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Inhibition of MALAT1 reduces tumor growth and metastasis and promotes drug sensitivity in colorectal cancer

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ABSTRACT

Human metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long non-coding RNA known to be highly expressed in several tumors. In colorectal cancer (CRC), MALAT1 promotes cell proliferation, metastasis, and invasion in vitro and in vivo. This study aimed to investigate the effect of MALAT1 on the proliferation, migration, and drug sensitivity of CRC cells in vitro and in vivo and the mechanisms involved therein. We observed increased expression of MALAT1 in six CRC cell lines compared to that in normal cells, suggesting its involvement in CRC progression. Downregulation of MALAT1 inhibited cell migration and induced apoptosis in vitro and inhibited tumor growth and metastasis in nude mice. Furthermore, MALAT1 silencing downregulated the expression of ATP-binding cassette transporters (ABC), breast cancer resistance protein (BCRP), and multi-drug resistance proteins including MDR1 and MRP1, resulting in decreased resistance of cancer cells to 5-FU. In addition, the metastasis and invasion of HCT-116 and HCT-116/5-FU cells were regulated via targeting miR-20b-5p. Based on these observations, we infer that inhibition of MALAT1 suppressed CRC progression and metastasis and improved the sensitivity of cancer cells to 5-FU. The present study proposes a new direction to investigate the molecular mechanisms underlying the invasion and metastasis of CRC, whereby the interaction between MALAT1 and miR-20b-5p could be a novel therapeutic target for CRC.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors with an increasing rate of incidence worldwide. Its carcinogenesis is a multistep process involving progressive disruption of epithelial cell differentiation, proliferation, apoptosis, and survival mechanisms [1,2]. Metastasis is the major cause of mortality in CRC patients [3,4]. Many tumors share the common mechanisms required for metastasis including enhanced motility and adhesion ability, which induce the secretion of proteolytic enzymes to degrade the extracellular matrix [5]. The molecular mechanisms of CRC development and metastasis remain elusive [6]. Currently, surgical resection, radiotherapy, and chemotherapy are the main treatment methods for CRC. Chemotherapeutic drugs can significantly inhibit the progression of CRC [7,8], but with repeated use, CRC tumor cells are prone to drug resistance, resulting in the failure of chemotherapy regimens. Drug resistance in tumors is mainly manifested in three aspects: reduced efficacy of drug action, enhanced DNA damage repair, and drug inactivation [9]. In addition, apoptosis inhibition, drug target change, epithelial-mesenchymal transition (EMT), epigenetics, tumor cell heterogeneity, and tumor stem cells play crucial roles in drug resistance [10,11]. Key molecules participating in drug resistance include ATP-binding cassette (ABC), breast cancer resistance protein (BCRP), and multi-drug resistance (MDR) proteins such as MDR1 and MRP1, and the PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways are also involved therein [12–14]. As the specific mechanism of chemotherapeutic drug resistance in CRC is still unclear, its investigation has important social and economic benefits for the development of novel chemotherapeutic drugs with improved treatment effects against CRC.

Coding RNA and non-coding RNA (ncRNA) constitute the two major families of RNA, and long non-coding RNA (lncRNA) research has recently gained attention. Thousands of ncRNA transcripts have been identified over the past several years and thus, the function of ncRNAs in tumor development has become an attractive research area [15,16]. A large number of small ncRNAs are involved in tumorigenesis by acting as oncogenes or suppressor genes [17]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an evolutionarily highly conserved lncRNA that lacks open reading frames and cannot be

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translated into protein in vivo. It plays essential roles in tumor development and drug resistance [18] and is overexpressed in liver, cervical, lung, and bladder cancer [19,20]. In CRC, MALAT1 promoted cancer cell proliferation, invasion, and migration in vitro by enhancing A-kinase anchor protein 9 expression [21]. Moreover, MALAT1 reduced the sensitivity of CRC cells to oxaliplatin and inhibited its therapeutic effect by downregulating the expression of microRNA (miR)-218 [22]. At the same time, lncRNAs impact the stemness maintenance of tumor cells. Downregulation of MALAT1 expression caused stemness-related genes to be suppressed and proliferation to be promoted in glioma stem cells [23]. On the other hand, miR-20b was shown to be lowly expressed in CRC tissues and further decreased in fluorouracil (5-FU)-resistant tissues and cells [24,25]. However, whether MALAT1 affects the stemness maintenance of CRC cells by interacting with miR-20b and mediating tumorigenesis is rarely reported.

We hypothesized that MALAT1, an important regulatory lncRNA, interacts with miR-20b, thereby regulating tumor growth, metastasis, and drug sensitivity. This study aimed to investigate the effect of MALAT1 on the proliferation, migration, and drug sensitivity of CRC cells in vitro and in vivo. The expression of MALAT1 and proteins related to drug resistance, metastasis, and apoptosis were also evaluated in HCT-116 cells and 5-FU-resistant HCT-116 (HCT-116/5-FU) cells.

2. Materials and methods

2.1. Cell culture and MALAT1 interference

Normal human colorectal epithelial cells NCM460 and CRC cell lines HCT-116, SW480, COLO205, LOVO, 5-FU-resistant HCT-116 (HCT-116/5-FU), and 5-FU-resistant SW480 (SW480/5-FU) were cultured in Dulbecco's modified Eagle medium (DMEM, Invitrogen, USA) containing 10% fetal bovine serum (FBS, Gibco, USA), 100 units/ml penicillin, and 100 g/ml streptomycin at 37 °C in humidified air with 5% CO_2. Specific small interfering RNAs (siRNAs) targeting MALAT1 (siRNA-MALAT1) and miR-20b-5p mimics were respectively transfected into HCT-116 and HCT-116/5-FU cells using Lipofectamine 2000 (11668–027, Invitrogen, USA) according to the manufacturer's instructions, and the cells were harvested 24 h after transfection. Non-transfected cells served as controls.

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

qRT-PCR was performed to detect the expression of MALAT1 (GAPDH as reference gene) and miR-20b-5p (U6 as reference gene) in CRC cell lines mentioned above. The total MALAT1 or miR-20b-5p of each group were extracted using Trizol (product code: 15596026, Ambion, American) and reverse-transcribed into cDNA using the M-MLV kit, and then the synthesized cDNA was subjected to qRT-PCR using YBR Green PCR kit (KM4101, KAPA Biosystems, USA) according to the manufacturer's instructions. The primer sequences are as follows: MALAT1-F 5′-GGTAACGATGGTGTCGAGGTC-3′, MALAT1-R 5′-CCAGC ATTACAGTTCTTGAACATG-3′; miR-20b-5p-F 5′-GGGCAAAGTGCTCAT AGT-3′, miR-20b-5p-R 5′-AACTGGTGTCGTGGAGTCGGC-3′; U6-F 5′-CTCGCTTCGGCAGCACA-3′, U6-R 5′-AACGCTTCACGAATTTG CGT-3′; GAPDH-F 5′-CCACTCCTCCACCTTTG-3′, GAPDH-R 5′-CACCAC CCTGTTGCTGT-3′. The data were collected using the QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems) and analyzed with the $2^{-\Delta\Delta Ct}$ method.

2.3. Transwell assays

After transfection, the cells were digested and placed into the upper chamber of 24-well plates at 5×10^4 cells/well in serum-free DMEM/F12. The lower chamber was filled with 0.75 ml of DMEM/F12 containing 10% FBS. After 24 h, the cells were fixed with 4% paraformaldehyde for 10 min, washed with phosphate-buffered saline (PBS), stained with 0.5% crystal violet for 30 min, and photographed. In each sample, invasive ability was quantified by counting crystal violet-stained cells.

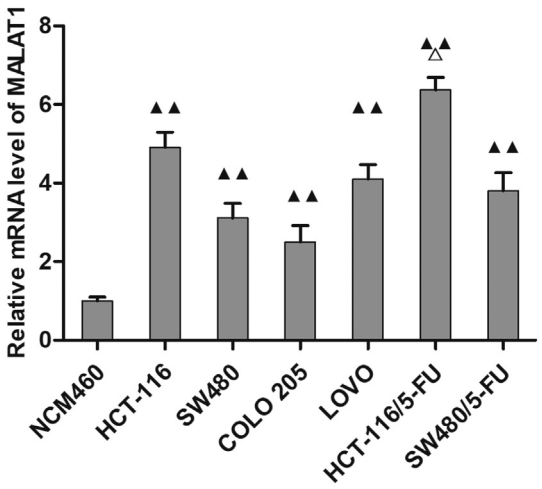

Fig. 1. MALAT1 mRNA expression in normal human colorectal epithelial cells NCM460 and CRC cell lines HCT-116, SW480, COLO205, LOVO, HCT-116/5-FU, and SW480/5-FU was detected by qRT-PCR. Data are shown as mean ± SD (n = 3). ▲▲P < .01 compared with NCM460 cells; △P < .05 compared with HCT-116 cells.

2.4. Western blot

Total proteins were extracted using radioimmunoprecipitation buffer (PAB180006, Bioswamp, China) and the protein concentration was evaluated using a bicinchoninic acid kit (PAB180007, Bioswamp, China). Proteins (20 μg) were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (IPVH00010, Millipore, USA). The membranes were placed in blocking buffer (Sigma-Aldrich) for 1 h at room temperature and incubated overnight at 4 °C with antibodies against EMT-associated proteins N-cadherin (1:1000, ab18203, abcam), vimentin (1:2000, ab137321, abcam), Snail (1:500, ab82846, abcam), E-cadherin (1:500, ab15148, abcam), α-catenin (1:10000, ab227181, abcam), and β-catenin (1:2000, ab16051, abcam); drug resistance-associated proteins MDR1 (1:2000, PAB30805, Bioswamp), MRP1 (1:1000, PAB33537, Bioswamp), BCRP (1:10000, ab108312, abcam), and ABC (1:1000, ab7360, abcam); apoptosis-associated proteins Bax (1:2000, PAB30040, Bioswamp), Bcl-2 (1:2000, PAB30041, Bioswamp), cytochrome-c (Cyt-c, 1:1000, ab90529, abcam), and caspase-3 (1:2000, ab136812, abcam); and GAPDH (1:2000, PAB36264, Bioswamp). Then, the membranes were washed with Tris-buffered saline and incubated in goat secondary antibody (1:20000, PAB160011, Bioswamp) for 2 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence kit (WBKLS0010, Millipore, USA).

2.5. Flow cytometry

Cells apoptosis was analyzed by annexin V/propidium iodide (PI) staining according to the manufacturer's instructions (BD, Franklin Lakes, USA). Cells were treated with SNP (Sigma-Aldrich) for 24 h, digested with ethylenediaminetetraacetic acid-free trypsin (Invitrogen, Carlsbad, CA, USA), and washed with cold PBS. Annexin V-fluorescein isothiocyanate (FITC) (5 μl) and PI (5 μl) were added and incubated for 1 h, and flow cytometry was carried out (Beckman Coulter, Brea, CA, USA).

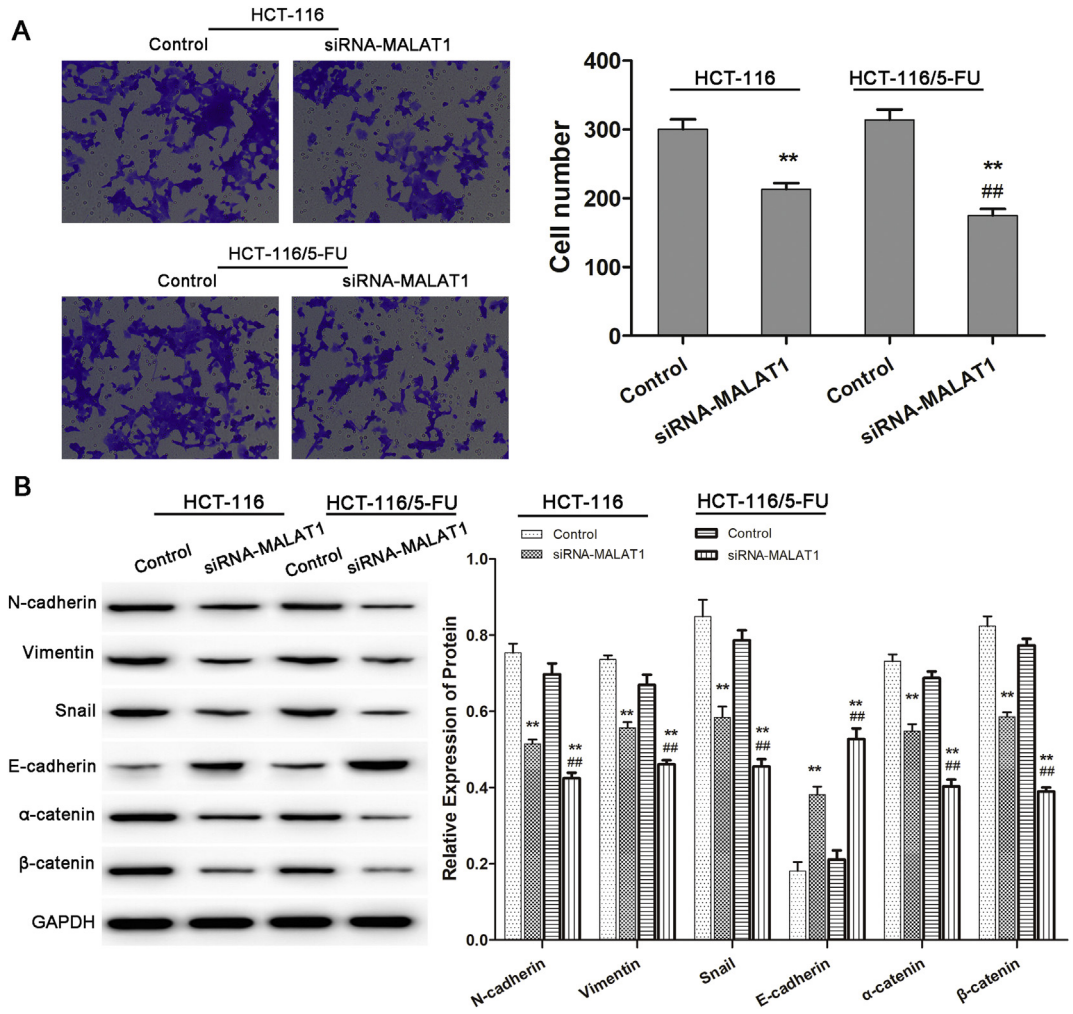

Fig. 2. MALAT1 silencing inhibited migration in HCT-116 and HCT-116/5-FU cells. (A) The migration of HCT-116 and HCT-116/5-FU cells were detected by Transwell assay. (B) The protein expression levels of N-cadherin, vimentin, Snail, E-cadherin, α-catenin, and β-catenin in HCT-116 and HCT-116/5-FU cells were detected by western blot. $^{**}P < .01$ compared with NCM460 cells; $^{\#\#}P < .01$ compared with MALAT1-silenced HCT-116 cells.

2.6. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cells were seeded into a 96-well plate at 5×10^4 cells/ml after transfection and incubated with different concentration of 5-FU (0, 5, 10, 20 μg/ml) for 48 h. After treatment, 20 μl of MTT (Sigma, St Louis, USA) at a concentration of 0.5 mg/ml was added and the cells were further incubated for 4 h at 37 °C. Thereafter, the culture medium was carefully removed, 150 μl of dimethyl sulfoxide (Sigma, St Louis, USA) was added to each well, and the plate was vibrated for 30 min at room temperature. A microplate reader (Sunrise Microplate Reader, TECAN, Switzerland) was used to measure the absorbance at 490 nm.

2.7. Luciferase reporter assay

TargetScan (www.targetscan.org) was used to analyze the target relationship between miR-20b-5p and MALAT1. The 3′-untranslated region (3'-UTR) of the MALAT1 gene was cloned and amplified by PCR. The product was inserted into the luciferase reporter pGL3 dual luciferase reporter vector (Addgen) to generate the wild-type (WT) MALAT1 3'-UTR. MALAT1 3'-UTR mutants (MUT) were prepared by mutating the predicted target sites using a site-directed mutagenesis kit (Takara Bio). Before luciferase activity analysis, 5×10^5 cells/well were seeded in 24-well plates and co-transfected with WT or MUT MALAT1 3'-UTR and miR-20b-5p mimics or negative controls using Lipofectamine 2000. Luciferase activity was measured by the Dual-Luciferase Reporter Assay System (Promega) after transfection for 48 h.

2.8. Animals

Thirty-two specific-pathogen-free male nude Balb/c mice (4 weeks old) weighing 16–20 g were purchased from the Laboratory Animal Centre at the Huazhong Agriculture University. All experimental procedures were carried out in accordance with the Chinese legislation regarding experimental animals. The animals were housed in opaque polypropylene cages in a standard 12 h/12 h light/dark cycle with food and water ad libitum at 22 ± 2 °C and 55–60% humidity. The mice were randomized into four groups: control, 5-FU, MALAT1-siRNA, and 5-FU + MALAT1-siRNA.

2.9. Tumor formation

HCT-116/5-FU cells (1×10^7 cells in 200 μl) were inoculated subcutaneously into the right axillary region of nude mice. 5-FU (5 mg/kg) was directly administered via intragastric injection into the mice in the 5-FU groups after 7 and 14 days of tumor growth. MALAT1-siRNA (10 μg) was directly administered via intragastric injection into the mice in the MALAT1-siRNA groups after 7, 9, 11, 14, and 16 days of tumor growth. PBS was used as a vehicle control. The mice were monitored every two days for tumor formation using calipers.

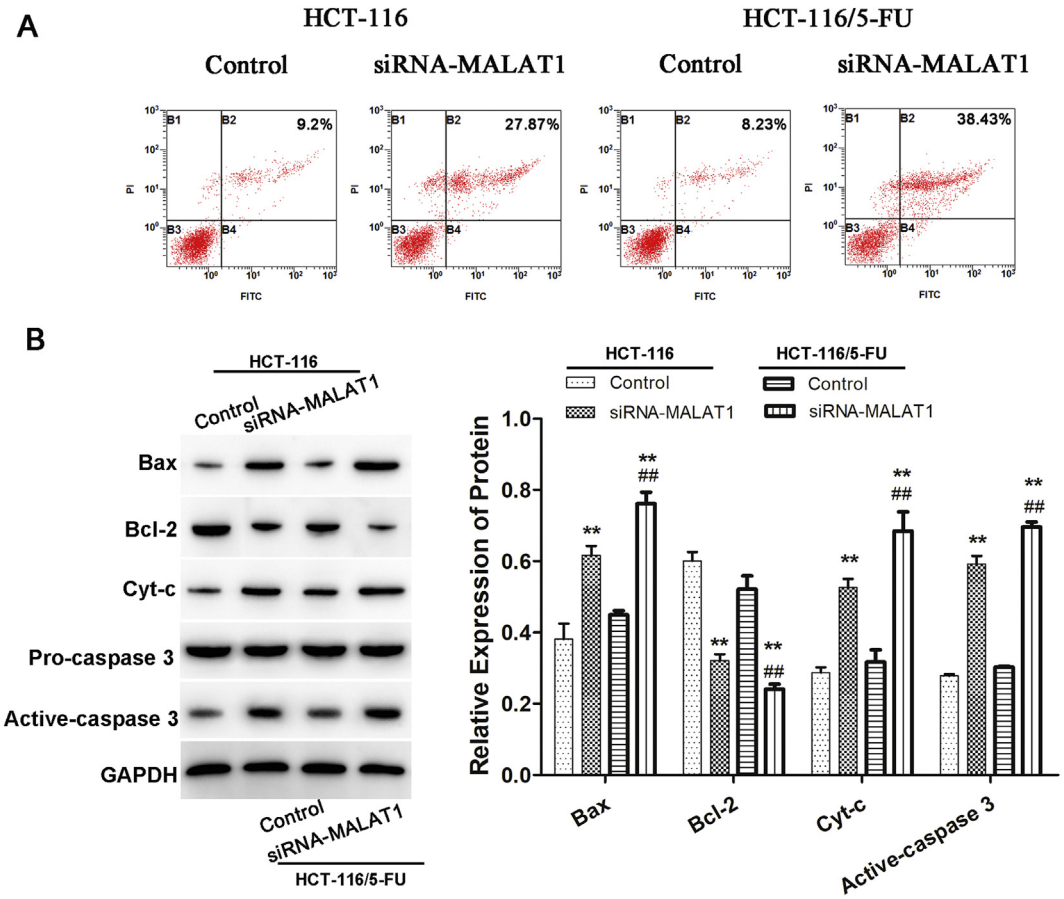

Fig. 3. MALAT1 silencing promoted apoptosis in HCT-116 and HCT-116/5-FU cells. (A) The percentage of apoptotic HCT-116 and HCT-116/5-FU cells were evaluated by annexin V-FITC/PI flow cytometry. (B) The protein expression levels of Bax, Bcl-2, Cyt-c, and caspase-3 in HCT-116 and HCT-116/5-FU cells were detected by western blot. Data are shown as mean ± SD (n = 3). $^{**}P < .01$ compared with control group; $^{\#\#}P < .01$ compared with MALAT1-silenced HCT-116 cells.

2.10. Immunohistochemistry (IHC)

The expression of Ki67 in tumor cells was evaluated by IHC, which was performed on 5-μm-thick tumor slices. The prepared tissues were subjected to antigen retrieval with citrate buffer (pH = 6) for 20 min. Then, the tissues were placed in 3% H_2O_2 for 10 min in a humidified chamber at room temperature to eliminate the activity of endogenous peroxidase, followed by three washes with PBS. The samples were incubated with Ki67 antibody (1:200, Bioswamp, PAB19919) in a humidified chamber overnight at 4 °C, followed by incubation with MaxVision TM HRP-Polymer anti-Mouse/Rabbit IHC (1:200, Bioswamp, PAB160022) in a humidified chamber at room temperature for 30 min. Diaminobenzidine was added and the core was counterstained with hematoxylin for 3 min. After staining, the slides were dehydrated in ethanol (75%, 85%, 95%, 100%) and xylene and mounted with neutral balsam. The results were quantified by Image-pro plus software via detect integrated option density.

2.11. Statistical analysis

Data are expressed as mean ± standard deviation (SD). One-way analysis of variance with post hoc test was performed to compare differences between multiple groups (more than two) and *t*-test was carried out for two groups using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). $P < .05$ was considered significantly different.

3. Results

3.1. MALAT1 was highly expressed in CRC cells

The expression levels of MALAT1 in all chosen CRC cell lines, especially those of HCT-116 and HCT-116/5-FU cells, were higher than that in normal human colorectal epithelial cells NCM460 (Fig. 1). HCT-116 and HCT-116/5-FU cells were chosen for the subsequent experiments because they exhibited the highest expression of MALAT1 among the CRC cell lines.

3.2. MALAT1 silencing inhibited migration of HCT-116 and HCT-116/5-FU cells in vitro

After siRNA-MALAT1 transfection, the migration and invasive abilities of HCT-116 and HCT-116/5-FU cells decreased significantly compared with those of non-transfected control cells. After MALAT1 silencing, the transfer numbers of HCT-116/5-FU cells were lower than HCT-116 cells (Fig. 2A). The expression levels of EMT-associated proteins N-cadherin, vimentin, Snail, α-catenin, and β-catenin decreased and E-cadherin was increased in siRNA-MALAT1-transfected cells significantly compared to those in control cells, and this effect was more pronounced in HCT-116/5-FU cells (Fig. 2B).

3.3. MALAT1 silencing promoted apoptosis of HCT-116 and HCT-116/5-FU cells in vitro

In contrast, MALAT1 silencing induced apoptosis in HCT-116 and

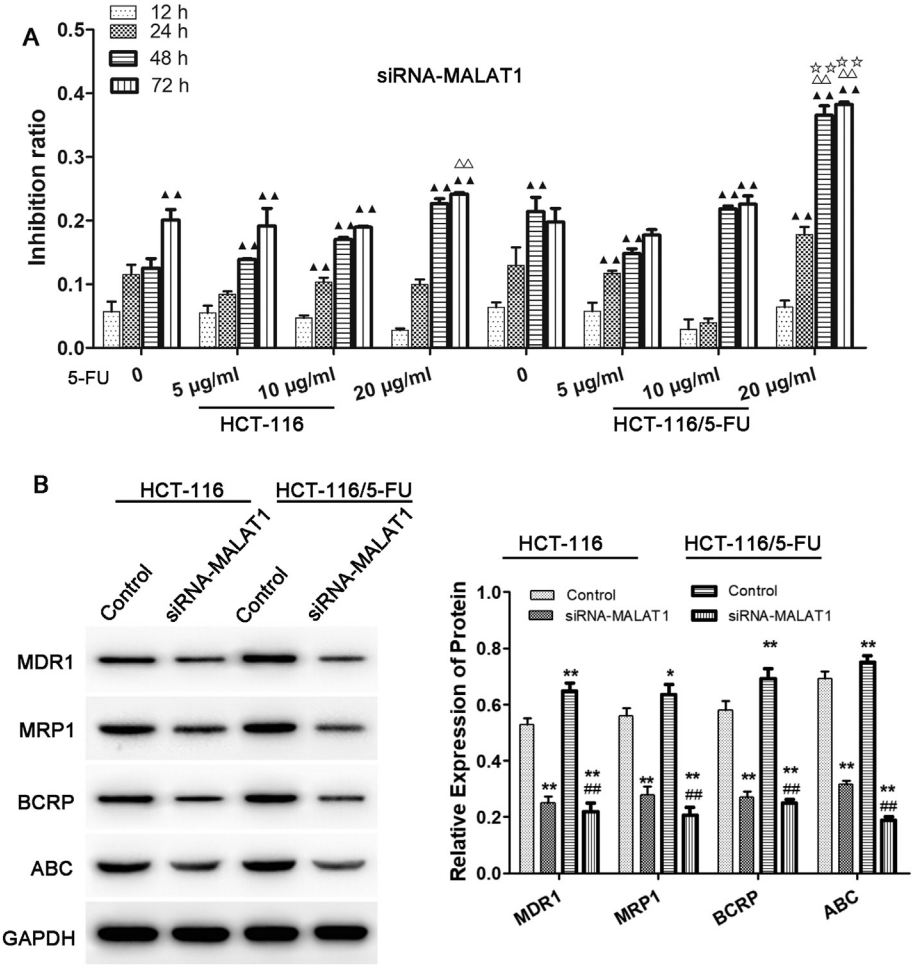

Fig. 4. Downregulation of MALAT1 reduced the resistance of HCT-116 and HCT-116/5-FU cells against 5-FU. (A) The inhibition of 5-FU to HCT-116 and HCT-116/5-FU cells. (B) The protein expression levels of MDR1, MRP1, BCRP, and ABC in HCT-116 and HCT-116/5-FU cells were detected by western blot. Data are shown as mean ± SD (n = 3). ▲▲P < .01 compared with 12 h group; △△P < .05 compared with 10 μg/ml group; ☆☆P < .05 compared with HCT-116 cells treated with 20 μg/ml 5-FU for 72 h group; **P < .01 compared with control group; ##P < .01 compared with MALAT1-silenced HCT-116 cells.

HCT-116/5-FU cells, and the apoptosis in HCT-116/5-FU cells was high than that in HCT-116 cells (Fig. 3A). The expression of pro-apoptotic proteins Bax, Cyt-c, and caspase-3 was upregulated, whereas that of the anti-apoptotic protein Bcl-2 was downregulated (Fig. 3B). These results showed that MALAT1 silencing promoted apoptosis while inhibiting the migration of HCT-116 and HCT-116/5-FU cells.

3.4. MALAT1 silencing reduced 5-FU resistance of HCT-116 and HCT-116/5-FU cells in vitro

To explore whether MALAT1 influences the resistance of CRC cells against 5-FU, HCT-116 and HCT-116/5-FU cells were transfected with siRNA-MALAT1 and treated with 5-FU (0, 5, 10, and 20 μg/ml) for 12, 24, 48 and 72 h. We found that MALAT1 silencing significantly inhibited the viability of HCT-116 cells and HCT-116/5-FU cells in time-dependent and dose-dependent manners. The inhibition of cell proliferation in HCT-116/5-FU cells treated with 20 μg/ml 5-FU for 48 h and 72 h was higher than that in HCT-116 cells (Fig. 4A). The expression levels of drug resistance-associated proteins MDR1, MRP1, BCRP, and ABC in HCT-116 and HCT-116/5-FU cells were downregulated by MALAT1 silencing, and the downregulation in HCT-116/5FU cells was higher than that in HCT-116 cells, indicating the suppression of 5-FU resistance (Fig. 4B).

3.5. Effect of MALAT1 on CRC cells by targeting miR-20b-5p

The expression levels of miR-20b-5p in CRC cell lines were lower than that in NCM460 cells (Fig. 5A). The luciferase activity in cells co-transfected with miR-20b-5p and WT MALAT1 3'-UTR reporter was markedly decreased (Fig. 5B), which verified that the MALAT1 targets miR-20b-5p, and the binding site was shown in Fig. 5C. Upregulation of miR-20b-5p evidently reduced the expression of MALAT1 in both HCT-116 and HCT-116/5-FU cells compared with that in the negative control group (Fig. 5D). Meanwhile, the migration and invasive abilities of HCT-116 and HCT-116/5-FU cells were diminished by miR-20b-5p (Fig. 5F). Altogether, these results indicated that miR-20b-5p is involved in the regulation of CRC cell metastasis via MALAT1.

3.6. MALAT1 silencing suppressed HCT-116/5-FU cell tumorigenesis in vivo

Ki67, a well-known proliferation marker, is widely used in the diagnosis of pathologic lesions, especially malignancies [26]. MALAT silencing markedly attenuated Ki67 expression (Fig. 6A and B), upregulated miR-20b-5p expression (Fig. 6C), and inhibited tumor growth (Fig. 6D). These results demonstrated that MALAT1 silencing suppressed tumorigenesis in HCT-116/5-FU cells by upregulating miR-20b-5p.

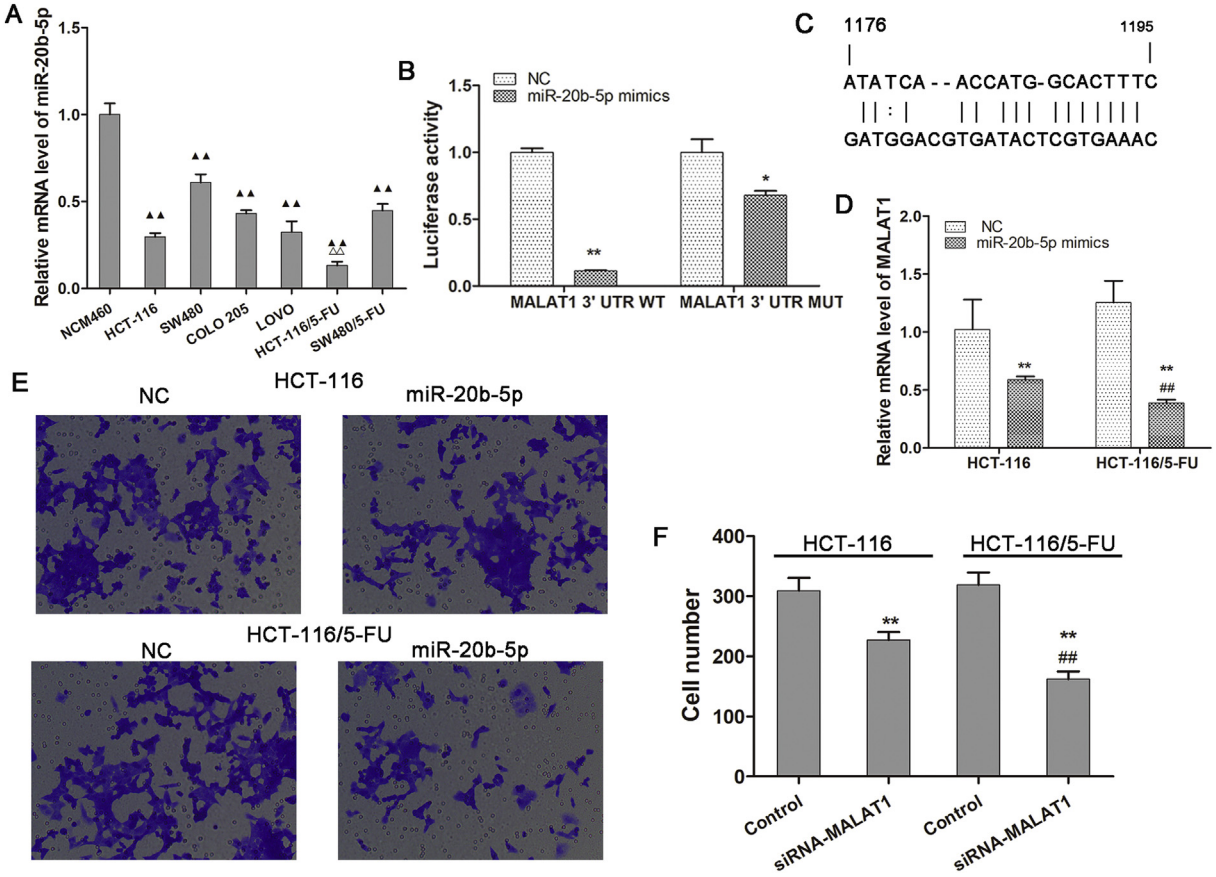

Fig. 5. Effect of MALAT1 on HCT-116 and HCT-116/5-FU cells via targeting miR-20b-5p. (A) The mRNA levels of miR-20b-5p in normal human colorectal epithelial cells NCM460 and CRC cell lines HCT-116, SW480, COLO205, LOVO, HCT-116/5-FU, and SW480/5-FU were detected by qRT-PCR. (B) Luciferase activity assay of HCT-116 and HCT-116/5-FU cells transfected with luciferase constructs containing WT and MUT MALAT1 3'-UTR. (C) The binding site of miR-20b-5p to MALAT1. (D) The mRNA levels of MALAT1 in HCT-116 and HCT-116/5-FU cells were detected by qRT-PCR. (D) miR-20b-5p inhibited the migration of HCT-116 and HCT-116/5-FU cells. NC = negative control. Data are shown as mean ± SD (n = 3). $^{▲▲}P < .01$ compared with NCM460 cells; $^{△△}P < .01$ compared with HCT-116 cells; $^{**}P < .01$ compared with control or NC group; $^{\#\#}P < .01$ compared with HCT-116 cells treated with miR-20b-5p mimics.

4. Discussion

CRC is one of the most common malignant diseases in humans and the third leading cause of cancer-related deaths worldwide [27]. Although recent advances in diagnostics and therapeutics have improved the clinical outcomes for early-stage CRC patients, a significant proportion of early-stage CRC patients still experience recurrence and metastasis. There are few reliable markers available to accurately predict metastasis in early-stage CRC patients, and CRC cells are prone to drug resistance, leading to the failure of chemotherapy. Therefore, effective treatment and prevention of metastasis and recurrence remain a challenge.

In recent years, growing evidence has indicated that the lncRNA MALAT1 contributes to tumor development in several types of human cancers, including lung, pancreatic, and colorectal cancer [21,28,29]. The elevated expression of MALAT1 plays an important role in tumor cell viability, migration, and invasiveness. MALAT1-deficient lung cancer cells showed impaired migration and formed fewer tumors in mice [30]. In addition, MALAT1 gene mutation was recently found in breast cancer and CRC [31,32]. In CRC, these mutations are enriched in the 3′-end of MALAT1, and MALAT1 overexpression induced the invasion of SW480 cells [31]. However, the effect of MALAT1 in CRC migration and its underlying mechanisms were previously unclear.

In this study, MALAT1 levels were higher in six CRC cell lines than that in normal human colorectal epithelial cells. In addition, MALAT1 affected the invasion and metastasis of HCT-116 and HCT-116/5-FU cells by targeting miR-20b-5p. Moreover, depletion of MALAT1 induced the sensitivity of cancer cells to 5-FU by inhibiting MDR1, MRP1, BCRP, and ABC. Different ABC transporters have been found to be upregulated in colon cancer, which facilitated the efflux of anti-cancer drugs out of cancer cells and decreased their therapeutic effects [33]. ABC transporters, such as MDR1/P-glycoprotein, MRP1, and BCRP, have been demonstrated to be increased in various tumors and were upregulated in tumor cells treated with cytotoxic agents [34,35]. Inhibiting ABC protein expression and suppressing the co-administration of modulators have been identified as effective approaches to sensitize drug-resistant cancer cells to anti-cancer drugs in vitro [36]. The present study supports that MALAT1 interference inhibited the resistance of HCT-116 cell lines against 5-FU via modulation of ABC transporters.

Our results also demonstrated that MALAT1 silencing significantly inhibited tumorigenesis and metastasis in CRC in vitro and in vivo. MALAT1 was first involved in tumor metastasis in non-small cell lung cancer patients, and MALAT1 knockdown in A549 lung cancer cells significantly inhibited cell migration without affecting cell proliferation [37]. Moreover, increased MALAT1 expression was observed in bladder cancer patients who developed metastases compared to those without metastases [38]. In this study, MALAT1 interference regulated the protein expression of N-cadherin, vimentin, Snail, E-cadherin, α-catenin, and β-catenin to inhibit EMT, in turn inhibiting metastasis and invasion of HCT-116 cells. Depletion of MALAT1 in HCT-116 cells induced the expression of the pro-apoptotic genes caspase-3, Bax, and Cyt-c and downregulated that of the anti-apoptotic gene Bcl-2. This effect of MALAT1 on cancer cell apoptosis has been reported in cervical cancer cells [20].

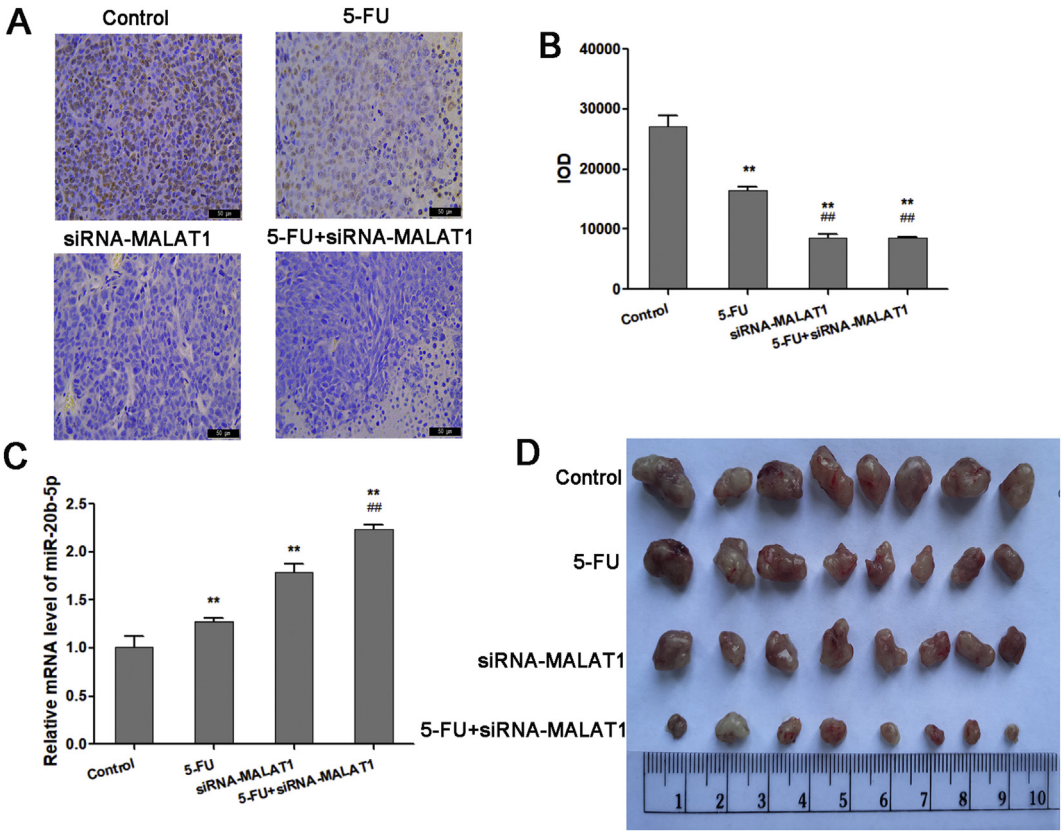

Fig. 6. MALAT1 silencing suppressed CRC tumorigenesis by upregulating miR-20b-5p in vivo. (A) The levels of Ki67 in CRC tumor tissues were detected by IHC and (B) qualified by Image-Pro Plus software. (C) The mRNA levels of miR-20b-5p in cancer tissues were detected by qRT-PCR. (D) MALAT1 silencing inhibited CRC tumorigenesis. Data are shown as mean ± SD (n = 3). $^{**}P < .01$ compared with control group; $^{\#\#}P < .01$ compared with 5-FU group.

Furthermore, our results showed that MALAT1 interference inhibited colorectal tumor growth by regulating the expression of Ki-67 and miR-20b-5p. miRNAs are closely related to tumor occurrence, and abnormal expression of miRNAs has been suggested to influence tumor progression. Recently, miR-20b has been reported to be related to the clinicopathological features of CRC [39]. In this study, the expression of miR-20b-5p in HCT-116 and HCT-116/5-FU cells were lower than that in normal cells, and MALAT1 interference induced the expression of miR-20b-5p in HCT-116/5-FU-induced tumor tissue. Fu et al. reported that the expression of miR-20b reduced 5-FU resistance to induce apoptosis in colon cancer by suppressing the ADAM9/EGFR pathway [24]. These findings further supported the view that the down-regulation of MALAT1 in CRC inhibited tumorigenesis by targeting miR-20b-5p.

In conclusion, MALAT1 expression was elevated in CRC cell lines, suggesting that MALAT1 may be involved in the progression of CRC. MALAT1 silencing suppressed CRC progression and metastasis and improved the sensitivity of HCT-116 to 5-FU. miR-20b-5p might act as a target of MALAT1 silencing to inhibit the development CRC.

Competing interests

The authors declare that they have no conflicts of interest regarding the contents of this article.

Acknowledgements

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