Metformin Suppresses the Proliferation and Promotes the Apoptosis of Colon Cancer Cells Through Inhibiting the Expression of Long Noncoding RNA-UCA1

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Jianbo Guo

Yan Li

He Duan

Lu Yuan

Department of General Surgery, The Fourth Affiliated Hospital of China Medical University, Shenyang, People's Republic of China

Purpose: LncRNA-UCA1 has been proven to facilitate the proliferation and metastasis of colon cancer. Whether metformin inhibits the progression of colon cancer by suppressing lncRNA-UCA1 remains unknown. In this research, we aimed to explore the role of Metformin playing in pathogenesis of colon cancer.

Materials and Methods: Using qRT-PCR, we measured the expression of five tumor-promoting lncRNAs in SW480 and SW620 colon cancer cells. Then, we conducted Western blotting and immunohistochemistry to evaluate the effects of MET or UCA1 knockdown or the combined MET+ UCA1 knockdown on the activities of the PI3K/AKT and ERK pathways in vitro and in tumor tissues obtained from tumor-bearing nude mice.

Results: The results from CCK-8 assays showed that MET dose-dependently and time-dependently inhibited the viability of the colon cancer cells in vitro. Flow cytometry revealed that MET promoted the apoptosis of the SW480 and SW620 cells. qRT-PCR showed that lncRNA-UCA1 had the highest expression among the five lncRNAs. Suppressing UCA1 expression by siRNA or shRNA could further enhance the metformin-mediated anticancer effects against colon cancer in vitro and in vivo. Metformin decreased the UCA1 expression and further inhibited the proliferation and promoted the apoptosis of the colon cancer cells, which were associated with inactivation of the PI3K/AKT and ERK signaling pathways in vitro 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

Correspondence: Lu Yuan
Department of General Surgery, The Fourth Affiliated Hospital of China Medical University, 4 Chongshan East Street, Shenyang, Liaoning 110032, People's Republic of China
Tel/Fax +86-2462043155
Email doc_yuan0629@yeah.net

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 (lncRNAs) 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 carcinoma-associated 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 effects of metformin on UCA1 expression in SW620 and SW480 colon cancer cells.

Materials and Methods

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 (Dojindo, Japan) according to the manufacturer's instructions. 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.

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 cytometry (Accuri C6; BD Biosciences; Becton, Dickinson and Company).

Western Blot Assay

For Western blotting, the total protein lysate from the cancer 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.

Immunohistochemistry (IHC) Staining

Tissue pieces were embedded in paraffin and sliced into 5- μ m 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 HRP-labeled 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.

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Cell Counting Kit-8 (CKK-8) Assay

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Table 1 Sequences of Primers and siRNA

β -actin	Forward Reverse	5'-CACCACACCTTCTACAATGAGC-3' 5'-GTGATCTCCTTCTGCATCCTGT-3'
UCA1	Forward Reverse	5'-CTCTCCATTGGGTTCACCATTC-3' 5'-GCGGCAGGTCTTAAGAGATGAG-3'
ATB	Forward Reverse	5'-CTTCACCAGCACCCAGAGA-3' 5'-AAGACAGAAAAACAGTCCGAGTC-3'
BCAR4	Forward Reverse	5'-ACAGCAGCTTGTTGCTCATCT-3' 5'-TTGCCTTGGGGACAGTTCAC-3'
SUMO1P3	Forward Reverse	5'-ACTGGGAATGGAGGAAGA-3' 5'-TGAGAAAG GATTGAGGGAAAAG-3'
CASC15	Forward Reverse	5'-CACACGCATGGAAAACCCAG-3' 5'-GAGGACCTGAGCTGT AAGCC-3'
siUCA1	Forward Reverse	5'-GGGAUACUAUUCGUAUGATT-3' 5'-UCAUACGAAUAGUAUUCCTT-3'
siNC	Forward Reverse	5'-UUCUCCGAACGUGUCACGUTT-3' 5'-ACGUGACACGUUCGGAGAATT-3'

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Lentivirus Production and Transduction

Short hairpin RNA (shRNA) targeting human lncRNA-UCA1 or a negative control was cloned into the LV-3 (pGLVH1/GFP) vector with resistance to puromycin (GenePharma, Shanghai, China). Cells were then transfected with lentiviruses or control virus (NC). The infected cancer cells were selected by using 4 μ g/mL puromycin for two weeks. As a result, the cancer cells expressing GFP were chosen as sh-UCA1 and sh-NC and then used for the subsequent assays.

Tumorigenesis Assays

Female BALB/c nude mice (4 weeks old) were obtained from the animal experimental 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 Proliferative Ability of Colon Cancer in vitro

To evaluate the antigrowth effects of metformin (MET) on colon cancer in vitro, we used the SW480 and SW620 cell lines in this study. A CCK-8 cellular viability assay was conducted to test whether MET exerts anticancer effects on the colon cancer cells. The results showed that MET significantly inhibited the viability of the SW480 and SW620 cells in a dose-dependent manner at 24 h in vitro ([Figure 1A and B](#); $n=3$). The IC₅₀ of MET on the SW480 and SW620 cells at 24 h was approximately 40 mM, which was used as the experimental concentration in the subsequent assays. In addition, MET significantly reduced the viability of the SW480 and SW620 cells in vitro in a 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 lncRNA-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 cellular viability assays were conducted to test the antitumor effects of metformin on the SW480 and SW620 cells in vitro. The results showed that MET exerts anticancer effects on colon cancer cells in vitro both dose-dependently (**A** and **B**) and time-dependently (**C** and **D**). Data are represented as the mean $\pm$ SD. *p-value<0.05; **p-value<0.01; ***p-value<0.001. One-way ANOVA is used in Figure 1.

which lncRNAs showed the highest expression. According to the results, we found that lncRNA-UCA1 showed the highest expression among the five lncRNAs, which indicated that lncRNA-UCA1 might play an important role in promoting colon cancer (Figure 3A; n=3). Next, we used qRT-PCR to study whether MET influences the expression of lncRNA-UCA1 in the SW480 and SW620 cells in vitro. The results showed that MET dose-dependently inhibited the expression levels of lncRNA-UCA1 in both the SW480 and SW620 cells (Figure 3B; n=3).

lncRNA-UCA1 Knockdown Facilitates the Anticancer 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

Figure 2 Metformin induces apoptosis in colon cancer cells in vitro. A flow cytometry-based method was conducted to test the effects of MET on the cellular apoptosis of the SW480 and SW620 cells in vitro. (A) Scatter plot (left) and histogram (right) of metformin induces apoptosis in SW480 cell. (B) Scatter plot (left) and histogram (right) of metformin induces apoptosis in SW620 cell. Early apoptotic cells are in the bottom-right field (Annexin V+/PI-). Late apoptotic cells are in the top-right field (Annexin V+/PI+). Data are represented as the mean $\pm$ SD. ***p-value<0.001.

was upregulated after treatment with MET and si-UCA1, indicating that the cancer cells showed low proliferation and were prone to apoptosis (Figure 4D).

LncRNA-UCA1 Knockdown Promotes the Inhibitory 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 down-regulated 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).

Figure 3 Metformin inhibits the expression of lncRNA-UCA1 in colon cancer in vitro. **(A)** Expression of the five lncRNAs reported to exert procancer effects in SW480 and SW620 cells was measured by qRT-PCR. The results showed that lncRNA-UCA1 has the highest expression level among these five candidate lncRNAs. **(B)** Metformin dose-dependently inhibited the expression of lncRNA-UCA1 in both the SW480 and SW620 cells. *p-value<0.05; **p-value<0.01; ***p-value<0.001. One-way ANOVA is used in **B**.

lncRNA-UCA1 Knockdown Promotes the Inhibitory Effects of Metformin on Colon Cancer and on the PI3K/AKT and ERK Signaling Pathways in vivo

To further evaluate whether lncRNA-UCA1 knockdown could improve the anticancer effects of MET against colon cancer in vivo, we established a SW480 cell line called sh-UCA1, in which 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 5 lncRNA-UCA1 knockdown promotes the inhibitory effects of metformin on the PI3K/AKT and ERK signaling pathways in colon cancer in vitro. Western blotting was conducted to evaluate the effects of MET or UCA1 knockdown or MET + UCA1 knockdown on the activation of the PI3K/AKT and ERK signaling 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 epithelial-mesenchymal transition in colon cancer and inhibit the IL-6-mediated invasion of cancer cells in vitro.²⁹ Sabit et al observed that metformin inhibited the growth of cancer cells through methylating tumor suppressor genes. Metformin inhibited the proliferation of both breast cancer and colon cancer in vitro. The researchers found that there was no RB (retinoblastoma protein) promoter methylation detected in colon cells, while RASSF1A was partially methylated.³⁰ The effects of long-term metformin therapy on the gut microbiome in nondiabetic patients were reported by Ma et al. These researchers found that metformin significantly changed the gut microbes in both tumor-bearing nude mice and human patients, which suggested that metformin-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 drug AICAR (5-Aminoimidazole-4-carboxamide-1- β -4-ribofuranoside) reduced AKT phosphorylation and

affected expression of p21 and PCNA in human cancer cells,³⁵ which is consistent with our findings that MET inhibits proliferation of colon cancer cells through regulating expression of p21 and PCNA. Moreover, Jin et al found that MET or AICAR promoted activation of AMPK,³⁶ an inhibitory regulator of AKT/mTOR axis, which indicates a possible mechanism of how MET inhibits colon cancer through affects AMPK. Many clinical investigations have demonstrated the effective anticancer effects of metformin on colon cancer. Hosono et al reported that patients who did not take nonsteroidal anti-inflammatory 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-UCA1 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 UCA1 knockdown significantly inhibited the proliferation of SW480 cells in a tumor-bearing mouse model. The anticancer effect of MET was further enhanced by UCA1 downregulation. (**C**) Immunohistochemistry was used to explore the effects of MET or UCA1 knockdown or MET + UCA1 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 decreased the proliferation and increased the apoptosis of BGC-823 cells in vitro. Furthermore, the researchers found that PI3K-Akt-mTOR participated in the UCA1-induced pro-cancer effects.⁴⁴ Yang et al observed that both inhibition of UCA1 expression and overexpression of UCA1 in bladder cancer cells significantly affected AKT expression and activity.⁴⁵ Sadek et al discovered a preliminary association between S-adenosylmethionine (SAM)-mediated downregulation of UCA1 and inhibition of the PI3K/AKT signaling pathway in hepatic cancer.⁴⁶ Wang et al found that hepatic cancer highly expressed UCA1, which was related to ERK activation. LncRNA-UCA1 promoted the progression of hepatic cancer via inhibiting miR-216b and activating the FGFR1/ERK signaling pathway.⁴⁷ In endothelial cells, silencing lncRNA-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 dose-dependently 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 anticancer effects of metformin and the regulation of metformin on lncRNA-UCA1 may provide promising therapeutic strategies for colon cancer.

Conclusion

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

Data Sharing Statement

The datasets and supporting materials generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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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.

References

1. Chen W-Q, Zhang S-W, Zou X-N, et al. Cancer incidence and mortality in China. 2006. *Chin J Cancer Res*. 2011;23(1):3-9. doi:10.1007/s11670-011-0003-9
2. Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014;383(9927):1490-1502. doi:10.1016/S0140-6736(13)61649-9
3. Li J, Yuan Y, Yang F, et al. Expert consensus on multidisciplinary therapy of colorectal cancer with lung metastases (2019 edition). *J Hematol Oncol*. 2019;12(1):16. doi:10.1186/s13045-019-0702-0

4. Decensi A, Puntoni M, Goodwin P, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res.* 2010;3(11):1451–1461. doi:10.1158/1940-6207.capr-10-0157
5. Evans JM, Donnelly LA, Emslie-Smith AM, et al. Metformin and reduced risk of cancer in diabetic patients. *BMJ (Clinical Research Ed).* 2005;330(7503):1304–1305. doi:10.1136/bmj.38415.708634.F7
6. Gandini S, Puntoni M, Heckman-Stoddard BM, et al. Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev Res.* 2014;7(9):867–885. doi:10.1158/1940-6207.capr-13-0424
7. Lee CK, Jung M, Jung I, et al. Cumulative metformin use and its impact on survival in gastric cancer patients after gastrectomy. *Ann Surg.* 2016;263(1):96–102. doi:10.1097/sla.0000000000001086
8. Faria J, Negalha G, Azevedo A, et al. Metformin and breast cancer: molecular targets. *J Mammary Gland Biol Neoplasia.* 2019;24(2):111–123. doi:10.1007/s10911-019-09429-z
9. Courtois S, Lehours P, Bessède E. The therapeutic potential of metformin in gastric cancer. *Gastric Cancer.* 2019;22(4):653–662. doi:10.1007/s10120-019-00952-w
10. Mayer MJ, Klotz LH, Venkateswaran V. Metformin and prostate cancer stem cells: a novel therapeutic target. *Prostate Cancer Prostatic Dis.* 2015;18(4):303–309. doi:10.1038/pcan.2015.35
11. Abdelsatir AA, Husain NE, Hassan AT, et al. Potential benefit of metformin as treatment for colon cancer: the evidence so far. *Asian Pac J Cancer Prev.* 2015;16(18):8053–8058. doi:10.7314/apjcp.2015.16.18.8053
12. Perkel JM. Visiting “noncodarnia”. *BioTechniques.* 2013;54(6):301,303–4. doi:10.2144/000114037
13. Huarte M. The emerging role of lncRNAs in cancer. *Nat Med.* 2015;21(11):1253–1261. doi:10.1038/nm.3981
14. Sun S, Gong C, Yuan K. LncRNA UCA1 promotes cell proliferation, invasion and migration of laryngeal squamous cell carcinoma cells by activating Wnt/ β -catenin signaling pathway. *Exp Ther Med.* 2019;17(2):1182–1189. doi:10.3892/etm.2018.7097
15. Chen L, Cao P, Wu Q, et al. Overexpression of LncRNA-UCA1 correlates with lung adenocarcinoma progression and poor prognosis. *Clin Lab.* 2019;65(3). doi:10.7754/Clin.Lab.2018.180739
16. Cui M, Chen M, Shen Z, et al. LncRNA-UCA1 modulates progression of colon cancer through regulating the miR-28-5p/HOXB3 axis. *J Cell Biochem.* 2019;120(5):6926–6936. doi:10.1002/jcb.27630
17. Zhong T, Men Y, Lu L, et al. Metformin alters DNA methylation genome-wide via the H19/SAHH axis. *Oncogene.* 2017;36(17):2345–2354. doi:10.1038/onc.2016.391
18. Yan L, Zhou J, Gao Y, et al. Regulation of tumor cell migration and invasion by the H19/let-7 axis is antagonized by metformin-induced DNA methylation. *Oncogene.* 2015;34(23):3076–3084. doi:10.1038/onc.2014.236
19. Yue B, Qiu S, Zhao S, et al. LncRNA-ATB mediated E-cadherin repression promotes the progression of colon cancer and predicts poor prognosis. *J Gastroenterol Hepatol.* 2016;31(3):595–603. doi:10.1111/jgh.13206
20. Ouyang S, Zheng X, Zhou X, et al. LncRNA BCAR4 promotes colon cancer progression via activating Wnt/ β -catenin signaling. *Oncotarget.* 2017;8(54):92815–92826. doi:10.18632/oncotarget.21590
21. Gou L, Liu M, Xia J, et al. BMP9 promotes the proliferation and migration of bladder cancer cells through up-regulating lncRNA UCA1. *Int J Mol Sci.* 2018;19(4):1116. doi:10.3390/ijms19041116
22. Zhang L, Wang P, Liu X, et al. LncRNA SUMO1P3 drives colon cancer growth, metastasis and angiogenesis. *Am J Transl Res.* 2017;9(12):5461–5472.
23. Jing N, Huang T, Guo H, et al. LncRNA CASC15 promotes colon cancer cell proliferation and metastasis by regulating the miR-4310/LGR5/Wnt/ β -catenin signaling pathway. *Mol Med Rep.* 2018;18(2):2269–2276. doi:10.3892/mmr.2018.9191
24. Xing Y, Ren S, Ai L, et al. ZNF692 promotes colon adenocarcinoma cell growth and metastasis by activating the PI3K/AKT pathway. *Int J Oncol.* 2019;54(5):1691–1703. doi:10.3892/ijo.2019.4733
25. Yu S, Guo H, Gao X, et al. Daphnoretin: an invasion inhibitor and apoptosis accelerator for colon cancer cells by regulating the Akt signal pathway. *Biomed Pharmacother.* 2019;111:1013–1021. doi:10.1016/j.biopha.2019.01.003
26. Zhou G, Yang J, Song P. Correlation of ERK/MAPK signaling pathway with proliferation and apoptosis of colon cancer cells. *Oncol Lett.* 2019;17(2):2266–2270. doi:10.3892/ol.2018.9857
27. Shen T, Cheng X, Xia C, et al. Erlotinib inhibits colon cancer metastasis through inactivation of TrkB-dependent ERK signaling pathway. *J Cell Biochem.* 2019;120(7):11248–11255. doi:10.1002/jcb.28400
28. Sena P, Mancini S, Benincasa M, et al. Metformin induces apoptosis and alters cellular responses to oxidative stress in Ht29 colon cancer cells: preliminary findings. *Int J Mol Sci.* 2018;19(5):1478. doi:10.3390/ijms19051478
29. Kang S, Kim BR, Kang MH, et al. Anti-metastatic effect of metformin via repression of interleukin 6-induced epithelial-mesenchymal transition in human colon cancer cells. *PLoS One.* 2018;13(10):e0205449. doi:10.1371/journal.pone.0205449
30. Sabit H, Abdel-Ghany S, Said M0, et al. Metformin reshapes the methylation profile in breast and colorectal cancer cells. *Asian Pac J Cancer Prev.* 2018;19(10):2991–2999. doi:10.22034/apjcp.2018.19.10.2991
31. Ma W, Chen J, Meng Y, et al. Metformin alters gut microbiota of healthy mice: implication for its potential role in gut microbiota homeostasis. *Front Microbiol.* 2018;9:1336. doi:10.3389/fmicb.2018.01336
32. Feng F, Zhang J, Fan X, et al. Downregulation of Rab27A contributes to metformin-induced suppression of breast cancer stem cells. *Oncol Lett.* 2017;14(3):2947–2953. doi:10.3892/ol.2017.6542
33. Bao B, Azmi AS, Ali S, et al. Metformin may function as anti-cancer agent via targeting cancer stem cells: the potential biological significance of tumor-associated miRNAs in breast and pancreatic cancers. *Ann Transl Med.* 2014;2(6):59. doi:10.3978/j.issn.2305-5839.2014.06.05
34. Montales MT, Simmen RC, Ferreira ES, et al. Metformin and soybean-derived bioactive molecules attenuate the expansion of stem cell-like epithelial subpopulation and confer apoptotic sensitivity in human colon cancer cells. *Genes Nutr.* 2015;10(6):49. doi:10.1007/s12263-015-0499-6
35. Mukhopadhyay S, Chatterjee A, Kogan D, et al. 5-Aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR) enhances the efficacy of rapamycin in human cancer cells. *Cell Cycle.* 2015;14(20):3331–3339. doi:10.1080/15384101.2015.1087623
36. Jin K, Ma Y, Manrique-Caballero C, et al. Activation of AMP-activated protein kinase during sepsis/inflammation improves survival by preserving cellular metabolic fitness. *FASEB J.* 2020. doi:10.1096/fj.201901900R
37. Hosono K, Endo H, Takahashi H, et al. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. *Cancer Prev Res.* 2010;3(9):1077–1083. doi:10.1158/1940-6207.capr-10-0186
38. Higurashi T, Hosono K, Takahashi H, et al. Metformin for chemoprevention of metachronous colorectal adenoma or polyps in post-polypectomy patients without diabetes: a multicentre double-blind, placebo-controlled, randomised Phase 3 trial. *Lancet Oncol.* 2016;17:473–483. S1470204515005653.
39. Anisimov V. Metformin for prevention and treatment of drug cancer: a reappraisal of experimental and clinical data. *Curr Drug Targets.* 2016;17(4):439–446. doi:10.2174/1389450116666150309113305
40. Vancura A, Bu P, Bhagwat M, et al. Metformin as an anticancer agent. *Trends Pharmacol Sci.* 2018;39(10):867–878. doi:10.1016/j.tips.2018.07.006

41. Wu J, Li W, Ning J, et al. Long noncoding RNA UCA1 targets miR-582-5p and contributes to the progression and drug resistance of bladder cancer cells through ATG7-mediated autophagy inhibition. *Onco Targets Ther.* 2019;12:495–508. doi:10.2147/OTT.S183940
42. Zhang T-H, Liang L-Z, Liu X-L, et al. LncRNA UCA1/miR-124 axis modulates TGFβ1-induced epithelial-mesenchymal transition and invasion of tongue cancer cells through JAG1/Notch signaling. *J Cell Biochem.* 2019;120(6):10495–10504. doi:10.1002/jcb.28334
43. Yang X, Liu W, Xu X, et al. Downregulation of long non-coding RNA UCA1 enhances the radiosensitivity and inhibits migration via suppression of epithelial-mesenchymal transition in colorectal cancer cells. *Oncol Rep.* 2018;40:1554–1564. doi:10.3892/or.2018.6573
44. Li C, Liang G, Yang S, et al. Dysregulated lncRNA-UCA1 contributes to the progression of gastric cancer through regulation of the PI3K-Akt-mTOR signaling pathway. *Oncotarget.* 2017;8(55):93476–93491. doi:10.18632/oncotarget.19281
45. Yang C, Li X, Wang Y, et al. Long non-coding RNA UCA1 regulated cell cycle distribution via CREB through PI3-K dependent pathway in bladder carcinoma cells. *Gene.* 2012;496:8–16. doi:10.1016/j.gene.2012.01.012
46. Sadek KM, Lebda MA, Nasr NE, et al. Role of lncRNAs as prognostic markers of hepatic cancer and potential therapeutic targeting by S-adenosylmethionine via inhibiting PI3K/Akt signaling pathways. *Environ Sci Pollut Res Int.* 2018;25:20057–20070. doi:10.1007/s11356-018-2179-8
47. Wang F, Ying H, He B, et al. Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway. *Oncotarget.* 2015;6(10):7899–7917. doi:10.18632/oncotarget.3219
48. Dexin Y, Changgeng F, Dajun S. Silence of lncRNA UCA1 represses the growth and tube formation of human microvascular endothelial cells through miR-195. *Cell Physiol Biochem.* 2018;49:1499–1511. doi:10.1159/000493454
49. Li P, Tong L, Song Y, et al. Long noncoding RNA H19 participates in metformin-mediated inhibition of gastric cancer cell invasion. *J Cell Physiol.* 2019;234:4515–4527. doi:10.1002/jcp.27269
50. Tian L, Sun X, Jiang X. UCA1 involved in the metformin-regulated bladder cancer cell proliferation and glycolysis. *Tumour Biol.* 2017;39. doi:10.1177/1010428317710823.
51. Deng J, Mueller M, Geng T, et al. H19 lncRNA alters methylation and expression of Hnf4α in the liver of metformin-exposed fetuses. *Cell Death Dis.* 2017;8:e3175. doi:10.1038/cddis.2017.392
52. Wu P, Tang Y, Fang X, et al. Metformin suppresses hypopharyngeal cancer growth by epigenetically silencing long non-coding RNA SNHG7 in FaDu cells. *Front Pharmacol.* 2019;10:143. doi:10.3389/fphar.2019.00143

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