ORIGINAL RESEARCH

RETRACTED ARTICLE: Metformin Suppresses the Proliferation and Promotes the Apoptosis of Colon Cancer Cells Through Inhibiting the Expression of Long Noncoding RNA-UCAI

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Purpose: LncRNA-UCA1 has been prove to fact the the production and metastasis of colon cancer. Whether metformin inhibit the progress in a colon cancer by suppressing lncRNA-UCA1 remains unknowns in this research, we aimed to explore the role of Metformin playing in pathogenesis of colon cancer.

Materials and Methods: using qRT-PCR, we cleasured the expression of five tumorpromoting lncRNAs in SW 80 and SW67 colon cancer cells. Then, we conducted Western blotting and immunohistoci unistry to evaluate the effects of MET or UCA1 knockdown or the combined MET+ UCA1 k okdown on the activities of the PI3K/AKT and ERK pathways in vitro and in a set tissues obtained from tumor-bearing nude mice.

s from 8 assays showed that MET dose-dependently and time-**Results:** The rest depend inhibite e viability of the colon cancer cells in vitro. Flow cytometry revealed that лЕТ рі noted 1 apoptosis of the SW480 and SW620 cells. qRT-PCR showed that RNA-U and the highest expression among the five lncRNAs. Suppressing UCA1 n by siRNA or shRNA could further enhance the metformin-mediated anticancer expi effects a just colon cancer in vitro and in vivo. Metformin decreased the UCA1 expression and further hibited the proliferation and promoted the apoptosis of the colon cancer cells, ich were associated with inactivation of the PI3K/AKT and ERK signaling pathways o and in the tumor tissues obtained from the mice.

Conclusion: These results indicated that metformin has potential anticancer properties and revealed the anticancer mechanisms of metformin against colon cancer via regulating lncRNA-UCA1.

Keywords: colonic neoplasms, metformin, urothelial cancer associated 1 noncoding RNA

Introduction

Colon cancer is a common malignant cancer in China.¹ Every year, more than 600,000 patients die from colon cancer worldwide.² Therapies for colon cancer include surgery, chemotherapy, targeted therapy and immune therapy. However, colon cancer is characterized by both aggressive behavior and a poor response to chemotherapy.³ Therefore, it is important to explore new strategies to treat colon cancer.

Metformin has been used as the first-line therapy for type II diabetes mellitus for decades. In addition, many epidemiological studies have observed that patients

© 2020 Guo et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php you hereby accept the free. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial uses of this work, please see paragraphs 4.2 and 5 of our Terms (http://www.dovepress.com/terms.php). treated with metformin had significantly lower rates of cancer than those not treated with metformin.^{4–7} Various laboratory results have also indicated that metformin possesses antitumoral properties, including effects against breast cancer,⁸ gastric cancer,⁹ prostate cancer,¹⁰ and colon cancer.¹¹

Long noncoding RNAs (IncRNAs) are a group of RNA transcripts containing more than 200 nucleotides that cannot be translated into protein products.¹² LncRNAs have important functions in the progression of many cancers.¹³ It has been reported that lncRNA-urothelial carcinomaassociated 1 (UCA1) plays a critical role in tumorigenesis, such as that of laryngeal squamous cell carcinoma,¹⁴ lung adenocarcinoma¹⁵ and colon cancer.¹⁶ Metformin has been proven to regulate the expression of lncRNAs through various mechanisms, such as altering DNA methylation via regulation of the lncRNA-H19 and SAHH axes¹⁷ and regulating tumor cell migration and invasion by interfering with the lncRNA-H19 and let-7 axes.¹⁸ Whether metformin exerts its antigrowth effects on colon cancer via regulating lncRNA-UCA1 remains unknown. In this work, we aimed to identify the anticancer effects of both metformin and lncRNA-UCA1 inhibition and the effect of metformin on UCA1 expression in SW620 and SW4 colon cancer cells.

Materials and Methods

Cancer Cell Lines

The SW480 and SW620 human color pancer cell has were purchased from ATCC (USA). The canor cells were cultured in DMEM (Thermo Loher Scientific, enc., Waltham, MA, USA) with 10% FL3 and 12 U/mL streptomycin and penicillin. All cells were neglited at 37°C with 5% CO₂.

Cell Counting Lit-8 (CCK-8) Assay

Cell viability as examined with CCK-8 assays (Dojindo, Japan) according to the manufacturer's instructions. A total of 5,000 can be cells were seeded in 96-well plates and treated with metformin at different concentrations (0, 20, 40, 80 mM) for 24 h. In addition, 40 mM metformin was used to treat the cancer cells for 12, 24 or 48 h. To test whether metformin could improve the lncRNA-UCA1 knockdown-mediated inhibition of the cellular viability of colon cancer cells in vitro, we used si-NC, si-NC + 40 mM metformin, si-UCA1 or si-UCA1 + 40 mM metformin to treat the cancer cells for 24 h.

Flow Cytometry-Based Method for Evaluating Apoptosis

Cells were treated with or without 40 mM metformin for 48 h. In addition, si-NC, si-NC + 40 mM metformin, si-UCA1 or si-UCA1 + 40 mM metformin was used to treat the cancer cells for 48 h. After 48 h, samples were collected for apoptotic cell analysis by using an Annexin V-FITC kit (Invitrogen, USA). Apoptotic rate (%) = the rate of the early apoptotic cells (bottom-right field) + the rate of late apoptotic cells (top-right field). The results were collected and evaluated by flow cytoperary to ccuri C6; BD Biosciences; Becton, Dickinson and Company

Western Blot Ass

For Western blotting, he tote protein state from the parated SDS-PAGE and cancer cells was pare PVDF me. rap (Millipore, Billerica, transferred to MA, USA). ne marbranes wei, blocked with 5% nonfat milk and incubated in e following primary antibodies at 4°C vernight: p21 (1:1000, CST, USA), PCNA (1:1000, CS , Bax (1:10, CST), Bcl-2 (1:1000, CST), cleavedcasp e 3 (1:10; CST), GAPDH (1:10,000; Abcam), phosphy AKT (Ser473) (1:1000; Abcam), total-AKT 200; Abcam), phosphor-ERK1/2 (1:1,000; Abcam), d totar-ERK (1:1000; Abcam). After the membranes were washed with TBST, they were incubated with HRPnjugated secondary antibody (1:2,500; Abcam). The GAPDH levels were used as internal standards.

Immunohistochemistry (IHC) Staining

Tissue pieces were embedded in paraffin and sliced into 5-µm sections on a microtome. After the samples were deparaffinized and dehydrated in gradient alcohol, ethylenediaminetetraacetic acid was used for antigen retrieval via a microwave oven for 20 min. The slides were treated with 5% BSA for 30 min to block the nonspecific antibody binding sites. Then, the slides were treated with primary antibodies overnight at 4°C. After being washed with TBST for 5 min three times, the slides were incubated with HRPlabeled secondary antibody at room temperature for 1 h. Then, diaminobenzene was used for visualization according to the instructions (Wuhan Goodbio Technology). The dilution rate of the antibodies used in this work was as follows: PCNA (1:5000; CST), phosphor-AKT (Ser473) (1:50; CST), phosphor-ERK1/2 (1:100; CST), and cleaved caspase 3 (1:100; CST) (HRP)-labeled secondary antibodies (Wuhan Goodbio Technology).

RNA Extraction and Real-Time PCR Analysis

RNA was isolated from the SW480 and SW620 cells with TRIzol reagent (Invitrogen, Carlsbad, CA). qRT-PCR-related reagent was purchased from Qiagen (USA). LncRNA expression was normalized to that of β -actin. The primers are shown in Table 1.

SiRNA Transfection and Plasmid Construction

SW480 and SW620 cells were transfected with siRNAs via Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's instructions. The lncRNA-UCA1 siRNA and negative control siRNA (si-NC) are shown in Table 1. The human lncRNA-UCA1 cDNA was cloned into the pcDNA3.1 vector.

Cancer Cell Lines

The SW480 and SW620 human colon cancer cell lines were purchased from ATCC (USA). The cancer cells were cultured in DMEM (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% FBS and 100 U/mL streptomycin and penicillin. All cells were incubated at 37° C with 5% CO₂.

Cell Counting Kit-8 (CCK-8) Assay

Cell viability was examined with CCK-8 assays (Dojil Japan) according to the manufacture s in ruction

Table I Sequences of Primers and sin A

β-actin	Forward Reverse	5'-CACCE A CETTETACAL SAGE-3' 5'-GTE ATETEE TETGEATCETGT-3'
UCAI	Forward Reverse	5'-GCC 2AGGTCTTAAGAGATGAG-3'
АТВ	Forward	4 ATCACC/ CACCCAGAGA-3' 5 GAC CAAAAACAGTTCCGAGTC- 3'
BCAR4	F ward verse	5CAGCAGCTTGTTGCTCATCT-3' 5'-TTGCCTTGGGGACAGTTCAC-3'
SUMO I P3	Forw d Reverse	5'-ACTGGGAATGGAGGAAGA-3' 5'-TGAGAAAG GATTGAGGGAAAAG-3'
CASC15	Forward Reverse	5'- CACACGCATGGAAAACCCAG-3' 5'- GAGGACCTGAGCTGT AAGCC-3'
siUCAI	Forward Reverse	5'-GGGAAUACUAUUCGUAUGATT-3' 5'-UCAUACGAAUAGUAUUCCCTT-3'
siNC	Forward Reverse	5'-UUCUCCGAACGUGUCACGUTT-3' 5'-ACGUGACACGUUCGGAGAATT-3'

A total of 5,000 cancer cells were seeded in 96-well plates and treated with metformin at different concentrations (0, 20, 40, 80 mM) for 24 h. In addition, 40 mM metformin was used to treat the cancer cells for 12, 24 or 48 h. To test whether metformin could improve the lncRNA-UCA1 knockdown-mediated inhibition of the cellular viability of colon cancer cells in vitro, we used si-NC, si-NC + 40 mM metformin, si-UCA1 or si-UCA1 + 40 mM metformin to treat the cancer cells for 24 h.

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ostern Birt Assay

For which blotting, the total protein lysate from the larger cells was prepared, separated by SDS-PAGE and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat milk and incubated in the following primary antibodies at 4°C overnight: p21 (1:1000, CST, USA), PCNA (1:1000, CST), Bax (1:1000, CST), Bcl-2 (1:1000, CST), cleaved-caspase 3 (1:1000; CST), GAPDH (1:10,000; Abcam), phosphor-AKT (Ser473) (1:1000; Abcam), total-AKT (1:1,000; Abcam), phosphor-ERK1/2 (1:1,000; Abcam), and total-ERK (1:1000; Abcam). After the membranes were washed with TBST, they were incubated with HRP-conjugated secondary antibody (1:2,500; Abcam). The GAPDH levels were used as internal standards.

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Lentivirus Production and Transduction

Short hairpin RNA (shRNA) targeting human lnc NA-UCA1 or a negative control was cloned into the E (pGLVH1/GFP) vector with resistance to huromycin (GenePharma, Shanghai, China) colls were the atransfected with lentiviruses or control virus (μ C). The infected cancer cells were selected by using 4 μ g/mL arromycin for two weeks. As a result the cancer cells expressing GFP were chosen as sh-UCA1 and on-NC and then used for the subsequent assault.

Tumorigen sis As

Female BALE result mice (4 weeks old) were obtained from the animal operimental ministry of China Medical University. All experimental protocols were approved by the Ethics Committee of China Medical University and were in accordance with the approved guidelines set by the Institutional Animal Care and Use Committee. For the tumorigenesis assay, single-tumor cell suspensions (2×10^6 cells) were injected into the right flanks of nude mice. The mice were divided into four groups (5 mice/group): sh-NC+PBS, sh-NC+MET, sh-UCA1+PBS, and sh-UCA1+MET. Mice in the sh-NC+MET and sh-UCA1 +MET groups were intraperitoneally injected with metformin at a dose of 100 mg/kg body weight per day. The mice were killed 30 days after injection, and the tumors were weighed.

Statistical Analysis

Data are presented as the mean \pm standard deviation (SD). Statistical differences were analyzed via Student's *t*-test or one-way analysis of variance (ANOVA). A significant difference was set at P<0.05.

Results Metformin Inhibits the proliferativ Ability of Colon Carcer in vitro

To evaluate the antigroup a effect of medication (MET) on colon cancer in vitro, we used and SW480 and SW620 cell lines in this study. A CCK is celluly viability assay was conducted to essenthether Micro xerts anticancer effects on the colon cancer alls. The results showed that MET significantly inhibited to viability of the SW480 and SW 20 cells in a dose-dependent manner at 24 h in vitro (Figure 1A and H in=3). The IC50 of MET on the SW480 and SU620 cells at 24 h was approximately 40 mM, which was used as the experimental concentration in the subsequent assays. In addition, MET significantly reduced and viability of the SW480 and SW620 cells in vitro in time-dependent manner (Figure 1C and D; n=3).

Metformin Induces Apoptosis in Colon Cancer in vitro

In this part, we conducted flow cytometry to evaluate whether MET could induce apoptosis in the SW480 and SW620 cells in vitro. According to the results from the CCK-8 assays, we chose 40 mM MET to conduct the subsequent experiments. The results from flow cytometry showed that MET significantly increased the apoptotic rates in the cancer cells compared with the control cells (Figure 2).

Metformin Inhibits the Expression of IncRNA-UCA1 in Colon Cancer in vitro

It has been reported that several lncRNAs, including lncRNA-ATB,¹⁹ LNCRNA-BCAR4,²⁰ lncRNA-UCA1,²¹ lncRNA-SUMO1P3²² and lncRNA-CASC15,²³ can facilitate the proliferation, progression or metastasis of cancer. To explore the relative expression of these five lncRNAs in the colon cancer cells, we conducted qRT-PCR to determine



Figure 1 Metformin inhibits the proliferative ability of colon cancer in vitro. A CCK-8 characteria viability assat the SW480 and SW620 cells in vitro. The results showed that MET exerts anticancer views on colon c dependently (**C** and **D**). Data are represented as the mean \pm SD. *p-value<0.05; **p-value 0.01; ***p-value

which lncRNAs showed the highest expressi Accor to the results, we found that lncRNAwed t Al s highest expression among the five here. NAs, inh indi cated that lncRNA-UCA1 might v an ortant role in A; n=3). promoting colon cancer (Figu t, we used qRT-PCR to study whether AET houseness the expression K620 cells in vitro. of lncRNA-UCA1 in the SW480 and The results showed nat MFT dose-dependently inhibited the expression level of lp ANA-UCA1 in both the SW480 (Fig. 3B; n= and SW620

LncRicA-Licza Enockdown Facilitates the Anticipcer Properties of Metformin Against the Colon Cancer Cells in vitro

To further explore whether MET exerts its anticancer property partially through inhibiting the expression of lncRNA-UCA1, we used siRNA to downregulate the lncRNA-UCA1 expression in the SW480 and SW620 cells. The results from qRT-PCR showed that both 40 mM MET and si-UCA1 significantly downregulated the expression of lncRNA-UCA1 in the cancer cells in vitro. In addition, 40 mM MET+si-UCA1 showed the most significant inhibition of lncRNA-UCA1 expression (Figure 4A; n=3). Next, we conducted a CCK-8 cellular viability assay to examine the influence of MET, siUCA1 and MET+si-UCA1 on the proliferation of the colon cancer cells. We found that lncRNA-UCA1 knockdown and MET significantly suppressed the proliferation of the cancer cells in vitro. Moreover, MET+si-UCA1 further depressed the viability of the SW480 and SW620 cells in vitro (Figure 4B; n=3). To explore whether lncRNA-UCA1 knockdown affects the apoptosis of colon cancer cells, we conducted a flow cytometry-based analysis to examine the effects of MET, si-UCA1 and MET+si-UCA1 on the apoptosis in vitro. We observed that both MET and si-UCA1 could promote the apoptosis of the cancer cells. However, MET+si-UCA1 further increased the proapoptotic effect on the SW480 and SW620 cells (Figure 4C; n=3). Then, we used Western blotting to explore the effects of MET and lncRNA-UCA1 on the expression of proliferation-related and apoptosis-related proteins, including p21, PCNA, Bax, Bcl-2 and cleaved caspase 3, in vitro. The results showed that PCNA and Bcl-2 expression in the cancer cells was downregulated and that the expression of p21, Bax and cleaved caspase 3

were conducted to test the antigrowth effects of metformin on

er cells in vitro both dose-dependently (**A** and **B**) and time-

<0.001. One-way ANOVA is used in Figure 1.





was upregulated after treatment with MEL and si-UCA1, indicating that the captor cells howed low proliferation and were prone to apoptorise figure 4D).

LncRNA UCA Knockdown Promotes the Inhibit of Effects of Metformin on the PI3K/AKT and ERK Signaling Pathways in Colon Cancer in vitro

Many researchers have reported the contribution of the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB or AKT)^{24,25} and mitogen-activated protein kinase (MAPK/ERK) signaling pathways^{26,27} to the progression of colon cancer, including promoting proliferation,

reducing apoptosis and facilitating metastasis. In this part, we conducted Western blotting to test the effects of MET and lncRNA-UCA1 on the activation of the PI3K/ AKT and ERK signaling pathways, including phosphor-AKT (Ser473) and phosphor-ERK1/2, in the SW480 and SW620 cells in vitro. We found that after treatment with 40 mM MET or si-UCA1 for 48 h, phosphor-AKT (Ser473) and phosphor-ERK1/2 were significantly downregulated in both the SW480 and SW620 cells in vitro (Figure 5). In addition, the ratios of phosphor-AKT to total AKT and phosphor-ERK1/2 to total ERK were decreased in both the SW480 and SW620 cells. The combination of MET and si-UCA1 further increased the inhibitory effects on the AKT and ERK signaling pathways (Figure 5).





LncRNA-UCAI Knockdown Promotes the Inhibitory Effects of Medormin on Colon Cancer and the PI3K/AKT and ERK Signaling in the ways in vivo

To further evaluate whether a cRNA-UCA1 knockdown could is prove the anticance, effects of MET against colon cancer in the we established a SW480 cell line called sh-UCA1, in thich lncRNA-UCA1 was downregulated. Twenty-eight days after subcutaneous transplantation into nude mice, we found that both MET injection and lncRNA-UCA1 knockdown could significantly inhibit the growth of the SW480 cells in vivo. In addition, the anticancer effects of MET treatment were further enhanced in the presence of low expression of lncRNA-UCA1 (Figure 6A and B).

Furthermore, we used immunohistochemistry to examine the expression levels of PCNA, phosphor-AKT, phosphor-ERK1/2, and cleaved caspase 3 in the tumor tissues. The results showed that MET and lncRNA-UCA1 knockdown decreased the expression of PCNA, phosphor-AKT and phosphor-ERK1/2. Moreover, MET and lncRNA-UCA1 knockdown increased the level of cleaved caspase 3 in vivo. The combination of MET and lncRNA-UCA1 knockdown showed the strongest anticancer effect against colon cancer in vivo (Figure 6C). These results were consistent with the data observed from the in vitro experiments, indicating that lncRNA-UCA1 knockdown promotes the inhibitory effects of metformin on colon cancer and on the activation of the PI3K/AKT and ERK signaling pathways in vivo.

Discussion

In this work, we found that metformin exerted anticancer effects against colon cancer in vivo and in vitro and that lncRNA-UCA1 was one of its targets in colon cancer. The role of the antidiabetic drug metformin in cancer



Figure 4 LncRNA-UCA1 knockdown facilitates the anticancer effects of metformin against colon cancer cells in vitro. (A) qRT-PCR was conducted to evaluate the effects of MET or UCA1 knockdown or MET + UCA1 knockdown on the expression of lncRNA-UCA1 in the SW480 and SW620 cells in vitro. (B) CCK-8 cellular viability assays were used to test the antiproliferative effects of MET or UCA1 knockdown or MET + UCA1 knockdown on colon cancer cells in vitro. (C) A flow cytometry-based method was conducted to test the effects of MET or UCA1 knockdown or MET + UCA1 knockdown on the cellular apoptosis of the SW480 and SW620 cells in vitro. (D) Western blotting was conducted to evaluate the effects of MET or UCA1 knockdown or MET + UCA1 knockdown on the expression of two proliferation-related markers (p21 and PCNA) and three apoptosis-related markers (Bax, Bcl-2 and cleaved caspase3) in colon cancer cells. *p-value<0.05; **p-value<0.01; ***p-value<0.001. One-way ANOVA is used in A-C.



Figure 5 LncRNA-UCAI knockdown promotes the inhibitory effects of metformin on the PI3K/AKT and ERK signaling path ways in an cancer in view. Western blotting was conducted to evaluate the effects of MET or UCAI knockdown or MET + UCAI knockdown on the activation of the PI3K/AKT and ERK signal pathways in vitro.

prevention and treatment is highly debated. Sena et al reported that MET exerted antiproliferative effects on colon cancer cells by promoting both autophagy and apoptosis in vitro.²⁸ Kang et al found that the interleukin (IL)-6 signaling pathway was highly associated with metastasis of colon cancer via analyzing clinical data obtained from the Cancer Genome Atlas. These researchers also observed that metformin could block the IL-6-induced epity and mesenchymal transition in colon cancer and inhibit the. ²⁹ Sa IL-6-mediated invasion of cancer cells ju et al observed that metformin inhibit a the owth a cancer cells through methylating tumo, upp Metformin inhibited the prolifer ion of both breast cancer and colon cancer in vitro. The archers four that there was no RB (retinoblastoma protein) comoter methylation detected in colon cros, while RASS. A was partially methylated.³⁰ The affects a long-term metformin therapy on the gut microb in no abetic patients were reported by Ma al The re-archers found that metformin signicantly hanged the gut microbes in both tumorbearing the ane-auman patients, which suggested that metform -induced changes in the gut microbiome may also participate in the anticancer properties of MET against colon cancer.³¹ In addition, metformin has been proven to show inhibitory effects on the cancer stem cells (CSCs) of breast,³² pancreatic,³³ prostate and colon cancer³⁴ through affecting specific pathways involved in cellular renewal, differentiation, metastasis and metabolism. Mukhopadhyay et al reported that metformin-like AICAR (5-Aminoimidazole-4-carboxamide-1-βdrug 4-ribofuranoside) reduced AKT phosphorylation and

sion of 21 and CNA in human cancer affected exp cells,³⁵ which is consister th our findings that MET inhibits proliferation of colon cancer cells through regulatession of 1 and PCNA. Moreover, Jin et al ound that MET or AICAR promoted activation of MPK,³⁶ are inhibitory regulator of AKT/mTOR axis, sh indicates a possible mechanism of how MET inhibits coron cancer through affects AMPK. Many clinical in tigations have demonstrated the effective anticancer effects of metformin on colon cancer. Hosono et al reported that patients who did not take nonsteroidal antiinflammatory drugs benefited from low-dose metformin (250 mg/day); metformin significantly reduced the development of aberrant crypt foci, polyps and adenomas compared with that of patients taking the placebo.37,38 Anisimov reported that treatment of human and murine colon cancer cells with a range of metformin concentrations (0-10 mM) decreased cells in S phase and increased apoptosis.³⁹ In this study we used 40 mM metformin to treat tumor-bearing nude mice, in consideration of the difficulty of achieving high in vivo concentrations of metformin.⁴⁰ In future study, we will put more factors into consideration when investigate the inhibitory effects of MET on colon cancer, including the effective concentration of MET in animal models, dosage regimen of MET, side effects and nonspecific. In summary, various studies indicate that metformin is safe, is associated with a low cost and might be used as a promising treatment for many kinds of cancer, including colorectal cancer.

Emerging evidence has shown that lncRNA-UCA1 plays a tumor-promoting role in various kinds of cancer,



Figure 6 LncRNA-UCAI knockdown promotes the inhibitory effects of metformin on colon cancer and on the PI3K/AKT and ERK signaling pathways in vivo. (A and B) Metformin and UCAI knockdown significantly inhibited the proliferation of SW480 cells in a tumor-bearing mouse model. The anticancer effect of MET was further enhanced by UCAI downregulation. (C) Immunohistochemistry was used to explore the effects of MET or UCAI knockdown or MET + UCAI knockdown on the expression of PCNA and cleaved caspase 3 and the activation of the PI3K/AKT and ERK signaling pathways in the tumor tissues obtained from a tumor-bearing nude mouse model. *p-value<0.05; **p-value<0.01. One-way ANOVA is used in **B**.

including colon cancer,¹⁶ lung cancer,⁸ bladder cancer,⁴¹ and tongue cancer.42 Cui et al observed that UCA1 was highly upregulated in malignant tissues and cancer cells and also had a positive correlation with tumor burden and the clinical staging of colon cancer. However, clinical results proved that miR-28-5p expression showed a negative relationship with the progression of colon cancer, and overexpression of this molecule significantly reduced the cellular proliferation and migration of colon cancer cells in vitro. Further experiments revealed that miR-28-5p was one of the targets of UCA1 in SW480 and HT116 cells.¹⁶ Yang reported that downregulation of UCA1 enhanced the radiosensitivity of colorectal cancer cells by inhibiting proliferation and promoting apoptosis and cell cycle arrest. Moreover, downregulation of UCA1 also suppressed the epithelial-mesenchymal transition in cancer cells.⁴³ In this work, we also found that UCA1 knockdown decreased the proliferation and promoted the apoptosis of colon cancer cells in vivo and in vitro. We also found that UCA1 knockdown decreased the expression levels of p-AKT and p-ERK1/2 in the tumor tissues obtained from the xenografted tumors. Li et al found that UCA1 was significantly upregulated in gastric cancer tissues, and the silencing of this molecule decreas proliferation and increased the apoptosis of BGC 823 cells in vitro. Furthermore, the researcher found PI3K-Akt-mTOR participated in the U/ Al-ind ced pr cancer effects.⁴⁴ Yang et al observed to t both judicition of UCA1 expression and overexpression of 1 in bladder cancer cells significantly affered AKT ex ression and activity.⁴⁵ Sadek et al discovered a reliminary association between S-adenosylmet conine (SAM mediated downregulation of UCA1 and inhibition of the N3K/AKT signaling pathway in http://wang et al found that hepatic cancer highly pressed / CA1, which was related Nation LncR CA1 promoted the progresto ERK a sion of spatic green via inhibiting miR-216b and activating the F 7.1/ERK signaling pathway.47 In endothelial cells, silencia IncRNA-UCA1 significantly reduced the proliferation and tube formation ability of microvascular endothelial cells in vitro, which was regulated by the UCA1/miR-195/MEK-ERK-mTOR signaling pathway.⁴⁸ Collectively, these results demonstrated the pivotal function of UCA1 in promoting cancer and regulating the PI3K/AKT and ERK signaling pathways, which is consistent with our findings.

In this work, we also reported that metformin dosedependently decreased the expression level of UCA1 in colon cancer in vitro. UCA1 knockdown significantly increased the anticancer effects of metformin in vitro and in vivo. Metformin has been reported to show an inhibitory role in regulating the expression of protumorigenic lncRNAs in tumor cells^{49,50} and nontumoral cells.^{51,52} Li et al found that metformin regulated the proliferation and glycolysis of bladder cancer by downregulating UCA1 expression, which is consistent with our study.⁴⁹

In this study, we found that metformin exerted an inhibitory influence on colon cancer in vivo and in vitro, which is associated with MET-mediated downregulation of lncRNA-UCA1. Although more ex vivo and in vivo evaluations are required, the an encer effect of metformin and the regulation of proformin on lncR) A-UCA1 may provide promising the appeutic strate feet or colon cancer.

Conclusion

These respects begest that he of min has potential anticancer properties and remained the anticancer mechanisms of metforming gainst color ancer via regulating lncRNA-UCA1.

Pata Shering Statement

And supporting materials generated during and/or analyzed during the current study are available neighbor corresponding author on reasonable request.

Acknowledgments

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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