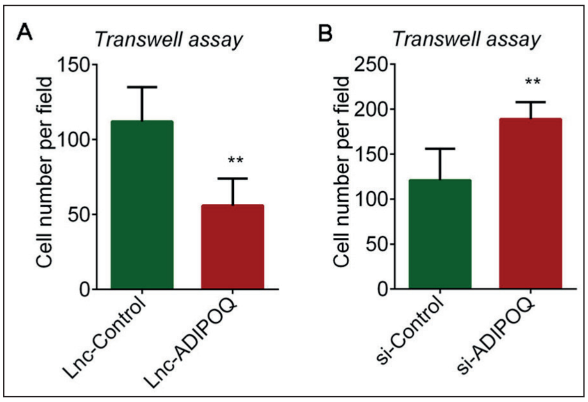

Figure 3. The migration ability of colorectal cancer cells was significantly inhibited after upregulating the ADIPOQ expression. **A**, The invasion of cells transfected with lnc-ADIPOQ was measured by transwell invasion assays. **B**, The invasion of cells transfected with si-ADIPOQ was measured by transwell invasion assays. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

ADIPOQ Can Sponge with MiR-219c-3p and Inhibit its Expression in Colorectal Cancer Cells

It has been previously suggested that LncRNAs can act as a competing sponge in regulating the biological functions of miRNAs. To investigate whether ADIPOQ was correlated miRNA, we used StarBase 2.0 to predict the target miRNA of ADIPOQ and found that miR-219c-3p is a target miRNA of ADIPOQ. Then, we used qRT-PCR analysis to detect the miR-219c-3p expressions of human colorectal cancer tissues and Caco-2 cells. Results showed that miR-219c-3p was highly expressed in colorectal carcinoma compared with adjacent normal tissues and was also upregulated in Caco-2 cells compared with HEK293 cells (Figure 4A and 4B). Correlation analysis was performed to investigate the expression relationship between ADIPOQ and miR-219c-3p. Results revealed that miR-219c-3p was remarkably negatively correlated with ADIPOQ, suggesting that miR-219c-3p might be regulated by ADIPOQ (Figure 4C). These results suggested the high expression of miR-219c-3p in colorectal cancer tissues and Caco-2 cell line, which was negatively correlated with ADIPOQ. Therefore, we assumed that ADIPOQ could modulate the migration and invasion of colorectal carcinoma through interacting with miR-219c-3p. To further confirm this, ADIPOQ-wt Luciferase reporter vector and ADIPOQ-mut 3'UTR Luciferase reporter vector were synthesized, and Luciferase reporter assay was performed (Figure 4D). Compared with the control, the Luciferase activity of Caco-2 cells that co-transfected with wild type ADIPOQ (ADIPOQ-wt) and miR-219c-3p mimic was significantly decreased ($p < 0.01$), and it was reversely increased after mutation at the binding site of ADIPOQ (ADIPOQ-mut) compared with ADIPOQ-wt ($p < 0.01$) (Figure 4E). These results demonstrated that ADIPOQ could directly bind to miR-219c-3p. Besides, ADIPOQ overexpression suppressed miR-219c-3p expression and ADIPOQ inhibition reversely facilitated miR-219c-3p expression in Caco-2 cells (Figure 4F, 4G). Additionally, we also transfected miR-219c-3p mimic and miR-219c-3p inhibitor into Caco-2 cells; the results revealed that miR-219c-3p mimic inhibited ADIPOQ expression and miR-219c-3p inhibitor increased ADIPOQ expression (Figure 4H, 4I). Taken together, these results demonstrated that ADIPOQ can directly sponge with miR-219c-3p.

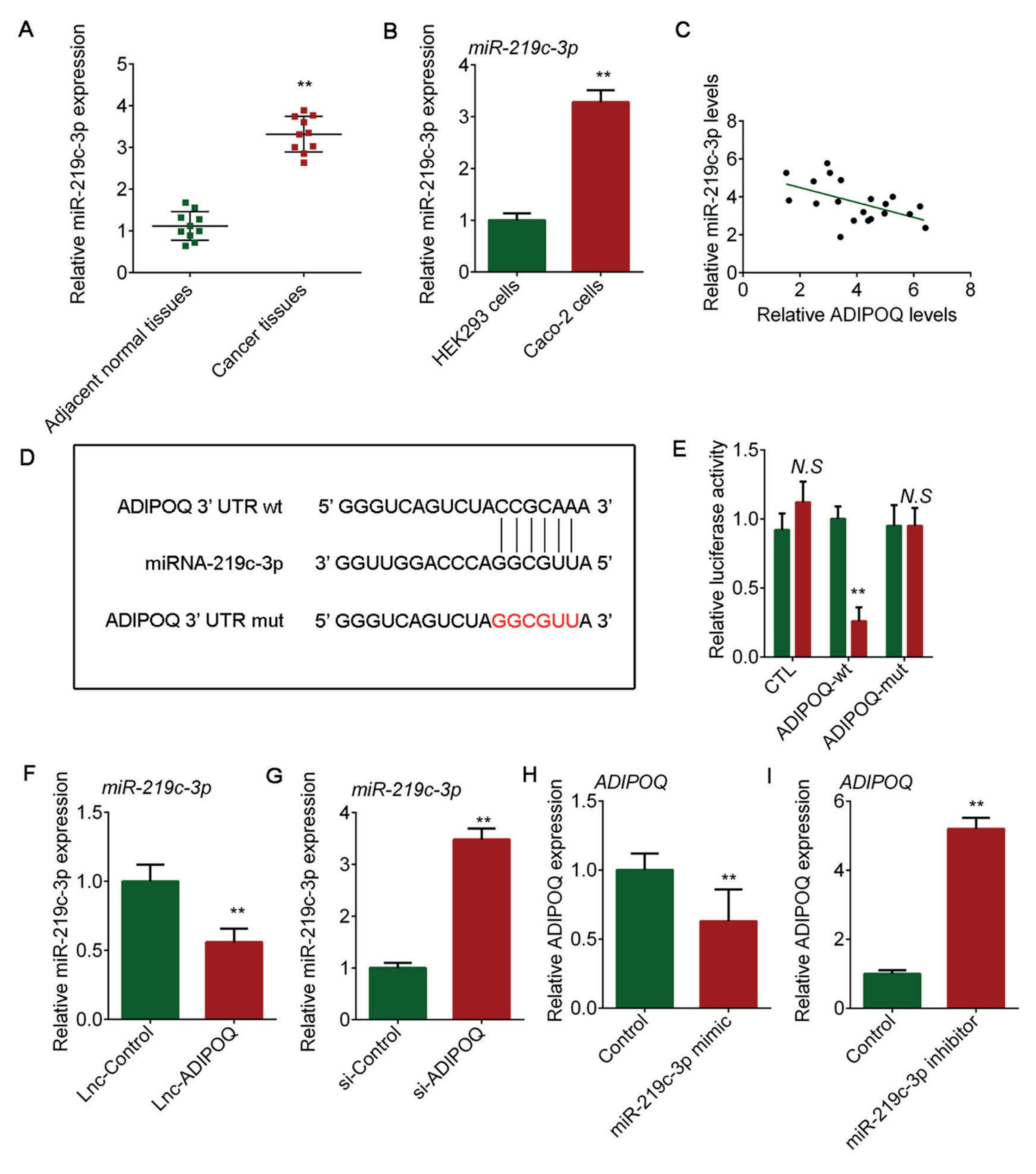

Figure 4. ADIPOQ can sponge with miR-219c-3p and inhibit its expression in colorectal cancer cells. **A**, Relative expression of ADIPOQ in colorectal cancer tissues and adjacent non-cancerous normal tissues. **B**, Relative expression of ADIPOQ in HEK293 cells and colorectal cancer cells. **C**, Correlation analysis was performed to determine the relationship between ADIPOQ and miR-219c-3p. **D**, Schematic illustration of the predicted miR-219c-3p binding sites and mutant sites in ADIPOQ. **E**, Relative luciferase activity of Caco-2 cells. **F-G**, qRT-PCR analysis of miR-219c-3p expression level in Caco-2 cells transfected with lentiviral ADIPOQ and si-ADIPOQ. **H-I**, Relative ADIPOQ expression was detected in Caco-2 cells after treated with miR-219c-3p mimics and miR-219c-3p inhibitor by RT-PCR. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

ADIPOQ Serve as ceRNA of MiR-219c-3p to Modulate the Expression of TP53

The tumor suppressor gene TP53 is one of the most frequently mutated genes in human cancer. To investigate whether miR-219c-3p interacts with TP53, we performed qRT-PCR analysis for TP53 in the presence of miR-219c-3p mimics or inhibitor. We observed decreased TP53 expression after Caco-2 cells were transfected with the miR-219c-3p mimics, which suggested that miR-219c-3p could downregulate TP53 expression (Figure 5A). To validate this mechanism, we cloned the mice TP53 3′-UTR into the Luciferase reporter vector and constructed miR-219c-3p binding mutants in which the putative miR-219c-3p binding sites GGUCC in the TP53 3′-UTR were mutated into CCAGG (Figure 5B). As expected, Dual-Luciferase report results showed that miR-219c-3p mimics significantly downregulated the TP53 expression whereas point mutations in the TP53 3′-UTR abrogated the suppressed effect of miR-219c-3p (Figure 5C). Then, we validated whether ADIPOQ can regulate TP53 expression via sponging with miR-219c-3p. The results showed that ADIPOQ could significantly increase TP53 expression; however, mutation of the binding site with ADIPOQ of miR-219c-3p eliminated the function effectively (Figure 5D). Conversely, inhibition of miR-219c-3p overcame the suppression of TP53 by ADIPOQ knockdown (Figure 5E). Taken together, these data demonstrated that ADIPOQ could serve as ceRNA for the miR-219c-3p to further modulate TP53.

Discussion

As a "bridge" between DNA and protein, the complicated regulatory role of RNA has long been neglected. In eukaryotic cells, protein-coding RNA (mRNA) only accounts for about 2% of the genome, and the rest large number of

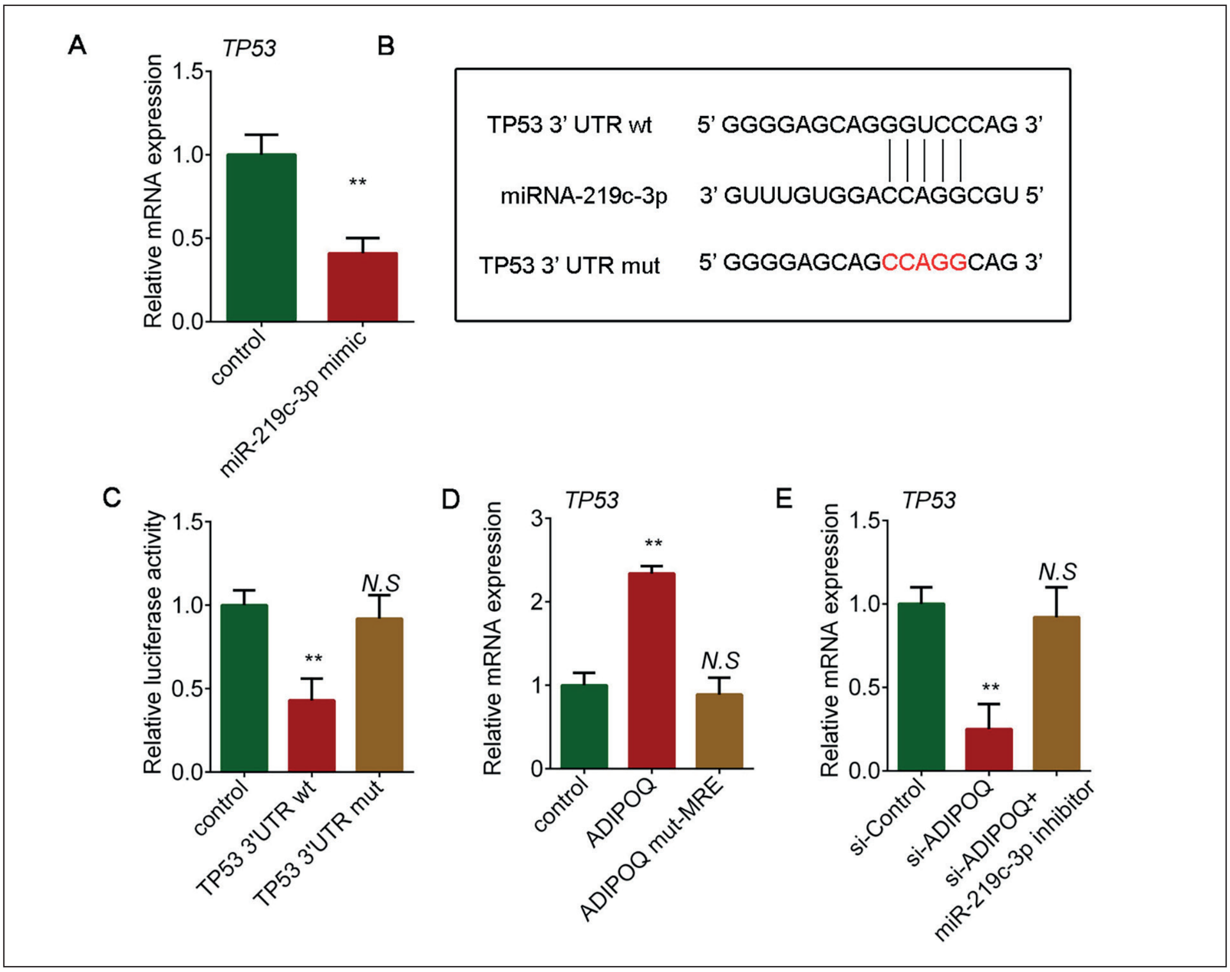

Figure 5. ADIPOQ modulated the expression of TP53 by sponging with miR-219c-3p. **A**, qRT-PCR analysis of TP53 mRNA expression level in Caco-2 cells treated with the miR-219c-3p mimics. **B**, Schematic illustration of the predicted TP53 binding sites and mutant sites in miR-219c-3p. **C**, Relative luciferase activity of Caco-2 cells. **D**, Relative mRNA expression levels of TP53 in Caco-2 cells transfected with ADIPOQ and ADIPOQ mut-MRE. **E**, Relative mRNA expression levels of TP53 in Caco-2 cells transfected with si-ADIPOQ, si-ADIPOQ and miR-219c-3p inhibitor by qRT-PCR analysis. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

transcripts are categorized to non-protein coding RNAs (ncRNAs)[27]. Unlike ribosomal RNA (rRNA) and transfer RNA (tRNA), which have been well-acknowledged, other non-coding RNAs (ncRNAs) were thought to be transcriptional "noises" once upon a time[28-30]. However, increasing research[31,32] have revealed that ncRNAs play a pivotal role in cellular process. In the past decade, miRNAs (20-24 nt) were most intensively studied among all the ncRNAs. These small miRNAs bind to the complementary site on the 3′ untranslated region (UTR) of targeting mRNAs called miRNA binding elements (MREs) and block protein translation or modulate mRNA stability on a post-transcriptional level[33]. However, different from miRNAs, the role of lncRNAs is barely explored. LncRNAs have transcripts longer than 200 nucleotides[33]. Due to their length, lncRNAs can fold into secondary or higher orders of structure, thus make it more flexible in targeting proteins or gene sites[34]. Further, because of the differential splicing and alternative transcription initiation sites, lncRNAs are becoming more and more complex[35].

CRC is one of the most common malignancies worldwide and is the fourth leading cause of cancer-related death[36]. Under most circumstances, symptoms of cancer are detected at an advanced stage, leading to poor prognosis[37,38]. Consequently, the exploration of new diagnostic and therapeutic biological targets for CRC is pivotal. In addition to microRNAs, emerging discoveries suggest that lncRNAs are also important factors in cell biology. It has been suggested that lncRNA dysregulation is associated with a variety of human diseases, including cancer[39,40]. Recently, emerging evidence demonstrates that lots of noncoding RNAs (ncRNAs) are closely associated with colon cancer tumorigenesis[19,41]. As an important member of ncRNA, lncRNAs have been reported to play a pivotal role in colon cancer development and progression[42]. However, the role and mechanism of ADIPOQ in colon cancer remained unknown.

Conclusions

In this study, we first validated the low expression of ADIPOQ in colorectal cancer and demonstrated its important role in proliferation of human colorectal cancer cells Caco-2. Besides, we found that altered expression of ADIPOQ could regulate the migration and invasion of Caco-2 cells. Using bioinformatics prediction, we screened out miR-219c-3p as a target miRNA of ADIPOQ followed by validation of the combination relationship between ADIPOQ and miR-219c-3p using Luciferase reporter assay. Furthermore, we found that miR-219c-3p can interact with ADIPOQ co-expression gene TP53 and downregulate the expression of TP53. Overexpression of ADIPOQ could significantly upregulate TP53 gene expression. However, mutagenesis of the miR-219c-3p recognition element in ADIPOQ alleviates the function effectively. Hence, we demonstrated that ADIPOQ served as ceRNA of miR-219c-3p to upregulate TP53 expression.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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