

# Inactivation of von Hippel-Lindau increases ovarian cancer cell aggressiveness through the HIF1 $\alpha$ /miR-210/VMP1 signaling pathway

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Received October 16, 2013; Accepted February 6, 2014

DOI: 10.3892/ijmm.2014.1661

**Abstract.** The inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene not only results in tumor initiation, but also mediates tumor metastasis. However, the mechanisms by which VHL inactivation leads to metastasis have not yet been well defined. In this study, the silencing of VHL in 3AO and SKOV3 ovarian cancer cells was found to promote cell motility and to increase the expression of matrix metalloproteinase (MMP)2, MMP9, hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ) and microRNA (miR)-210. The suppression of HIF-1 $\alpha$  with its inhibitor 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) in VHL-silenced 3AO cells antagonized the pro-migratory activity induced by the VHL deficiency and reversed the upregulation of MMP2, MMP9, HIF-1 $\alpha$  and miR-210; however, it had no obvious effect on the VHL protein level. The introduction of miR-210 inhibitor into VHL-silenced 3AO cells resulted in similar changes as those induced by YC-1. Furthermore, vacuole membrane protein 1 (VMP1) was found to be diminished by VHL silencing in a HIF-1 $\alpha$ /miR-210-dependent manner. Taken together, our data demonstrate that the loss of VHL stimulates ovarian cancer cell migration by stabilizing HIF-1 $\alpha$ , upregulating miR-210 and decreasing VMP1 expression. These results indicate that the aberrant signaling of the VHL/HIF-1 $\alpha$ /miR-210/VMP1 pathway may be involved in ovarian cancer aggressiveness.

## Introduction

E3 ubiquitin ligase von Hippel-Lindau (VHL) has been established as a crucial gatekeeper, inhibiting tumorigenesis (1);

VHL is inactivated in a number of tumors through diverse mechanisms. VHL deficiency has been recently identified as a driver of cancer metastatic colonization (2). The tumor-suppressive function of VHL lies in its role as a mediator of the ubiquitin/proteasome-dependent degradation of the  $\alpha$ -subunit of hypoxia inducible factor (HIF) in the presence of oxygen (3,4). The pro-metastatic effect of VHL inactivation is also ascribed to the loss of the control of HIF and its transcriptional targets implicated in metastasis (2).

HIF-1 is the main member of the HIF transcription factor family, implicated in crucial aspects of cancer biology, including cancer cell invasion, by transcriptionally activating a number of protein-coding genes (5). Recently, non-coding microRNAs (miRs) emerged as a new class of HIF-1-regulated targets (6). Among the HIF-1-responsive miRs, miR-210 is the master hypoxamir ubiquitously stimulated by HIF-1 in various cell types (7,8). Of note, miR-210 has been classified into VHL-regulated and HIF-dependent miRs in renal cancer (9). By targeting a large spectrum of genes, miR-210 participates in a wide range of cellular functions, including the cell cycle, cell proliferation and apoptosis (10-12). miR-210 has been linked to cancer invasion and metastasis by suppressing vacuole membrane protein 1 (VMP1) expression (13). Initially defined as an autophagy-related membrane protein highly expressed in pancreatitis (14), VMP1 has been established to be a negative regulator of the cancer-relevant cell cycle, cell-cell adhesion, cell invasion and metastasis (15).

In epithelial ovarian oncogenesis, the *VHL* gene has been shown to be inactivated either by aberrant promoter methylation (16) or by the loss of heterozygosity of loci on the short arm of chromosome 3 (3p) (17). HIF-1 consists of a constitutively expressed HIF-1  $\beta$ -subunit and an oxygen- and growth-factor-regulated HIF-1  $\alpha$ -subunit and is overexpressed in ovarian cancer biopsies and correlates with patient prognosis (18). As regards miR-210, its aberrant overexpression has been found in a variety of cancers (19,20), apart from epithelial ovarian cancer, in which miR-210 is exceptionally deleted (21). VMP1 has been reported to be an inhibitor of the metastasis and proliferation of hepatocellular carcinoma and a protective factor against invasive ductal carcinoma (22,23). However, to date, no relation between VMP1 and ovarian cancer has been reported. Based on these published results, an aberrant signaling transduction

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**Key words:** ovarian cancer, von Hippel-Lindau, microRNA-210

initiating from VHL inactivation to HIF-1 $\alpha$ , miR-210 and VMP1 possibly exists and is involved in cancer progression. To date, the mechanisms through which VHL inactivation mediates ovarian cancer metastasis have not been well defined. It is necessary to explore whether there is a signaling pathway composed of VHL, HIF-1 $\alpha$ , miR-210 and VMP1 exerting pro-metastatic effects in ovarian cancer. Therefore, in this study, we silenced VHL expression using siRNA in ovarian cancer cells, examined the signaling among HIF-1 $\alpha$ , miR-210 and VMP1, and observed the resultant changes in cellular biology.

## Materials and methods

**Cell culture.** The human ovarian cancer cell line, 3AO, was obtained from the Shandong Academy of Medical Sciences (Jinan, China). SKOV3 cells were obtained from the Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (Gibco-BRL, Grand Island, NY, USA) supplemented with 10% newborn bovine serum in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C. For HIF-1 $\alpha$  inhibition, the cells were pre-treated with 50 mM of 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) (Sigma Chemical Co., St. Louis, MO, USA) for 24 h before total RNA or protein extraction.

**siRNA transfection.** The 3AO and SKOV3 cells were transfected with siRNA specific to VHL (obtained from Shanghai GenePharma Co., Ltd., Shanghai, China) using X-tremeGENE siRNA transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) in accordance with the manufacturer's instructions. The siRNA sequences used to silence the human *VHL* gene were as follows: siVHL-A, 5'-GUCUCAUUCUCAGAGUAAATT-3'; and siVHL-B, 5'-AACUGAAUUAUUUGUGCCAUCTT-3'. A scrambled siRNA (5'-UUCUCCGAACGUGUCACGUTT-3') was used in parallel experiments as a negative control. The cells were plated onto 6-well plates and were grown to 40-50% confluence at the time of transfection. For each sample, 1  $\mu$ g of siRNA and 5  $\mu$ l of transfection reagent were incubated in 100  $\mu$ l of serum- and antibiotics-free medium for 5 min, followed by mixing the solutions together and incubating at room temperature for a further 20 min; the resultant solution was layered over the cells at 37°C for the indicated periods of times.

**Total RNA isolation and quantitative reverse transcription PCR (qRT-PCR).** Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) was used to synthesize the first-strand cDNA. Quantitative PCR was performed on a Bio-Rad CFX-96 real-time PCR system using a SYBR-Green Master Mix (Takara Biotechnology Co., Ltd., Dalian, China). Thermal cycling conditions were as follows: 95°C for 30 sec, ensuing 40 cycles of 95°C for 5 sec and 60°C for 31 sec. Melting curve analysis was performed at the end of the cycles. Relative expression levels, normalized to those of  $\beta$ -actin, were calculated automatically based on the formula  $RQ=2^{-\Delta\Delta C_t}$ . All samples were assayed in triplicate, and 3 independent experiments were conducted. The sequences of the primers

were as follows: VHL forward, 5'-GCAGGCGTCCAAGAGTACG-3' and reverse, 5'-CGGACTGCGATTGCAGAAAG-3'; HIF-1 $\alpha$  forward, 5'-ATCCATGTGACCATGAGGAAATG-3' and reverse, 5'-TCGGCTAGTTAGGGTACACTTC-3'; and  $\beta$ -actin forward, 5'-TCCCTGGAGAAGAGCTACGA-3' and reverse, 5'-AGCACTGTGTTGGCGTACAG-3'.

**Transfection with miR-210 inhibitor, small RNA isolation and qRT-PCR.** A total of 100 nM of miR-210 inhibitor provided by RiboBio Co., Ltd. (Guangzhou, China) were transfected into the 3AO and SKOV3 cells seeded into 6-well plates using the X-tremeGENE siRNA transfection reagent (Roche Molecular Biochemicals) according to the manufacturer's instructions. A negative control was used in parallel experiments. After 48 h of transfection, small RNA was isolated with RNAiso as a small RNA reagent (Takara Biotechnology Co., Ltd.) and reverse-transcribed using stem-loop reverse transcription primers for miR-210 or U6 with the RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. Quantitative PCR was performed using SYBR-Green Master Mix (Takara Biotechnology Co., Ltd.). For the normalization of miR-210, snRNA U6 was used.

**Western blot analysis.** The cells were lysed with RIPA buffer containing complete protease inhibitor mixture (Roche Molecular Biochemicals), incubated for 30 min on ice before they were scraped, transferred to microfuge tubes and centrifuged at 12,000 rpm, 4°C for 30 min. The supernatants were collected and protein concentrations were detected using the Bradford Protein Assay kit (Bio-Rad, Hercules, CA, USA). Proteins were then boiled for 5 min. Following SDS-PAGE, the proteins were transferred onto nitrocellulose membranes (Pall Life Science, Ann Arbor, MI, USA). The membranes were blocked for 60 min at room temperature in 5% non-fat milk prior to incubation overnight at 4°C with the following antibodies: rabbit anti-VHL polyclonal antibody (1:1,000; Cell Signaling Technology, Beverly, MA, USA), mouse anti-HIF-1 $\alpha$  polyclonal antibody (1:500; Abcam Inc., Cambridge, MA, USA), rabbit anti-matrix metalloproteinase (MMP)2 polyclonal antibody (1:100; Epitomics, Burlingame, CA, USA), rabbit polyclonal antibody against MMP9 (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), rabbit polyclonal antibody against VMP1 (1:1,000) and mouse anti- $\beta$ -actin polyclonal antibody (1:1,000) (both from Cell Signaling Technology). The blots were then reacted with horseradish peroxidase (HRP)-conjugated goat anti-mouse/anti-rabbit immunoglobulin (IgG) (Pierce, Rockford, IL, USA) for 60 min at room temperature. The membranes were washed 5 times with Tris-buffered saline containing 0.05% Tween-20 between incubations. An enhanced chemiluminescence kit (Santa Cruz Biotechnology, Inc.) was used to visualize the signal of immunoreactive bands.

**Cell migration assay.** Ovarian cancer cells ( $1 \times 10^5$ ) in 100  $\mu$ l of RPMI-1640 medium without serum were seeded into Millicell modified Boyden chambers with an 8- $\mu$ m pore size (Millipore, Bedford, MA, USA), and were then placed in a 24-well plate containing 500  $\mu$ l of medium with 20% fetal bovine serum per well. Following 24 h of incubation at 37°C under 5% CO<sub>2</sub>, non-migrating cells remaining on the upper surface of the filter were removed with a cotton swab. Migrated cells were

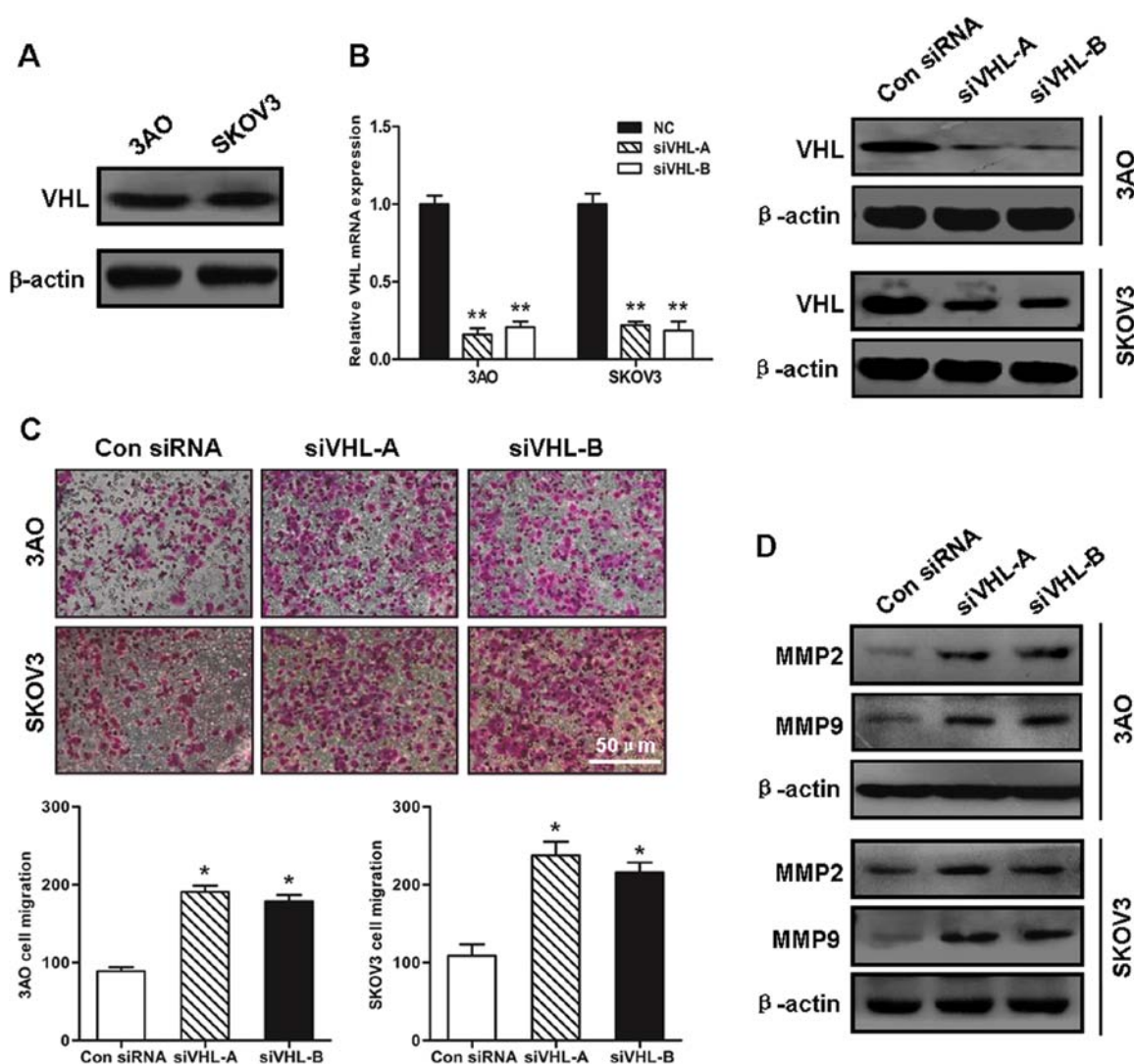


Figure 1. Loss of von Hippel-Lindau (VHL) promotes aggressiveness of ovarian cancer cells. (A) Western blot analysis revealed that the 3AO and SKOV3 cells expressed moderate levels of VHL protein.  $\beta$ -actin was used as an internal control. (B) Detection of interference efficiency of siVHL. Left panel, 3AO and SKOV3 cells were transfected with siVHL for 48 h, and the VHL mRNA level was examined by qRT-PCR. Right panel, cells were transfected with siVHL for 72 h, and the VHL protein level was measured by western blot analysis. A scrambled siRNA was used as a negative control (Con siRNA). (C) Silencing of VHL enhanced the motility of 3AO and SKOV3 cells. Cells were harvested 24 h after siRNA transfection, and placed into Millicell chambers for 12 h to examine the *in vitro* migration ability. (D) Suppression of VHL increased the expression of matrix metalloproteinase (MMP)2 and MMP9. Western blot analysis revealed that the protein level of MMP2 and MMP9 was elevated in the VHL-silenced cells. Data shown are the means  $\pm$  standard deviation (SD) of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , as shown by Student's t-test.

fixed with 5% glutaric dialdehyde and stained with Giemsa. The number of migrated cells was counted in 5 random fields under an inverted microscope at x200 magnification.

**Statistical analysis.** The SPSS18.0 statistical software package was used for statistical analysis. All assays were independently conducted at least 3 times and data are presented as the means  $\pm$  standard deviation (SD). Differences between groups were analyzed using the Student's t-test with a values of  $P < 0.05$  and  $P < 0.01$  considered to indicate statistically significant and highly significant differences, respectively.

## Results

**VHL silencing facilitates ovarian cancer cell migration.** The 3AO and SKOV3 ovarian cancer cell lines, which express the

VHL protein (Fig. 1A), were then used to examine the effects of VHL silencing on ovarian cancer cell migration. qRT-PCR and western blot analysis verified that the transient transfection of the synthetic siRNAs effectively inhibited VHL expression (Fig. 1B). The number of cells transfected with siVHL that had migrated was 2-fold greater than that of the cells transfected with the control siRNA (Fig. 1C). In parallel with the increase in cell motility, the MMP2 and MMP9 protein levels were substantially elevated when VHL was knocked down, indicating the augmentation of invasive potential induced by the loss of VHL (Fig. 1D).

**Downregulation of VHL increases HIF-1 $\alpha$  and miR-210 expression.** Since VHL is involved in the ubiquitination and degradation of the HIF-1 $\alpha$  protein, we then examined the changes in HIF-1 $\alpha$  protein levels following the silencing of

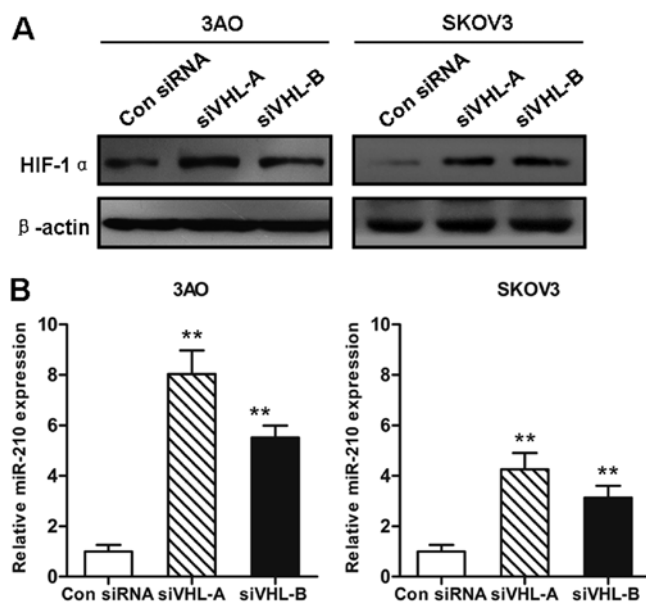


Figure 2. Inhibition of von Hippel-Lindau (VHL) promotes the expression of hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ) and miR-210. (A) Western blot revealed that transfection with siVHL for 72 h upregulated the protein level of HIF-1 $\alpha$ . (B) qRT-PCR indicated that the miR-210 level was significantly enhanced following transfection with siVHL for 48 h. Data are presented as the means  $\pm$  standard deviation (SD) from 3 independent experiments. \* $P$ <0.05, \*\* $P$ <0.01, as shown by Student's t-test. Con siRNA, scrambled control siRNA.

VHL. In line with previously published data (3), accumulated HIF-1 $\alpha$  protein was detected by western blot analysis in both the 3AO and SKOV3 cells transfected with siVHL for 72 h; the effects were more evident in the 3AO than in the SKOV3 cells (Fig. 2A). Based on the finding that miR-210 is a direct target of HIF-1 $\alpha$ , the relevance between the loss of VHL and the miR-210 expression level was further investigated. As expected, a significant increase in the miR-210 expression level was induced by the VHL deficiency. The augmentation in miR-210 expression in the VHL-deficient 3AO cells was almost twice that observed in the SKOV3 cells (Fig. 2B).

**HIF-1 $\alpha$ /miR-210 mediate VHL inactivation-induced migration.** To determine the function of increased HIF-1 $\alpha$  expression in the enhanced cell migration induced by VHL silencing, the VHL-silenced 3AO cells were treated with the HIF-1 $\alpha$  inhibitor, YC-1. *In vitro* cell migration assay revealed that compared with the untreated VHL-silenced 3AO cells, treatment with 50 mM YC-1 for 24 h decreased the number of migrated cells by almost 2-fold (Fig. 3A). The expression-enhancing effect of VHL silencing on HIF-1 $\alpha$ , MMP2 and MMP9 was effectively antagonized by YC-1 (Fig. 3B). Additionally, the increase in miR-210 expression induced by VHL silencing was greatly diminished by YC-1 (Fig. 3C), verifying that miR-210 is a downstream target of HIF-1 $\alpha$ .

To further explore the role of upregulated miR-210 in the enhanced cell migration induced by VHL silencing, VHL and miR-210 were simultaneously inhibited by co-transfection with siVHL and miR-210 inhibitor into 3AO cells (Fig. 4A). The inhibition of miR-210 abolished the contribution of VHL silencing to the enhanced cell motility (Fig. 4B) and to the increased expression of MMP2 and MMP9 (Fig. 4C); these changes were similar to those induced by YC-1. Of note,

neither YC-1, nor miR-210 inhibitor had an effect on VHL protein levels, indicating that HIF-1 $\alpha$  and miR-210 are downstream targets of VHL.

**VHL/HIF-1 $\alpha$ /miR-210 downregulate VMP1, promoting ovarian cancer cell migration.** miR-210 has been shown to mediate hypoxia-induced hepatocarcinoma cell migration and invasion by directly targeting VMP1 (13). Therefore, we investigated the involvement of VMP1 in the VHL/HIF-1 $\alpha$ /miR-210 pathway. When VHL alone was silenced, the VMP1 protein level was substantially diminished (Fig. 5A and B). By contrast, VMP1 protein expression was markedly augmented with HIF-1 $\alpha$  alone was inhibited. The suppressive effects of VHL silencing on VMP1 expression were reversed by the YC-1 and miR-210 inhibitors (Fig. 5A and B), indicating that VMP1 is a downstream effector of the VHL/HIF-1 $\alpha$ /miR-210 pathway (Fig. 5C).

**The VHL/HIF-1 $\alpha$ /miR-210/VMP1 pathway promotes cell motility independent of epithelial-mesenchymal transition (EMT).** Since EMT is a critical mechanism initiating cancer invasion and metastasis, and HIF-1 $\alpha$  is an important mediator of EMT, we examined the potential implication of the loss of VHL in inducing EMT in ovarian cancer cells. When VHL was knocked down, cell morphology did not undergo a typical EMT change from a cobble-stone shape to a dispersed fibroblastoid shape (Fig. 6A). Consistently, the protein level of the epithelial marker, E-cadherin, and the mesenchymal marker, vimentin, remained unaltered when VHL was silenced (Fig. 6B). These observations suggest that the pro-motility function of the VHL downregulation is irrelevant to EMT.

## Discussion

The VHL protein has long been characterized as an important gatekeeper, inhibiting tumor initiation (24,25). The crucial aspect of VHL function is to act in a ubiquitin ligase complex targeting the prolyl-hydroxylated  $\alpha$ -subunit of HIF-1 (26) for ubiquitination and further proteolysis under normoxic conditions (3). During tumor development and progression, the decrease or inactivation of VHL stabilizes the  $\alpha$ -subunit of HIF, which then heterodimerizes with the  $\beta$ -subunit and gives rise to HIF (27). The increase in HIF levels results in the transcriptional activation of a number of genes, to facilitate the adaptation of angiogenesis and cell metabolism to the hypoxic microenvironment (28). VHL has been shown to negatively regulate tumor metastasis (29). The anti-metastatic mechanism of VHL is realized through controlling the degradation of the  $\alpha$ -subunit of HIF and influencing the transcription of its target genes associated with metastasis. Nevertheless, the VHL/HIF-1 $\alpha$  axis has been extensively examined in certain types of cancer, particularly renal cell carcinoma (30). Evidence to verify the role of VHL/HIF-1 $\alpha$  in ovarian cancer is limited, even though a negative correlation has been found between the VHL expression level and the nuclear expression of HIF-1 $\alpha$  in ovarian clear cell carcinomas tissues (17). Furthermore, the detailed mechanisms through which VHL functions as a suppressor of tumor metastasis remain to be clarified. The direct cellular and molecular evidence provided in this study indicates that in ovarian cancer cells, the depletion of VHL

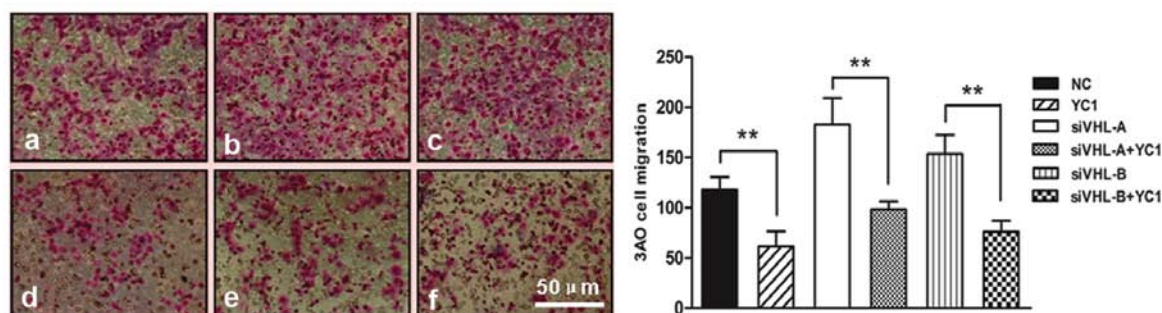
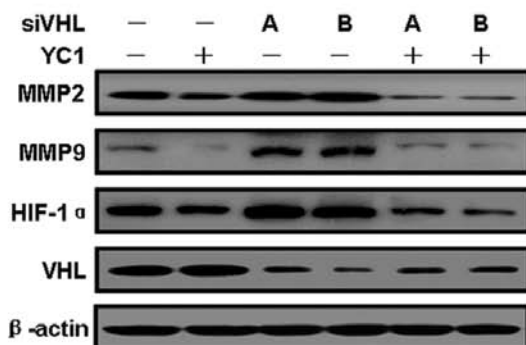
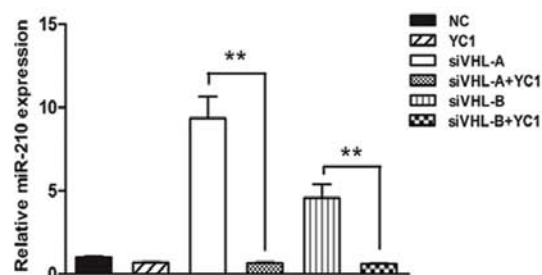
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Figure 3. Hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ) mediates the effects of von Hippel-Lindau (VHL) silencing on cell migration and invasion. (A) *In vitro* migration assay showed that the number of migrated cells significantly increased when VHL was knocked down, which was reversed by the HIF-1 $\alpha$  inhibitor, YC-1. (a) negative control (NC); (b) siVHL-A; (c) siVHL-B; (d) YC-1; (e) siVHL-A + YC-1; (f) siVHL-B + YC-1. (B) Western blot analysis revealed that YC-1 inhibited HIF-1 $\alpha$ , matrix metalloproteinase (MMP)2 and MMP9 protein expression in VHL-silenced cells. (C) The level of miR-210 was measured by qRT-PCR. The miR-210 expression level was attenuated by YC-1 in the VHL-silenced cells. Values are presented as the means  $\pm$  standard deviation (SD) from 3 independent experiments. \* $P$ <0.05, \*\* $P$ <0.01, as shown by Student's *t*-test.

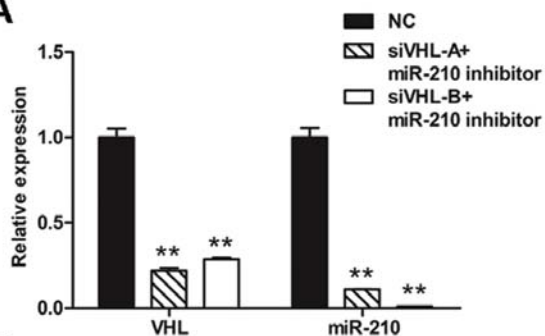
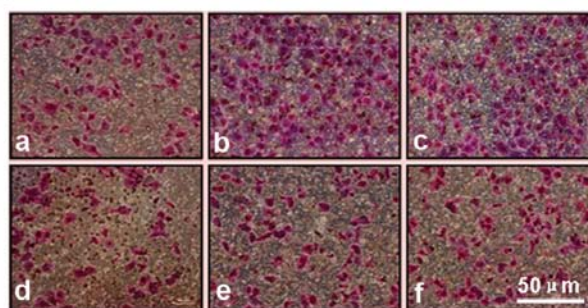
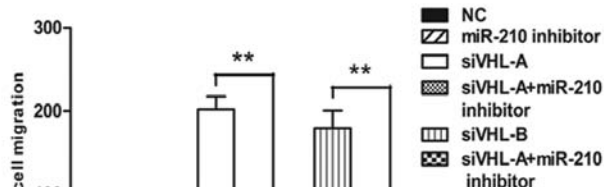
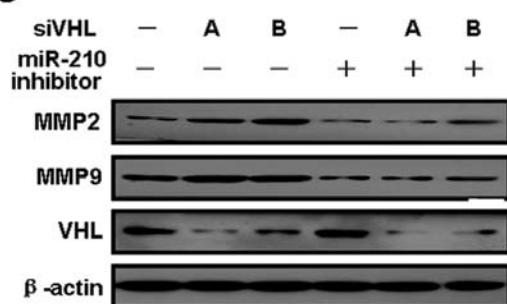
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Figure 4. miR-210 is involved in the migration and invasion potential of von Hippel-Lindau (VHL)-silenced cells. (A) The level of VHL mRNA and miR-210 was examined by qRT-PCR in cells co-transfected with siVHL and miR-210 inhibitor simultaneously. (B) *In vitro* migration assay revealed that miR-210 inhibitor repressed the migration ability of VHL-silenced cells. (a) negative control (NC); (b) siVHL-A; (c) siVHL-B; (d) miR-210 inhibitor; (e) siVHL-A + miR-210 inhibitor; (f) siVHL-B + miR-210 inhibitor. (C) miR-210 inhibitor downregulated the protein expression of matrix metalloproteinase (MMP)2 and MMP9. Data are presented as the means  $\pm$  standard deviation (SD) from 3 independent experiments. \* $P$ <0.05, \*\* $P$ <0.01, as shown by Student's *t*-test.



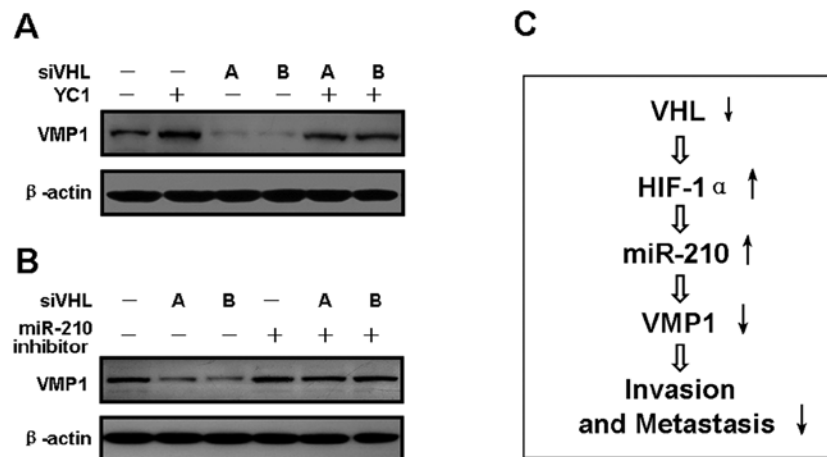


Figure 5. von Hippel-Lindau (VHL) silencing affects the vacuole membrane protein 1 (VMP1) expression level, dependent on hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ )/miR-210. (A) Silencing of VHL decreased VMP1 protein level, which was reversed by YC-1 (HIF-1 $\alpha$  inhibitor). (B) miR-210 inhibitor reversed the decrease in VMP1 expression induced by VHL silencing. (C) The suggested model of the VHL/HIF-1 $\alpha$ /miR-210/VMP1 pathway in ovarian cancer cells.

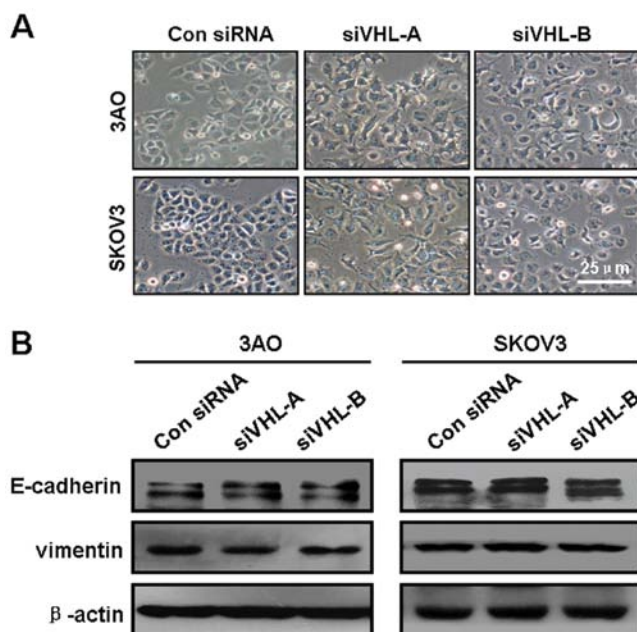


Figure 6. Loss of von Hippel-Lindau (VHL) was insufficient to induce epithelial-mesenchymal transition (EMT) in ovarian cancer cells. (A) Cell morphology of 3AO and SKOV3 cells transiently transfected with siRNA for 24 h. The images were captured under a phase contrast microscopy (x100 magnification). (B) Western blot analysis of E-cadherin and vimentin in VHL-silenced cells. No significant changes in the protein level were observed. Con siRNA, scramble control siRNA.

enhances cell motility and the invasion potential by stabilizing HIF-1 $\alpha$  protein.

HIF modulates cellular functions by acting as a transcription factor. Recent studies have revealed that its transcriptional targets are not merely confined to protein-coding genes, as several non-coding miRs have been found as a new class of HIF-responsive molecules. Among the HIF-regulated miRs, including miR-21 (31) and miR-373 (32), miR-210 is unique as it is consistently upregulated by HIF in diverse cell types. An increase in the miR-210 level has been reported in a number

of tumor types and correlates with poor prognosis (33). Exceptionally, the diminished expression of miR-210 has been found in more than half of ovarian cancer patients (21). However, miR-210 cannot be simply assumed as a candidate tumor-suppressor gene in ovarian cancer. In SKOV3 ovarian cancer cells, miR-210 has been shown to be constantly induced by hypoxia (21,34), and is considered a crucial factor for tumor development and progression (35,36), indicating that miR-210 may play a certain role under a specific temporal-spatial condition without necessarily maintaining a high level of expression. The observations made in this study indicate that miR-210 is essential for the deficient VHL-induced aggressiveness of ovarian cancer cells, indicating that miR-210 potentially possesses pro-malignant activity in ovarian cancer.

Consistent with previously published data (37), the miR-210 expression level was found to be dependent on HIF-1 $\alpha$  protein expression, which was stabilized by the loss of VHL. miR-210 targets a complex set of genes involved in a number of processes, including cell metabolism, cell cycle, proliferation and apoptosis (8). Moreover, miR-210 has been reported to promote the migration and invasion capability of hepatocellular carcinoma cells by directly repressing the autophagy-associated protein, VMP1 (13). VMP1 inhibition had been associated with the enhanced invasion and metastatic potential of cancer cells (15). In agreement with these findings, we found that VMP1 was a downstream effector of VHL in restraining ovarian carcinoma malignant progression, since the loss of VHL suppressed the VMP1 protein level in a HIF-1 $\alpha$ /miR-210-dependent manner.

EMT has been regarded as a significant mechanism implicated in cancer metastasis, featured by an increase in cell motility and invasion capability, cell morphological changes from a cobble-stone shape to a fibroblastoid shape, a decrease in the levels of the epithelial marker, E-cadherin, and an increase in the levels of the mesenchymal marker, vimentin. VHL is capable of elevating the expression of E-cadherin in clear-cell renal cell carcinoma (34,35), and the silencing of VHL promotes EMT in lung cancer cells (36). However, no obvious EMT phenomena were observed after the silencing of VHL in this study. We assumed that VHL may restrain ovarian cancer cell metastasis independent of EMT.

In conclusion, we found that VHL suppresses the invasion and migration capability of ovarian cancer cells by promoting the suppressive effects of HIF-1 $\alpha$ /miR-210 on VMP1. Although the role of the aberrant signaling pathway composed of VHL/HIF-1 $\alpha$ /miR-210/VMP1 in ovarian cancer metastasis and invasion has not been extensively defined, the results reported in this study provided new insight into the mechanisms behind ovarian cancer progression.

## Acknowledgements

This study was supported by a grant from the National Natural Science Foundation of China (no. 30973429).

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