RETRACTED ARTICLE: Silencing UNC5B antisense IncRNA 1 represses growth and metastasis of human Colon cancer cells via raising miR-622

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ABSTRACT

Background: Colon cancer is the most frequently lethal cancer in digestive system. Herein, we tested the influences of UNC5B antisense IncRNA 1 (UNC5B-AS1) on colon cancer cell growth and metastasis, along with the regulatory function of UNC5B-AS1 in microRNA-622 (miR-622) expression.

Methods: Firstly, UNC5B-AS1 expression in colon cancer tissues and corresponding normal tissues were tested. Then, the influences of silencing UNC5B-AS1 by sh-UNC5B-AS1 transfection on colon cancer HCT116 and Caco-2 cell viability, proliferation, migration, invasion and apoptosis, as well as miR-622 expression were assessed, respectively. Subsequently, whether miR-622 attended to the influences of silencing UNC5B-AS1 on HCT116 and Caco-2 cells were probed. Finally, the activities of AMPK and PI3K/AKT pathways in cells were analysed.

Results: UNC5B-AS1 had high expression level in colon cancer tissues. Silencing UNC5B-AS1 repressed HCT116 and Caco-2 cell proliferation, migration and invasion, but boosted cell apoptosis. Moreover, silencing UNC5B-AS1 raised miR-622 expression in HCT116 and Caco-2 cells. miR-622 inhibitor transfection weakened the influences of silencing UNC5B-AS1 on HCT116 and Caco-2 cells. Besides, Silencing UNC5B-AS1 suppressed AMPK and PI3K/AKT pathways via raising miR-622.

Conclusion: Silencing UNC5B-AS1 repressed colon cancer growth and metastasis might be through raising miR-622 expression and suppressing AMPK and PI3K/AKT pathways.

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Introduction

Cancer is a major public health issue worldwide. Colon cancer is the most frequently lethal cancer in digestive system [1]. In 2019, 101420 new cases were diagnosed with colon cancer and 51020 colon cancer-related deaths are predicted to occur in the United States [1]. The importance and influence of cellular epigenetic modification on cancer initiation and development has been widely studied in recent years [2,3]. Many types of epigenetic modifications have been found to take part in the occurrence of colon cancer [4,5]. We believe that probing the molecular mechanism related to epigenetic modification on colon cancer cell growth and metastasis is of great important sense for the diagnosis and therapy of colon cancer.

Long non-coding RNAs (lncRNAs) are RNA transcripts in cells without protein-coding capabilities, but emerge gene mediatory activities at post-transcriptional level [6]. As a key type of epigenetic modification, aberrant expression of lncRNAs has been reported to engage in the initiation and advancement of many cancers, covering colon cancer [7,8]. UNC5B antisense IncRNA 1 (UNC5B-AS1) is a recently confirmed lncRNA in human papillary thyroid cancer [9]. It has been discovered to be up-regulated in papillary thyroid cancer and be correlated with the clinical-pathological features of papillary thyroid cancer [10,11]. Moreover, knockdown of UNC5B-AS1 has been testified to exhibit anti-proliferative and anti-metastatic activities in papillary thyroid cancer cells [10]. Until now, there is no any literature can be searched concerning the influences of UNC5B-AS1 in other cancers. Whether UNC5B-AS1 takes part in the initiation and development of colon cancer is still needed to be probed.

Apart from lncRNAs, microRNAs (miRNAs) are another class of regulatory RNAs in cells, which also engage in adjusting cellular gene expression and cancer development [12]. Moreover, it was proved that lncRNAs emerged in gene expression regulatory activities usually via modulating miRNAs expression [13]. miRNA-622 (miR-622) has been discovered to be down-regulated in colon cancer tissues and repress colon cancer occurrence and metastasis through declining oncogenes expressions and raising tumour suppressive genes expressions [14,15].

In the current research, we analysed the UNC5B-AS1 expression in colon cancer tissues and corresponding normal tissues. Then, the influences of silencing UNC5B-AS1 in colon cancer HCT116 and Caco-2 cell proliferation, apoptosis, migration and invasion, along with the internal mechanisms...
related to miR-622, were probed. In our opinion, the outcomes of our research will offer novel experimental evidence for comprehending the critical role of UNC5B-AS1 in colon cancer growth and metastasis.

Materials and methods

Clinical samples

Twenty-five patients with colon cancer were selected in this study, which subjected to any therapy prior to surgery. Tumour tissues and para-carcinoma tissues were collected during surgery. The research was encouraged by the Medical Ethics Committee of our hospital. These participants signed the informed consents before surgery.

Cell lines

HCT116 and Caco-2 cells were both received from Procell Inc., (CL-0096 and CL-0050, Wuhan, China). HCT116 cells were grown in MoCoy’s 5A medium (PM150710, Procell Inc.,) including 10% fetal bovine serum (FBS, F8687, Sigma-Aldrich, MO, USA) at 37°C with 5% CO2 and 95% air. Caco-2 cells were grown in Minimum Essential Medium (MEM, PM150410, Procell Inc.,) including 20% FBS at 37°C with 5% CO2 and 95% air.

Quantitative reverse transcription PCR (qRT-PCR)

Total RNAs were isolated through exploiting RNAiso Plus (9108, Takara, Beijing, China). For testing UNC5B-AS1 expression, High Capacity RNA-to-cDNA™ kit (4387406, Applied Biosystem, CA, USA) was done for compounding cDNA. qRT-PCR was carried out using TaqMan™ Non-coding RNA Assay (4426961, Applied Biosystem) and compared to GAPDH expression. For testing miR-622 expression, mirVana™ qRT-PCR miRNA Detection kit was carried out in the light of the manufacturer’s instruction and compared to U6 snRNA expression. Consequences were computed via adopting 2−ΔΔCt method [16]. The correlative sequences used in qRT-PCR as followed: UNC5B-AS1, Forward: 5′-GAT CCT GCC TCA GGG AAA-3′, Reverse 5′-GCTCAA GAG GTT GGG ACT-3′; GAPDH, Forward 5′-GCG AGT CAA CGG ATT TG-3′, Reverse 5′-ATG ACC CCC AGC TCT CTC CAT-3′; miR-622, Forward 5′-ACA CTC CAG CTG GGA CAG TCT GCT GAG GT-3′, Reverse 5′-TGG TGT CTT GGG TTC GCT G-3′; U6, Forward 5′-CTC GTC GCT GCA GCA CA-3′, Reverse 5′-AAC GCT TCA CCA ATT TGC GT-3′.

Plasmids or miRNA transfection

The short-hairpin RNA targeting UNC5B-AS1 (5′-GGC GGA TCG CAG ACC CTA A-3′) was inserted into U6/GFP/Neo plasmid (BioVector NTCC Inc., Beijing, China) to form sh-UNC5B-AS1, U6/GFP/Neo plasmid carrying a non-targeting sequence served as a control (sh-NC, 5′-TTC GGG TCA TCC GAT GGG CC-3′). miR-622 inhibitor (5′-GCU CCA ACC UCA GCA GAC UGU-3′) and NC inhibitor (5′-CGA UUG GAC ACG GGG CCA AUC-3′) were achieved from GenePharma Corporation (Shanghai, China). Lipofectamine 3000 reagent (L3000-150, Invitrogen, CA, USA) was done for cell transfection. Transfection efficiencies were tested by qRT-PCR.

Cell counting kit-8 (CCK-8) assay

CCK-8 assay (HK-K0301, MedChem Express, NJ, USA) was applied to assay the viabilities of HCT116 and Caco-2 cells. After incubation, 10μl CCK-8 kit solution was supplemented to react with cells for 1 h at 37°C. After that, a Microplate Reader (Packard Instrument Company, CT, USA) was done for the detection of the absorbance at 450 nm.

Bromodeoxyuridine (Brdu) incorporation assay

Brdu (HY-15910, MedChem Express) incorporation assay was done for evaluating HCT116 and Caco-2 cell proliferation. Cells were fostered in 6-well plate (1×10⁵ cells/well) for 21 h. Subsequently, Brdu (1 mg/mL) was supplemented into the culture medium for 3 h at 37°C. Afterward, the number of Brdu positive (+) cells in each group was counted.

Cell apoptosis assay

For assaying HCT116 and Caco-2 cell apoptosis, Annexin V/PE apoptosis kit (C1065, Beyotime, Shanghai, China) was done. Cells were gathered as well as rinsed with kit buffer. Subsequently, 5μl Annexin V/PE solution was utilised for reacting 20 min under the darkness at 20–25°C. The percentage of apoptotic cells was assessed through flow cytometer (Attune Nxt, Thermo Fisher Scientific, MA, USA).

Cell migration and invasion assay

The migratory and invasive capacities of HCT116 and Caco-2 cells were assayed as earlier described [17].

Western blotting

RIPA Lysis Buffer (HY-K1001, MedChem Express) along with Protease Inhibitor Cocktail (P1010, Beyotime Biotechnology) was done for the separation of total proteins. The correlative procedures were executed on the basis of the earlier described [17]. Primary antibodies, including Cyclin D1 (ab226977), CDK6 (ab151247), Bax (ab53154), Caspase 3 (ab32351), p-AMPK (ab80039), p-AMPK (ab23875), t-PI3K (ab191606), p-PI3K (ab182651), t-AKT (ab8805), p-AKT (ab38449), β-actin (ab8227) and second antibodies (ab205718, ab205719) were all received from Abcam Biotechnology (MA, USA).

Statistical analysis

Graphpad 6.0 software was applied in statistical analysis, and consequences were emerged as mean ± SD. Analysis of difference was done utilising Student’s t-test or one-way analysis
silenced (p < .01). Results of Figure 3(A) presented that silence of UNC5B-AS1 notably lowered the viabilities of HCT116 and Caco-2 cells (p < .05 or p < .01). The percentages of BrdU+ cells were both reduced after silencing UNC5B-AS1 (Figure 3(B), p < .01). By contrast with sh-NC group, levels of Cyclin D1 and CDK6 in HCT116 and Caco-2 cells were declined in sh-UNC5B-AS1 group (Figure 3(C), p < .01). In addition, Figure 3(D) pointed out that silencing UNC5B-AS1 significantly elevated HCT116 and Caco-2 cell apoptosis (p < .01), along with the elevation of Bax and Cleaved-caspase 3 in HCT116 and Caco-2 cells (Figure 3(E), p < .01). The observations uncovered that silencing UNC5B-AS1 could repress colon cancer HCT116 and Caco-2 cell proliferation, but elevate cell apoptosis.

Silencing UNC5B-AS1 repressed Colon cancer cell migratory and invasive abilities

Influences of silencing UNC5B-AS1 in HCT116 and Caco-2 cell migratory and invasive capacities were assayed. Figure 4(A) showed that silencing UNC5B-AS1 obviously declined HCT116 and Caco-2 cell migration (p < .01). The percentages of invaded HCT116 and Caco-2 cells were also lowered by sh-UNC5B-AS1 transfection (Figure 4(B), p < .01). These consequences expounded that silencing UNC5B-AS1 could repress colon cancer HCT116 and Caco-2 cell migration and invasion.

Silencing UNC5B-AS1 raised miR-622 expression in colon cancer cells

Followed by sh-UNC5B-AS1 transfection, the miR-622 expressions in HCT116 and Caco-2 cells were tested. As shown in Figure 5, silencing UNC5B-AS1 dramatically raised the miR-622 expression in HCT116 and Caco-2 cells (p < .01), which hinted that elevation of miR-622 might engage in the influences of silencing UNC5B-AS1 in colon cancer cell behaviours.

miR-622 inhibitor transfection weakened the influences of silencing UNC5B-AS1 in colon cancer cell behaviours

miR-622 inhibitor was transfected into HCT116 and Caco-2 cells to knock down the miR-622 expression (Figure 6(A), p < .01). Data in Figure 6(B) showcased that miR-622 inhibitor transfection notably weakened the silencing UNC5B-AS1-caused HCT116 and Caco-2 cell viability reduction (p < .05). By contrast with sh-UNC5B-AS1 + NC inhibitor group, the percentages of BrdU+ HCT116 and Caco-2 cells were elevated in sh-UNC5B-AS1 + miR-622 inhibitor group (Figure 6(C), p < .05), along with the raising levels of Cyclin D1 and CDK6 in HCT116 and Caco-2 cells (Figure 6(D), p < .05). Besides, Figure 6(E–G) presented that silencing UNC5B-AS1-caused HCT116 and Caco-2 cell apoptosis elevation, migration inhibition and invasion reduction were also mitigated by miR-622 inhibitor transfection (p < .05). Relative to sh-UNC5B-AS1 + NC inhibitor group, the Bax and Cleaved-caspase 3 expression in HCT116 and Caco-2 cells were declined in sh-UNC5B-AS1 + miR-622 inhibitor group (Figure 6(H), p < .05 or p < .01).
Taken together, the above discoveries illuminated that silencing UNC5B-AS1 exhibited anti-tumour influences in colon cancer cells might be achieved via raising miR-622 expression.

**Silencing UNC5B-AS1 suppressed AMPK and PI3K/AKT pathways in colon cancer cells via raising miR-622**

Finally, the influences of sh-UNC5B-AS1 and/or miR-622 inhibitor transfection in AMPK and PI3K/AKT pathways were assayed. As presented in Figure 7A and B), sh-UNC5B-AS1 transfection significantly suppressed AMPK and PI3K/AKT pathways in HCT116 and Caco-2 cells via declining p/t-AMPK, p/t-PI3K and p/t-AKT levels (p < .01). Besides, miR-622 inhibitor transfection notably weakened the suppressive effects of sh-UNC5B-AS1 on AMPK and PI3K/AKT pathways in HCT116 and Caco-2 cells through raising p/t-AMPK, p/t-PI3K and p/t-AKT expression (p < .05 or p < .01). These above outcomes illustrated that silencing UNC5B-AS1 could suppress AMPK and PI3K/AKT pathways in colon cancer cells via raising miR-622.

**Discussion**

The accumulation of genetic and epigenetic modification has been demonstrated to conduce to the evolution of colon cancer [5,18]. Herein, we discovered that UNC5B-AS1 had high expression level in colon cancer tissues. Silencing UNC5B-AS1 repressed colon cancer cell proliferation, migration and invasion, but elevated cell apoptosis. Furthermore, silencing UNC5B-AS1 raised miR-622 expression in colon cancer cells. Repression of miR-622 attended to the influences of silencing UNC5B-AS1 in colon cancer cell proliferation, migration, invasion and apoptosis. Besides, silencing UNC5B-AS1 suppressed AMPK and PI3K/AKT pathways in colon cancer cells via raising miR-622.

It is predicted that exceeding 90% genes in mammalian cells are transcribed into non-coding RNAs [19]. Among them, IncRNAs take part in modulating many cellular physiological courses, such as chromosome dosage compensation, epigenetic modification, gene transcription and translation, as well as cell cycle conversion and cell fate decision [19,20]. The critical roles of IncRNAs in the cancer occurrence and progression are still widely studied [7]. Earlier literatures reported that UNC5B-AS1 was highly expressed in papillary
thyroid cancer and exhibited oncogenic activity [9–11]. In this study, we discovered that by contrast with corresponding normal tissues, UNC5B-AS1 had high expression in colon cancer tissues. Followed by silence of UNC5B-AS1, the proliferation of colon cancer cells was lowered. Cyclin D1 and CDK6 are important cell cycle regulatory factors [21]. We found that the Cyclin D1 and CDK6 protein levels were both declined in colon cancer cells after silencing UNC5B-AS1. Besides, silencing UNC5B-AS1 elevated HCT116 and Caco-2 cell apoptosis, along with the enhancement of Bax and Cleaved-caspase 3, two key pro-apoptotic molecules in cells [22]. These outcomes illustrated that UNC5B-AS1 also exhibit oncogenic roles in colon cancer and silencing UNC5B-AS1 could repress colon cancer cell growth, but elevate cell apoptosis.

Cancer metastasis is the main reason for poor prognosis of colon cancer [23]. Thus, limiting metastasis of colon cancer is also deemed to a main goal in remedying colon cancer. The migratory and invasive abilities of cancer cells determine the possibility of cancer metastasis [24]. In the current research, we discovered that silencing UNC5B-AS1 also repressed colon cancer HCT116 and Caco-2 cell migration and invasion, which illustrated that UNC5B-AS1 also engaged in the modulation of colon cancer metastasis and silencing UNC5B-AS1 could repress colon cancer metastasis. Moreover, in light of many other lncRNAs, such as lncRNA-activated by TGF-β (ATB) [25], lncRNA-cancer susceptibility 15 (CASC15) [26] and lncRNA-SUMO1P3 [27] also engaged in adjusting colon cancer growth and metastasis, we can put forward that colon cancer growth and metastasis are modulated by many lncRNAs that may from a very complicated regulatory network.

A mass of researches have testified that lncRNA served as a competingingendogenoud RNA (ceRNA) that be interacted with miRNA, thereby exhibiting the functions in heterogeneous cancers [28,29]. Additionally, evidences have showcased that IncRNA was mainly passed three ways to regulate miRNA expression. One of the most momentous ways is that IncRNA can exert the function of endogenous miRNA sponge to restrain the expression of miRNA and then affect the malignant biological behaviour of tumour cells [30]. Until now, it is not clear whether UNC5B-AS1 exerts oncogenic role via modulating miRNAs. Earlier studies reported that miR-622 was lowery expressed in colon cancer cells and

Figure 4. Silencing UNC5B-AS1 repressed colon cancer cell migration and invasion. HCT116 and Caco-2 cells were subjected to sh-UNC5B-AS1 transfection. (A) Cell migratory and (B) invasive abilities were assayed. p-values were computed utilising ANOVA along with Tukey’s post-hoc test and hinted as asterisk (*) in figure. **p < .01.

Figure 5. Silencing UNC5B-AS1 raised miR-622 expression in colon cancer cells. HCT116 and Caco-2 cells were subjected to sh-UNC5B-AS1 transfection. The miR-622 expression were tested, respectively. miR-622: microRNA-622. p-values were computed utilising ANOVA along with Tukey’s post-hoc test and hinted as asterisk (*) in figure. **p < .01.
exerted tumour inhibitory activity by reducing oncogenes expression and elevating tumour suppressive genes expression [14,31]. Herein, we discovered that silencing UNC5B-AS1 raised miR-622 expression in HCT116 and Caco-2 cells, which indicated a negative correlation between miR-622 and UNC5B-AS1. What’s more, knockdown of miR-622 by miR-622 inhibitor transfection notably weakened the influences of silencing UNC5B-AS1 on HCT116 and Caco-2 cell proliferation, migration, invasion and apoptosis. Therefore, we guessed that UNC5B-AS1 emerged its functions might through sponge of miR-622. Nonetheless, more researches are still needed to certify this conjecture.

AMPK and PI3K/AKT pathways are proved to be activated in colon cancer [32,33]. It is reported that unusual activations of AMPK and PI3K/AKT pathways contribute to growth, metastasis and drug resistance of colon cancer [34–36]. Hereon, silencing UNC5B-AS1 hampered AMPK and PI3K/AKT pathways in HCT116 and Caco-2 cells. Knockdown of miR-622
weakened the suppressive influences of silencing UNC5B-AS1 on AMPK and PI3K/AKT pathways in HCT116 and Caco-2 cells. These outcomes illustrated that UNC5B-AS1 silencing repressed colon cancer growth and metastasis might be via raising miR-622 meanwhile restraining AMPK and PI3K/AKT pathways. To our best knowledge, the regulation of AMPK and PI3K/AKT pathways are complicated. On the basis of several previous researches [35,36], we speculated that miR-622 affected AMPK and PI3K/AKT pathways might through adjusting the up-stream interrelated genes expression of these two pathways. Further researches are still necessary to probe this issue.

Conclusion

Taken together, this research affirmed the oncogenic activity of UNC5B-AS1 in colon cancer and analysed the regulatory function of UNC5B-AS1 on miR-622. Silencing UNC5B-AS1 repressed colon cancer growth and metastasis might be realised via raising miR-622 expression and impeding AMPK and PI3K/AKT pathways. Further researches are still worthy for the comprehensive analysis the lncRNA adjusting network in colon cancer, which is of great importance for comprehending the epigenetic modification of colon cancer.

Disclosure statement

The authors declare that there is no conflict of interest.


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