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MicroRNA-493-5p suppresses colorectal cancer progression *via* the PI3K-Akt-FoxO3a signaling pathway

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Abstract. – OBJECTIVE: MicroRNA493-5p (miR-493-5p) appears to have an essential role in the abnormal cell proliferation and migration observed in the development and progression of various cancers. However, the function and mechanism of action of miR-493-5p in colorectal cancer (CRC) is unclear.

PATIENTS AND METHODS: MiR-493-5p expression was analyzed in CRC patient tissue samples and cell lines by fluorescence quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). SW480 and Caco-2 cells were transfected with miR-493-5p mimics or treated with the phosphoinositide 3-kinase (PI3K) agonist 740Y-P. Cell proliferation was determined by colony formation and cell proliferation assays and cell migration and invasion by transwell migration and wound-healing assays. The Luciferase reporter assay was used to verify the association between the expression of miR-493-5p and PI3K activity. Expression levels of PI3K, protein kinase B(Akt), and forkhead box O 3a (FoxO3a) proteins were measured by Western blot analysis and immunofluorescence assay.

RESULTS: MiR-493-5p expression was significantly downregulated in CRC tissue samples and cell lines which was associated with progression of CRC. The proliferation, migration, and invasion of CRC cells were inhibited by miR-493-5p overexpression. The finding that miR-493-5p upregulation decreased PI3K, Akt, and FoxO3a protein expression revealed that it directly targets PI3K. Additionally, the miR-493-5p-mediated suppression of CRC cell proliferation, migration and invasion was counteracted by the PI3K agonist, indicating that miR-493-5p suppresses CRC progression by inhibiting the PI3K-Akt-FoxO3a signaling pathway.

CONCLUSIONS: MiR-493-5p suppresses the proliferation, migration, invasion, and progression of CRC via the PI3K-Akt-FoxO3a signaling pathway.

Key Words:
MiR-493-5p, Colorectal cancer, PI3K-Akt-FoxO3a signaling pathway, Migration, Progression.

Introduction

Colorectal cancer (CRC) is a malignancy with high heterogeneity and poor prognosis, accounting for approximately 10.2% of new cancer diagnoses and 9.2% of cancer deaths worldwide in 2018[1]. Surgical resection and radiotherapy/chemotherapy are the main treatment strategies for non-metastatic CRC patients. Metastasis of CRC often results in progression and death. According to clinical reports, approximately 25% of CRC patients have distant organ metastasis[2] resulting in a decrease in the 5-year survival rate to 12.5% in these patients[3,4]. Previous studies have implicated various underlying mechanisms in cancer progression, including tumor cell invasion, migration, proliferation, and growth[5,6]. Accordingly, elucidating the mechanisms associated with CRC initiation and progression would be beneficial for the diagnosis and treatment of CRC.

MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression[7], and are also involved in various cellular processes, such as cell proliferation, differentiation, apoptosis, and invasion[8,9]. MiR-6803-5p promotes colorectal cancer cell proliferation and invasion through the tyrosine-protein phosphatase receptor type O (PTPRO)/Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) axis[10]. MiR-566 promotes migration and invasion in colon cancer

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cells by directly targeting protein serine kinase H1 (PSKH1)[11]. Many miRNAs, such as miR-346-5p[12], miR-144[13], and miR-92a[14], are abnormally expressed and promote the occurrence and development of CRC, which suggests that miRNAs can interact with cancer-related target genes and signaling pathways.

MiR-493-5p, derived from miR-493 and consisting of 22 nucleotides, has been found to suppress tumor growth in a variety of cancer cell types, including osteosarcoma[15], non-small cell lung cancer[16], and liver cancer. In human breast cancer, miR-493-5p reduces invasiveness and tumorigenicity by binding to fructosyltransferase 4[17]. Wang et al[18] also reported that miR-493-5p promotes apoptosis while suppressing proliferation and invasion in liver cancer cells by targeting vesicle associated membrane protein 2. In addition, epigenetic demethylation of miR-493-5p activates the expression of Dickkopf WNT signaling pathway inhibitor 2, thereby promoting angiogenesis and tumor metastasis[19]. However, the action of miR-493-5p in the regulation of CRC progression remains unknown.

The phosphoinositide-3-kinase (PI3K)/protein kinase B(Akt) pathway has been found to be involved in tumor progression as it promotes angiogenesis and metastatic migration of cancer cells[20]. Forkhead box O3a (FoxO3a), functions downstream of the PI3K-Akt pathway and its phosphorylation and translocation can induce apoptosis and inhibit cell proliferation[21]. Tenbaum et al[22] found that β-catenin induces resistance to inhibitors of PI3K and Akt, thereby blocking the action of FoxO3a and promoting colon cancer metastasis. Furthermore, the inhibition of PI3K-Akt-FoxO3a signaling by a quinazoline-like compound was found to suppress tumor progression in experimental CRC[23]. Notably, Liang et al[24] found that the upregulation of miR-493-5p can block hepatocellular carcinoma growth and metastasis by activating the PI3K-Akt-FoxO3a signaling pathway. However, the roles of both the PI3K-Akt-FoxO3a pathway and miR-493-5p in CRC remain undefined.

We speculated that the PI3K-Akt-FoxO3a signaling pathway is activated in CRC progression due to downregulation of miR-493-5p. The objective of this study was to investigate the potential roles of miR-493-5p, and PI3K-Akt-FoxO3a in CRC cell proliferation and migration and provide a theoretical basis and treatment strategy for stent restenosis.

Patients and Methods

Patients and Samples

A total of 56 primary CRC samples with matched adjacent normal tissue samples were obtained from patients diagnosed with CRC who received surgical treatment in Henan Provincial People's Hospital and People's Hospital of Zhengzhou University (Zhengzhou, China) between 2015 and 2017. The patients' characteristics, including age, sex, body mass index (BMI), tumor size and differentiation, tumor node metastasis (TNM) stage, lymph node metastasis, and liver metastasis, were recorded. All studies were approved by the Ethical Committee of the Henan Provincial People's Hospital & People's Hospital of Zhengzhou University and all the patients provided written informed consent.

Cell Culture and Transfection

Human CRC-derived cell lines (HCT116, HT29, SW480, SW620, Caco2, and DLD1) were purchased from the Chinese Academy of Sciences (Haidian, Beijing, China) and the normal human fetal colon cell line (FHC) was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cell lines were maintained in dental restorative materials soaked in culture medium (Millipore Corp., Billerica, MA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA) and 100 μg/mL streptomycin in an a constant temperature incubator with a humidified atmosphere and in 5% CO_2 at 37°C. SW480 and Caco-2 cells were transfected with either miR-493-5p mimics or their corresponding negative control (NC) mimics using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfection efficiency was verified by RNA extraction and quantitative real-time PCR (qRT-PCR). The PI3K agonist 740Y-P (25 μmol/L) was used to study the role of PI3K and PI3K signaling in the activity of miR-493-5p.

Cell Proliferation Assay

The cell proliferation of SW480 and Caco-2 cells was measured by the MTT assay. After relevant transfection with miR-493-5p mimics or NC mimics for 48 h, cells were seeded into 96-well plates at 1×10^4 cells per well and incubated in a humidity-controlled incubator for 4 h. Cell proliferation was measured at 0, 12, 24, and 48 h using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan). Cell

viability was determined by measuring absorbance at 450 nm on a microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

Wound-Healing Assays

For the wound-healing assay, SW480 and Caco-2 cells were seeded in a six-well plate at 1×10^4 cells/well and transfected with miR-493-5p mimics or NC mimics. Subsequently, 48 h later, the cells were harvested upon reaching 90% confluence. A scratch wound was made through the cell layer with a sterilized pipette tip. After 24 h of incubation, the cells in the scratch wounded areas were examined under the microscope. The extent of wound-healed scratched area of was used as a measure of the migration ability of the cells.

Transwell Migration Assay

The migratory potential of SW480 and Caco-2 cells was measured by the transwell migration assay. Briefly, the cells were seeded in the upper chamber on an 8 μm pore size insert (BD Biosciences, Franklin Lakes, NJ, USA). After starving the cells in serum-free medium for 24 hours, the cell suspension was transferred to the apical chamber. The cells on the upper surface membrane were cleaned using a cotton swab, fixed with 70% ethanol, and stained with 0.5% crystal violet for 30 min at room temperature. The number of migrated cells were counted under light microscopy.

Dual-Luciferase Reporter Assay

The homo sapiens (has)-miR-493-5p target was identified by the TargetScan online software (http://www.targetscan.org). The PI3K 3'-UTR-Luciferase reporter plasmids containing wild-type or mutant-type reporter 3′-UTR of PI3K (PI3K-WT and PI3K-MUT) were synthesized. pGL3 control vectors (Invitrogen, Waltham, MA, USA) were inserted in the plasmid. After culturing for 48 h, the relative Luciferase activity of each construct was measured using the Dual-Luciferase Reporter (DLR™) assay system (Promega, Madison, WI, USA).

Western Blot Analysis

Total protein was extracted from SW480 and Caco-2 cells using RIPA lysis buffer (Beyotime, Shanghai, China). Briefly, the bicinchoninic acid assay (BCA; Beyotime, Songjiang Shanghai, China) method was used to determine the protein concentration. Equal amounts of protein (40 μg/lane) were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% milk in Tris-Buffered Saline and Tween-20 (TBST) buffer at room temperature, the blots were probed with primary antibodies against PI3K (ab40776, Abcam, Cambridge, MA, USA), Akt (ab38449, Abcam, Cambridge, MA, USA), and FoxO3a (ab47285, Abcam, Cambridge, MA, USA) at 4°C overnight. This was followed by incubation with horseradish-peroxidase conjugated secondary rabbit or mouse antibody (Beyotime, Shanghai, China). The proteins were visualized using the enhaced chemiluminescence (ECL) detection kit (Thermo Fisher, Waltham, ME, USA).

RNA Extraction and Quantitative Real Time-PCR (qRT-PCR)

Total RNA was extracted from tissue samples and cells using TRIzol reagent (Yaji Mall, Minhang, Shanghai, China). The purity and concentration of the extracted RNA were analyzed using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher, Scientific Inc., Waltham, MA, USA). The total RNA was stored at -80°C. MiR-493-5p was reverse transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA was reverse transcribed into cDNAs using the PrimeScript RT Reagent Kit (TaKaRa Bio, Inc., Otsu, Shiga, Japan) following the manufacturer's protocol. The miR-493-5p expression level was determined using the Prime Script miRNA RT-PCR Kit (Applied Biosystems Life Technologies, Foster City, CA, USA). The relative expression of miR-493-5p was calculated using the $2^{-\Delta\Delta Ct}$ method.

Immunofluorescence Assay

The cells on glass coverslips were fixed using pre-cooled acetone and rinsed three times with phosphate-buffered saline (PBS). The cells were then incubated with 3% H_2O_2 for 10 min at room temperature in a humidified chamber to remove endogenous peroxidase activity. Then, the cells were stained with the primary antibodies against PI3K (ab40776, Abcam, Cambridge, MA, USA), Akt (ab38449, Abcam, Cambridge, MA, USA), and FoxO3a (ab47285, Abcam, Cambridge, MA, USA) at 4°C overnight. Then, the cells were incubated with HRP-conjugated secondary antibody (ZSGB-BIO, Xicheng, Beijing, China) for 45 min. The nuclei were counterstained with a 4′,6-diamidino-2-phenylindole (DAPI) solution

for 5 min in the dark. Images were acquired with a Nikon Ni-U microscope (Nikon Corp., Tokyo, Japan).

Statistical Analysis

Statistical analysis was performed with SPSS 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Data were presented as the mean ± standard deviation (SD). Repeated measures analysis of variance (ANOVA) were used to compare means across one or more variables based on repeated observations. The difference was analyzed using the Student's *t*-test. Multiple group comparisons were performed using ANOVA with Least Significant Difference (LSD) test. A *p*-value < 0.05 was considered statistically significant.

Results

Lower Levels of MiR-493-5p are ssociated With Poor CRC Prognosis

Analysis of the levels of miR-493-5p by qRT-PCR revealed that miR-493-5p expression was significantly lower in CRC tissue than in the adjacent normal tissue ($p<0.05$, Figure 1A). Analysis of the correlations between miR-493-5p expression and CRC patient data showed that increased miR-493-5p expression was significantly associated with tumor size (in mm) ($p<0.001$), differentiation ($p<0.001$), TNM stage ($p<0.001$), lymphatic metastasis ($p<0.05$), and liver metastasis ($p<0.05$) (Table I), but showed no association with age, sex, and BMI ($p>0.05$, Table I). In addition, the expression of miR-493-5p was significantly reduced in various CRC cell lines, namely HCT116, HT29, SW480, SW620, Caco2, and DLD1, compared with normal human colorectal mucosa cell lines (Figure 1B). These results indicate that miR-493-5p is downregulated in both CRC tissue and CRC cell lines and is particularly associated with tumor progression and poor prognosis of CRC.

MiR-493-5p Suppresses Proliferation, Migration, Colony Formation, and Invasion in CRC Cells

As shown in Figure 2A, transfection of SW480 and Caco-2 cells with miR-493-5p mimics significantly upregulated the expression of miR-493-5p ($p<0.01$; Figure 2A). The viability in both cell lines was significantly reduced ($p<0.01$, Figure 2B; $p<0.01$, Figure 2C) compared with the NC mimics-transfected cells. In contrast to the NC mimics, the upregulation of miR-493-5p greatly decreased the colony-forming capacity of CRC cells and significantly reduced invasion of SW480 ($p<0.01$, Figure 2D) and Caco-2 ($p<0.01$, Figure 2E) cells when transfected with miR-493-5p mimics. In addition, the overexpression of miR-493-5p promoted the migration (SW480: $p<0.01$, Figure 3A; Caco-2: $p<0.01$, Figure 3B) and invasion (SW480: $p<0.01$, Figure 3C; Caco-2: $p<0.01$, Figure 3D) of CRC cell lines compared with NC mimics. These results detect that miR-493-5p can contribute to colorectal carcinogenesis.

The PI3K-Akt-FoxO3a Pathway Is Targeted by MiR-493-5p

Potential targets of miR-493-5p were predicted using TargetScan 7.2. TargetScan analysis revealed that miR-493-5p targets PI3K (Figure 4A). To confirm this prediction, we used Luciferase

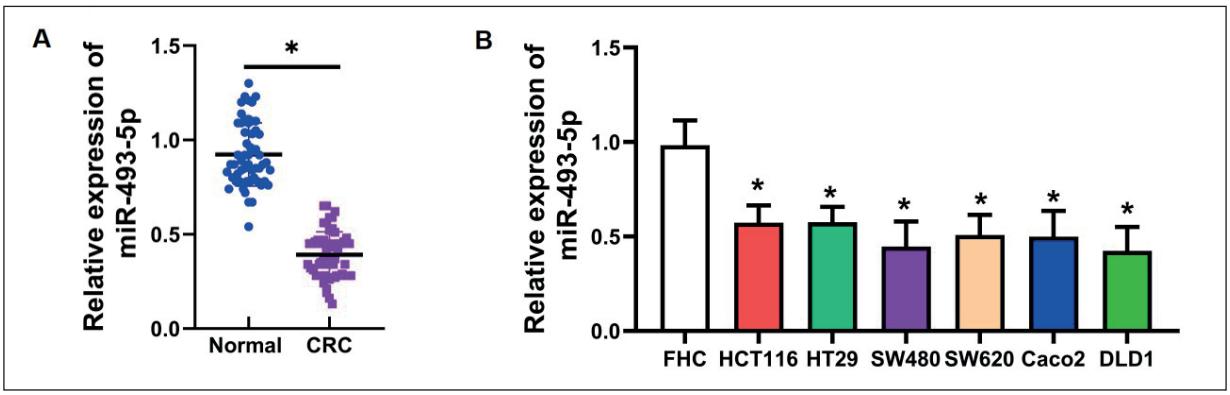

Figure 1. Downregulation of miR-493-5p in CRC tissue samples and CRC cell lines. **A,** Expression of miR-493-5p in 56 pairs of CRC tissue samples was determined by qRT-PCR analysis. **B,** Expression of miR-493-5p in CRC cell lines was measured by qRT-PCR analysis. Data are expressed as the mean ± S.E.M. *$p<0.01$ was considered as significant.

Table I. Correlation between miR-493-5p levels and clinicopathological characteristics.

Characteristic	No. of patients (n)	$\bar{x} \pm SD$	*t*	*p*
Gender			1.395	0.169
Male	37	0.38 ± 0.11		
Female	19	0.42 ± 0.14		
Age, years			0.204	0.839
< 50	29	0.39 ± 0.12		
≥ 50	27	0.39 ± 0.13		
BMI, kg/m^2			0.274	0.785
< 23	18	0.40 ± 0.14		
≥ 23	35	0.39 ± 0.11		
Tumor size, mm			4.593	< 0.001
≤ 5	36	0.44 ± 0.12		
> 5	20	0.31 ± 0.07		
Differentiation			5.848	< 0.001
Well/Moderate	33	0.45 ± 0.11		
Poor	23	0.30 ± 0.07		
TNM stage			9.208	< 0.001
0 & I & II	30	0.48 ± 0.09		
III & IV	26	0.29 ± 0.06		
Lymph node metastasis			2.477	0.016
Negative	23	0.44 ± 0.12		
Positive	33	0.36 ± 0.11		
Liver metastasis			2.324	0.024
Negative	40	0.41 ± 0.12		
Positive	16	0.33 ± 0.10		

reporter assays which showed that miR-493-5p repressed the activity of pGL3-PI3K-WT in both SW480 cells (p<0.01, Figure 4B) and Caco-2 cells (p<0.01, Figure 4C). Western blot analysis further showed that the protein levels of PI3K, Akt, and FoxO3a were significantly reduced in cells transfected with miR-493-5p mimics compared with cells transfected with NC mimics (SW480:

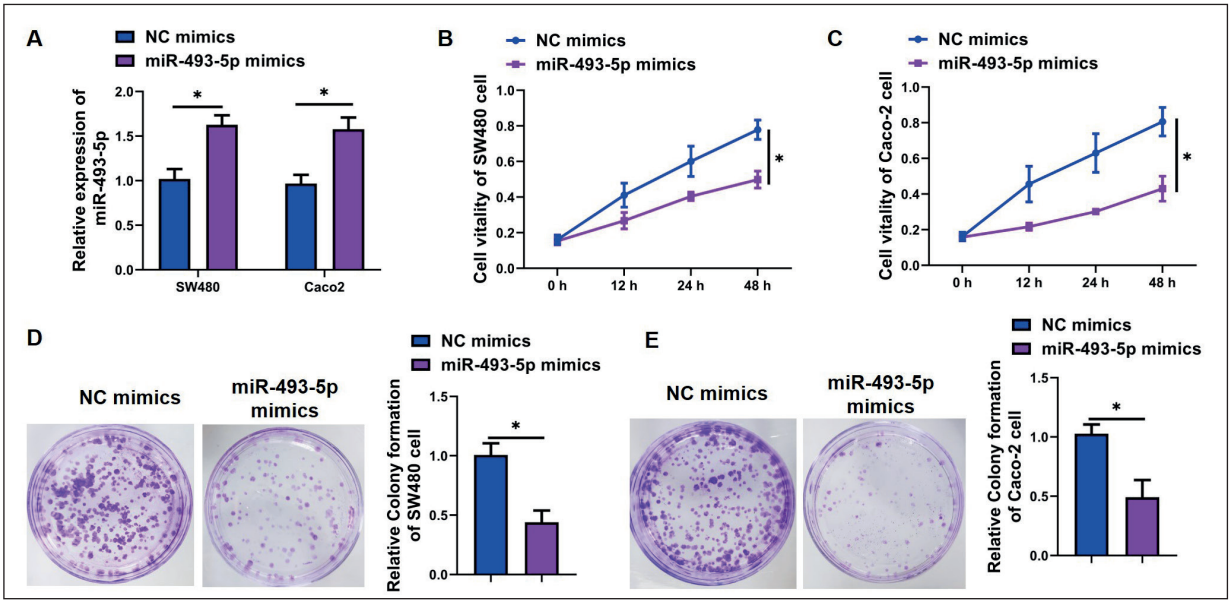

Figure 2. Overexpression miR-493-5p inhibits cell proliferation and viability in both SW480 and Caco-2 cells. **A,** Expression of miR-493-5p in SW480 and Caco-2 cells was measured by qRT-PCR analysis. **B,** and **C,** Cell viability of SW480 and Caco-2 cells was determined by CCK-8 assay. **D,** and **E,** Clonogenic capacity of SW480 and Caco-2 cells was assessed by cell colony formation assays. Representative images of the SW480 and Caco-2 cells are shown as photomicrographs (400×). Data are expressed as the mean ± S.E.M. *p<0.01 was considered as significant.

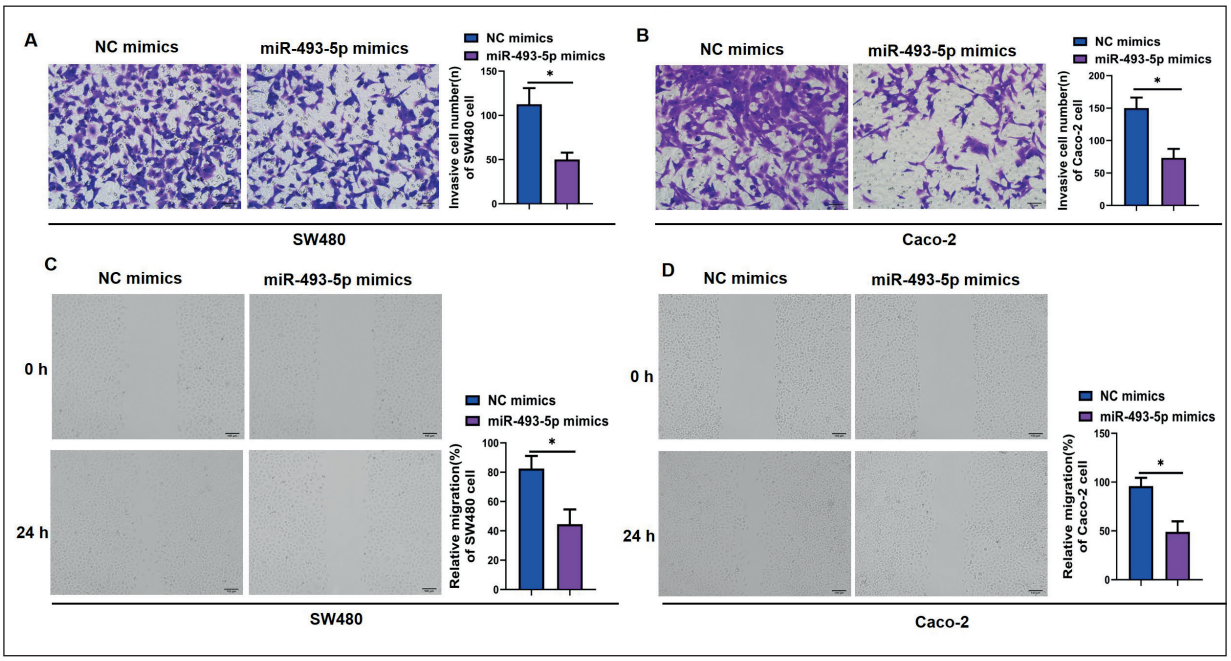

Figure 3. Overexpression of miR-493-5p inhibits migration and invasion of both SW480 and Caco-2 cells. **A,** and **B,** Invasion capability of SW480 and Caco-2 cells was evaluated by the transwell invasion assays. **C,** and **D,** Migratory capability of SW480 and Caco-2 cells was evaluated by the wound healing assays. Representative images of the SW480 and Caco-2 cells are shown as photomicrographs (400×). Data are expressed as the mean ± S.E.M. *p<0.01 was considered as significant.

p<0.01, Figure 4D and E; Caco-2: p<0.01, Figure 4D and F). Meanwhile, the immunofluorescence assay showed that expression of PI3K, Akt, and FoxO3a expressions was decreased with miR-493-5p mimics compared with NC mimics (SW480: p<0.01, Figure 4G; Caco-2: p<0.01, Figure 4H).

MiR-493-5p Suppresses Proliferation, Migration, Colony Formation, and Invasion by Targeting the PI3K-Akt-FoxO3a Pathway

We further analyze the role of PI3K-Akt-FoxO3a pathway in mediating the effects of miR-493-5p in CRC progression through treatment of the cells with the PI3K agonist 740Y-P. The analysis revealed that, while miR-493-5p expression did not change after treatment with the PI3K agonist (p>0.05, Figure 5A), the levels of PI3K and Akt were decreased (p<0.01, Figure 5B, C and D), implying that the PI3K agonist only affects the PI3K-Akt-FoxO3a pathway. The cell proliferation (SW480: p<0.01, Figure 5E; Caco-2: p<0.01, Figure 5G) and viability (SW480: p<0.01, Figure 5F and I; Caco-2: p<0.01, Figure 5H and J) of both cell lines were significantly increased after treatment with the PI3K agonist compared with the miR-493-5p mimics. In addition, the results of the wound-healing and transwell invasion assay indicated that the suppressing effects of miR-493-5p on CRC migration (SW480: p<0.01, Figure 6C; Caco-2: p<0.01, Figure 6D) and invasion (SW480: p<0.01, Figure 6A; Caco-2: p<0.01, Figure 6B) was counteracted by the PI3K agonist.

Discussion

The main cause of mortality from CRC is uncontrolled tumor growth and metastasis[25]. This study demonstrated that miR-493-5p is downregulated in CRC tissue samples and CRC cell lines, and such downregulation correlated with CRC progression. On the other hand, the upregulation of miR-493-5p expression was found to reduce CRC cell proliferation, viability, invasion, and migration *in vitro*. Moreover, the results showed that the PI3K-Akt-FoxO3a pathway was directly targeted by miR-493-5p, and the expression of PI3K, Akt and FoxO3a was inhibited by overexpression of miR-493-5p. In addition, the suppression of CRC progression by miR-493-5p was counteracted by the PI3K agonist. Taken together,

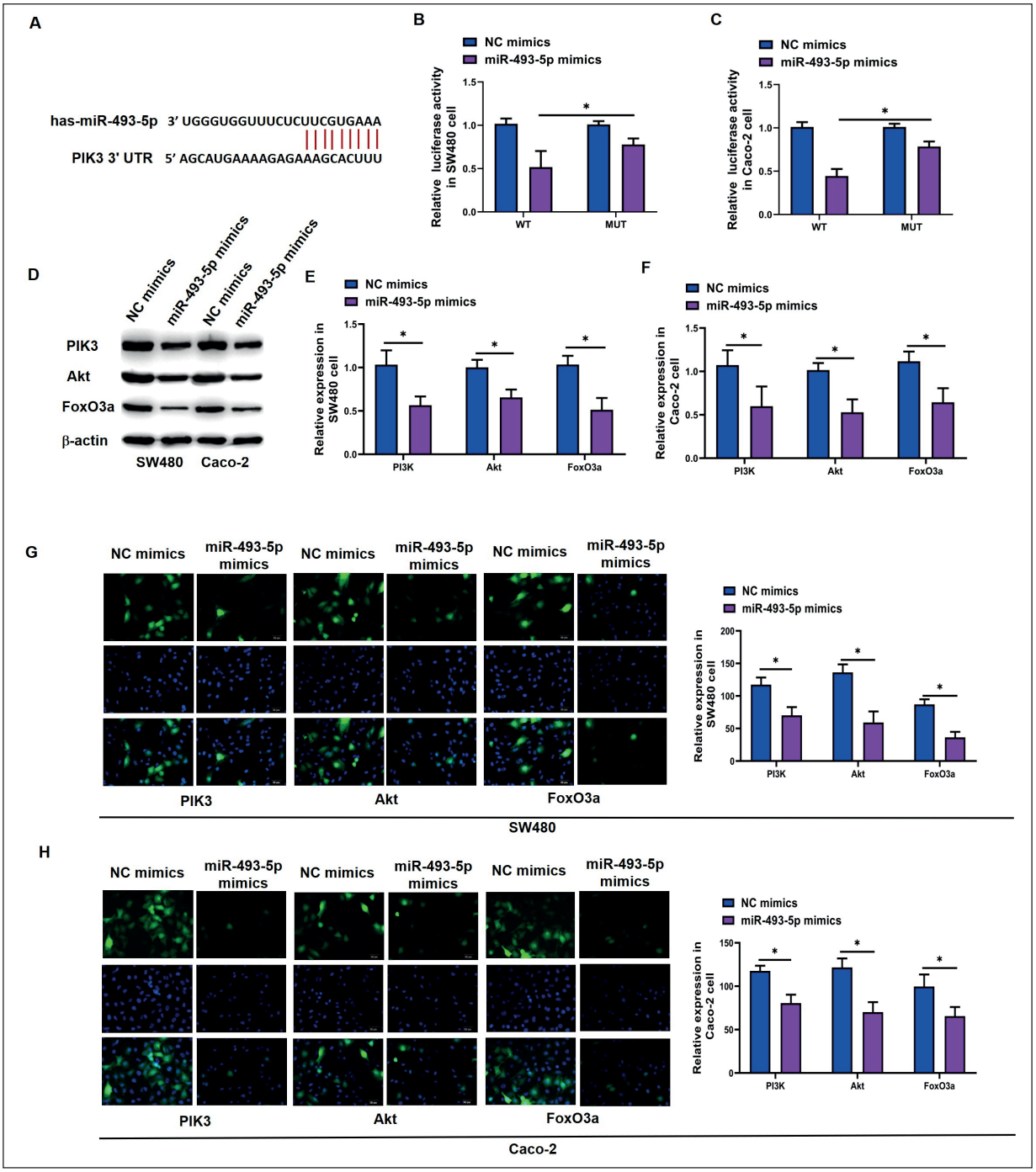

Figure 4. PI3K-Akt-FoxO3a is the target pathway of miR-493-5p. **A,** The binding site of miR-493-5p in the 3'UTR of PIK3 was predicted by TargetScan. **B,** and **C,** Luciferase activity of PI3K was detected by the Dual-Luciferase reporter assay. **D, E,** and **F,** Expression of PI3K, Akt, and FoxO3a in SW480 and Caco-2 cells was determined by Western blot analysis. **G,** and **H,** Luciferase activity of PI3K, Akt, and FoxO3a in SW480 and Caco-2 cells was evaluated by immunofluorescence assay. Representative images of the SW480 and Caco-2 cells are shown as photomicrographs (400×). Data are expressed as the mean ± S.E.M. **p*<0.01 was considered as significant.

these findings suggest that miR-493-5p counteracts the progression, proliferation, and migration of CRC through the PI3K-Akt-FoxO3a signaling pathway.

A number of reports have suggested that miR-493-5p, which has been identified as a biomarker and possible therapeutic target on various tumor types, might participate in tumor metastasis and

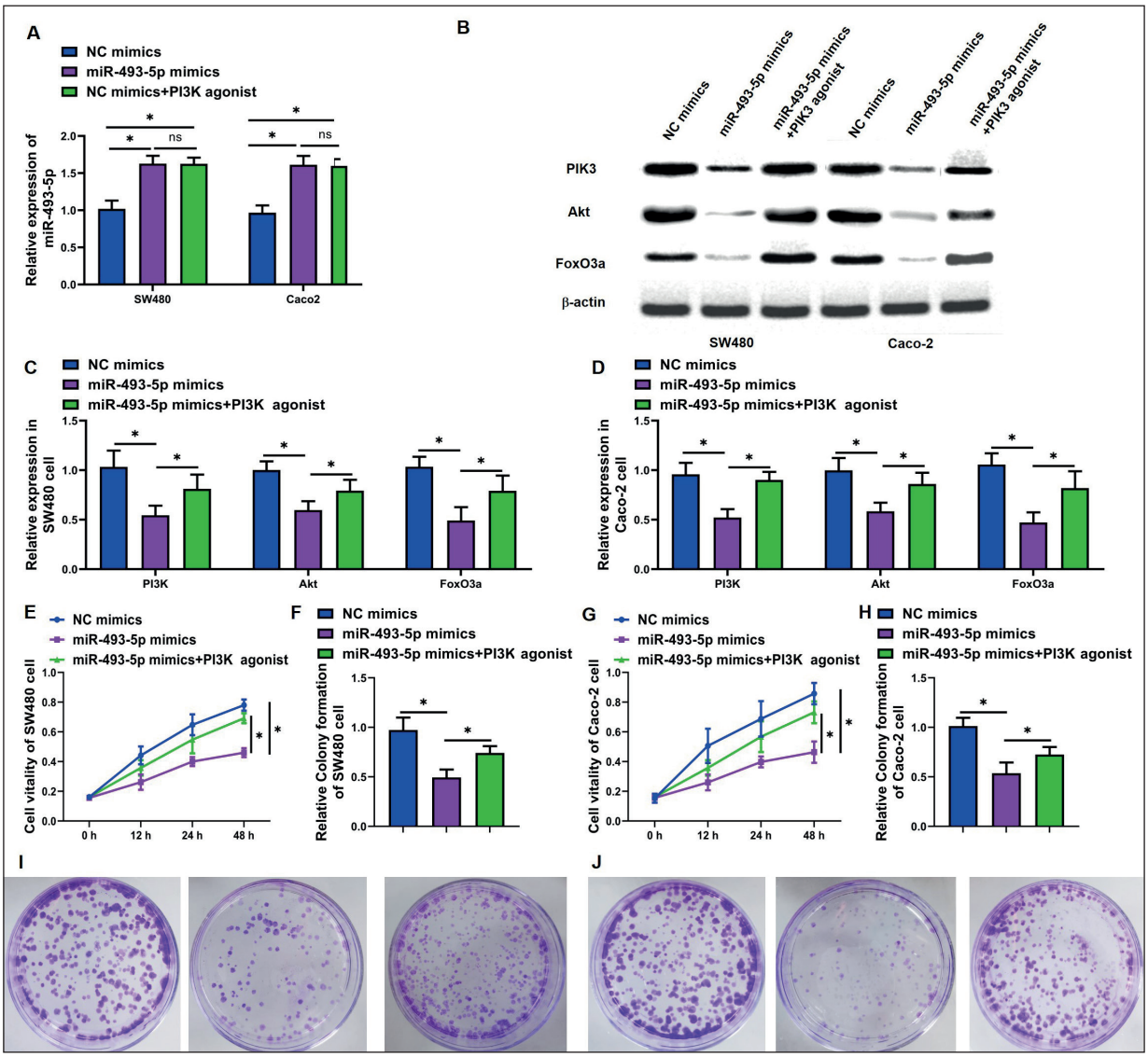

Figure 5. MiR-493-5p inhibits cell proliferation and viability *via* the PI3K-Akt-FoxO3a pathway. **A,** Expression of miR-493-5p in SW480 and Caco-2 cells was measured by qRT-PCR analysis. **B, C,** and **D,** Expression of PI3K, Akt, and FoxO3a in SW480 and Caco-2 cells was determined by Western blot analysis. **E,** and **G,** Cell viability of SW480 and Caco-2 cells was evaluated by CCK-8 assay. (**F, I,** and **H, J**). Clonogenic capacity of SW480 and Caco-2 cells was assessed by colony formation assay. Representative images of the SW480 and Caco-2 cells are shown as photomicrographs (400 ×). Data are expressed as the mean ± S.E.M. ***p*<0.01 was considered as significant. ns, *p*>0.01 was considered as non-significant.

progression[17,18,26,27]. In this study, we found that miR-493-5p was significantly downregulated in CRC tumor tissue samples compared with normal tissues, and this downregulation was associated with clinical and pathological features, such as increased tumor size, reduced differentiation, TNM stage, lymphatic metastasis, and liver metastasis. These findings suggest that miR-493-5p, acting as a tumor suppressor in human CRC, might play an important role in the progression, metastasis, and prognosis in patients with CRC. This is supported by previous studies[18,27] that have investigated the role of miR-493-5p in other types of human tumors.

Uncontrolled proliferation, migration, and invasion are early events that are critical for cancer development[12,28]. Wu et al[29] reported that miR-493-5p can contribute to the proliferation, invasion, and migration of cervical cancer cells. Here, we found that miR-493-5p was also significantly downregulated in several CRC cell lines, which is consistent with the results of the clinical samples.

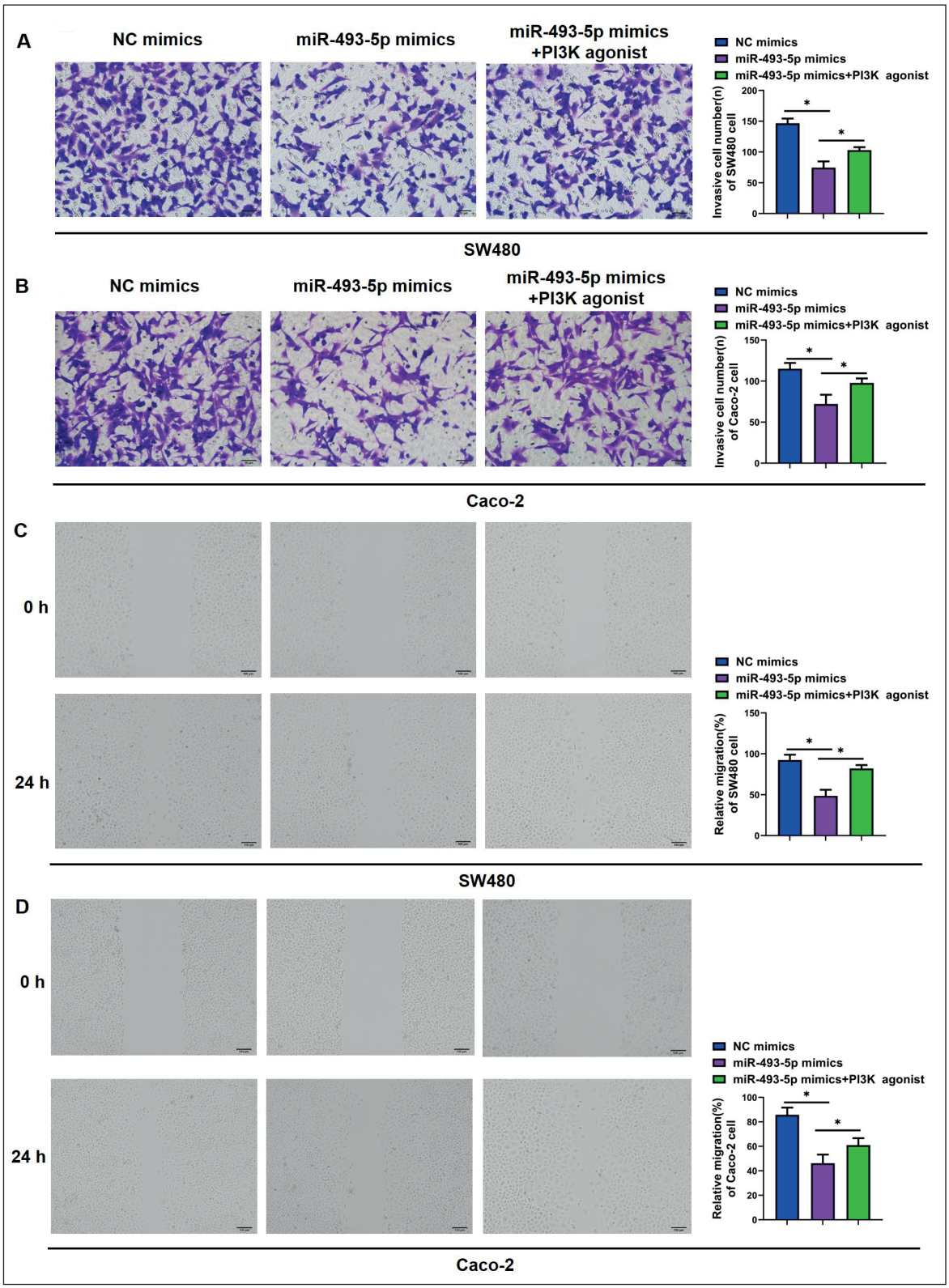

Figure 6. MiR-493-5p inhibits cell migration and invasion *via* the PI3K-Akt-FoxO3a pathway. **A,** and **B,** Invasion capability of SW480 and Caco-2 cells was evaluated by the transwell invasion assays. **C,** and **D,** Migratory capability of SW480 and Caco-2 cells was evaluated by the wound-healing assay. Representative images of SW480 and Caco-2 cells are shown as photomicrographs (400 ×). Data are expressed as the mean ± S.E.M. *$p<0.01$ was considered as significant.

We also demonstrated that the overexpression of miR-493-5p significantly reduced cell viability and colony formation in CRC cells, whereas increased miR-493-5p expression increased migration, as well as invasion, as shown by the wound-healing and transwell assay results. This suggests that miR-493-5p could act as a negative regulator in the pathogenesis of CRC, as well as CRC progression and metastasis.

The PI3K-Akt signaling pathway is activated in various human cancers including CRC[30,31]. This pathway is involved in many aspects of cell growth and survival in CRC[32,33]. The nuclear transcription FOXO3a is a downstream substrate of the PI3K-Akt pathway and is closely associated with cell proliferation, apoptosis, and tumorigenesis[34]. Our results using the online software TargetScan to predict the targets of miR-493-5p revealed that PI3K was directly targeted by miR-493-5p. Unexpectedly, the results of our Western blot analysis and immunofluorescence assays indicated that the upregulation of miR-493-5p reduces the levels of PI3K, Akt, and FOXO3a proteins. This is consistent with previous studies which found that the inhibition of the PI3K-Akt-FoxO3a survival axis could be involved in the metastasis of CRC[35]. This work thus elucidated the biological association between miR-493-5p expression and the PI3K-Akt-FoxO3a signaling pathway in CRC, which offers an avenue to further investigate potential treatments for CRC.

To further explain the role of the PI3K-Akt-FoxO3a pathway and miR-493-5p in the suppression of CRC progression, we investigated the effect of treating miR-493-p5 expressing and non-expressing cells with the PI3K agonist 740Y-P. This research showed that the inhibitory effects of miR-493-5p on the proliferation, migration, colony formation, and invasion of CRC cells were markedly reversed by the PI3K agonist, indicating that PI3K-Akt-FoxO3a signaling plays an important role in the inhibitory effects of miR-493-5p on CRC progression and growth. According to the relevant literature, activated PI3K indirectly phosphorylates Akt which can result in translocation of FoxO3a. Subsequently, FoxO3a proteins specifically bind to 14-3-3 chaperones, producing a conformational change and causing the translocation of FoxO3a from the nucleus to the cytosol[23]. These changes in FeoxO3a modulate cell cycle and apoptotic responsive genes[36] which can participate in tumor cell proliferation and migration[23]. In addition, it has been found that FoxO3a regulates the G1 to S checkpoint in the cell cycle, altering transcription of the cyclin D gene, which is involved in the proliferation of transformed intestinal epithelial cells and has been implicated in CRC tumor progression[23,37]. Together, these findings further indicate that miR-493-5p suppresses the progression of CRC through the regulation of the PI3K-Akt signaling pathway and induces abnormal expression of FOXO3a.

Conclusions

We showed that miR-493-5p functions as a tumor suppressor in CRC patients and CRC cell lines. Overexpression of miR-493-5p inhibited CRC cell proliferation, invasion, and migration by regulating the PI3K-Akt-FoxO3a signaling pathway. This is the first study providing evidence of the role and regulatory mechanism of miR-493-5p in CRC progression and suggests the potential application of miR-493-5p in the diagnosis and treatment of CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethical Statement

All analyses were based on previous published studies; thus, no ethical approval and patient consent are required.

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