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Original Article

LncRNA SLC16A1-AS1 participates in the initiation and progression of colorectal cancer by regulating MAP3K9 expression through targeting miR-515-5p

Wanxing Xu[2], Suzhen Bi[1], Meichun Xing[1]

[1]Precision Research Center for Refractory Diseases, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201620, China; [2]Department of Laboratory Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201620, China

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Abstract: Objective: To investigate the role of long non-coding RNA (lncRNA) SLC16A1-AS1 in the initiation and progression of colorectal cancer (CRC). Methods: Cell viability was tested using Cell Counting Kit-8 (CCK-8). Cell invasion and migration were evaluated using Transwell assays, and apoptosis was determined by flow cytometry. Gene expression was tested by qRT-PCR or Western blot. The targeting relationship between miR-515-5p and MAP3K9 was verified using bioinformatics tools, RNA immunoprecipitation (RIP) experiments, and dual-luciferase reporter assays. Results: Both lncRNA SLC16A1-AS1 and MAP3K9 were upregulated in CRC cells, while miR-515-5p expression was downregulated. Overexpression of miR-515-5p and silencing of lncRNA SLC16A1-AS1 inhibited CRC cell proliferation, suppressed cell invasion and migration, and promoted cell apoptosis. The targeting relationship between lncRNA SLC16A1-AS1 and miR-515-5p, as well as between MAP3K9 and miR-515-5p, were confirmed by bioinformatics, RIP assays, and luciferase reporter assays. Conclusion: lncRNA SLC16A1-AS1 contributes to the initiation and progression of CRC by modulating miR-515-5p to regulate MAP3K9 expression, providing potential insights for CRC treatment.

Keywords: Long non-coding RNA SLC16A1-AS1, microRNA-515-5p, mitogen-activated protein kinase 9, colorectal cancer, proliferation, invasion, apoptosis

Introduction

Colorectal cancer (CRC) accounts for roughly 10% of all cancers and is a leading cause of cancer-related mortality globally. Roughly 20% of patients present with metastatic CRC at diagnosis, and up to 50% of patients with localized CRC eventually develop metastases, posing significant challenges in clinical management [1]. The progression of CRC is driven by various pathophysiological mechanisms, including aberrant cell differentiation, uncontrolled cell proliferation, distant metastasis, invasion of adjacent tissues, and resistance to apoptosis. These processes are influenced by a complex interplay of numerous environmental and genetic factors, involving a sedentary lifestyle, smoking, gut microbiota and obesity [2]. Early-onset CRCs are characterized by a diverse range of mutations that promote cancer development, higher mucosal histological incidence, distinctive DNA methylation profiles, more distant metastatic sites, and lower survival rates [2]. Despite some advances in targeted therapies for CRC in recent years, their clinical efficacy remains limited, and they are neither curative nor cost-effective [3].

Recent research has highlighted the important role of long non-coding RNA (lncRNA) in tumorigenesis and cancer progression. LncRNA are defined as RNA transcripts longer than 200 nucleotides that do not encode proteins. With advancements in molecular biology, lncRNAs has emerged as key players in oncology, offering new insights into cancer mechanisms [4]. Among them, lncRNA SLC16A1-AS1 has garnered considerable attention as a novel tumor-associated lncRNA. Increasing evidence suggests that SLC16A1 plays a crucial role in regu-

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lating tumor growth and metastasis. Specifically, SLC16A1-AS1, an antisense RNA to SLC16A1, is abnormally expressed in multiple cancer types [4]. Recent findings have emphasized the deregulated expression of SLC16A1-AS1 across multiple tumor types, functioning either as a tumor suppressor or an oncogene in the development of different cancers [5, 6]. The expression level of SLC16A1-AS1 is closely related to the clinical characteristics and prognosis of various tumors. For example, in CRC, high expression of SLC16A1-AS1 is associated with reduced patient survival, indicating its potential prognostic value [7]. SLC16A1-AS1 promotes CRC progression by regulating key signaling pathways, such as the MAPK signaling pathway, and modulating various microRNAs (miRNAs), thus influencing cell proliferation, migration, and invasion [8]. These functional characteristics suggest that SLC16A1-AS1 could serve as both a diagnostic and prognostic marker for CRC, as well as a potential target for therapeutic intervention.

SLC16A1-AS1 has also been reported to be overexpressed in hepatocellular carcinoma (HCC), where it plays an oncogenic role, potentially via its interaction with the miRNA (miR)-411/Microtubule Interacting and Transport Domain Containing 1 (MITD1) axis [5]. In contrast, SLC16A1-AS1 is down-regulated in triple-negative breast cancer (TNBC), and this suppression can inhibit cell cycle progression and TNBC cell proliferation by modulating the miR-182/Programmed Cell Death Protein 4 (PDCD4) axis [9]. However, studies on the role of lncRNA SLC16A1-AS1 in CRC are limited, and the specific mechanism of action remains largely underexplored, which underscores the novelty of this study.

miR-515-5p is another key regulator in cancer, with its expression levels differing across various cancer types and being closely associated with cancer prognosis and progression. For example, Zhao et al. revealed that inhibiting miR-515-5p expression in non-small cell lung cancer (NSCLC) cells restored the inhibitory effect of MAPKAPK5-AS1 silencing on NSCLC progression [10]. Similarly, Mao et al. disclosed that miR-515-5p expression was reduced in CRC cells and tissues, and overexpression of miR-515-5p partially counteracted the tumor-promoting effect of circ_0040809 [11]. Zhang et al. further identified that circPGPEP1 promoted CRC progression by regulating the NFAT5/miR-515-5p axis [12]. Although miR-515-5p's involvement in the CRC has been studied, its specific mechanism of action and targets are not fully understood.

In this study, we used the DIANA TOOLS bioinformatics platform to predict the interaction between miR-515-5p and SLC16A1-AS1. Our results suggest the presence of possible binding sites between the two. Furthermore, to explore the downstream regulatory mechanisms of miR-515-5p, we conducted further bioinformatics analysis and identified mitogen-activated protein kinase kinase kinase 9 (MAP3K9) as a potential target of miR-148a-3p, and elevated MAP3K9 levels are found in both lung adenocarcinoma tissues and cell lines. Reduced miR-148a-3p expression, due to promoter methylation, may contribute to lung cancer metastasis by modulating MAP3K9 [13]. Besides, bioinformatics analysis implies that MAP3K9 may also be targeted by miR-125b-5p, which inhibits proliferation, glycolysis, and facilitates apoptosis in laryngeal squamous cell carcinoma by directly targeting MAP3K9 [14]. Although studies specifically targeting MAP3K9 in CRC is limited, given its crucial role in the MAPK signaling pathway and its aberrant expression in other cancers [15], we hypothesize that MAP3K9 may also play an important role in CRC. Therefore, this study focused on investigating the impact of the SLC16A1-AS1/miR-515-5p/MAP3K9 axis in CRC, aiming to provide novel insights and strategies for CRC diagnosis and treatment.

Materials and methods

Ethical statement

The Ethics Committee of Shanghai Tenth People's Hospital's approved this study (Ethics number: 2021KN94), and all patients provided written informed consent.

Study subjects

From July 2016 to July 2021, CRC and matched non-cancerous tissues were collected from 70 patients with CRC who underwent initial surgical resection at Shanghai Tenth People's Hospital. The tissues were preserved at -80°C for future use. No patients received any treatment before surgery.

Cell culture

The human CRC cell line SW480 was sourced from the American Type Culture Collection (ATCC, VA, USA). The selection of SW480 cells was based on their extensive use in colorectal cancer research and their well-defined molecular characteristics, making them a suitable model for studying the role of lncRNA SLC16A1-AS1 in CRC [16]. Cells were cultured in monolayer within cell culture flasks using RPMI 1640 medium (A33823, Thermo Scientific™, Shanghai, China) containing 10% (v/v) fetal bovine serum (10091155, Gibco™, Shanghai, China) and 1% penicillin/streptomycin (15140148, Gibco™, Shanghai, China) at 37°C in a 5% CO_2 environment [17].

Cell transfection and grouping

si-negative control (NC) and si-SLC16A1-AS1 were used for gene silencing. The mimic NC, miR-515-5p mimic, inhibitor NC, and miR-515-5p inhibitor were used to regulate the expression of miR-515-5p. oe-NC and oe-MAP3K9 are overexpression plasmids. All reagents were provided by GenePharma (Shanghai, China). Cell transfection for each reagent was performed using the Lipofectamine 2000 reagent (Invitrogen, 11668019; Shanghai, China) [18].

Cell counting kit-8 (CCK-8)

After successful transfection, SW480 cells (2×10^3 cells/well) were seeded into 96-well plates. According to the manufacturer's protocol, CCK-8 (Dojindo; Tokyo, Japan) was adopted to measure cell number and viability, with absorbance was measured at 450 nm using a spectrophotometer [19].

Transwell assay

Matrigel-coated transwell chambers filled with serum-free medium were employed for invasion assays. A total of 1×10^4 cells were introduced into the upper chamber, while the lower chamber was filled with Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum as a chemotactic agent. After incubation at 37°C for 24 hours, cells were immobilized in methanol for 30 minutes and then stained with 0.5% crystal violet for 30 minutes. Cells were then counted using a microscope and counting chamber (× 100, Olympus, Tokyo, Japan). For migration experiments, Matrigel was not added, and the remaining steps were consistent with the invasion experiments [20].

Flow cytometry

Apoptosis was determined using the ApoDETECT Annexin V-fluorescein isothiocyanate (FITC) kit (331200, Invitrogen, CA, USA) according to the manufacturer's instructions. SW480 cells were digested with EDTA-free trypsin, washed with phosphate-buffered saline (PBS), and centrifuged at 1000 rpm for 5 min. The cell sediment was collected, and the cell density was modified to 1-5 × 10^6 cells/mL by adding 1 × binding buffer. A total of 5 μL of Annexin V-FITC and 10 μl of propidium iodide were added, and apoptosis was analyzed by flow cytometry [21].

Real-time quantitative polymerase chain reaction (PCR) analysis

Total RNA was isolated from tissues or cells employing Trizol reagent (15596018; Invitrogen, CA, USA). cDNAs were synthesized using the RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Scientific™, Shanghai, China) for SLC16A1-AS1 and MAP3K9, while the Transcriptor First Strand cDNA Synthesis Kit (04897030001, Roche, Indianapolis, IN, USA) for miR-515-5p. Quantitative PCR detection of miR-515-5p was performed using the SYBR Green real-time PCR kit (218076; Qiagen, Germany). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with U6 and GAPDH serving as internal reference genes. The primer sequences applied are listed in **Table 1**.

Western blotting

Cells were lysed using RIPA buffer (Beyotime, China) supplemented with a mixture of protease inhibitors. Equal amounts of proteins were loaded onto 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The lysed proteins were subsequently transferred onto a PVDF membrane, which was blocked with 5% BSA. The membranes were subjected to overnight incubation with anti-MAP3K9 (1:500, ab228752, Abcam, USA) and GAPDH (1:500, ab8245, Abcam, USA) at 4°C. Afterward, the membranes were incubated with the appropriate secondary antibody

Table 1. qRT-PCR primers

Gene	Primer sequence (5'-3')
SLC16A1-AS1	F: GGGAGACTTAGGCACAAATTAACC
	R: ATGTTGGTGTGCTTGAAATCTTCC
miR-515-5p	F: CGGGTTCTCCAAAAGAAAGCA
	R: CAGCCACAAAAGAGCACAAT
MAP3K9	F: GAG TGC GGC AGG GAC GTA
	R: CCC CAT AGC TCC ACA CAT CAC
U6	F: GCTTCGGCAGCACATATACTAAAAT
	R: CGCTTCACGAATTTGCGTGTCAT
GAPDH	F: CCAGGGCTGCTTTTAACTCT
	R: GGACTCCACGACGTACTCA

Note: SLC16A1-AS1: Solute Carrier Family 16 Member 1 Antisense RNA 1; MAP3K9: Mitogen-Activated Protein Kinase Kinase Kinase 9; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

(1:2000, ab181236, Abcam, USA) for 1 hour at room temperature. Visualization of the protein bands was achieved using a gel imaging system (G: BOXChemiXR5, SYNGENE, UK) and quantitative analysis of protein expression levels was conducted using ImageJ (National Institutes of Health, Bethesda, USA) [22].

Dual luciferase reporter gene analysis

SW480 cells were seeded into 48-well plates and co-transfected with miR-515-5p mimics with NC and reporter plasmids containing either mutant (MUT) or wild-type (WT) MAP3K9 sequence. Luciferase activity was measured 48 hours post-transfection using a luciferase detection system (Promega, USA), following the manufacturer's protocols [23].

RNA binding protein immunoprecipitation (RIP)

RIP was implemented using the RNA Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA, USA). Cell lysates were incubated overnight with recombinant NC anti-human IgG antibody (1:100, ab181236, Abcam, USA) or human anti-Ago2 antibody (1:100, ab186733, Abcam, USA) conjugated magnetic beads. The levels of SLC16A1-AS1, miR-515-5p, and MAP3K9 in the immunoprecipitates were determined by reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis [24].

Data analysis

Statistical analyses were conducted using SPSS 20.0 software (IBM, New York, USA). Data were described as mean ± standard deviation (SD). For paired data, such as cancerous and normal tissues from CRC patients, a paired t-test was used; for independent data between different experimental groups, such as different transfection groups, an unpaired t-test was applied. Pearson's correlation analysis was used to assess the correlation between genes. Statistical significance was set at $P < 0.05$.

Results

SLC16A1-AS1 was upregulated in CRC and correlated with shorter survival in CRC patients

Previous findings have shown that SLC16A1-AS1 is up-regulated in HCC and contributes to the promotion of HCC cell proliferation [25]. In this study, qRT-PCR confirmed that SLC16A1-AS1 expression was significantly higher in CRC tissues compared to normal tissues (NT) ($P < 0.05$, **Figure 1A**).

To assess the clinical relevance of SLC16A1-AS1 in CRC, 70 CRC samples were grouped into a high expression group (n = 36) and a low expression group (n = 34) based on SLC16A1-AS1 expression, using the median as the cutoff. Chi-square test indicated a potential correlation between high SLC16A1-AS1 expression and the occurrence of CRC (**Table 2**). Further investigation into the prognostic significance of SLC16A1-AS1 revealed that patients with high SLC16A1-AS1 expression had a significantly shorter overall survival, as shown by Kaplan-Meier analysis ($P = 0.011$, **Figure 1B**).

Summing up, SLC16A1-AS1 is up-regulated in CRC and is associated with shorter survival in CRC patients.

SLC16A1-AS1 silence inhibited CRC progression

As indicated by qRT-PCR results, SLC16A1-AS1 expression was significantly reduced in the si-SLC16A1-AS1 group, implying successful transfection ($P < 0.05$, **Figure 2A**). CCK-8 experiments displayed a decrease in cell viability in the si-SLC16A1-AS1 group ($P < 0.05$, **Figure 2B**). Transwell experiments also demonstrated a decrease in cell invasion and migration abilities in si-SLC16A1-AS1 group ($P < 0.05$, **Figure 2C**, **2D**). Furthermore, flow cytometry analysis showed an increase in cell apoptosis rate in si-SLC16A1-AS1 group ($P < 0.05$, **Figure 2E**).

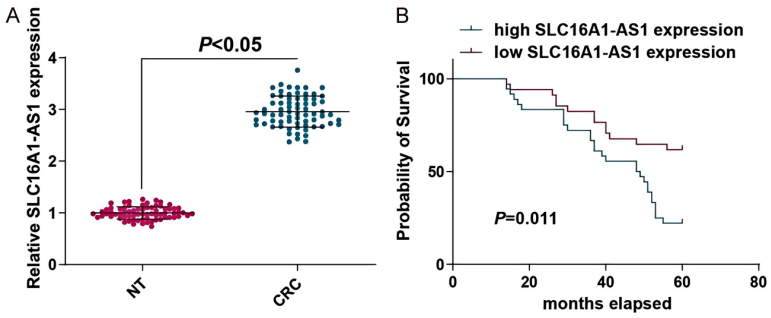

Figure 1. SLC16A1-AS1 is upregulated in CRC and correlated with shorter survival of CRC patients. Note: (A) SLC16A1-AS1 expression in CRC tissues; (B) Survival curve of CRC patients with high/low SLC16A1-AS1 expression. Comparison between the two groups was performed by paired-sample t-test, N = 70. CRC: Colorectal Cancer; SLC16A1-AS1: Solute Carrier Family 16 Member 1 Antisense RNA 1.

Table 2. Relationship between SLC16A1-AS1 expression and clinical characteristics of CRC patients

Parameter	Case	SLC16A1-AS1		*P* value
		High expression (n = 36)	Low expression (n = 34)	
Age (years)				0.3403
> 50	52	25	27	
≤ 50	18	11	7	
Gender				0.6417
Male	37	20	17	
Female	33	16	17	
Tumor stage				0.0337
I-II	47	20	27	
III-IV	23	16	7	
Lymph node metastasis				0.0173
Positive	39	25	14	
Negative	31	11	20	
Tumor size (cm)				0.016
> 3	33	22	11	
≤ 3	37	14	23	

Note: For measurement data, analysis was performed by chi-square test.

These results suggest that SLC16A1-AS1 is upregulated in CRC, and reducing its expression can inhibit CRC progression.

miR-515-5p overexpression inhibited CRC cell proliferation and migration, and promoted apoptosis

Previous studies have shown that miR-515-5p is down-regulated in prostate cancer [26]. In this study, qRT-PCR results revealed that miR-515-5p expression was also downregulated in CRC (P < 0.05, **Figure 3A**). miR-515-5p-mimic group exhibited an increase in the expression, indicating successful transfection (P < 0.05, **Figure 3B**). CCK-8 assay indicated that cell viability was significantly reduced in the miR-515-5p mimic group (P < 0.05, **Figure 3C**). Transwell assay demonstrated that cell invasion and migration abilities were markedly diminished in the miR-515-5p mimic group (P < 0.05, **Figure 3D**, **3E**). Furthermore, flow cytometry experiments showed an increased cell apoptosis rate in miR-515-5p mimic group (P < 0.05, **Figure 3F**).

The above findings confirm that miR-515-5p is down-regulated in CRC, and overexpressing miR-515-5p can suppress the migration and proliferation of CRC cells and facilitate apoptosis.

Targeting relationship between SLC16A1-AS1 and miR-515-5p

The relationship between SLC16A1-AS1 and miR-515-5p was first predicted using the DIANA TOOLS bioinformatics platform, which indicated the existence of a possible binding site between SLC16A1-AS1 and miR-515-5p (**Figure 4A**).

To experimentally validate this prediction, a dual-luciferase reporter assay was performed. SW480 cells were co-transfected with SLC16A1-AS1-WT or SLC16A1-AS1-MUT plasmids with either mimic NC or miR-515-5p mimic. The results showed that miR-515-5p suppressed SLC16A1-AS1-WT's luciferase activity of SLC16A1-AS1-MUT, while no significant effect was observed with the SLC16A1-AS1-MUT plasmid (P < 0.05, **Figure 4B**). The results of RIP disclosed that the expression of both miR-515-5p and SLC16A1-AS1 was elevated following Ago2 treatment, which demonstrated the existence of a binding site between the two (P < 0.05, **Figure 4C**).

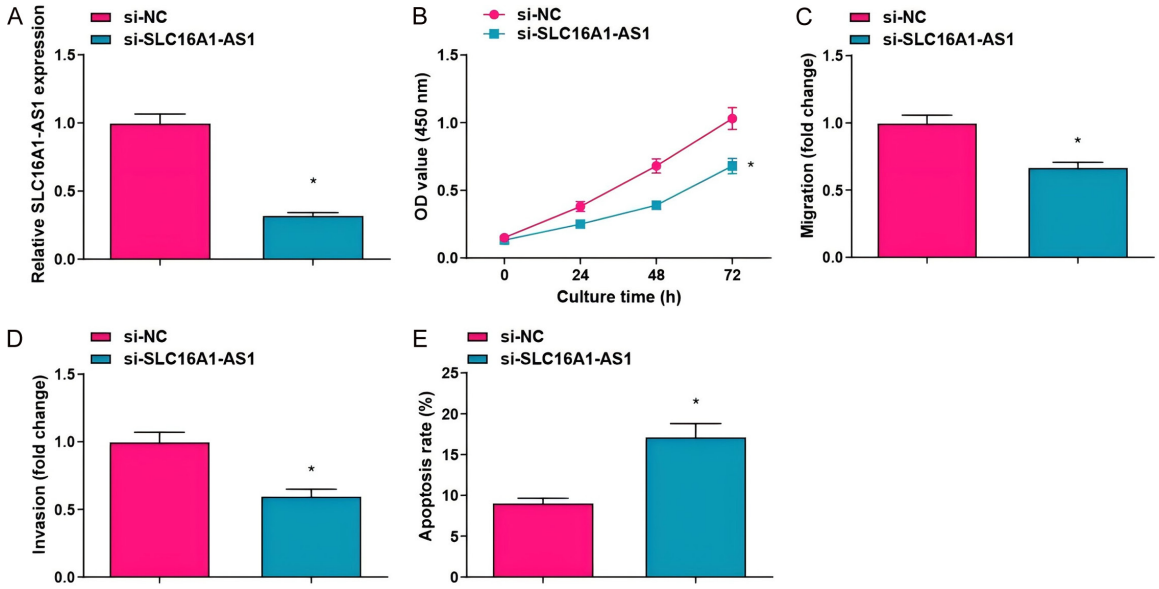

Figure 2. Silencing SLC16A1-AS1 inhibited CRC progression. Note: (A) SLC16A1-AS1 expression detected by qRT-PCR; (B) Cell viability detected by CCK-8; (C, D) Cell migration and invasion ability detected by Transwell assays; (E) Cell apoptosis detected by Flow cytometry. Data are expressed as the mean ± standard deviation and compared between the two groups with independent samples t-test, n = 3; *P < 0.05, compared with the si-NC group. CRC: Colorectal Cancer; SLC16A1-AS1: Solute Carrier Family 16 Member 1 Antisense RNA 1; CCK-8: Cell Counting Kit-8.

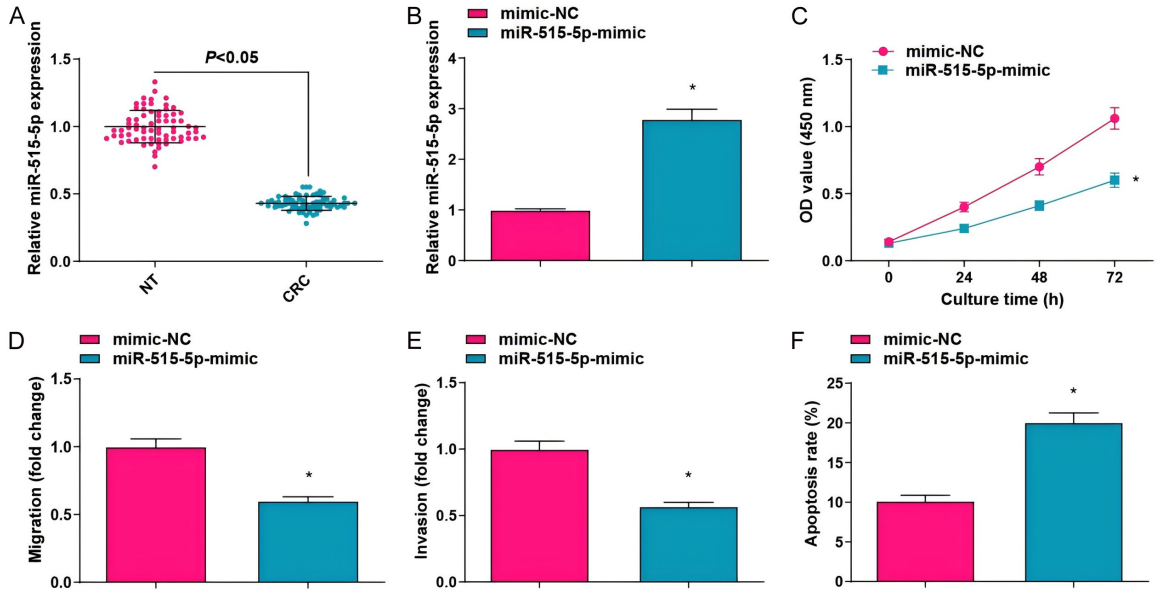

Figure 3. miR-515-5p overexpression inhibited CRC cell proliferation and migration and promoted apoptosis. Note: (A) miR-515-5p expression in NT and CRC tissues; (B) Successful transfection approved by qRT-PCR; (C) Cell viability detected by CCK-8; (D, E) Cell invasion detected by Transwell assay; (F) Cell apoptosis detected by Flow cytometry. Data are expressed as mean ± standard deviation and compared between the two groups with independent samples t-test. (A) N = 70, n = 3 for the rest of the graphs; *P < 0.05, compared with mimic-NC group. CRC: Colorectal Cancer; CCK-8: Cell Counting Kit-8; MiR-515-5p: MicroRNA-515-5p.

Additionally, qRT-PCR was performed to examine the expression level of miR-515-5p in SW480 cells transfected with si-NC and si-SLC16A1-AS1, and the results unveiled that downregulation of SLC16A1-AS1 promoted miR-515-5p expression (P < 0.05, **Figure 4D**).

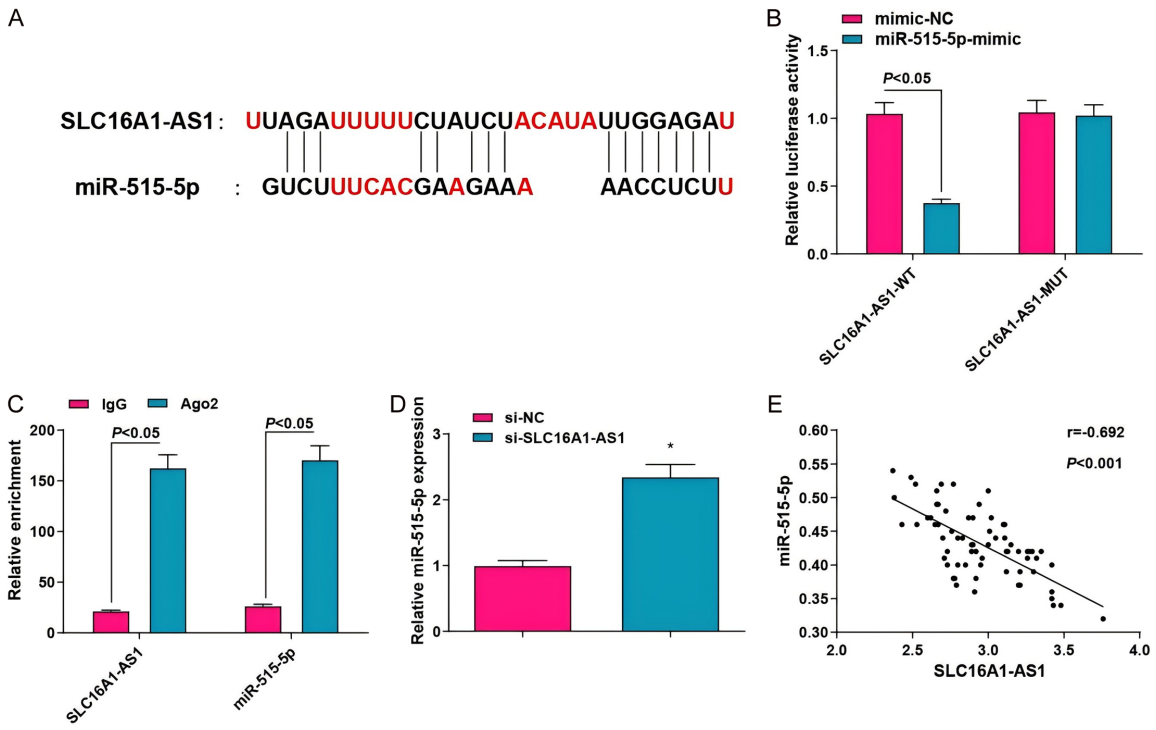

Figure 4. Relationship between SLC16A1-AS1 and miR-515-5p. Note: (A) Prediction of binding sites between SLC16A1-AS1 and miR-515-5p using the DIANA website; (B) The binding between SLC16A1-AS1 and miR-515-5p validated by Dual-luciferase reporter experiment; (C) The enrichment of SLC16A1-AS1 and miR-515-5p detected by RIP; (D) The expression level of miR-515-5p detected by qRT-PCR; (E) Correlation between SLC16A1-AS1 and miR-515-5p examined by Pearson correlation analysis. Data are expressed as mean ± standard deviation and compared between two groups using independent sample t-tests. (E) N = 70; the other figures, n = 3; **P* < 0.05, compared to the si-NC group. SLC16A1-AS1: Solute Carrier Family 16 Member 1 Antisense RNA 1; MiR-515-5p: MicroRNA-515-5p; RIP: RNA immunoprecipitation.

Analysis of the correlation between the two genes yielded that SLC16A1-AS1 was negatively correlated with miR-515-5p (r = -0.692, P < 0.001, **Figure 4E**).

These findings provide strong evidence that SLC16A1-AS1 directly interacts with miR-515-5p and functions as a negative regulator of miR-515-5p expression.

Targeting relationship between miR-515-5p and MAP3K9

To ascertain the downstream regulatory mechanism of miR-515-5p, we first screened for potential binding sites between MAP3K9 and miR-515-5p using the Target bioinformatics platform (**Figure 5A**). Their targeting relationship was further validated through dual-luciferase reporter experiments and RIP experiments. The dual-luciferase experiment uncovered that upregulation of miR-515-5p inhibited the luciferase activity of MAP3K9-WT (P < 0.05) but had no significant effect on MAP3K9-MUT (**Figure 5B**). The RIP experiment results disclosed that under Ago2 treatment, the expressions of both miR-515-5p and MAP3K9 were increased, suggesting the presence of binding sites between them (all P < 0.05, **Figure 5C**).

qRT-PCR and Western blot analyses indicated that MAP3K9 was upregulated in CRC tissues (all P < 0.05, **Figure 5D**). Upon miR-515-5p overexpression, MAP3K9 expression was significantly decreased (all P < 0.05, **Figure 5E**). Correlation analysis displayed a negative correlation between miR-515-5p and MAP3K9 (r = -0.673, P < 0.001, **Figure 5F**). Furthermore, the si-SLC16A1-AS1 group exhibited decreased MAP3K9 expression compared to the si-NC group, suggesting that silencing of SLC16A1-AS1 inhibited MAP3K9 expression (all P < 0.05, **Figure 5G**). Correlation analysis of the two genes showed a positive association between SLC16A1-AS1 and MAP3K9 (r = 0.656, P < 0.001, **Figure 5H**).

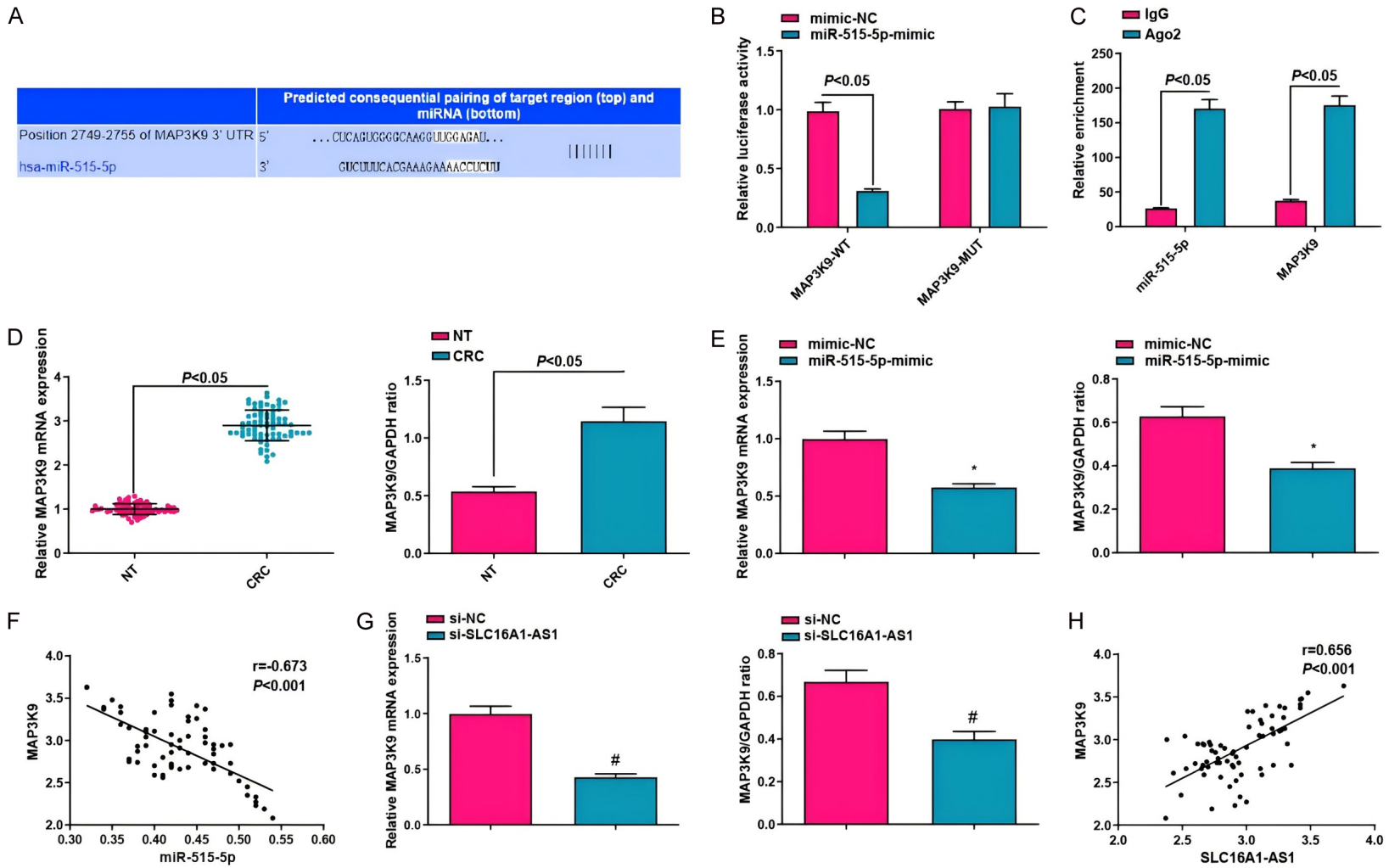

Figure 5. Relationship between miR-515-5p and MAP3K9. Note: (A) Prediction of the binding site between miR-515-5p and MAP3K9 by Target website; (B) The binding between miR-515-5p and MAP3K9 verified by Dual luciferase reporter assay; (C) The enrichment of miR-515-5p and MAP3K9 detected by RIP; (D) The expression of MAP3K9 in CRC tissues detected by qRT-PCR and Western blot; (E) MAP3K9 expression in RCR cells detected by qRT-PCR and Western blot; (F) Correlation between miR-515-5p and MAP3K9 determined by Pearson correlation analysis; (G) MAP3K9 expression detected by qRT-PCR and Western blot; (H) Correlation between SLC16A1-AS1 and MAP3K9 determined by Pearson correlation analysis. Data are expressed as mean ± standard deviation and compared between two groups using independent sample t-tests. (D, F, H) N = 70; the rest of the figures, n = 3; *P < 0.05 compared with the mimic-NC group; #P < 0.05 compared with the si-NC. MiR-515-5p: MicroRNA-515-5p; MAP3K9: Mitogen-Activated Protein Kinase Kinase Kinase 9; RIP: RNA immunoprecipitation.

The above results demonstrate that miR-515-5p directly targets MAP3K9, and that SLC16A1-AS1 may modulate MAP3K9 expression through miR-515-5p.

SLC16A1-AS1 promoted CRC initiation and progression by regulating MAP3K9 expression through targeting miR-515-5p

To verify that SLC16A1-AS1 contributes to the initiation and progression of CRC by modulating MAP3K9 expression through targeting miR-515-5p, we first confirmed the transfection efficiency in different groups (all $P < 0.05$, **Figure 6A**), with qRT-PCR results confirming successful transfection. The subsequent experiments disclosed that cell migration, invasion and proliferation abilities were significantly increased in the si-SLC16A1-AS1 + miR-515-5p-inhibitor group and the si-SLC16A1-AS1 + oe-MAP3K9 group, while apoptosis was reduced compared to the corresponding NC groups. The results of the above experiments could indicate that both down-regulation of miR-515-5p and overexpression of MAP3K9 could reverse the effects of SLC16A1-AS1 silence on the initiation and progression of CRC (all $P < 0.05$, **Figure 6B-E**).

Taken together, SLC16A1-AS1 promotes the initiation and progression of CRC by modulating the expression of MAP3K9 through targeting miR-515-5p.

Discussion

Colorectal cancer (CRC) is a malignant tumor of the digestive system and one of the most prevalent cancers globally, with its incidence still increasing. The onset and progression of CRC is a multifaceted process encompassing genetic mutations in cancer cells as well as various factors within the tumor microenvironment [27]. Despite recent advancements in targeted therapies, their clinical effectiveness remains limited without a cure, and costly [3]. This study focuses on the SLC16A1-AS1/miR-515-5p/MAP3K9 axis in CRC, aiming to identify new potential targets for the diagnosis and treatment of CRC patients.

SLC16A1-AS1 exhibits dual functions in different types of cancer: in HCC and NSCLC, it can act as an oncogene, promoting cancer cell proliferation, migration, and invasion, while in others, it can exert tumor-suppressive effects through particular miRNA axes [7]. For example, in liver cancer, SLC16A1-AS1 promotes cell proliferation and malignant progression by interacting with miR-141 [25]; in oral squamous cell carcinoma, it inhibits cell proliferation through the miR-5088-5p regulatory axis [28]. These functional differences across cancer types suggest that the role of SLC16A1-AS1 is influenced by the specific tumor microenvironment and its molecular network. Its role in CRC is markedly different from other cancers, further validating its potential as a CRC-specific therapeutic target.

Studies have shown that SLC16A1-AS1 is strongly associated with tumorigenesis and various metabolic processes. SLC16A1 modulates lactate chemotaxis, and SLC16A1-AS1 acts through multiple mechanisms to influence the metabolic processes of tumor cells and has implications for cancer prognosis [4]. Additionally, SLC16A1-AS1 regulates cell functions such as migration, proliferation, and invasion through different signaling pathways and molecules [29]. Elevated SLC16A1-AS1 expression in HCC cell lines and tissues correlates with poor prognosis, with evidence suggesting that its expression is regulated by miR-411 and promotes cancer cell proliferation through methylation of miR-141 [5, 25]. Similarly, in CRC, our findings show increased SLC16A1-AS1 expression in cancer tissues, which is associated with shorter patient survival, supporting its potential as a prognostic marker. Moreover, reducing SLC16A1-AS1 expression inhibited CRC progression, indicating that SLC16A1-AS1 could be a promising therapeutic target in CRC.

In exploring the mechanism of action of SLC16A1-AS1, we identified a potential link between it and miR-515-5p. A study has reported that the expression of miR-515-5p is down-regulated in HCC, and overexpression of miR-515-5p has been found to suppress HCC cell invasion and migration *in vivo* and *in vitro*, whereas knockdown of miR-515-5p is counterproductive [30]. These findings indicate that miR-515-5p holds promise as a potential target for treating HCC. Furthermore, miR-515-5p expression is notably lower in BC cells, and miR-515 overexpression suppresses cellular proliferation, viability, and induces apoptosis by directly targeting CBX4 [31]. Similarly, in this study, we found that miR-515-5p is down-regulated in CRC, where it inhibits cell migration and proliferation while promoting apoptosis.

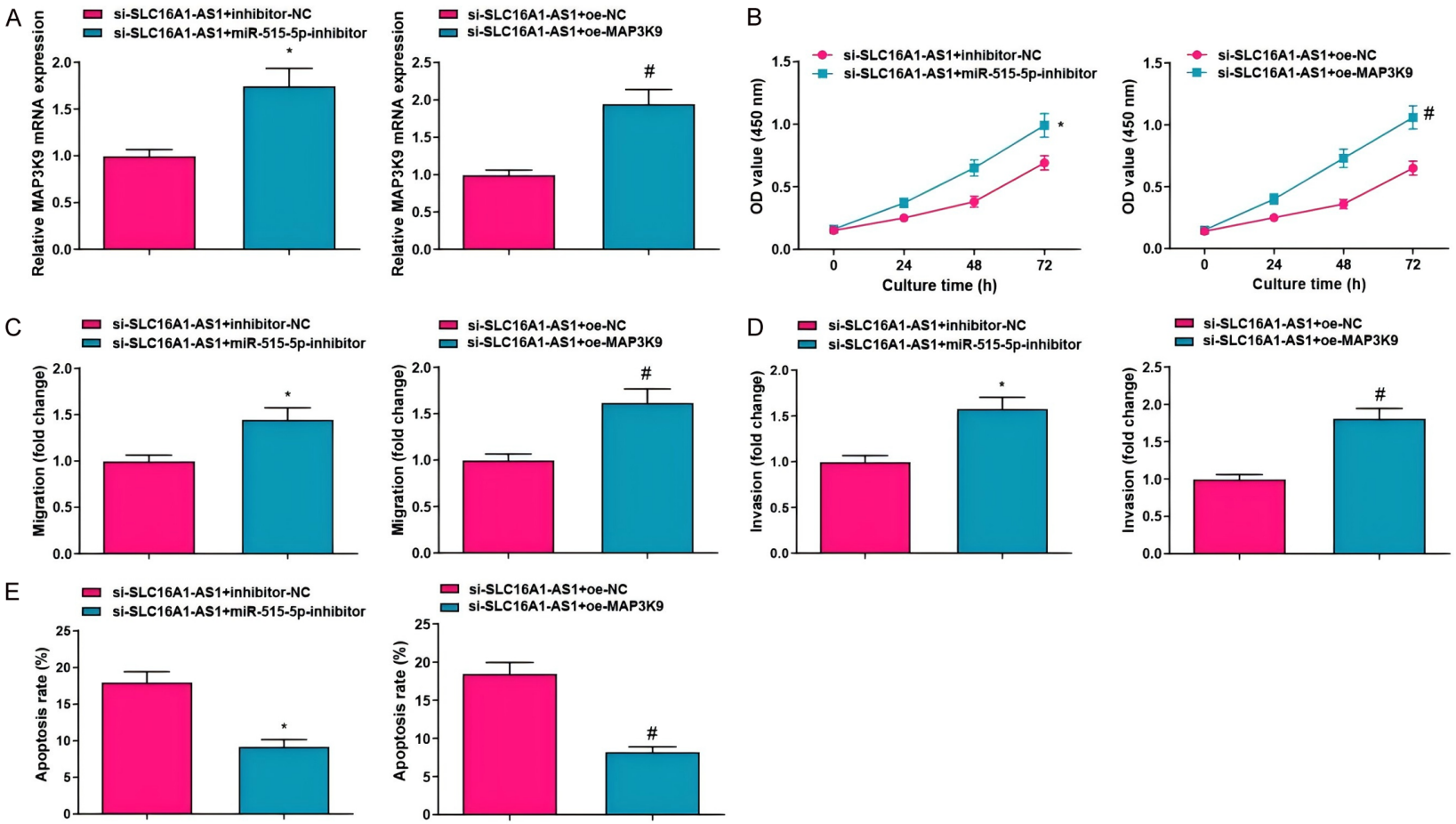

Figure 6. SLC16A1-AS1 is involved in the initiation and progression of CRC by regulating the expression of MAP3K9 through targeting miR-515-5p. Note: (A) Successful transfection validated by qRT-PCR; (B) Cell viability detected by CCK-8; (C, D) Cell migration and invasion ability detected by Transwell assay; (E) Cell apoptosis detected by Flow cytometry. Data are expressed as mean ± standard deviation (n = 3) and compared between the two groups using independent samples t-test. **P* < 0.05, compared with si-SLC16A1-AS1 + inhibitor-NC group; #*P* < 0.05, compared with si-SLC16A1-AS1 + oe-NC group. SLC16A1-AS1: Solute Carrier Family 16 Member 1 Antisense RNA 1; MiR-515-5p: MicroRNA-515-5p; MAP3K9: Mitogen-Activated Protein Kinase Kinase Kinase 9; CRC: Colorectal Cancer; CCK-8: Cell Counting Kit-8.

Further assays displayed a possible binding site between SLC16A1-AS1 and miR-515-5p, with SLC16A1-AS1 negatively regulating miR-515-5p expression. Moreover, miR-515-5p was found to target MAP3K9, a key signaling molecule involved in tumorigenesis. Previous study have shown that MAP3K9 is a direct target of miR-7, which inhibits NF-κB and MEK/ERK pathways, thus suppressing pancreatic cancer cell proliferation and inducing apoptosis [32]. MAP3K9 is also a target gene of miR-148a in cutaneous squamous cell carcinoma (CSCC) cells, where its overexpression inhibits cell proliferation and metastasis by down-regulating the expression of MAP3K9 [33]. In our study, the expression of MAP3K9 was up-regulated in CRC tissues, and its expression decreased following upregulation of miR-515-5p. Correlation analysis yielded a negative correlation between miR-515-5p and MAP3K9. In addition, silencing SLC16A1-AS1 suppressed MAP3K9 expression, and SLC16A1-AS1 was positively correlated with MAP3K9. To further confirm the role of SLC16A1-AS1 in CRC initiation and progression, we performed qRT-PCR, which revealed that both down-regulation of miR-515-5p and overexpression of MAP3K9 could reverse the effects of SLC16A1-AS1 silence on CRC. These results demonstrate that SLC16A1-AS1 contributes to CRC progression by regulating miR-515-5p, which in turn targets MAP3K9, providing insight into the molecular mechanisms underlying CRC pathogenesis.

Conclusion

This study demonstrates the crucial role of lncRNA SLC16A1-AS1 in CRC and its mechanism of action. LncRNA SLC16A1-AS1 influences CRC progression by regulating miR-515-5p expression, which subsequently targets MAP3K9. This discovery not only deepens our understanding of the molecular mechanisms driving CRC pathogenesis, but also offers a potential therapeutic target for CRC treatment. Given the complexity of cancer development, which involves the interplay of multiple molecules and signaling pathways, further research in this area is essential. Our ongoing investigations aim to enhance therapeutic outcomes and improve the prognosis for CRC patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Meichun Xing, Precision Research Center for Refractory Diseases, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201620, China. E-mail: history15sci@163.com

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