Wnt5a promotes vasculogenic mimicry and epithelial-mesenchymal transition via protein kinase Cα in epithelial ovarian cancer

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Abstract. Epithelial ovarian cancer is one of the most common causes of cancer-related death in women. The majority of epithelial ovarian cancer patients present with metastasis at the time of initial diagnosis. Studies have demonstrated that vasculogenic mimicry (VM) is highly correlated with metastasis and invasiveness, and epithelial-mesenchymal transition (EMT) is pivotal in VM formation. Wnt5a, a member of the Wnt protein family, can activate the non-canonical Wnt signaling pathway mediating cancer initiation and progression. Thus, the present study aimed to investigate the relationship between Wnt5a and VM and its mechanism in epithelial ovarian cancer. The present results showed that Wnt5a staining was significantly correlated with metastasis in epithelial ovarian cancer. The correlation between the expression of Wnt5a and VM or protein kinase Cα (PKCα) indicated that Wnt5a was associated with VM and may be linked to the PKC pathway. *In vitro* experiments revealed that Wnt5a enhanced the vasculogenic capacity, motility and invasiveness of ovarian cancer cells; however, the PKCα inhibitor blocked these effects. Western blot analysis showed that changes in Wnt5a expression coincided with changes in PKC expression and that PI3K and Snail expression increased along with Wnt5a upregulation. However, no change was observed in β-catenin levels, indicating that Wnt5a may mediate EMT and VM in ovarian cancer cells via the PKCα pathway.

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Introduction

Epithelial ovarian cancer is one of the most common causes of cancer-related death in women. The survival rate of ovarian cancer patients has not improved although diagnosis and treatment have advanced significantly in recent years. Therefore, advances in ovarian cancer treatment must be supported by a deep understanding of its biological features. Vasculogenic mimicry (VM) was derived from aggressive uveal melanoma microcirculation by Maniotis *et al* (1). VM is the ability of aggressive cancer cells to form vasculogenic-like networks *in vivo*. Endothelial cells are not apparent in VM vessels upon CD31 immunostaining, while cells lining the VM vessels are, as expected of cells of hepatic origin and periodic acid-Schiff (PAS)-positive. The presence of red blood cells indicates that blood circulates in VM vessels (2). VM has been observed in several malignant tumor types, such as breast, prostate and liver cancer, glioma, melanoma and bidirectional differentiated malignant tumors (3). Our previous studies demonstrated that the prognosis of patients with VM is significantly worse than that of patients without VM (4), and these findings suggest that epithelial-mesenchymal transition (EMT) is important in tumor progression and VM (5). However, the molecular mechanism of VM remains unclear.

The Wnt families are crucial for tumorigenesis and chick embryo development; these families include canonical (β-catenin-dependent) Wnt signaling pathways and non-canonical (β-catenin-independent) Wnt signaling pathways (6). Our studies showed that activation of the Wnt-β-catenin signaling pathway in colon cancer cells is associated with VM (7). However, the function of the non-canonical Wnt pathway in VM is unidentified. Wnt5a is an important non-canonical Wnt pathway that was first identified in *Drosophila* developmental studies (8). Dissanayake *et al* (9), and Weeraratna *et al* (10) reported that Wnt5a signals can activate phospholipase C via frizzled. This activation causes phospholipid turnover in the membrane, thereby releasing calcium from intracellular stores and increasing protein kinase C (PKC) activity. The authors also induced Wnt5a overexpression and downregulation, and conducted microarray analysis to demonstrate that Wnt5a/

PKC stimulates melanoma cell motility by inducing genes involved in melanoma EMT. However, the function of Wnt5a/PKC in epithelial ovarian cancer is unknown. The present study aimed to confirm the relationship between Wnt5a and VM and its mechanism in epithelial ovarian cancer.

Materials and methods

Tissue samples. Seventy-nine cases of ovarian cancer were selected from Tianjin Cancer Hospital and Tianjin Medical University General Hospital between 1991 and 1999. All specimens were obtained by tumor surgical operation, were formalin-fixed and processed for paraffin embedding, and were sectioned and stained with hematoxylin and eosin, and diagnosed by two pathologists.

A total of 79 patients with epithelial ovarian cancer were enrolled in this study, and their clinicopathological characteristics are summarized in Table I. The ages ranged from 21 to 80 years (median age, 54.4 years); 28 patients (35.4%) were <50 years and 51 patients (64.6%) were ≥50 years of age. The size of tumors ranged from 1 to 20 cm (median, 5.6 cm); 41 cases (51.9%) were <5 cm, 38 cases (48.1%) were ≥5 cm. The FIGO stages at initial diagnosis were as follows: stage I in 24 cases (30.4%), stage II in 12 cases (15.2%), and stage III in 43 cases (54.4%). The histological types were serous tumors in 54 cases (68.4%), mucous tumors in 11 cases (13.9%), and endometrioid tumors in 14 cases (17.7%). Distant metastasis was detected in 43 cases (54.4%), and ascites was present in 56 cases (70.9%). The study protocol was approved by the Ethics Committee of Tianjin Medical University.

Table I. Wnt5a expression in 79 epithelial ovarian cancer cases and the correlation with clinicopathological characteristics.

Variables	Cases n=79	Wnt5a expression: Negative n (%)	Wnt5a expression: Positive n (%)	χ^2	P-value
Age, years					0.802
<50	28	20 (71.4)	8 (28.6)	0.189	
≥50	51	34 (73.8)	17 (26.2)		
Tumor size (cm)					0.226
<5	41	31 (75.6)	10 (24.4)	2.074	
≥5	38	23 (60.5)	15 (39.5)		
Histological type					0.581
Serous	54	35 (64.8)	19 (35.1)	1.086	
Mucous	11	8 (72.7)	3 (27.3)		
Endometrioid	14	11 (78.6)	3 (21.4)		
FIGO stage					0.115
I	24	17 (70.8)	7 (29.2)	4.320	
II	12	11 (91.7)	1 (8.3)		
III	43	26 (60.5)	17 (39.5)		
Metastasis					0.008[a]
Absent	36	31 (86.1)	5 (13.9)	7.419	
Present	43	23 (53.5)	20 (46.5)		
Ascites					0.292
Absent	23	18 (78.3)	5 (21.7)	1.472	
Present	56	36 (64.3)	20 (35.7)		

[a]Statistically significant.

Inmunohistochemistry. The sections were pretreated with a microwave, blocked and incubated using a series of antibodies: Wnt5a antibody (dilution 1:100; R&D Biosystems, Minneapolis, MN, USA) and the protein kinase Cα (PKCα) antibody (dilution 1:100; Zhongshan Chemical Co., Beijing, China). The staining systems used in the present study were PV6000 and Elivision Plus (Zhongshan Chemical Co.).

Cell lines. The cells used in this study were human ovarian adenocarcinoma cells OVCAR3 and SKOV3 (American Type Culture Collection, Rockville, MD, USA). The cells were cultured in a mixture of RPMI-1640 medium, antibiotics and 10% fetal bovine serum (FBS) (both from HyClone, Thermo Scientific), and were incubated at 37˚C in a 5% CO_2 incubator.

Plasmid constructs and generation of stable cell clones. The plasmids carrying Wnt5a and Wnt5a shRNA (shWnt5a) as well as a control scrambled plasmid were purchased from GeneChem (Shanghai, China). Wnt5a shRNA and the control scrambled plasmid were labeled with GFP. The vectors were transfected into cells by percutaneous ethanol injection (cat. no. 23966; Polysciences, Inc.). More than 60% efficiency was utilized in the transient transfection experiments. We used G418 (600 mg/l) in the stable transfection experiments.

Western blot analysis. The antibodies used were Wnt5a (dilution 1:100; R&D Biosystems); Snail, PI3K (dilution 1:200; Santa Cruz, Dallas, TX, USA); PKCα, E-cadherin, vimentin, β-catenin (dilution 1:100), GAPDH and β-actin (dilution 1:2,000) (all from Zhongshan Chemical Co.). GAPDH or β-actin were used as a protein-loading control.

Wound assay to assess cell motility. SKOV3 and OVCAR3 cells ($1x10^5$) were plated in 12-well plates for 24 h and were then transfected with the plasmids. PMA (200 nM) or PKC inhibitor (1 μM) (Calbiochem) was added to the conditioned medium. After 48 h when the cells reached 90% confluency, sterile pipette tips were used to scratch the wound uniformly. Then, the medium was replaced with fresh RPMI-1640 without FBS. The cells were photographed with a microscope (Nikon, Japan) and counted in several pre-marked areas at 0, 4, 8, 12 and 24 h.

Transwell assay. Transwell chambers (6.5 mm) (Corning Costar, Cambridge, MA, USA) with polycarbonate membranes (with 8.0-μm pores) were treated with 10 μl Matrigel. SKOV3, SKOV3-Wnt5a, OVCAR3 and OVCAR3-pGFP-shWnt5a cells ($5x10^4$/well) were incubated in the upper chamber at 37˚C in a 5% CO_2 incubator, and the medium, serum-free, was added to the lower chamber to allow the cells to migrate. Cells from the top of the Transwell chambers were removed by a cotton swab, and the cells that had migrated to the lower surface were fixed with 4% formaldehyde and stained with crystal violet. Cells in

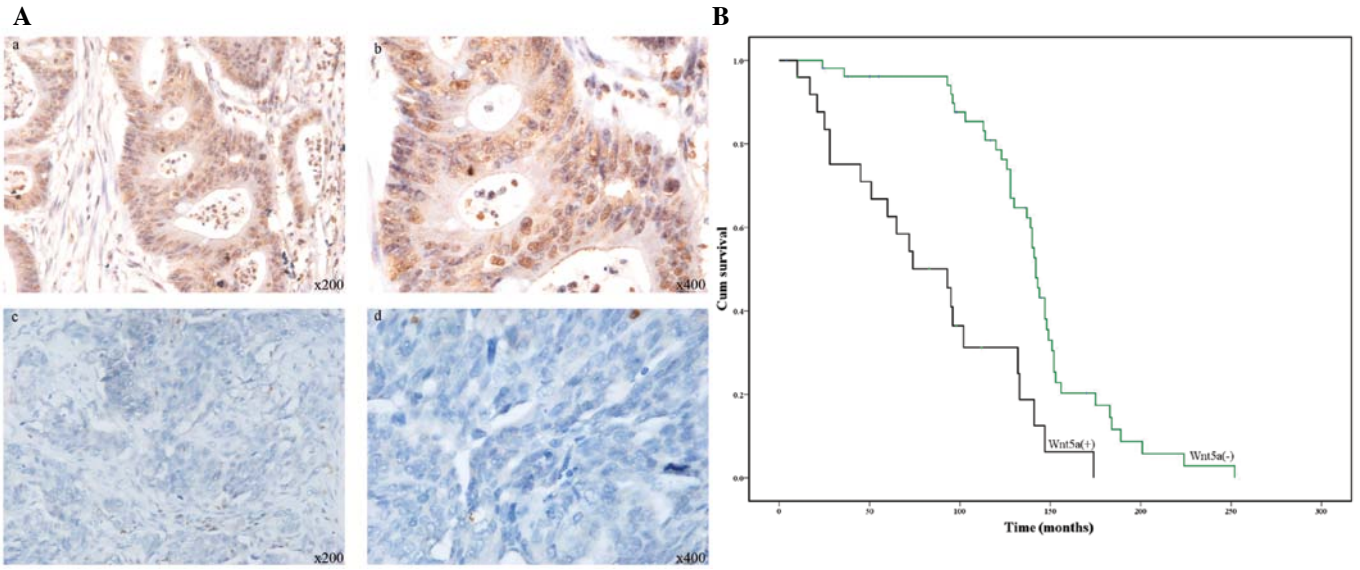

Figure 1. Expression of Wnt5a and its prognostic significance in epithelial ovarian cancer. (A) Representative immunohistochemical staining of Wnt5a-positive (a, x200; b, x400 magnification) and Wnt5a-negative (c, x200; d, x400 magnification) epithelial ovarian cancer tissues. (B) Kaplan-Meier survival analysis of patients categorized according to Wnt5a expression. Wnt5a-negative patients had prolonged survival when compared with the survival of Wnt5a-positive patients (P=0.00).

the lower chamber were counted in three random microscopic fields using an inverted microscope (Nikon, Japan).

3D culture. A total of 50 μl of Matrigel basement membrane matrix and cells (as mentioned above) were coated on a 96-well plate for 4 h at 37˚C. Then, 50 μl of SKOV3 or OVCAR3 cells ($1x10^5$) was added. The cells were incubated for 24 h at 37˚C in a 5% CO_2 incubator. Tube-like structures of cells that formed after 6 h were counted under an inverted microscope (Nikon, Japan).

Immunofluorescence. The antibodies used were E-cadherin and vimentin (as mentioned above). Cells were observed under a confocal microscope (Nikon, Japan).

Statistical analysis. All data were evaluated by SPSS version 17.0. Data are representative of at least triplicate independent determinations. The relationship between Wnt5a expression and clinicopathological characteristics and the expression of VM and PKCα was analyzed using Chi-square and Pearson's correlation test. For univariate survival analysis, survival curves were obtained using the Kaplan-Meier method. Differences in survival curves were assessed according to the log-rank test. GraphPad Prism 6 (GraphPad software) was used for western blotting and cellular function analysis. Differences were considered significant at values of P<0.05.

Results

Wnt5a expression is significantly associated with VM and PKCα expression in epithelial ovarian cancer and clinicopathological characteristics. Immunohistochemistry was used to evaluate Wnt5a protein expression in the ovarian cancer cases. Wnt5a was distributed in the cytoplasm and nucleus (Fig. 1A). Of the 79 ovarian cancer cases in the present study, 25 (31.6%) exhibited positive Wnt5a expression and 54 (68.4%) exhibited negative Wnt5a expression. Groups were classified as positive or negative according to Wnt5a expression, and SPSS was used to analyze the relationship between Wnt5a and clinicopathological characteristics. The results showed that Wnt5a expression was not correlated with age, tumor size, histological type, FIGO stage and the presence of ascites, but was highly correlated with cancer metastasis (Table I, P=0.008), indicating that Wnt5a promotes tumor metastasis. The mean survival time of the 79 ovarian cancer patients was 137 months (range, 4-252 months), and Wnt5a-negative patients were found to have a longer survival than Wnt5a-positive patients (Fig. 1B, P=0.00).

We assessed the relationship between Wnt5a and VM in ovarian cancer. Our previous study showed that the prognosis of patients with VM was significantly worse than the prognosis of patients without VM (4). The expression of Wnt5a

Table II. Correlation of Wnt5a expression with VM and PKCα in 79 epithelial ovarian cancer cases.

		Wnt5a expression			
Variables	Cases n=79	Negative n (%)	Positive n (%)	χ^2	P-value
VM					0.000[a]
Negative	56	46 (82.1)	10 (17.9)	16.906	
Positive	23	8 (34.8)	15 (65.2)		
PKCα					0.000[a]
Negative	44	40 (90.1)	4 (9.1)	23.356	
Positive	35	14 (40.0)	21 (60.0)		

VM, vasculogenic mimicry; PKCα, protein kinase Cα. [a]Statistically significant.

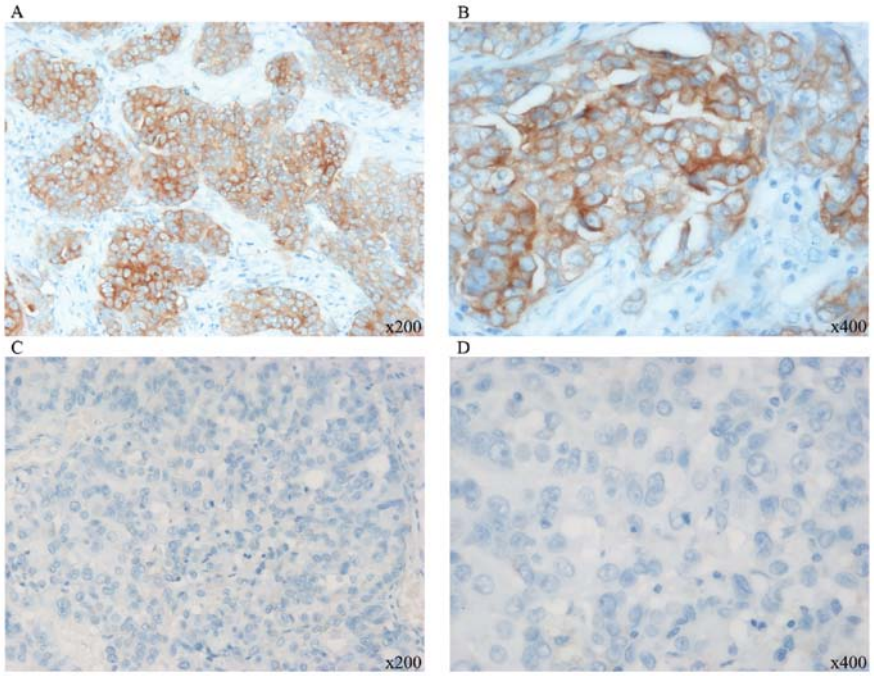

Figure 2. Representative immunohistochemical staining of PKCα-positive (A, x200; B, x400 magnification) and PKCα-negative (C, x200; D, x400 magnification) epithelial ovarian cancer cases. PKCα, protein kinase Cα.

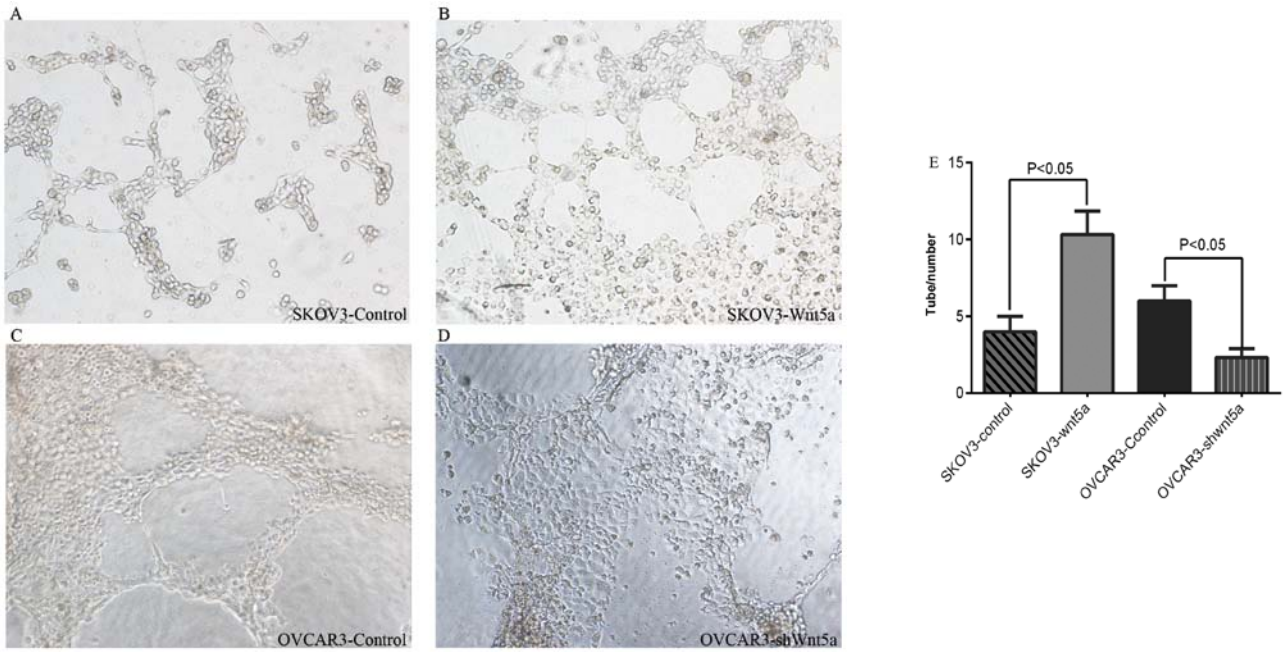

Figure 3. Change in tube formation capacity of ovarian cancer cells *in vitro*. (A) SKOV3 cells displayed a lower tube formation capacity than (C) OVCAR3 cells in the 3D culture experiments. Wnt5a enhanced the tube formation capacity of SKOV3 cells (B and E; P<0.01), and shWnt5a weakened this capacity in the OVCAR3 cells (D and E; P<0.05).

in ovarian cancer cases with or without VM was analyzed, and Wnt5a staining was shown to be significantly correlated with VM (Table II, P=0.000). Since the non-canonical Wnt5a pathway may induce PKCα activation (9,11), the expression of PKCα in ovarian cancer and its relationship with Wnt5a was determined. PKCα was expressed via a cytoplasmic staining pattern (Fig. 2). Of the 79 ovarian cancer cases, 35 (44.3%) exhibited positive PKCα expression and 44 (55.6%) exhibited negative expression. The correlation of Wnt5a and PKCα expression was analyzed, and Wnt5a staining was found to be significantly correlated with PKCα (Table II, P=0.000). The results indicate that Wnt5a expression is associated with VM and may trigger PKCα pathway activation.

Wnt5a enhances the vasculogenic capacity of ovarian cancer cells in vitro. SKOV3 and OVCAR3 cells were used in this

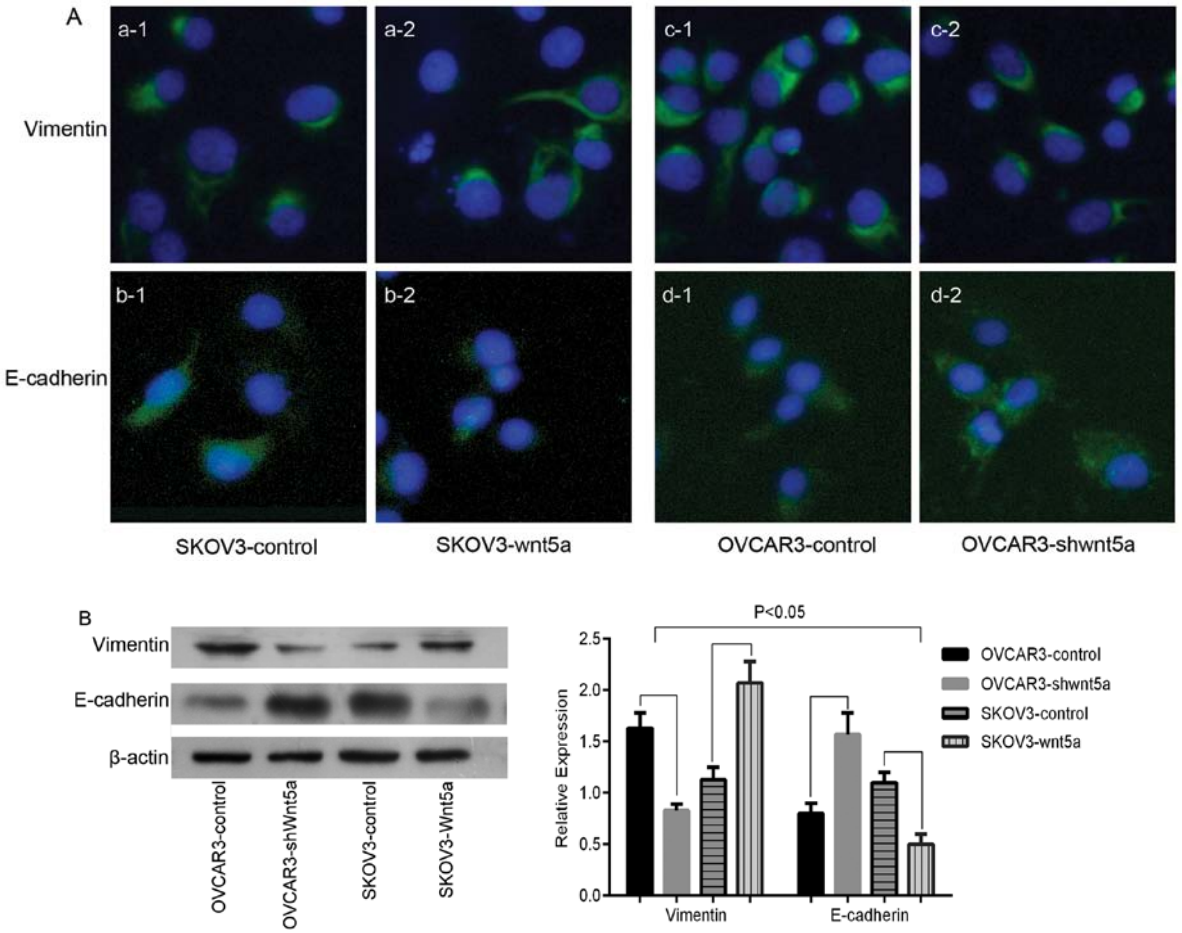

Figure 4. Changes of in the levels of EMT-associated proteins *in vitro*. (A) Immunofluorescence assays showed that Wnt5a enhanced the expression of vimentin (a-1 and a-2) and inhibited the expression of E-cadherin (b-1 and b-2). In contrast, shWnt5a inhibited the expression of vimentin (c-1 and c-2) and enhanced the expression of E-cadherin (d-1 and d-2). (B) Western blot analysis confirmed this correlation. The results were statistically significant (P<0.05). EMT, epithelial-mesenchymal transition.

study to verify the association of Wnt5a in ovarian cancer cells with VM *in vitro*. The endogenous expression of Wnt5a in the cell line SKOV3 was significantly lower than that in the cell line OVCAR3. Thus, SKOV3 cells were infected with the Wnt5a plasmid and OVCAR3 cells with the shWnt5a plasmid. Stably transfected cells were used in the following study.

Three-dimensional culture is recognized as an important method by which to evaluate VM *in vitro*. Thus, the well-established Matrigel culture was used to investigate vasculogenic capacity in ovarian cancer cell lines. OVCAR3, a poorly differentiated ovarian cancer line, displayed higher vasculogenic capacity than SKOV3, a well-differentiated cell line (Fig. 3). However, OVCAR3 cells transfected with Wnt5a shRNA displayed low vasculogenic capacity (Fig. 3), and SKOV3 cells transfected with Wnt5a cDNA exhibited high vasculogenic capacity (Fig. 3). This result indicates that Wnt5a can enhance the vasculogenic capacity of ovarian cancer cells *in vitro*.

Wnt5a enhances EMT in ovarian cancer cells in vitro. Our previous studies demonstrated that EMT is a critical step in VM. E-cadherin and vimentin are recognized as EMT-associated proteins (3,5). Immunofluorescence was performed to investigate the changes in the expression levels of these two proteins after *in vitro* Wnt5a transfection. The result revealed that vimentin expression was increased and E-cadherin expression was decreased in the SKOV3 cells after Wnt5a upregulation (Fig. 4A). By contrast, vimentin expression was decreased and E-cadherin expression was increased in OVCAR3 cells after shWnt5a transfection (Fig. 4A). We also detected the protein expression levels of vimentin and E-cadherin in the two ovarian cancer cell lines. Western blot analysis confirmed the results of the immunofluorescence analysis (Fig. 4B). The results were statistically significant (P<0.05). These findings indicate that Wnt5a can enhance EMT in ovarian cancer cells *in vitro*.

Wnt5a increases the motility and invasiveness of ovarian cancer cells in vitro. Cell motility is closely related to tumor metastasis, and Wnt5a was found to be positively correlated with melanoma motility and invasiveness (9-11). The effect of Wnt5a on the motility and invasiveness of ovarian cancer cells *in vitro* was investigated. Cell migration was evaluated by a wound healing assay, also known as the 'scratch' assay, and cell invasion was examined by Transwell assay. SKOV3 cells were transfected with the Wnt5a plasmid and OVCAR3 cells were transfected with the shWnt5a plasmid based on endogenous Wnt5a expression. The results showed that Wnt5a overexpression enhanced cell migration and invasiveness in the SKOV3 cells following transfection with Wnt5a (Fig. 5,

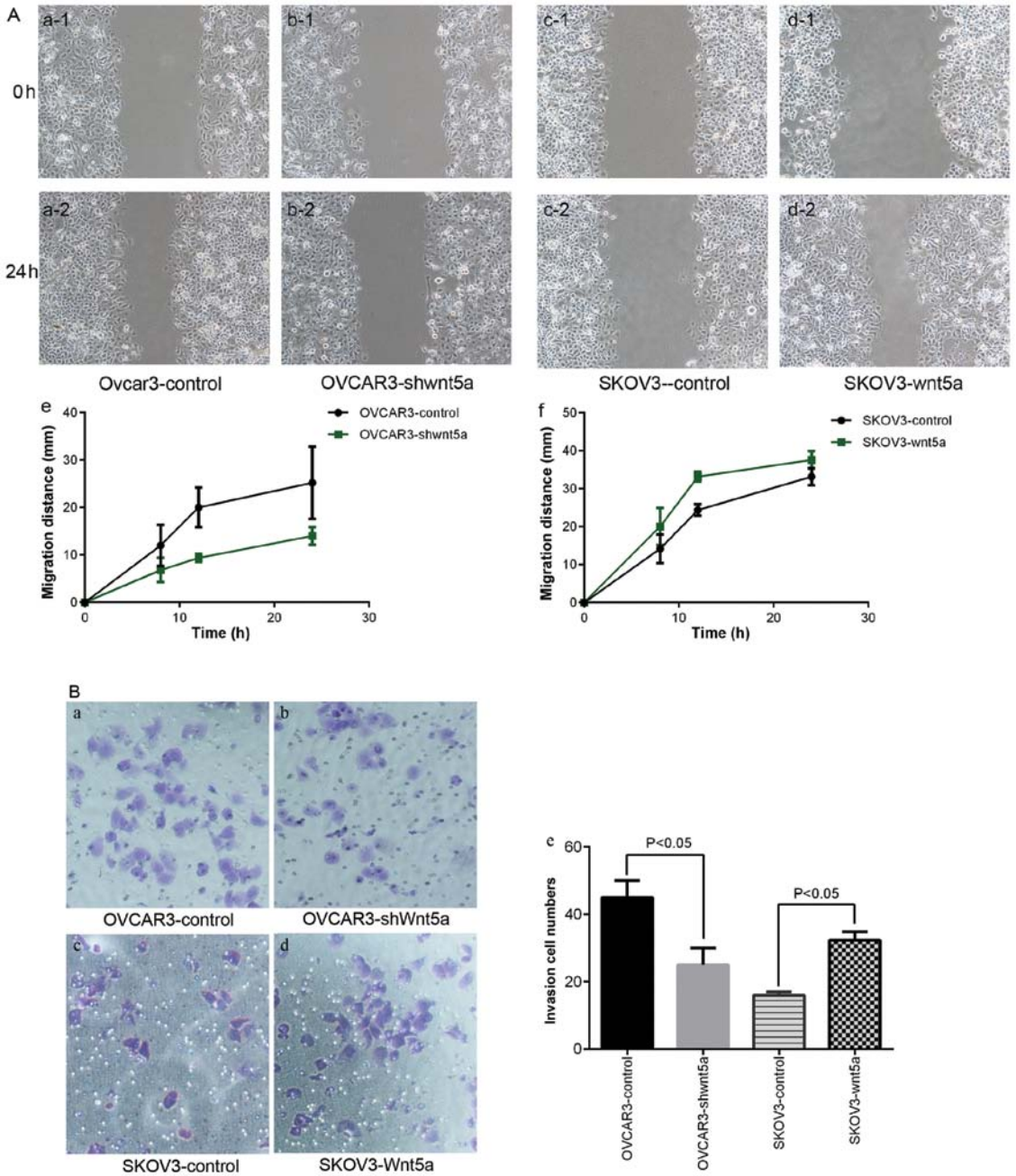

Figure 5. Increase in the motility and invasiveness of ovarian cancer cells via Wnt5a. (A) Wound healing assays revealed that OVCAR3-control cells displayed higher motility (a-1 and a-2) than OVCAR3-shWnt5a cells (b-1 and b-2) (magnification, x100). SKOV3-Wnt5a cells displayed higher motility (d-1 and d-2) than SKOV3-control cells (c-1 and c-2). The results were statistically significant (e and f; P<0.05). (B) Transwell assays showed that Wnt5a downregulation inhibited OVCAR3 cell invasion (a, b and e; P<0.05) and Wnt5a upregulation evidently enhanced SKOV3 cell invasion (c, d and e; P<0.05) (magnification, x200).

P<0.05). These findings corresponded with the weakened motility and invasiveness in the OVCAR3 cells transfected with shWnt5a (Fig. 5, P<0.05). Thus, Wnt5a can enhance the motility and invasiveness of ovarian cancer cells.

Wnt5a promotes ovarian cancer EMT and VM via the PKCα pathway in vitro. The non-canonical pathway signaled by Wnt5a is involved in PKC activation. Dissanayake *et al* (9), and Weeraratna *et al* (10) demonstrated the importance of PKC signaling in melanoma metastasis. Therefore, we hypothesized that PKC is critical to the effect of Wnt5a on ovarian cancer cells. The effect of Wnt5a expression on PKCα expression was investigated via western blot analysis. The results showed that changes in Wnt5a expression coincided with changes in PKCα expression (Fig. 6A, P<0.05). The addition of a PKCα inhibitor (1 μM) to SKOV3-Wnt5a cells significantly reduced cell motility (Fig. 6B, P<0.05). This result indicates that PKCα is critical to the effect of Wnt5a on ovarian cancer cells *in vitro*.

Finally, EMT-associated proteins were assessed in the two ovarian cancer cell lines at the protein level after cells were transfected with the Wnt5a plasmids. The findings showed that PI3K and Snail expression increased with Wnt5a upregulation (Fig. 6C), but not β-catenin, indicating that Wnt5a may mediate EMT and VM in ovarian cancer cells via PKCα.

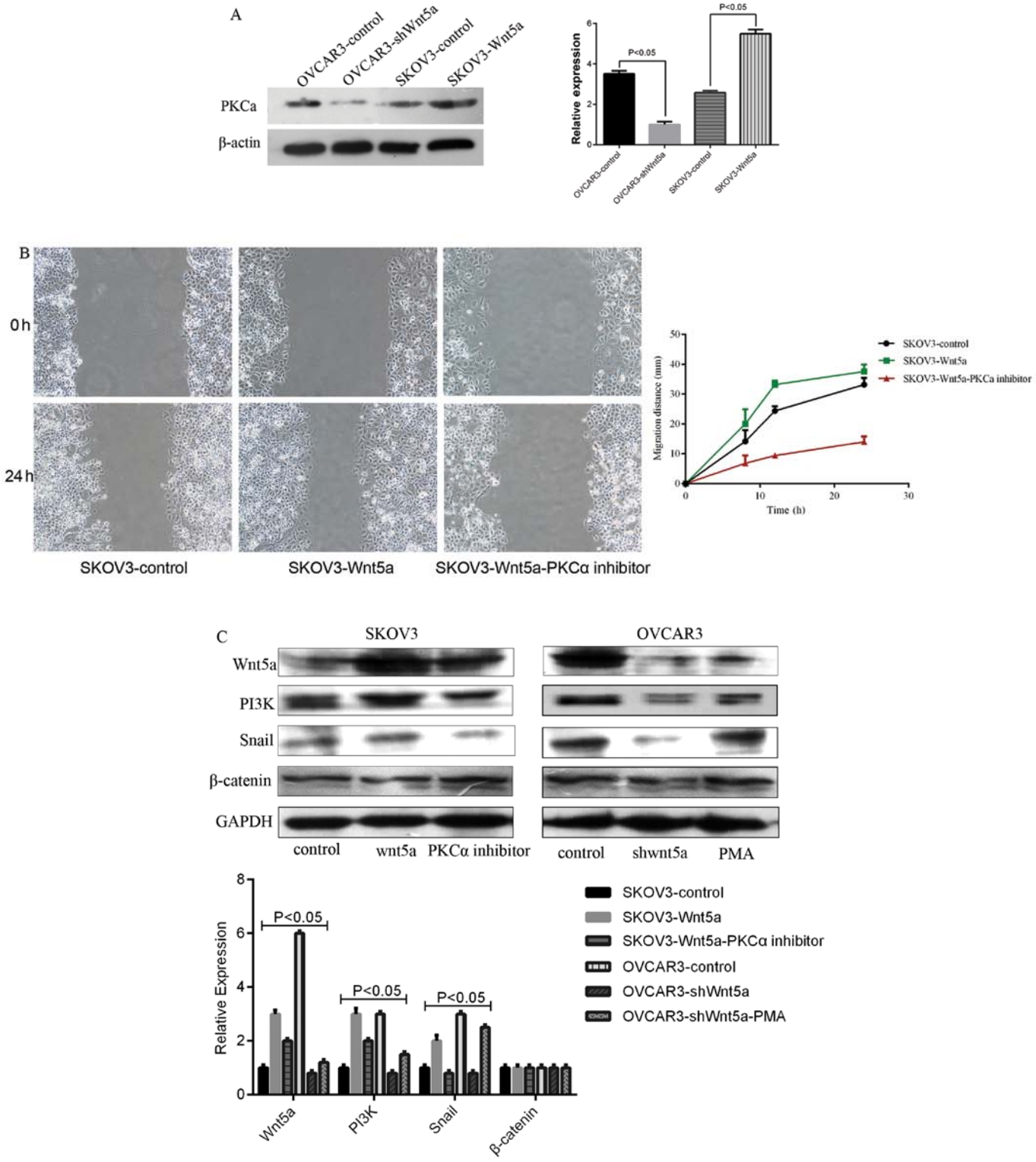

Figure 6. Effect of Wnt5a on ovarian cancer cells via the PKCα pathway. (A) PKCα expression coincided with Wnt5a expression (P<0.05). (B) PKCα inhibitor decreased the effect of Wnt5a in regards to the motility of ovarian cancer cells (P<0.05). (C) Western blot analysis revealed that the expression levels of snail and PI3K coincided with Wnt5a expression (P<0.05). However, β-catenin was unaffected by Wnt5a expression. PKCα, protein kinase Cα.

Discussion

VM was derived from aggressive uveal melanoma microcirculation by Maniotis *et al* (1). Our previous studies have shown that VM is present in ovarian cancer and that the prognosis of patients with VM is significantly worse than that of patients without VM (4). EMT is pivotal in malignant tumor progression and VM formation (12). EMT is a reversible dedifferentiation process that converts epithelial cancer cells into dedifferentiated cells with additional mesenchymal features. This process is characterized by the loss of epithelial traits and the acquisition of mesenchymal phenotypes (13). Our laboratory reported that EMT contributes to VM formation and that EMT-associated transcription factors are upregulated

in VM-forming tumor cells, such as twist1, DKK-1, ZEB1, β-catenin and Snail/Slug (5,14-16).

Wnt families are crucial in tumorigenesis and chick embryo development; these families include canonical (β-catenin-dependent) Wnt signaling pathways and non-canonical (β-catenin-independent) Wnt signaling pathways. Alterations that affect Wnt pathway proteins on the cell membrane, in the cytoplasm, and in the nucleus are involved in ovarian cancer tumorigenesis. Wnt/β-catenin target genes regulate cell proliferation and apoptosis, thereby mediating cancer initiation and progression. The Wnt/β-catenin pathway is a major signaling pathway that may be involved in EMT (17).

Research has shown that the non-canonical (β-catenin-independent) Wnt signaling pathways are important in embryonic development and tumor progression (6,9,10). Wnt5a, a member of the Wnt protein family, can activate the non-canonical Wnt signaling pathway. However, the function of Wnt5a in tumors remains controversial. Wnt5a has been described as a tumor suppressor in various malignancies (18,19); however, increasing evidence suggests that it has pro-migratory and pro-invasive effects in other tumors (19,20). A correlation between Wnt5a expression and increased tumor aggressiveness has been observed in human melanoma biopsies (9,10,21), suggesting that Wnt5a is involved in the progression of tumors to malignant stages. We hypothesized that other factors affect the function of Wnt5a. In the present study, the expression of Wnt5a in 79 ovarian cancers at different stages was examined by immunohistochemistry. Wnt5a expression was found to be correlated with tumor metastasis. The presence of VM was investigated in 79 ovarian cancers and was determined to be significantly correlated with Wnt5a expression. The effect of Wnt5a on ovarian cancer cells *in vitro* was studied by establishing models of upregulated or downregulated Wnt5a expression. Tube formation is recognized as a universal *in vitro* VM evaluation method. Wnt5a upregulation promoted tube formation, whereas Wnt5a downregulation inhibited it. This finding indicated the important function of Wnt5a in VM. Our previous studies also showed that EMT is a critical step in VM. E-cadherin and vimentin are recognized as characteristic EMT proteins (3,5). The results of the present study showed that the upregulation of Wnt5a enhanced mesenchymal characteristics and increased the motility and invasiveness of ovarian cancer cells and that Wnt5a downregulation enhanced epithelial traits and decreased motility and invasiveness. This finding suggests that Wnt5a is crucial for VM via EMT. These results are consistent with findings that Wnt5a increases cell invasiveness in vector-transfected melanoma cells constitutively overexpressing Wnt5a (10).

Several studies have shown that the Wnt non-canonical pathways, such as the Wnt/Ca^{2+} pathway, are involved in the regulation of tumor cell motility and migration through PKC activation (9,10). PKCα is composed of isoforms (11) with variable regulatory regions and conserved catalytic domains. Several isoforms have complex and occasionally opposite functions in the melanogenesis, proliferation and transformation of human melanocytes. Numerous studies have consistently shown that phorbol esters serve dual functions as growth promoters of normal melanocyte proliferation and as inhibitors of melanoma cell growth via the activation of different sets of PKC isoforms (22). Other studies have demonstrated the involvement of Wnt5a in the progression and metastasis of several malignant diseases via PKC (9,11,23). Wnt5a may also be involved in EMT in ovarian cancer. Thus, we examined PKCα expression in 79 ovarian cancer cases. Immunohistochemical staining showed that PKCα expression coincided with Wnt5a expression. This result suggests that Wnt5a is important in ovarian cancer via the PKC pathway. A PKCα inhibitor was used to upregulate Wnt5a cells *in vitro*. The results of a clinical study of ovarian cancer patients revealed that the effect of Wnt5a was blocked and matched. This finding is consistent with those of previous studies, which found that Wnt5a overexpression promotes PKCα activation. The proinvasive effects of Wnt5a are supposedly predominantly mediated by PKCα, and this mediation is associated with EMT, an event related to tumor progression (24-26).

Palma-Nicolas (27) previously demonstrated that the *in vitro* treatment of retinal pigment epithelium cells with a-thrombin induces cell proliferation through the joint activation of PKC and mitogen-activated protein kinase pathways. Exogenously treated Wnt5a induces the phosphorylation of PKCα, PKC-pan and PI3K/Akt, which is a downstream modulator of PKC. This result suggests that PKC and PI3K/Akt activation is associated with Wnt5a-mediated, premalignant transformation (28). In the present study, the results of the western blotting revealed that PI3K increased with Wnt5a upregulation but that β-catenin was not affected. This result indicates that Wnt5a may mediate EMT and VM in ovarian cancer cells via PKCα and PI3K. However, the mediation is β-catenin-independent. The results also showed that Snail expression increased along with upregulated Wnt5a expression and decreased with increased vimentin level. E-cadherin expression was decreased in ovarian cancer cells with increased Wnt5a expression. The transfection of vectors that overexpressed Snail into squamous carcinoma cells has been shown to upregulate Wnt5a expression (29), suggesting that Wnt5a and Snail induce a positive feedback loop, as with PKC and Wnt5a (9).

In summary, Wnt5a expression was correlated with VM in ovarian cancer and was associated with poor patient prognosis. Wnt5a overexpression increased tube formation, enhanced mesenchymal characteristics, and increased the motility and invasiveness of ovarian cancer cells. Wnt5a also promoted EMT via PKCα but did not affect the β-catenin pathway. Wnt5a promoted ovarian cancer and could be a novel prognostic marker of ovarian cancer in the future.

Acknowledgements

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