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Role of visfatin in promoting proliferation and invasion of colorectal cancer cells by downregulating SDF-1/CXCR4-mediated miR-140-3p expression

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Abstract. – OBJECTIVE: Visfatin is significantly upregulated in colorectal cancer (CRC). However, its exact role in CRC progression and the regulatory mechanism involved in this process have not been fully illuminated. The aim of this study was to investigate the roles of visfatin in CRC progression and the potential molecular mechanism.

MATERIALS AND METHODS: *In vitro*, two CRC cell lines (DLD-1 and SW480) were transfected with visfatin, si-visfatin, and their control vectors. Some cells were transfected with miR-140-3p mimics or miRNA negative control. Cell Counting Kit-8 and transwell invasive assays were used to detect cell proliferation and invasion ability. Luciferase reporter assays were performed to confirm whether CXC motif chemokine receptor 4 (CXCR4) directly targets miR-140-3p. Western blotting and qRT-PCR analyses were respectively conducted to evaluate the protein and mRNA levels of stromal cell-derived factor-1 (SDF-1) and CXCR4. *In vivo*, DLD-1 cells transfected with visfatin construct or vector control were inoculated into nude mice. After 5 weeks, the mice were sacrificed, and the tumor nodules were weighed. The expression of visfatin, SDF-1, and CXCR4 in tumor tissues was detected via immunohistochemistry analysis.

RESULTS: *In vitro*, the transfection of visfatin promoted the proliferation and invasion of CRC cells, as well as upregulated the expression of SDF-1/CXCR4. MiR-140-3p directly targets the 3'untranslated region of CXCR4. MiR-140-3p expression was downregulated by treatment with visfatin, and miR-140-3p exerted similar effects to those of visfatin knockdown on the proliferation and invasion of CRC cells. *In vivo*, visfatin stimulated CRC tumor growth and downregulated miR-140-3p expression, whereas it upregulated SDF-1/CXCR4 expression.

CONCLUSIONS: Visfatin promotes CRC progression by downregulating the SDF-1/CXCR4-mediated expression of miR-140-3p both *in vitro* and *in vivo*.

Key Words:
Visfatin, Colorectal cancer, SDF-1/CXCR4, MiR-140-3p, Proliferation, Invasion.

Introduction

Colorectal cancer (CRC) is one of the most common malignant types of tumors. It ranks fourth among the most prevalent types of cancer and is a major cause of cancer-related deaths worldwide. Annually, there are approximately 1.2 million newly diagnosed patients with CRC[1]. Currently, chemotherapy remains one of the main therapies for the treatment of CRC. Although tremendous progress in the diagnosis and treatment of cancer has been achieved over the past few decades, the overall survival rate and prognosis of patients with cancer remains very poor. Considering the increasing incidence and low cure rate of patients with CRC, understanding the underlying pathological mechanisms of CRC occurrence and development is urgently required.

Visfatin is a newly discovered adipokine secreted by visceral fat tissue[2] and serves as a positive regulator in the progression of multiple biological progresses, including energy metabolism, angiogenesis, inflammation, and cell longevity[3-6]. Visfatin is significantly overexpressed within malignant tissues and associated with the pathogenesis of various types of cancers, such as colon cancer, breast cancer, gastric cancer, glioblastoma, endometrial cancer, and prostate cancer[7]. In breast cancer, the increased serum levels of visfatin are

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positively correlated with short disease-free and overall survival[8]. The detailed mechanism of visfatin-induced tumor progression may be explained by the fact that visfatin is able to induce epithelial-mesenchymal transition (EMT)[9] and activate some important tumor-related signaling pathways, such as the PI3K/Akt pathway, nuclear factor-κB (NF-κB)/Snail-1/EMT pathway, and MAPK/ERK1/2 pathway[10,11].

Clinical research found an evident increase in the extracellular expression of visfatin in stage T4 tumor tissues when compared with visfatin expression in initial stage II-III tumor tissues[12], and this elevation in the levels of visfatin was a powerful risk factor for both advanced and early CRC[13]. Therefore, visfatin is regarded as a promising potential biomarker for CRC[14]. However, the exact regulatory role of visfatin in CRC occurrence and development remains unclear.

Chemokines, a family of small cytokines, are present in the microenvironment of all tumors[15] and serve as a key moderator in tumor cell metastasis and invasion[16]. Stromal cell-derived factor-1 (SDF-1) is a member of the CXC subfamily of chemokines. The major function of these chemokines is the regulation of various biological processes, including immune response, angiogenesis, hematopoiesis, and tumor development[17,18]. In earlier reports, the high levels of SDF-1 was confirmed to be correlated with CRC tumor progression; thus, SDF-1 is considered a key negative prognostic indicator of CRC[19,20]. CXC motif chemokine receptor 4 (CXCR4), a receptor for SDF-1, plays a critical role in cell survival, proliferation, and migration by affecting several major signaling pathways[21]. Upregulation of CXCR4 is implicated in the development of a wide variety of metastatic diseases, such as prostate[22], breast[23], melanoma[24], and lung[25]. In earlier reports, the levels of SDF-1 and CXCR4 were markedly increased in osteosarcoma[26]. Based on the function of visfatin on CRC progression, we hypothesized that the role and regulatory mechanisms of visfatin in CRC may refer to the changes in the levels of SDF-1/CXCR4.

MicroRNAs (miRNAs), a class of small endogenous non-coding RNAs involved in the regulation of gene expression, have a function in the development of various cancers. Several miRNAs are associated with the development of CRC, including miR-375[27], miR-17-5p[28], miR-30b[29], miR-153[30], miR-224[31], etc. In the present study, we found that miR-140-3p is a potential target of CXCR4 using a bioinformatics analysis tool (TargetScan). The important role of miR-140-3p in inhibiting tumorigenesis has been revealed in squamous cell lung cancer[32] and breast cancer[33]. In CRC, a significant decrease in miR-140-3p expression was observed. Overexpression of miR-140-3p was found to suppress the proliferation, migration, and invasion, and promote the apoptosis of CRC cells[34]. However, whether miR-140-3p is associated with the regulatory role of CXCR4 in CRC progression should be further investigated.

Based on the above findings, understanding the influence of visfatin on CRC progression and exploring the potential molecular mechanism will be necessary for the prevention and treatment of CRC. To our knowledge, previous research studies have revealed the abnormal expression of visfatin in patients with CRC, while few studies investigated the regulatory role of visfatin in the occurrence and development of CRC. The present study was the first to investigate the impact of visfatin on the proliferation and invasion of CRC cells both *in vitro* and *in vivo*, as well as the potential mechanisms involved in this process.

Materials and Methods

Cell Culture and Transfection

CRC cells DLD-1 and SW480 (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen Corporation, Carlsbad, CA, USA) in a 5% CO_2 atmosphere at 37°C. For transfection, the cells were seeded into plates and transfected with pEGFP-N1 (vector control), pEGFR-N1/visfatin (visfatin construct), siRNA negative control (si-NC), and si-visfatin using Lipofectamine 2000 (Invitrogen Corporation). Some cells were transfected with miR-140-3p mimics and its negative control microRNA (miR-NC; Genepharma, Shanghai, China).

Cell Proliferation Assay

The cell proliferation capacity of CRC cells was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) according to the instructions provided by the manufacturer. Briefly, the cells were seeded in 96-well plates and mixed with the CCK-8 solution. Following incubation for another 2 h, the absorbance was detected at 450 nm using a microplate reader (Dynex, Chantilly, VA, USA).

Transwell Assay

The invasion assays were performed in Boyden chambers (8-μm pore size using Matrigel Matrix (BD Biosciences, San Jose, CA, USA) as previously reported[35]. The resulting values were calculated based on three independent experiments.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Analysis

Total RNA was extracted using the TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA), and the corresponding concentration was determined by measuring the optical density at 260 nm. cDNA was prepared using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). qRT-PCR was performed using the Bio-Rad qPCR instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with the following thermocycling conditions: 95°C for 15 s, followed by 95°C for 5 s, and finally 62°C for 30 s for 40 cycles. The sequences of primers were as follows: SDF-1, forward: TGGTAGAGAGGTGCTGGTGT, reverse: TACGTCTGCAGCCATTTGGT. CXCR4, forward: GGCAGAGGAGTTAGCCAAGAT, reverse: GTGGGCTAAGGGCACAAGAG; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward: TGAAGGTCGGAGTCAACGGA, reverse: TGACAAGCTTCCCGTTCTCA; miR-140-3p, forward: ACACTCCAGCTGGGAGGCGGGGCGCCGCGGGA, reverse: CTCAACTGGTGTCGTGGA; and U6, forward: CTCGCTTCGGCAGCACA, reverse: AACGCTTCACGAATTTGCGT. The relative levels of the genes were normalized to those of GAPDH using the $2^{-\Delta\Delta Ct}$ method. For the quantification of miR-140-3p, U6 was utilized for normalization.

Western Blotting Analysis

Protein was extracted using lysis buffer and quantified through a bicinchoninic acid assay. Subsequently, equal amounts of protein samples were separated using 10% bicinchoninic acid and placed on a polyvinylidene difluoride membrane. The membrane was incubated with the primary antibodies, including monoclonal antibodies against SDF-1, CXCR4, and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1:1,000 dilution at 4°C overnight, followed by incubation with a horseradish peroxidase-conjugated secondary antibody (Abcam, Cambridge, UK) at 25°C for 2 h. The bound antibodies were evaluated using the Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Luciferase Reporter Assay

A full-length human CXCR4 wild type (WT) 3'untranslated region (3'UTR) containing the putative miR-140-3p targeting sequence was cloned into a firefly Luciferase reporter vector psiCHECK™ (Promega, Madison, WI, USA), and the CXCR4 mutant (Mut) 3'UTR plasmid served as a control. Subsequently, the CRC cells were transiently co-transfected with miR-140-3p mimics or control and the WT or Mut CXCR4 3'UTR plasmid with the Renilla Luciferase-expressing vector pRL-TK (Promega, Madison, WI, USA) as a spiked-in control. Following 48 h, the Luciferase activity was detected using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Animal Tumor Xenograft Model

Twelve nude mice aged 5-6 weeks (SLAC Laboratory Animal Co., Ltd, Shanghai, China) were housed in a standard rearing environment. The animals were randomly classified into two groups (10 mice per group). DLD-1 cells (1×10^6) stably transfected with a visfatin construct or vector control were subcutaneously inoculated into the posterior flank of nude mice. Tumor growth was assessed once every 3 days. The tumor volume was calculated based on the following formula: $V = 0.5 \times D \times d^2$, where V is the volume, D is the longitudinal diameter, and d is the latitudinal diameter. Subsequently, the mice were sacrificed at 5 weeks post injection and the tumor nodules were weighed, photographed, and embedded in paraffin for the immunohistochemical analysis of visfatin, SDF-1, and CXCR4 protein expression. All animal experimental procedures were approved by the Ethics Committee of the First People's Hospital of Yunnan Province, the Affiliated Hospital of Kunming University of Science and Technology (Kunming, China).

Immunohistochemical Staining

Tumor tissue sections extracted from nude mice were dried, dewaxed, rehydrated, and treated with antigen retrieval. After blocking in goat serum (Boster, Wuhan, China), the sections were incubated with visfatin, SDF-1, and CXCR4 antibody (1:200; Cell Signaling Technology Inc., Danvers, MA, USA) at 4°C overnight. After washing, the sections were incubated with horseradish peroxidase-polymer-conjugated secondary antibodies. Finally, the sections were placed in a solution of 0.5 mg/ml diaminobenzidine for 10 min. After washing, images of three randomly

positive areas in each section were captured by an Olympus BX51 microscope (Olympus, Tokyo, Japan).

Statistical Analysis

All values are presented as the mean ± standard error of the mean. The Statistical Product and Service Solution (SPSS) version 19.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis. The differences between the two groups or multiple groups were analyzed by two-tailed unpaired Student's *t*-test or one-way analysis of variance with a post-hoc test. A p-value < 0.05 denoted statistically significant difference.

Results

Visfatin Promotes the Proliferation and Invasion of CRC Cells

Visfatin is highly expressed in primary CRC compared with nonneoplastic mucosa[36], suggesting its potential involvement in the development of CRC. Therefore, we initially investigated the effects of visfatin on the proliferative and invasive ability of CRC cells *in vitro*. Based on the statistical results of the cell proliferation assay, we found that the overexpression of visfatin significantly promoted the cell proliferation of both DLD-1 and SW480 cells compared with the control group ($p < 0.05$, Figure 1A). By contrast, the knockdown of visfatin by its specific siRNA contributed to an evident inhibition of CRC cell proliferation ($p < 0.05$, Figure 1B). Meanwhile, the transwell assay was applied to investigate the impact of visfatin on the invasion of CRC cells *in vitro*. Clearly, the percentage of invading DLD-1 and SW480 cells in visfatin group was markedly higher than that observed for control, and transfection of CRC cells with si-visfatin contributed to an antipodal result ($p < 0.01$, Figures 1C, D). Collectively, the above findings revealed that visfatin serves as a tumor promoter by stimulating the proliferation and invasion of CRC cells *in vitro*.

Visfatin Upregulates the Expression of SDF-1/CXCR4 in CRC Cells

Based on a previous report, it is established that chemokine SDF-1 and its receptor CXCR4 are overexpressed in CRC tissues, and have been considered as the key negative prognostic indicators in CRC[37]. We next measured the expression levels of SDF-1/CXCR4 in DLD-1 and SW480 cells *in vitro* to further reveal the potential mechanism of visfatin-mediated CRC progression. As shown in Figure 2A, the transcriptional levels of SDF-1/CXCR4 were significantly upregulated in CRC cells overexpressing visfatin *vs.* control cells ($p < 0.05$). These levels were markedly decreased following the silencing of visfatin ($p < 0.01$). The same trend was also observed in the results of the Western blotting analysis (Figure 2B). Thus, visfatin increases the expression of SDF-1/CXCR4 at both the mRNA and protein levels in CRC cells.

MiR-140-3p Directly Targets CXCR4 in CRC Cells

According to the results of the bioinformatics analysis using the TargetScan tool, we hypothesized that CXCR4 may contain a putative binding site with miR-140-3p (Figure 3A). To identify whether miR-140-3p directly targets the 3'UTR of CXCR4, we next established a Luciferase reporter gene vector with the downstream target Luciferase gene, CXCR4-3'-UTR-WT and CXCR4-3'-UTR-Mut. Next, DLD-1 cells were transiently co-transfected with miR-143-3p mimics/NC and the dual-reporter encoding vector. As shown in Figure 3B, the treatment with miR-140-3p mimics led to a significant downregulation of CXCR4 by targeting the 3'UTR of WT CXCR4 ($p < 0.01$); there was no evident effect found on Mut CXCR4. We transfected CRC cells with miR-140-3p mimics and miR-NC and measured the levels of CXCR4 to further identify the correlation between miR-140-3p and CXCR4. We found that the relative mRNA and protein expression of CXCR4 was significantly suppressed by the transfection of miR-140-3p mimics in DLD-1 and SW480 cells ($p < 0.01$) (Figures 3C, D). Taken together, the above findings supported that miR-140-3p may directly target CXCR4 in CRC cells.

Overexpression of MiR-140-3p Inhibits the Proliferation and Invasion of CRC Cells

Considering the direct target of miR-140-3p on CXCR4 and the high expression of CXCR4 induced by visfatin in CRC cells, visfatin may be implicated in regulating the expression of miR-140-3p. As expected, the overexpression of visfatin resulted in a major reduction of miR-140-3p expression in CRC cells ($p < 0.001$) (Figure 4A), whereas the knockdown of visfatin caused an opposite effect ($p < 0.01$) (Figure 4B). These data uncovered the negative regulatory role of visfatin on miR-140-3p. Cell proliferation and transwell invasion assays were subsequently performed to

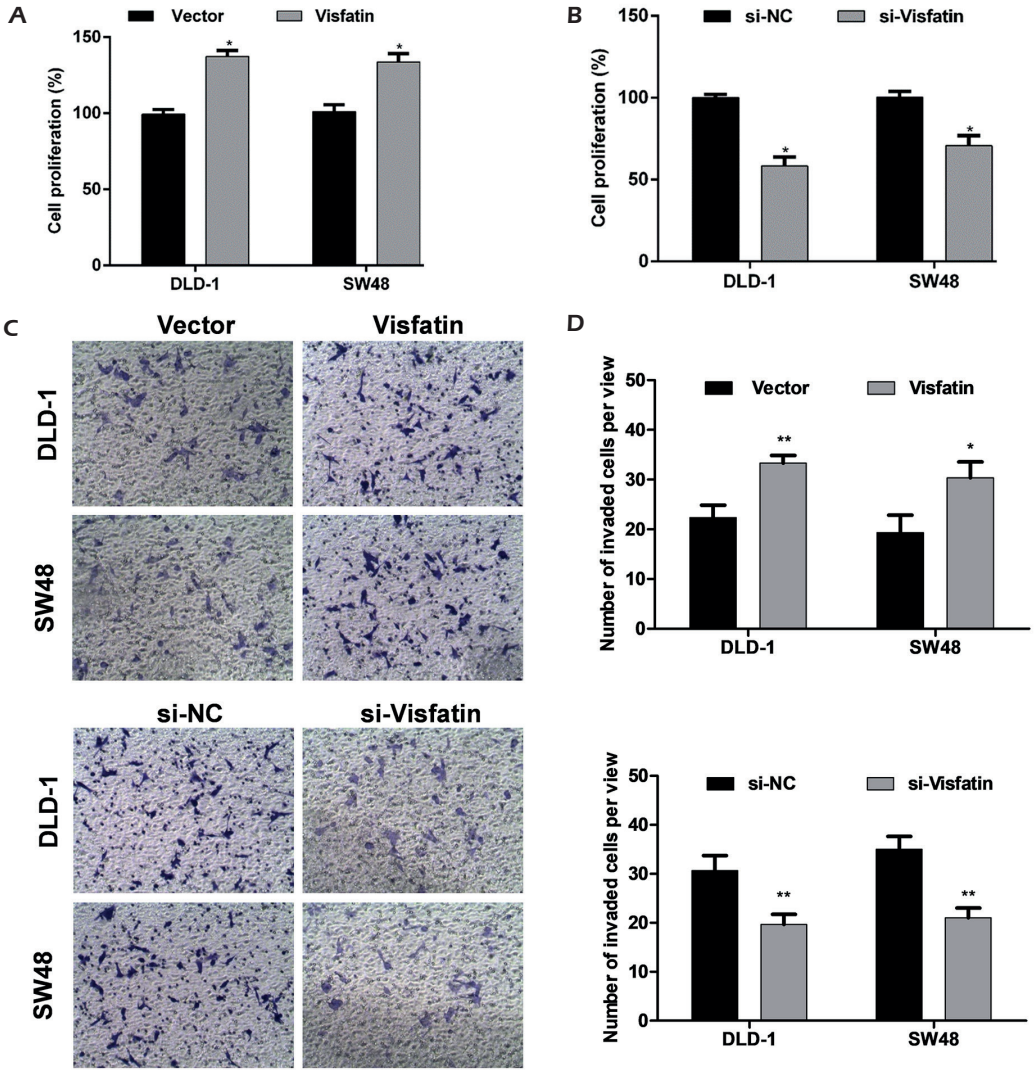

Figure 1. Visfatin promotes the proliferation and invasion of CRC cells. DLD-1 and SW480 cells were transfected with visfatin or si-visfatin and their negative control for 48 h. Subsequently, CCK-8 and transwell assays were performed. **A,** Quantification of cell proliferation in DLD-1 and SW480 cells transfected with the visfatin construct or vector control, detected by CCK-8 assay. **B,** Quantification of cell proliferation in DLD-1 and SW480 cells transfected with si-visfatin or si-NC, detected by CCK-8 assay. **C,** Representative images of cell invasion in DLD-1 and SW480 cells transfected with visfatin or si-visfatin and their negative control, detected by transwell assay. Representative images of the transwell assay results captured under 100× original magnification. **D,** Quantification of invading cells in DLD-1 and SW480 cells transfected with visfatin or si-visfatin and their negative control. *$p < 0.05$, **$p < 0.01$.

confirm the exact role of miR-140-3p in CRC progression. Compared with control, the CRC cells transfected with miR-140-3p mimics led to an evident inhibition of CRC progression, as represented by a reduction in the number of proliferating ($p < 0.01$) (Figure 4C) and invading cells ($p < 0.05$) (Figure 4D). Collectively, the above findings indicated that visfatin downregulates the expression of miR-140-3p and the latter suppresses the proliferation and invasion of CRC cells.

Visfatin Promotes CRC Tumor Growth In Vivo by Downregulating SDF-1/CXCR4-Mediated Expression of MiR-140-3p

Considering that visfatin was able to promote the proliferation and invasion of CRC cells *in vitro*, we hypothesized that visfatin could exert similar pro-tumor effects *in vivo*. Therefore, DLD-1 cells transfected with visfatin construct or vector control were inoculated into nude mice. At 5 weeks post injection, the tumor size in the mice treated

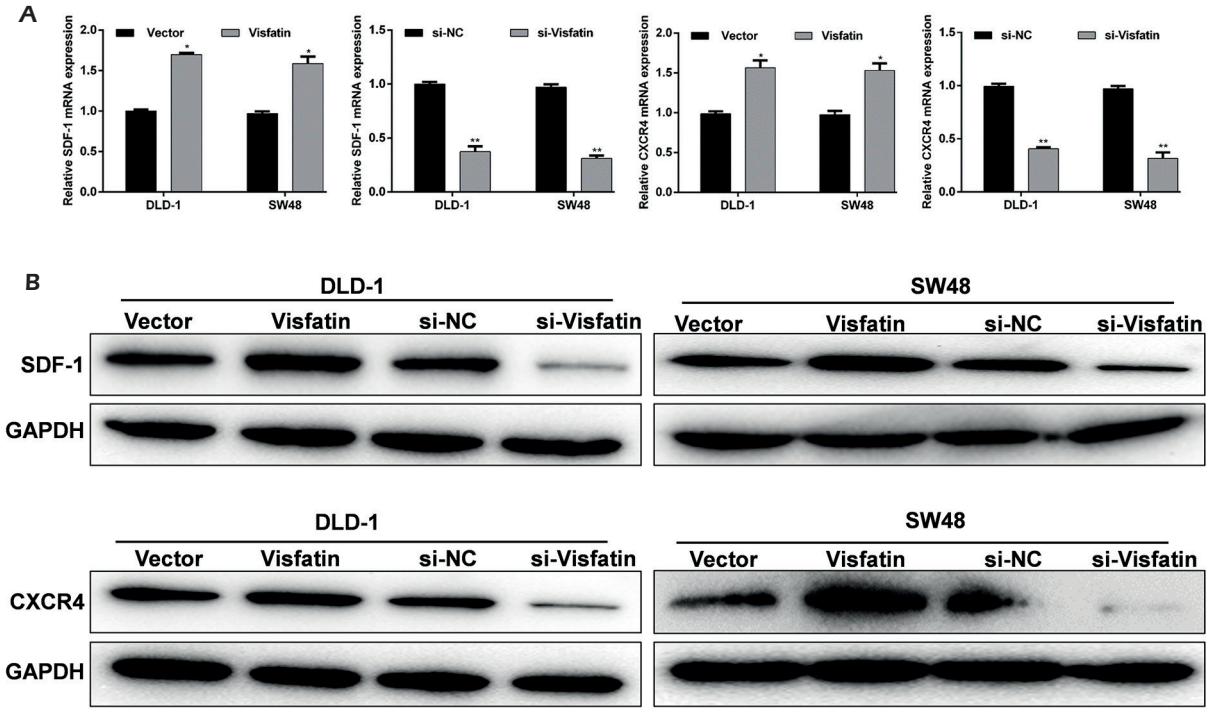

Figure 2. Visfatin upregulates SDF-1/CXCR4 expression in CRC cells. DLD-1 and SW480 cells were transfected with visfatin or si-visfatin and their negative control for 48 h. Subsequently, qRT-PCR and western blotting were performed. **A,** The relative mRNA expression of SDF-1/CXCR4 in DLD-1 and SW480 cells transfected with visfatin or si-visfatin and their negative control, detected by qRT-PCR. **B,** The relative protein expression of SDF-1/CXCR4 in DLD-1 and SW480 cells transfected with visfatin or si-visfatin and their negative control, detected by Western blotting. *$p < 0.05$, **$p < 0.01$.

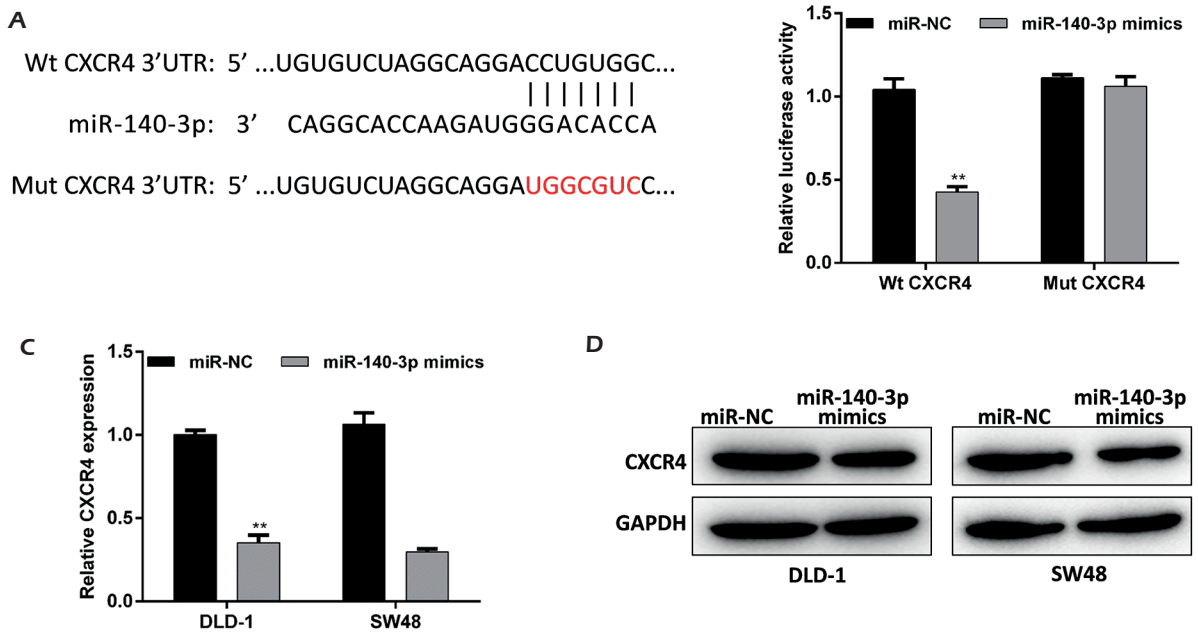

Figure 3. MiR-140-3p directly targets CXCR4 in CRC cells. **A,** Bioinformatics analysis showed the predicted binding site between miR-140-3p and CXCR4. **B,** The relative Luciferase activity of DLD-1 cells co-transfected with CXCR4-3'-UTR-WT or Mut and miR-140-3p mimics or miR-NC, detected by luciferase reporter assay. **C,** The relative CXCR4 mRNA expression in DLD-1 and SW480 cells transfected with miR-140-3p mimics or miR-NC, detected by qRT-PCR. **D,** The relative CXCR4 protein expression in DLD-1 and SW480 cells transfected with miR-140-3p mimics or miR-NC, detected by Western blotting assay. **$p < 0.01$.

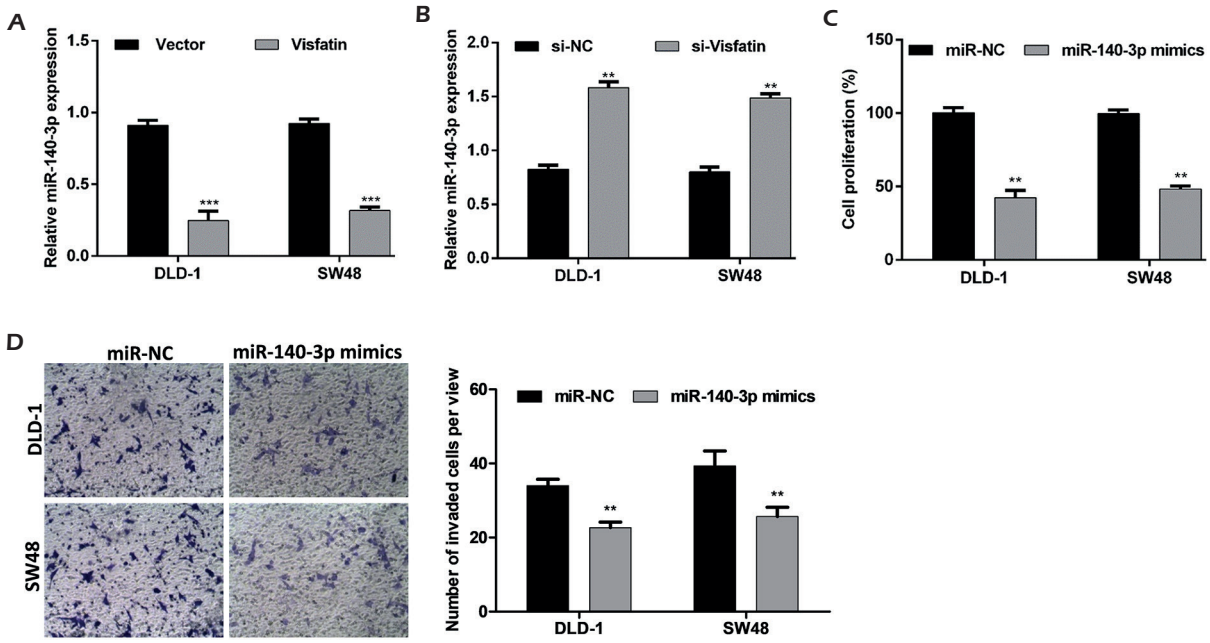

Figure 4. Overexpression of miR-140-3p promotes the proliferation and invasion of CRC cells. **A,** The relative expression of miR-140-3p in DLD-1 and SW480 cells transfected with visfatin construct or vector control, detected by qRT-PCR. **B,** The relative expression of miR-140-3p in DLD-1 and SW480 cells transfected with si-visfatin or si-NC, detected by qRT-PCR. **C,** Quantification of cell proliferation in DLD-1 and SW480 cells transfected with miR-140-3p mimics or miR-NC, detected by CCK-8 assay. **D,** Representative images of cell invasion in DLD-1 and SW480 cells transfected with miR-140-3p mimics or miR-NC, detected by transwell assay, and the corresponding quantification results. Representative images of transwell results captured under 100× original magnification. *$p < 0.05$, **$p < 0.01$.

with visfatin was markedly larger than that measured in control mice (Figure 5A). Similarly, the average tumor weight in the visfatin group was also markedly increased compared with that noted in the control group ($p < 0.01$) (Figure 5B), suggesting the positive role of visfatin in CRC tumor growth. This result was in accordance with previous *in vitro* findings. Moreover, the tumor tissues obtained from the visfatin group also exhibited a significant attenuation of miR140-3p expression ($p < 0.01$) (Figure 5C) (revealed by qRT-PCR), and a well-marked augment of visfatin, SDF-1, and CXCR4 expression (Figure 5D) (validated by immunohistochemical staining). These data verified that visfatin acts as a critical promoter of CRC progression by activating the SDF-1/CXCR4 axis *via* downregulation of miR-140-3p.

Discussion

As one of the most serious malignancies, CRC is associated with high morbidity and mortality worldwide[38,39]. Among various risk factors for the occurrence of cancer, obesity is considered an important risk factor for CRC and colorectal adenoma[40,41]. Some adipokines secreted from adipose tissues, such as adiponectin, interleukin-6, resistin, visfatin, leptin, and tumor necrosis factor-α are generally related to the risk of cancer at different sites[42,43]. Of those, visfatin is a newly discovered adipokine, which is remarkably upregulated in the blood of patients with CRC and some CRC cell lines[44,45]. Moreover, the targeted inhibition of visfatin improved resistance to doxorubicin in human CRC cells[46]. Based on these findings, visfatin may be implicated in the development and progression of CRC. However, the exact role of visfatin in regulating the malignant behaviors of CRC and the underlying molecular mechanism have not been thoroughly illuminated.

To investigate the role of visfatin in CRC progression, we initially transfected two CRC cell lines (DLD-1 and SW480) with visfatin, si-visfatin, and their respective negative control. We subsequently determined the proliferative and migratory ability of these cells using CCK-8 and transwell assays. As expected, transfection of

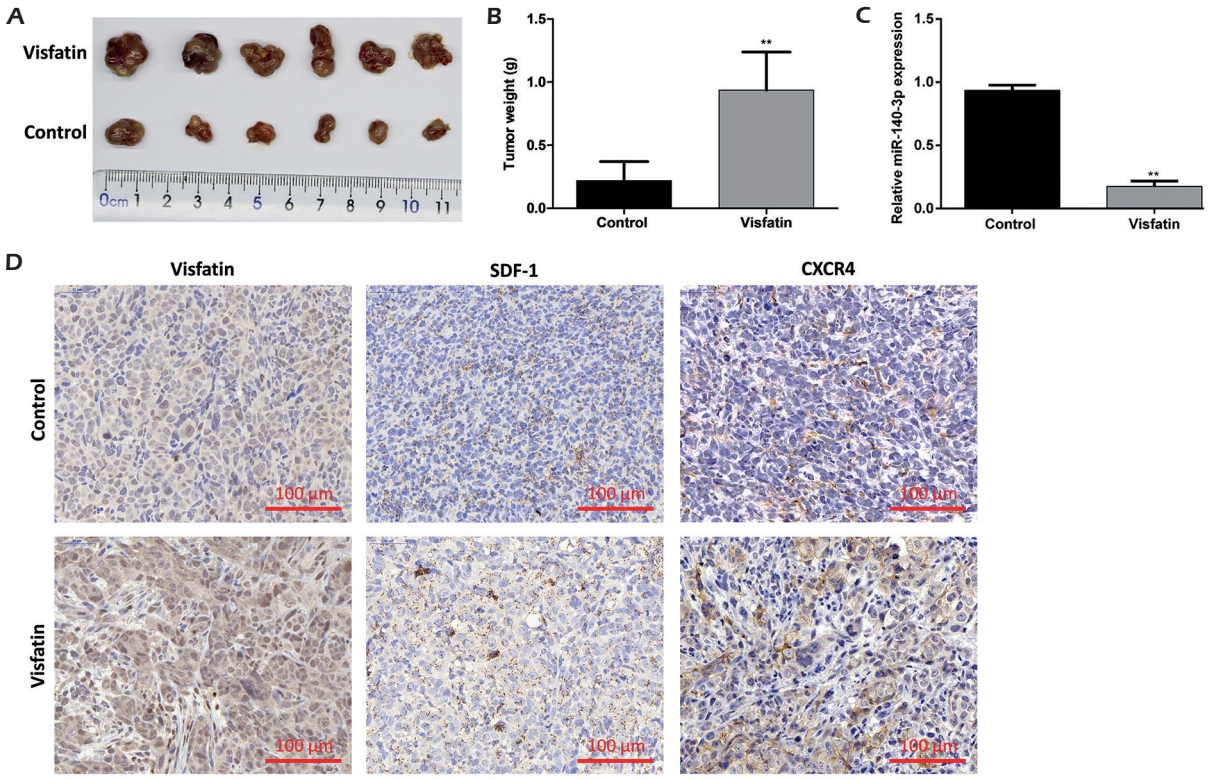

Figure 5. Visfatin promotes CRC tumor growth *in vivo* by downregulating the SDF-1/CXCR4-mediated expression of miR-140-3p. Nude mice were inoculated with DLD-1 cells transfected with visfatin construct or vector control. The mice were sacrificed at the designated time- point and the *in-situ* CRC was isolated. **A,** Photograph of CRC tumor harvested from the mice within 5 weeks. **B,** The tumor weight curve of the mice within 5 weeks. **C,** The relative expression of miR-140-3p in CRC tumors harvested from the mice, detected by qRT-PCR. **D,** Immunohistochemical staining of visfatin, SDF-1, and CXCR4 in CRC tumors harvested from the mice. Scale bar represents 100 μm. $^{**}p < 0.01$.

visfatin caused a major increase in the number of proliferating and invading DLD-1 and SW480 cells compared with the control. By contrast, silencing of visfatin resulted in an opposite effect, suggesting that visfatin acts as a positive regulator of CRC progression *in vitro*. Many studies have demonstrated the participation of visfatin in tumorigenesis and metastasis in multiple types of human cancer. Okumura et al[47] observed that the knockdown of visfatin could effectively inhibit the growth of non-small cell lung cancer by decreasing the intracellular levels of adenosine triphosphate (ATP). Meanwhile, administration of visfatin can also stimulate the migratory and invasive behavior of osteosarcoma cells *via* the induction of EMT and the activation of NF-κB/interleukin-6 signals[10,48]. Our result was highly consistent with these previous findings, providing support and supplement for the positive regulatory function of visfatin in tumor progression.

Furthermore, we noted that visfatin exerts a positive effect on the expression of SDF-1/CXCR4 in both DLD-1 and SW480 cells. This was partly in line with the results of a previous study showing that visfatin upregulates the expression of SDF-1 in CRC cells *via* the induction of transcriptional activation[49]. We next investigated the potential target for CASC15 (Cancer susceptibility candidate 15) through bioinformatics analysis to further understand the contribution of CXCR4 in CRC progression. The results revealed a potential binding site between CXCR4 and miR-140-3p. Further analysis through Luciferase reporter assay found that transfection of miR-140-3p mimics induced a significant decrease in WT CXCR4 activity in CRC cells compared with the miR-NC group; there were no significant changes observed in Mut CXCR4 activity. Similarly, as demonstrated by qRT-PCR and Western blotting, the overexpression of miR-140-3p resulted in a well-marked suppression of CXCR4 expression at both the mRNA and protein levels, whereas miR-NC did not exert an effect. Collectively, the above data suggest an antagonistic effect between miR-140-

3p and CXCR4. A close antagonistic role of miR-140-3p was revealed by Dong et al[50], showing that the overexpression of miR-140-3p repressed the growth and invasion of non-small cell lung cancer by directly targeting ATP8A1. Kong et al[51] found that miR-140-3p could act as an inhibitor of lung cancer progression by targeting ATP6AP, thereby enhancing the p53 signaling axis. Therefore, miR-140-3p may be a promising marker for therapies against cancer. Moreover, we established that overexpression of visfatin contributed to a marked decrease in miR-140-3p expression; conversely, silencing of visfatin exerted the opposite effects. This finding revealed the negative regulatory function of visfatin on miR-140-3p and the potential participation of miR-140-3p in CRC progression. To verify this, cell proliferation and transwell invasion assays were performed in DLD-1 and SW480 cells after transfection with miR-140-3p. The corresponding results demonstrated that CRC cells transfected with miR-140-3p mimics presented a marked attenuation of their proliferation and invasion similar to those observed in cells with knockdown of visfatin. Overall, this is a novel finding with regards to the antagonistic effect between miR-140-3p and visfatin.

The CRC cells transfected with the visfatin construct were inoculated into nude mice *in vivo* to further study the relationship between visfatin, miR-140-3p, and SDF-1/CXCR4. We noted that transfection of visfatin significantly promoted CRC tumor growth *in vivo*, as manifested by the reduction in tumor size and the lower tumor weight. This finding implied the vital role of visfatin in the progressive growth of lung tumors. Our observation is consistent with the results of several studies. Park et al[52] supported that visfatin could promote tumor growth in breast cancer by activating the NF-κB signaling pathway. Meanwhile, accelerated growth of endometrial carcinoma tumor in BALB/c nude mice was confirmed following stimulation with visfatin[53]. Based on qRT-PCR and immunohistochemical staining, we found that visfatin contributed to a major increase in the expression levels of miR-140-3p, SDF-1, and CXCR 4; this was highly consistent with the results of the *in vitro* experiment. Based on the *in vivo* and *in vitro* experiments, our study is the first to reveal that visfatin promotes CRC progression by downregulating the SDF-1/CXCR4-mediated expression of miR-140-3p. Nevertheless, further research studies are warranted to investigate the detailed regulatory mechanism involved in this process.

Conclusions

We revealed that visfatin has the ability to suppress the proliferative and invasive capabilities of CRC cells by downregulating the SDF-1/CXCR4-mediated expression of miR-140-3p both *in vivo* and *in vitro*. Although the exact molecular mechanisms remain to be fully elucidated, these findings assist us in deeply understanding the function of visfatin in CRC progression and may lay the foundation for the use of visfatin in therapeutic strategies against CRC.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

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