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MiR-543 inhibits proliferation and metastasis of human colorectal cancer cells by targeting PLAS3

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Abstract. – OBJECTIVE: Colorectal cancer (CRC) has a very high morbidity and mortality worldwide. Related studies have shown that microRNA-543 (miR-543) is involved in the development of many cancers, including CRC. The purpose of this study was to explore the potential molecular mechanism of miR-543's involvement in the development of CRC.

PATIENTS AND METHODS: QRT-PCR and Western blot were used to detect the expression of proliferation and migration-related proteins, signal transduction and transcriptional activator 3 and protein inhibitor of activated signal transducer and activators of transcription 3 (PIAS3). Cell proliferation and metastasis were measured by MTT, transwell and Western blot. The binding sites of miR-543 and PIAS3 were predicted by TargetScan database and verified by double-luciferase report experiment.

RESULTS: The expression of miR-543 was high in CRC tissues and cell lines, while the mRNA and protein levels of PIAS3 were decreased. Meanwhile, a negative correlation between miR-543 and PIAS3 was also observed in CRC tissues. Moreover, the downregulation of miR-543 led to the inhibition of viability and the expression of proliferation and migration related proteins. Subsequently, miR-543 depletion also blocked cell migration and invasion. MiR-543 inhibits the expression of PISA3. Furthermore, downregulation of PIAS3 undermined the miR-543 depletion-mediated suppression effect on SW480 and LOVO cells. Notably, loss of miR-543 downregulated STAT3 activity, which was rescued by PIAS3 ablation.

CONCLUSIONS: MiR-543 participated in cell proliferation and metastasis by targeting PIAS3 in CRC.

Key Words:
MiR-543, PIAS3, Colorectal cancer, Proliferation, Metastasis.

Abbreviations

CRC = Colorectal cancer; PIAS3 = Protein inhibitor of activated signal transducer and activators of transcription 3; CASC2 = cancer susceptibility candidate 2; STAT3 = signal transducer and activators of transcription 3; SD = standard deviation; microRNA-543 = miR-543.

Introduction

Colorectal cancer (CRC) is the most common cause of morbidity and mortality worldwide, with annual deaths estimated at 700,000[1-3]. Although a great progress in the treatment of CRC has been made, the molecular mechanisms in the development of CRC remain largely unknown.

MicroRNAs (miRNAs) could act as biomarkers in the diagnosis of CRC[4-7]. Cong et al[8] revealed that the overexpression of miR-760 is decreased in CRC tissues and suppresses CRC cell proliferation, migration and invasion by inhibiting FOXA1 expression *in vitro*. Previous scholars[9] also suggested that miR-506 is downregulated *in vivo*, and that miR-506 also shows the suppressive effect on proliferation, migration and invasion of CRC cells. In another report[10], miR-942 is significantly increased in CRC and results in cell cycle progression in SW480 cells through Wnt signaling pathway. Wang et al[11] revealed that miR-496 promotes migration and epithelial-mesenchymal transition by targeting Ras association domain family member 6 in CRC. Researchers have revealed that miR-543 promotes colorectal cancer proliferation and metastasis by targeting Krüppel-like Factor-4[12]. Moreover, the downregulation of miR-543 ex-

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pression increases the sensitivity of colorectal cancer cells to 5-Fluorouracil through the PTEN/PI3K/AKT pathway[13]. Sun et al[14] also disclosed that miR-543 is increased in CRC tissues and acts as a tumor promoter role in metastasis of CRC. These lines of evidence suggest that miR-543 may promote the development of CRC. PIAS3 has been reported to be a direct target of miR-18a to participate in cancer susceptibility candidate 2 (CASC2)-mediated suppression effect on cell proliferation and tumor growth *in vivo*[15]. Moreover, the interaction among CASC2, miR-18a, and PIAS3 could regulate the expression of signal transducer and activators of transcription 3 (STAT3). However, whether miR-543 regulates cell proliferation and metastasis in CRC by targeting PIAS3 has not been reported. In this report, we sought to investigate the roles of miR-543 and PIAS3 in the development of CRC.

Patients and Methods

Clinical Samples and Cell Transfection

Tumor and adjacent samples were obtained from CRC patients (n=39) at Jingjiang People's Hospital. CRC patients were diagnosed and classified through pathological examination on the basis of the World Health Organization classification system. The inclusion criteria were as follows: the patient had CRC, as confirmed from pathological diagnosis; no radiotherapy or chemotherapy was delivered prior to the surgery. The patients with any other primary disease were excluded and patients who accepted local or systemic treatment before surgery were excluded. All patients and volunteers gave their written informed consent and the study was approved by the Changhai Hospital, Navy Military Medical University of PLA. CRC cell lines SW480, SW620, LOVO, HT29, and human normal colonic epithelial cell line NCM460 were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) solution, in a humidified atmosphere containing 5% CO_2 at 37°C. The miR-543 mimic (miR-543), miR-con, miR-543 inhibitor (anti-miR-543), miR-con inhibitor (anti-miR-con), small interfering RNA for PIAS3 (si-PIAS3), and negative RNA control (si-NC) were introduced into SW480 and LOVO cells using Liposome 3000 (Thermo Fisher Scientific, Waltham, MA, USA). The transfected cells were used for following the experiments.

qRT-PCR Assay

Total RNA from cultured cells and tissues of CRC patients was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed using First-Strand RT-PCR kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. qPCR was performed with the PCR Master Mix (Roche, Mannheim, Germany) on the ABI-7500 platform (Biosystems, Foster City, CA, USA). U6 and β-actin were used as internal controls. PIAS3-F, 5'-GAGCCGACATCCAAGGTTTAG-3' and PIAS3-R, 5'-GACAGCGAAGTTTCCATAATCC-3'; β-actin-F, 5'-GAAGGTGAAGGTCGGAGTC-3' and β-actin-R, 5'-GAAGATGGTGATGGGATTTC-3'; miR-543-F, 5'-TGGCAAAGGAGCAGATTAGTAGG-3' and miR-543-R, 5'-CTGCCACAAGCCACTAGAGGATAAGA-3'; U6-F, 5'-CTCGCTTCGGCAGCACA-3' and U6-R, 5'-AACGCTTCACGAATTTGCGT-3'.

MTT Assay

Cell viability was evaluated by MTT assay. MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added to each well at 24 h, 48 h, and 72 h of the culture period. These cells were incubated for another 4 h at 37°C, and then, treated with 150 µL dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) for 10 min. Absorbance was examined at 490 nm using a Multiskan plate reader (Thermo Fisher Scientific, Waltram, MA, USA).

Western Blot Assay

The cells were lysed using RIPA buffer (Beyotime, Shanghai, China) and the proteins were determined by BCA assay kit (Beyotime, Shanghai, China), and then, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Next, the proteins were transfected to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After that, the membranes were incubated with primary antibodies (anti-PIAS3, anti-CDK4, anti-CyclinD1, anti-MMP-2, and anti-MMP-9; Abcam, Cambridge, MA, USA) at 4°C for overnight, and then, incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h

at 37°C. All protein bands were visualized with enhanced chemiluminescence (ECL) kit (Beyotime, Shanghai, China).

Dual-Luciferase Reporter Assay

The wild type or muted 3'UTR of PIAS3 was inserted into the p-GL3-reporter plasmid (Promega, Madison, WI, USA) and the Luciferase reporter plasmids PIAS3-WT and PIAS3-MUT were constructed. Next, the PIAS3-WT or PIAS3-MUT and miR-543 mimic or miR-con were co-transfected into SW480 and LOVO cells using Liposome 3000. Luciferase activity was measured with the Dual-Glo™ assay (Biosystems, Pleasanton, CA, USA).

Transwell Assay

Transwell assay was used to detect cell migration and invasion abilities in SW480 and LOVO cells. For cell invasion assay, the membranes of the upper compartments were Matrigel (BD Bioscience, Franklin Lakes, NJ, USA) pre-coated. Un-coated ones were used for cell migration assay. Cells seeded into the upper transwell chamber (Corning, Corning, NY, USA) were cultured and cultured with FBS free medium. Complete growth medium with 10% FBS was added to the lower chamber. Then, the invaded and migrated cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet and counted in 5 randomly picked views under the microscope (Nikon Corp., Tokyo, Japan).

Statistical Analysis

The results were presented as the mean ± standard deviation (SD) and processed using GraphPad 7.0 statistical software (GraphPad, La Jolla, CA, USA). For comparing the differences between them, paired Student's *t*-test and one-way analysis of variance followed by post-hoc test were used. Kaplan-Meier survival assay and log-rank test were used to assess the relationship between miR-543 level and prognosis of CRC patients. *p*-value less than 0.05 was considered to be statistically significant.

Results

MiR-543 Expression Is Dramatically Elevated, While PISA3 Is Downregulated in CRC Tissues and Cells

In this study, we obtained tumor tissues and adjacent tissues from 39 CRC patients. qRT-PCR was used to examine the expressions of miR-543 and PISA3 in CRC i*n vivo*. We found that the level of miR-543 was upregulated in CRC patients compared with that of adjacent group (Figure 1A). Based on the median of miR-543 expression in CRC tumor tissues, the patients were divided into low and high miR-543 expression groups, and the high miR-543 expression was associated with a poor prognosis (Table I). Kaplan-Meier survival curves showed that the patients in high miR-543 level group had a shorter survival time than those in low miR-543 level group (**Supplementary Figure 1**). By contrast, the mRNA level of PISA3 was decreased in CRC tumor tissues (Figure 1B). Interestingly, a negative relationship between miR-543 and PISA3 expression was observed in CRC tumor tissues (Figure 1C). We further examined the expressions of miR-543 and PISA3 in CRC cell lines and human normal colonic epithelial cell line NCM460. Consistent with the data *in vivo*, the level of miR-543 was higher in CRC cell lines compared with that of NCM460 (Figure 1D). Similarly, a decrease of PISA3 at mRNA and protein levels was also observed in CRC cell lines (Figure 1E and 1F). These data suggested that miR-543 and PISA3 may play important roles in the development of CRC.

Silencing of MiR-543 Inhibits Cell Proliferation and Metastasis

To explore the role of miR-543 in cell viability and metastasis of CRC, we knocked down the expression of miR-543 in SW480 and LOVO cells using miR-543 inhibitor (anti-miR-543). As displayed in Figure 2A and 2B, the introduction of anti-miR-543 was able to induce the decrease of miR-543 expression, indicating that anti-miR-5 could be used for the subsequent loss-of-function exploration. MTT assay further showed that anti-miR-543 markedly hampered the cell viability relative to the anti-miR-con group (Figure 2C and 2D). Western blot further indicated that the expressions of proliferation-related protein CDK4 and CyclinD1 were also inhibited in anti-miR-543 group (Figure 2E-2H). Subsequently, our results also suggested that loss of miR-543 led to the reduction of cell migration and invasion (Figure 3A-3D; 100X, 100X). Western blot further confirmed that the protein levels of metastasis-related protein MMP-2 and MMP-9 were also downregulated in cells introduced with anti-miR-543.

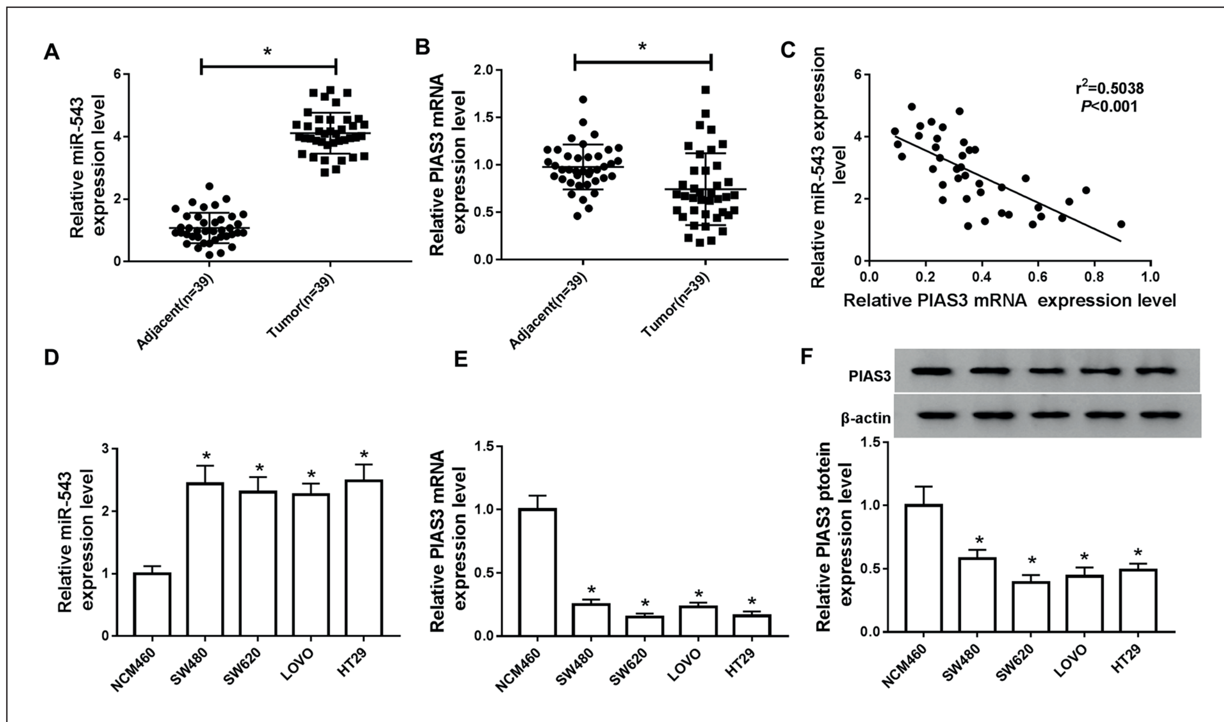

Figure 1. The expressions of miR-543 and PIAS3 in CRC tissues and cells. **A,** and **B,** qRT-PCR was performed to measure the levels of miR-543 and PIAS3 in CRC patients (n=39) tumor tissues and matched adjacent tissues. **C,** The expression of the correlation between miR-543 and PIAS3 in CRC tumor tissues. **D,** The expression of miR-543 in CRC cells. **E,** and **F,** qRT-PCR and Western blot were carried out to examine the mRNA and protein levels of PIAS3 in CRC cells, respectively. *$p < 0.05$.

Table I. Correlation between miR-543 expression and clinical clinicopathological parameters of CRC patients.

Parameter	Case	miR-543 expression		*p*-value[a]
		Low (n = 20)	High (n = 19)	
Age (years)				0.648
≤ 60	15	7	8	
> 60	24	13	11	
Gender				0.621
Female	18	10	8	
Male	21	10	11	
Tumor size				0.006*
≤ 5 cm	17	13	4	
> 5 cm	22	7	15	
TNM stages				0.037*
I-II	23	15	8	
III-IV	16	5	11	
Lymphatic metastasis				0.038*
Negative	21	14	7	
Positive	18	6	12	
Vascular invasion				0.584
Absent	25	12	13	
Present	14	8	6	
Distant metastasis				0.013*
M_0	26	17	9	
M_1	13	3	10	

CRC: Colorectal cancer; TNM, tumor-node-metastasis; *$p < 0.05$ [a]Chi-square test.

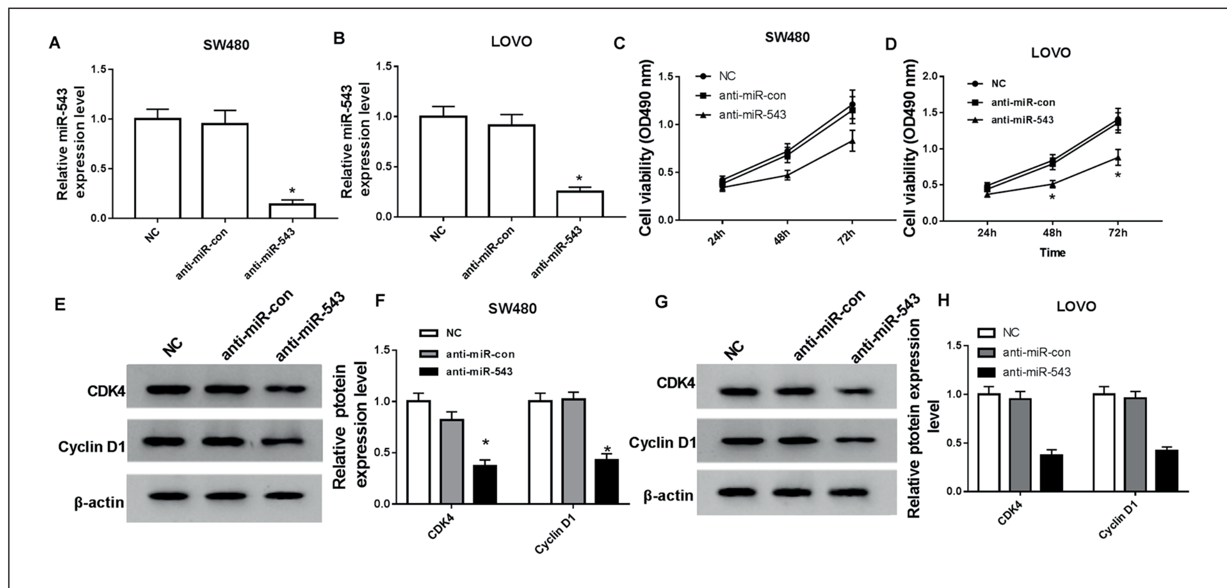

Figure 2. The effect of miR-543 downregulation on cell proliferation. **A,** and **B,** The expression of miR-543 in SW480 and LOVO cells transfected with anti-miR-con and anti-miR-543. **C,** and **D,** MTT assay was used to analyze cell viability. **E-H,** Western blot was conducted to determine the protein level of proliferation-related protein CDK4 and Cyclin D1. *$p < 0.05$.

MiR-543 Negatively Regulates the Expression of PISA3

Then, we predicted the binding sites between miR-543 and PISA3 using TargetScan database (Figure 4A). Subsequently, Luciferase reporter assay was implied to confirm the relationship between them. The Luciferase activity of miR-543 mimic-transfected cells in PISA3-WT group was decreased, while there was no evident change in PISA3-MUT (Figure 4B and 4C). These data demonstrated that PISA3 might be a direct target of miR-543. Additionally, further investigation of PISA3 expression in CRC cells transfected with miR-543 mimic or inhibitor was conducted. The results revealed that the protein level of PISA3 was inhibited by miR-543 mimic while it was promoted by anti-miR-543 (Figure 4D and 4E).

Loss of PISA3 Undermines the Effect of MiR-543 Degradation on CRC Cells

Next, the rescue-of-function experiment was performed to address the role of miR-543/PISA3 axis in CRC progression. MTT assay showed that the downregulation of PISA3 strikingly rescued the miR-543 depletion-mediated suppression of cell viability (Figure 5A and 5B). Meanwhile, cell migration and invasion abilities were also promoted in anti-miR-543+si-PIAS3 group in comparison with anti-miR-543+si-con group (Figure 5C and 5D). To explore the molecular mechanisms of this process, Western blot was used to evaluate the expression of proliferation or metastasis-related protein. The results stated that siRNA-mediated knockdown of PIAS3 undermined the miR-543 ablation-mediated effect on expressions of PIAS3, CDK4, CyclinD1 MMP-2, and MMP-9 (Figure 5E and 5H). These data revealed that PIAS3 might be involved in miR-543-mediated effect on cell proliferation and metastasis.

Interference of PIAS3 Mediated by MiR-543 depletion affects STAT3 Activation in CRC cells

PIAS3 could regulate the activation of STAT3 in various cancers. Finally, to uncover whether miR-543 is associated with the PIAS3-mediated STAT3, Western blot was conducted to determine the SATA3 expression. As expected, we found that the depletion of miR-543 blocked the protein expression of p-SATA3, which was restrained by knockdown of PIAS3 in SW480 cells (Figure 6A and 6B). Besides, the siRNA-mediated knockdown of PIAS3 also recapitulated the loss of miR-543-mediated p-SATA3 downregulation in LOVO cells (Figure 6C and 6D). Those data disclosed that miR-543/PIAS3 axis might promote cell proliferation, metastasis, and STAT3 activation in CRC.

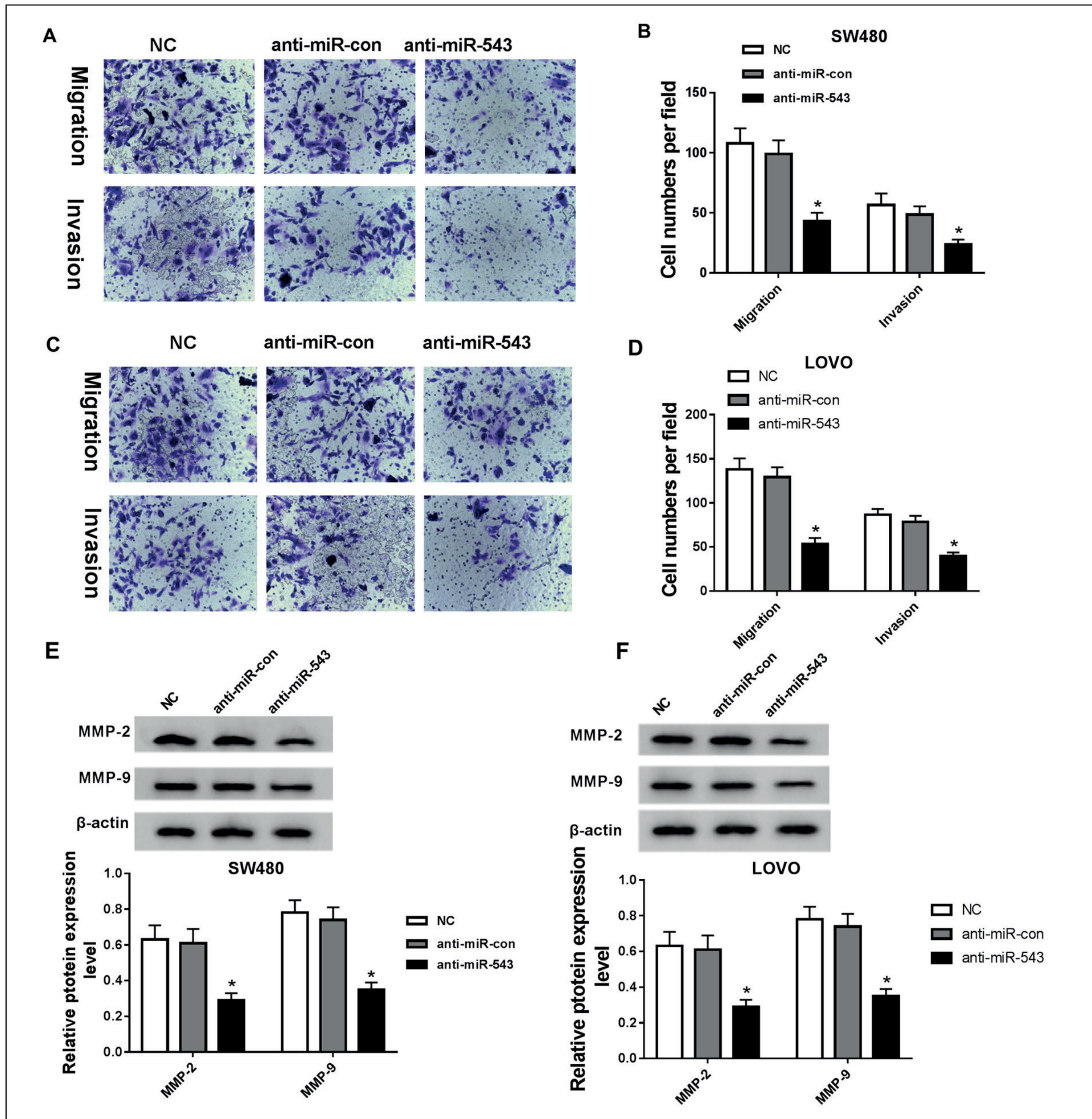

Figure 3. The effect of miR-543 depletion on cell migration and invasion. **A-D,** Cell migration and invasion abilities were determined by the transwell assay (Magnification of figure 3A/3C is 100×). **E,** and **F,** Western blot was used to determine the protein levels of metastasis-related protein MMP-2 and MMP-9. *$p < 0.05$.

Discussion

MiRNAs have been widely reported[16-19] to have an implication in the progression and metastasis of human tumors. Recent evidence showed that miR-543 is upregulated in metastatic prostate cancer cell line C4-2B. Further function experiments indicated that the overexpression of miR-543 promotes the proliferation and metastasis of cancer cells[20]. In gastric cancer, high expression of miR-543 shows a positive association with tumor size, clinical grade, TNM stage and lymph node metastasis[21]. miR-543 enhances gastric cancer cell proliferation by targeting and regulating silent information regulator 1. In osteosarcoma, miR-543 promotes osteosarcoma cell proliferation and glycolysis by partially suppressing protein arginine methyltransferase 9 and stabilizing HIF-1α protein[22]. In prostate cancer, HCG11 upregulation could inhibit cell

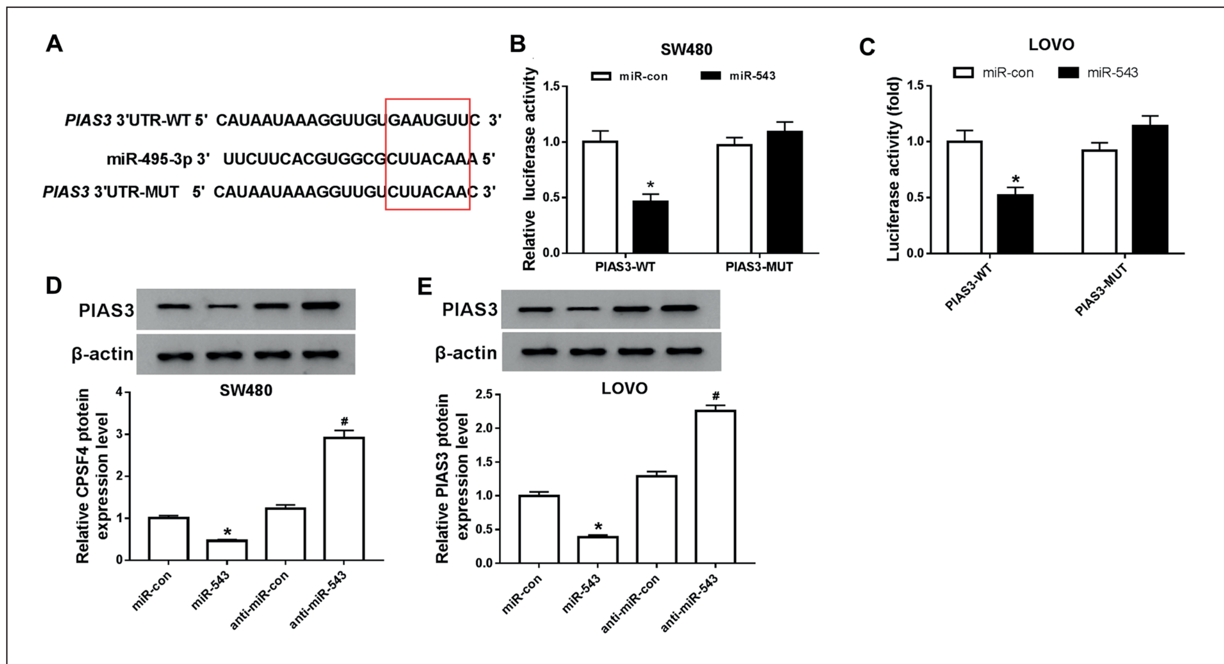

Figure 4. PIAS3 is a direct target of miR-543. **A,** The binding sites between miR-543 and PIAS3. **B,** and **C,** Luciferase activity was determined in cells introduced with miR-543 or miR-con and PIAS3-WT or PIAS3-MUT. **D,** and **E,** The protein level of PIAS3 in cells transfected with miR-543 mimic, anti-miR-543, and negative controls. $*p < 0.05$.

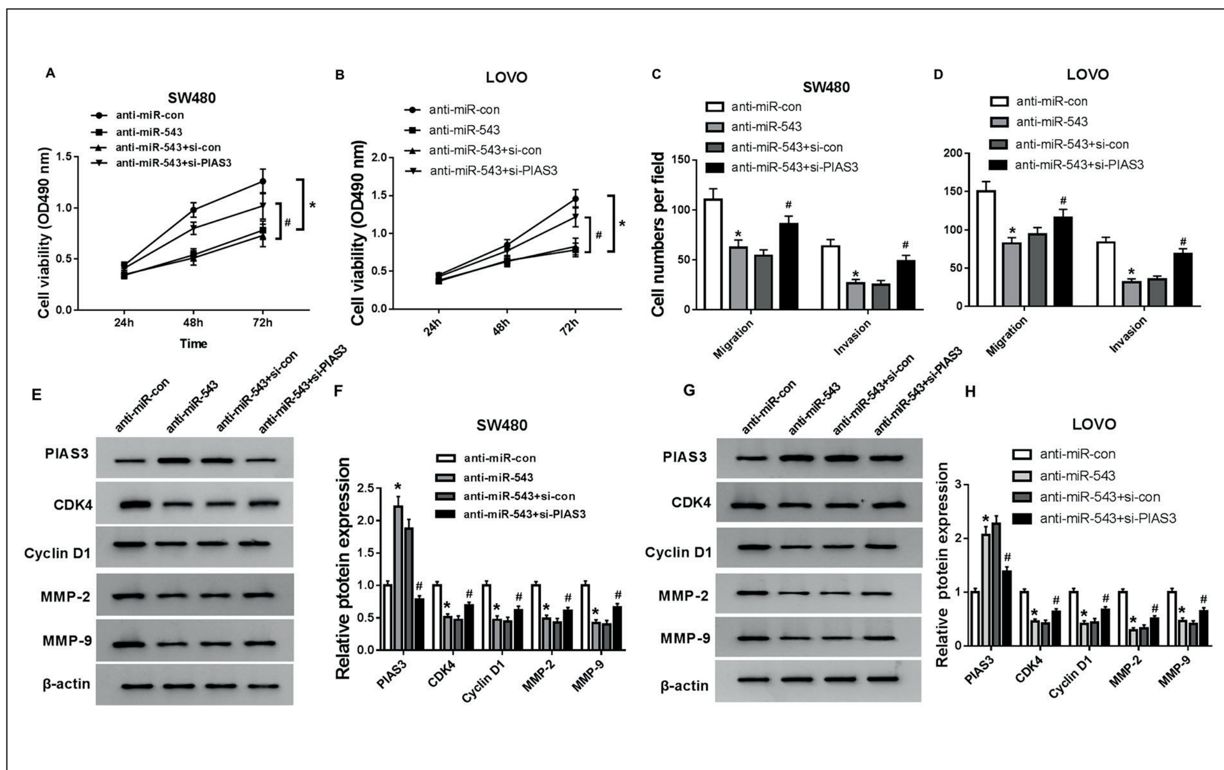

Figure 5. The effect of PIAS3 ablation and miR-543 knockdown on cell proliferation and metastasis. **A-D,** Cell viability and metastasis were determined by MTT assay and Transwell assays. **E,** and **F,** Western blot was employed to detect the expressions of proliferation or metastasis-related protein and PIAS3. $*p < 0.05$.

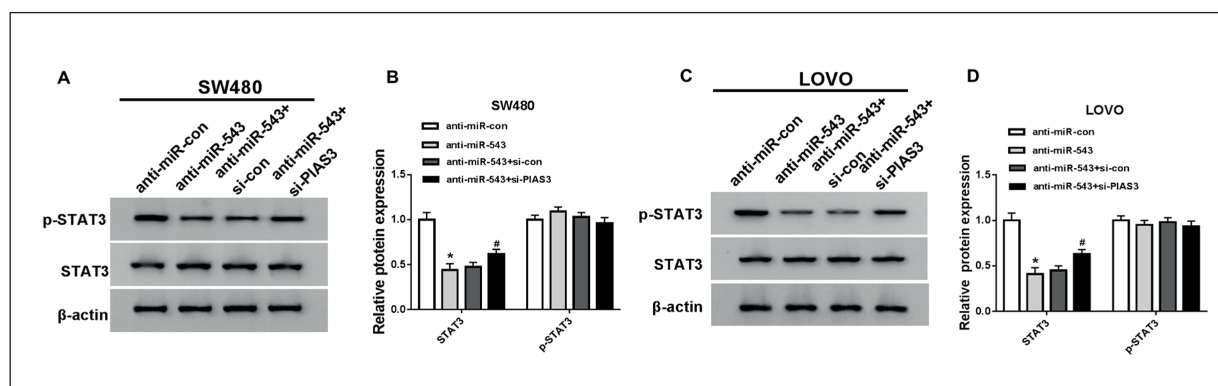

Figure 6. The effect of PIAS3 ablation and miR-543 knockdown on STAT3 activity. **A-D,** Expression of STAT3 and P-STAT3 in SW480 and LOVO cells transfected with anti-miR-543 and si-PIAS3. $*p < 0.05$.

proliferation, invasion and migration, whereas inducing cell apoptosis by downregulating miR-543 expression[23]. Recently, miR-543 is shown to be upregulated in CRC tissues and contributes to cell proliferation and metastasis of CRC *in vitro*[12]. Our results also unveiled that miR-543 was highly expressed in CRC tissues, which was consistent with the previous report[12]. Meanwhile, loss of miR-543 also inhibits cell proliferation, migration, and invasion, confirming that miR-543 could act as an oncogene in the progression of CRC. PIAS3 was downregulated in CRC tissues and cell lines, which was negatively correlated with miR-543 expression. Interestingly, our data further suggested that PIAS3 may be a potential target of miR-543. Therefore, we thought that PIAS3 might participate in miR-543 depletion-mediated function in CRC.

PIAS3 is an endogenous suppressor of signal transducer and activator of transcription 3 (STAT3) signaling[24]. Overexpression of PIAS3 in cancer cells suppresses the transcriptional activity of STAT3 and tumor growth[25,26]. In colon cancer, PIAS3 weakens the miR-181b-mediated promotion on the growth of colon cancer[25]. In human osteosarcoma, miR-199a-5p contributes to the tumor progression through downregulating PIAS3 expression, leading to the STAT3 activation and cell proliferation[27]. Prior studies showed that miR-18a modulates STAT3 activity through negative regulation of PIAS3 during gastric adenocarcinogenesis[28]. In multiple myeloma cells, miR-21 also promotes the STAT3 signal pathway blocking the expression of PIAS3[29]. PIAS3 is correlated with CRC[15,30]. In our results, we demonstrated that the knockdown of PIAS3 abated the effect of the miR-543 depletion on CRC cells. We further evaluated the regulation of miR-543/PIAS3 axis on the STAT3 signaling pathway in the following experiments. The results enhanced the conclusion that miR-543 degradation may repress CRC proliferation and metastasis through PIAS3-mediated inhibition of the STAT3 signaling pathway.

However, this study only investigated the role of miR-543/PIAS3 axis in cell proliferation and metastasis *in vitro*. The function of miR-543/PIAS3 axis should be authenticated *in vivo* functional experiments. In addition, the clinical samples used in this study are still small. More CRC samples should be collected to explore the relationship between miR-543 and the other clinical characteristics of CRC in the future.

Conclusions

Our data provided a new clue that miR-543 depletion suppressed cell proliferation and metastasis by upregulating PIAS3 in CRC. Moreover, the present study elucidated a novel miRNA-mRNA regulatory network that is miR-543/PIAS3 signaling pathway in CRC, which may help to lead a better understanding of the pathogenesis of CRC. It is for the first time revealed the role of the miR-543/PIAS3 axis in the metastasis of CRC, which offers further evidence that miR-543 is associated with a worse prognosis CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding
No funding was received.

Availability of Data and Materials
The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The present study was approved by the Ethical Review Committee of Changhai Hospital.

References

1) Issa IA, Noureddine M. Colorectal cancer screening: an updated review of the available options. World J Gastroenterol 2017; 23: 5086-5096.
2) Chen DL, Lu YX, Zhang JX, Wei XL, Wang F, Zeng ZL, Pan ZZ, Yuan YF, Wang FH, Pelicano H, Chiao PJ, Huang P, Xie D, Li YH, Ju HQ, Xu RH. Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a ceRNA for microRNA-215 to regulate ZEB2 expression. Theranostics 2017; 7: 4836-4849.
3) Hadjipetrou A, Anyfantakis D, Galanakis CG, Kastanakis M, Kastanakis S. Colorectal cancer, screening and primary care: A mini literature review. World J Gastroenterol 2017; 23: 6049-6058.
4) Strubberg AM, Madison BB. MicroRNAs in the etiology of colorectal cancer: pathways and clinical implications. Dis Model Mech 2017; 10: 197-214.
5) Baltruskeviciene E, Schveigert D, Stankevicius V, Mickys U, Zvirblis T, Bublevic J, Suziedelis K, Aleknavicius E. Down-regulation of miRNA-148a and miRNA-625-3p in colorectal cancer is associated with tumor budding. BMC Cancer 2017; 17: 607.
6) Yan G, Li C, Zhao Y, Yue M, Wang L. Downregulation of microRNA6295p in colorectal cancer and prevention of the malignant phenotype by direct targeting of low density lipoprotein receptor related protein 6. Int J Mol Med 2019; 44: 1139-1150.
7) Rapado-González Ó, Álvarez-Castro A, López-López R, Iglesias-Canle J, Suárez-Cunqueiro MM, Muinelo-Romay L. Circulating microRNAs as promising biomarkers in colorectal cancer. Cancers (Basel) 2019; 11: 898.
8) Cong K, Li CG, Wei YH, Zhang K, Xu HB. MicroRNA-760 inhibits the biological progression of colorectal carcinoma by directly targeting FOXA1 and regulating epithelial-to-mesenchymal transition and PI3K/AKT signaling pathway. Eur Rev Med Pharmacol Sci 2019; 23: 5730-5740.
9) Huang M, Xie X, Song X, Gu S, Chang X, Su T, Liang B, Huang D. MiR-506 suppresses colorectal cancer development by inhibiting orphan nuclear receptor nr4a1 expression. J Cancer 2019; 10: 3560-3570.
10) Fasihi A, Soltani BM, Ranjbaran ZS, Bahonar S, Norouzi R, Nasiri S. Hsa-miR-942 fingerprint in colorectal cancer through Wnt signaling pathway. Gene 2019; 712: 143958.
11) Wang H, Yan B, Zhang P, Liu S, Li Q, Yang J, Yang F, Chen E. MiR-496 promotes migration and epithelial-mesenchymal transition by targeting RASSF6 in colorectal cancer. J Cell Physiol 2020; 235: 1469-1479.
12) Zhai F, Cao C, Zhang L, Zhang J. miR-543 promotes colorectal cancer proliferation and metastasis by targeting KLF4. Oncotarget 2017; 8: 59246-59256.
13) Liu G, Zhou J, Dong M. Down-regulation of miR-543 expression increases the sensitivity of colorectal cancer cells to 5-fluorouracil through the PTEN/PI3K/AKT pathway. Biosci Rep 2019; 39: BSR20190249.
14) Sun J, Zhou J, Dong M, Sheng W. Dysregulation of MicroRNA-543 expression in colorectal cancer promotes tumor migration and invasion. Mol Carcinog 2017; 56: 250-257.
15) Huang G, Wu X, Li S, Xu X, Zhu H, Chen X. The long noncoding RNA CASC2 functions as a competing endogenous RNA by sponging miR-18a in colorectal cancer. Sci Rep 2016; 6: 26524.
16) Qadir MI, Faheem A. miRNA: A Diagnostic and therapeutic tool for pancreatic cancer. Crit Rev Eukaryot Gene Expr 2017; 27: 197-204.
17) Tan W, Liang G, Xie X, Jiang W, Tan L, Sanders AJ, Liu Z, Ling Y, Zhong W, Tian Z, Lin W, Gong C. Incorporating microrna into molecular phenotypes of circulating tumor cells enhances the prognostic accuracy for patients with metastatic breast cancer. Oncologist 2019; 24: e1044-e1054.
18) Yau TO, Tang CM, Harriss EK, Dickins B, Polytarchou C. Faecal microRNAs as a non-invasive tool in the diagnosis of colonic adenomas and colorectal cancer: a meta-analysis. Sci Rep 2019; 9: 9491.
19) Mansoori B, Mohammadi A, Naghizadeh S, Gjerstorff M, Shanehbandi D, Shirjang S, Najafi S, Holmskov U, Khaze V, Duijf PHG, Baradaran B. miR-330 suppresses EMT and induces apoptosis by downregulating HMGA2 in human colorectal cancer. J Cell Physiol 2020; 235: 920-931.
20) Du Y, Liu XH, Zhu HC, Wang L, Ning JZ, Xiao CC. MiR-543 promotes proliferation and epithelial-mesenchymal transition in prostate cancer via targeting RKIP. Cell Physiol Biochem 2017; 41: 1135-1146.
21) Li J, Dong G, Wang B, Gao W, Yang Q. miR-543 promotes gastric cancer cell proliferation by targeting SIRT1. Biochem Biophys Res Commun 2016; 469: 15-21.
22) Zhang H, Guo X, Feng X, Wang T, Hu Z, Que X, Tian Q, Zhu T, Guo G, Huang W, Li X. MiRNA-543 promotes osteosarcoma cell proliferation and glycolysis by partially suppressing PRMT9 and stabilizing HIF-1alpha protein. Oncotarget 2017; 8: 2342-2355.

23) Wang YC, He WY, Dong CH, Pei L, Ma YL. LncRNA HCG11 regulates cell progression by targeting miR-543 through regulating AKT/mTOR pathway in prostate cancer. Cell Biol Int 2019 Jun 22. doi: 10.1002/cbin.11194. Epub ahead of print.
24) He T, McColl K, Sakre N, Chen Y, Wildey G, Dowlati A. Post-transcriptional regulation of PIAS3 expression by miR-18a in malignant mesothelioma. Mol Oncol 2018; 12: 2124-2135.
25) Pan X, Feng J, Zhu Z, Yao L, Ma S, Hao B, Zhang G. A positive feedback loop between miR-181b and STAT3 that affects Warburg effect in colon cancer via regulating PIAS3 expression. J Cell Mol Med 2018; 22: 5040-5049.
26) Song X, Shu XH, Wu ML, Zheng X, Jia B, Kong QY, Liu J, Li H. Postoperative resveratrol administration improves prognosis of rat orthotopic glioblastomas. BMC Cancer 2018; 18: 871.
27) Wang C, Ba X, Guo Y, Sun D, Jiang H, Li W, Huang Z, Zhou G, Wu S, Zhang J, Chen J. MicroRNA-199a-5p promotes tumour growth by dual-targeting PIAS3 and p27 in human osteosarcoma. Sci Rep 2017; 7: 41456.
28) Wu W, Takanashi M, Borjigin N, Ohno SI, Fujita K, Hoshino S, Osaka Y, Tsuchida A, Kuroda M. MicroRNA-18a modulates STAT3 activity through negative regulation of PIAS3 during gastric adenocarcinogenesis. Br J Cancer 2013; 108: 653-661.
29) Xiong Q, Zhong Q, Zhang J, Yang M, Li C, Zheng P, Bi LJ, Ge F. Identification of novel miR-21 target proteins in multiple myeloma cells by quantitative proteomics. J Proteome Res 2012; 11: 2078-2090.
30) Yu T, Lu Q, Ou X, Cao D, Yu Q. Association of sedentary behavior with the expression levels of biomarkers in colorectal cancer: clinical analysis of 228 patients. Tohoku J Exp Med 2014; 232: 167-176.