DOI:http://dx.doi.org/10.7314/APJCP.2014.15.3.1489

RESEARCH ARTICLE

Differential Wnt11 Expression Related to Wnt5a in High- and Low-grade Serous Ovarian Cancer: Implications for Migration, Adhesion and Survival

Farnaz Jannesari-Ladani[1], Ghamartaj Hossein[1]*, Narges Izadi-Mood[2]

Abstract

Wnt is a powerful signaling pathway that plays a crucial role in cell fate determination, survival, proliferation and motility during development, in adult tissues and cancer. The aims of the present study were three fold: i) to assess Wnt11 immunoexpression and its possible relationship with Wnt5a in high- and low-grade human serous ovarian cancer (HGSC and LGSC) specimens; ii) to assess Wnt11 expression levels in Wnt5a overexpressing SKOV-3 cells; iii) to reveal the role of Wnt11 in viability, adhesion, migration and invasion of SKOV-3 cells using recombinant human Wnt11 (rhWnt11). Immunohistochemistry revealed a significant difference in Wnt11 expression between HGSC and LGSC groups (p=0.001). Moreover, a positive correlation was observed between Wnt5a and Wnt11 expression in the HGSC (r=0.713, p=0.001), but not the LGSC group. The expression of Wnt11 was decreased by 35% in Wnt5a overexpressing cells (SKOV-3/Wnt5a) compared to mock controls. Similarly Wnt11 expression levels were decreased by 47% in the presence of exogenous Wnt5a compared to untreated cells. In the presence of rhWnt11, 31% increased cell viability (p<0.001) and 21% increased cell adhesion to matrigel (p<0.01) were observed compared to control. Cell migration was increased by 1.6-fold with rhWnt11 as revealed by transwell migration assay (p<0.001). However, 45% decreased cell invasion was observed in the presence of rhWnt11 compared to control (p<0.01). Our results may suggest that differential Wnt11 immunoexpression in HGSC compared to LGSC could play important roles in serous ovarian cancer progression and may be modulated by Wnt5a expression levels.

Keywords: Epithelial ovarian cancer - Wnt11 - Wnt5a - adhesion - migration - viability

Asian Pac J Cancer Prev, **15 (3)**, 1489-1495

Introduction

Wnt signaling plays important roles in embryonic development, tissue differentiation, and cancer. In both normal and malignant tissue, Wnt family members are often expressed combinatorially, although the significance of this is not understood (Cha et al., 2009). Wnt molecules trigger gene transcription via at least three signaling pathways: the canonical or β-catenin dependent, and two non-canonical pathways. When Wnts bind to their trans-membrane receptors, Frizzleds (Fzd) and coreceptors, LRP5/6, signal transduction begins on the canonical pathway. Once stabilized, non-degraded β-catenin molecules move to the nucleus where they activate TCF-LEF-dependent gene transcription. In the absence of Wnt signals, the cytoplasmic β-catenin is subjected to phosphorylation in the APC-Axin-GSK3β-complex then to subsequent proteasomal degradation. Upon non-canonical Wnt signals, the JNK/AP1 dependent, planar cell polarity (PCP) and the PKC/CAMKII/NFAT dependent Ca^{2+} pathways are activated (Mikels and Nusse, 2006). The Wnt pathways regulate morphogenesis by controlling cell adhesion and migration via downstream effectors that regulate epithelial cell interaction with basement membrane; processes that when corrupted, lead to tumorigenesis (Amin and Vincan, 2012). This leads to multiple biochemical changes that enable epithelial cells to assume a mesenchymal cell phenotype (Amin and Vincan, 2012).

Wnt11 is best known for its role during development, for example, it is required for convergent extension movements during gastrulation (Tada et al., 2002) and kidney morphogenesis (Majumdar et al., 1999). In addition, cell-based assays have demonstrated that Wnt11 promotes cardiac differentiation (Eisenberg and Eisenberg, 1999), increases proliferation, migration and transformation of intestinal epithelial cells, reduces apoptosis in breast cancer cells and increases cell viability in chinese hamster ovary (CHO) cells (Railo et al., 2008). The signals downstream of Wnt11 are not fully characterised (Lin et al., 2007) . Wnt11 has been reported to inhibit JNK and NF-kappaB (Railo et al., 2008),

[1]*Department of Animal Physiology, Developmental Biology Laboratory, School of Biology, University College of Science, University of Tehran,* [2]*Department of Pathology, Mirza Koochak Khan Hospital, Tehran University of Medical Sciences, Tehran, Iran *For correspondence: ghossein@khayam.ut.ac.ir*

activate PKC and JNK (Flaherty et al., 2008) and activate cAMP response element binding protein (CREB) family members (Zhou et al., 2007). Wnt11 does not appear to stabilise β-catenin and is frequently found to inhibit canonical Wnt/β-catenin signaling (Maye et al., 2004; Railo et al., 2008). A role for Wnt11 in human cancer was first suggested by its high expression in gastric and renal cell carcinoma cell lines, as well as some primary colorectal adenocarcinomas (Kirikoshi et al., 2001). It is highly expressed in some colon cancers (Ueno et al., 2009) and many prostatic tumours express high levels of Wnt11 (Zhu et al., 2004). In contrast, Wnt11 levels are low in hepatocellular carcinomas which plays a tumour suppressor role in this cancer (Toyama et al., 2010). Wnt11 expression has been reported in ovarian cancer cell lines (Ricken et al., 2002), but its role in ovarian cancer remains unknown.

Wnt5a is also able to activate non-canonical signaling and to inhibit the activation of the canonical signaling pathway (McDonald and Silver, 2009). Wnt5a have been reported to be associated with invasion, scattering and promotion of EMT in some cancer cells (Medrek et al., 2009). However, there is conflicting evidence as to whether Wnt5a has a tumor-promoting or - suppressing role in different cancer types which suggests it could play a context-dependent role in human cancers (Pukrop and Binder, 2008). Wnt5a is expressed in ovarian cancer cell lines (Ricken et al., 2002; Bitler et al., 2011) and ovarian cancer specimens (Matei et al., 2002; Badiglian-Filho et al., 2009). However these studies reports controversial role of Wnt5a in epithelial ovarian cancer (EOC), suggesting that Wnt5a is a predictor of poor prognosis for ovarian cancer (Matei et al., 2002; Badiglian Filho et al., 2009) and may play a key role in increased chemosensitivity to anticancer drugs of ovarian cancer cells (Peng et al., 2011). On the other hand, a recent study reports that loss of Wnt5a predicts poor outcome in EOC patients and Wnt5a suppresses the growth of EOC cells by triggering cellular senescence (Bitler et al., 2011).

Little is known about the mechanism or biological significance of Wnt-Wnt protein interaction (Uysal-Onganer and Kypta, 2011). Non-canonical Wnt signaling plays a key role in both convergence and extension movements and midline convergence of organ precursors (Liu et al., 2009). Wnt11 and Wnt5a are both required for the initiation of embryonic axis formation and show physical interaction with each other in xenopus (Cha et al., 2009). Wnt11 and Wnt5a are also two of several Wnts expressed during palate development, where they have been anticipated to play a role in convergent extension like movements, such as when the medial edge epithelium is intercalated into the midline epithelial seam (Uysal-Onganer and Kypta, 2011). To date there is no report regarding Wnt11 expression in ovarian cancer specimen and its role or Wnt11/Wnt5a interaction in ovarian cancer remains to be determined.

The present study for the first time sought to clarify whether there is a relationship between Wnt11 and Wnt5a in high- and low-grade human serous ovarian cancer specimens. Next, an interaction between Wnt11 and Wnt5a expression was assessed by using exogenous Wnt5a or transfection of Wnt5a gene in human ovarian cancer cell line SKOV-3.Finally, we sought to determine the role of Wnt11 in viability, migration, invasion and adhesion of SKOV-3 cell line in the presence of rhWnt11.

Materials and Methods

Immunohistochemistry

Ovarian cancer specimens were obtained from surgeries performed between 1990-2012 at Women University Hospital. All samples were examined by two independent and experienced gynecological pathologists for histological diagnosis and grade. The patients (age=31-68 Median=52) were divided in two groups: high grade serous ovarian cancer (HGSC, n=49) and low grade serous ovarian cancer (LGSC, n=11). Serous ovarian carcinoma specimens were sectioned (5μm) and mounted on Vectabond (Vector laboratories Ltd. Peterbrough, UK.) coated slides. Sections were deparaffinized in three changes of xylene and rehydrated in a graded series of ethanol finishing in distillated water. For antigen retrieval, slides were placed in 0.01 M citrate-buffer pH 6.0 and heated for 20 min at 90°C. Endogenous peroxidases were quenched by incubating with 3% H_2O_2 in 10% methanol for 10 min at room temperature (RT). Subsequently, non-specific binding sites were blocked with 4% BSA for one hour; then sections were incubated overnight at 4˚C with the following antibodies obtained from Abcam, Cambridge, UK: mouse monoclonal anti human Wnt5a antibody (1:500) and rabbit polyclonal anti human Wnt11 (1:100), diluted in 4% BSA+0.1% Triton X-100. Immunoreactivities were visualized by exposing the cells to HRP LSAB reagents (Dako France S.A.S) and revealed with diaminobenzidine tetra hydrochloride (DAB) (Vector laboratories Ltd. Peterbrough, UK). The sections were counterstained with hematoxylin, rinsed and mounted with Vectamount (Vector Laboratories Ltd. Peterbrough, UK). The samples were analyzed semiquantitatively as 0 (0-10% positive tumor cells), 1+(11-50% positive tumor cells), 2+(<50% positive tumor cells).

Cell culture and transfection of Wnt5a

SKOV-3 cells were a kind gift from Dr. AH Zarnani (Avicenna Research Center, Tehran, Iran). Cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS) and pencillin/streptomycin antibiotics obtained from Life Technologies GmbH (Darmstadt, Germany) at 37°C in 5% CO_2 atmosphere under 90-95% humidity. The pcDNA3.1 (+)-Wnt5a plasmid encoding the full length of human Wnt5a was obtained from Origene (MD, USA). The coding region was cloned and inserted into pIRES2-EGFP plasmid (Clontech, CA, USA).The sequences of the plasmid were verified by DNA sequencing. SKOV-3 cells were transfected with pIRES2-EGFP-Wnt5a or pIRES2-EGFP using Lipofectamine 2000 (Invitrogen, CA, USA) according to the protocol of the manufacturer. Stably transfected clones were selected 24 hours later by adding the selection reagent G418 (500 µg/mL; Sigma-Aldrich, Germany). Selection was continued for 14 days, with the medium being refreshed every other day.

Transfectants with vector pIRES2-EGFP-Wnt5a were named as SKOV3/Wnt5a. The stable clones were then evaluated for Wnt5a expression by western blot analysis as described previously.

For treatment with recombinant human Wnt5a (rhWnt5a), 500×10^3 of SKOV-3 cells were seeded in 6-well plate, after cells were reached confluency, rhWnt5a (500ng/ml, R and D Systems, USA) was added in free serum medium for 24h. Then cells were harvested on ice to prepare cell lysate for further analysis.

Western blotting

Following antibodies were used: mouse monoclonal anti human Wnt5a antibody (1:1500) and rabbit polyclonal anti human Wnt11 (1:1500) were obtained from Abcam (Cambridge, UK). Rabbit polyclonal anti human GAPDH antibody (1:1000, Abcam, Cambridge, UK) was used as internal control. Cells were grown to 80% confluency, lysed with RIPA buffer (150mM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris, pH 8.0), then sonicated, shaked for 10 minutes at RT and centrifuged at 10,000×g for 10 min. The supernatant was quantitated using the Bradford protein quantitation assay (Merck, Darmstadt, Germany). Wnt5a and Wnt11 were detected by using 5 μg and 30 μg of each lysate, respectively and run out on SDS-PAGE 10% Tris-glycine and transferred onto PVDF membrane. The membranes were probed with anti-mouse IgG-HRP or anti-rabbit-IgG-HRP antibodies and bands were visualized by using ECL system (Millipore, MA, USA). Bands were quantified by densitometric analysis, using AlphaEaseFC software (Miami, USA). Results were expressed relative to SKOV-3 cells (for rhWnt5a treatment) or mock cells (for SKOV-3/Wnt5a) set as arbitrary value=1.0.

Cell survival assay

Cell survival was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. SKOV-3 cells were seeded at 8000 cells/wells in 96-well plates in medium-free serum with rhWnt11 600 ng/ml (R D Systems, USA). After 48h, 1:10 MTT solution (5mg/ml) was added to each well followed by incubation at 37ºC for 3 h, then medium was removed and formazan crystals were revealed by adding 100μL dimethylsulfoxide (DMSO) to each well, followed by gentle 10 min shaking at RT and absorbance (A) value was measured at 570 nm with Elisa reader (Awarnesse, USA). Individual samples were analyzed in quadruplicate against a background of blank wells. Cell survival from three independent experiments was expressed as percentage *(A value of treated cells/untreated SKOV-3 cells named as control×100)***.**

Cell adhesion assays

A 96 wells plastic plate was coated with 4 μg/well matrigel (BD Bioscience, MA, USA) at 4°C for overnight. After washing wells twice with Phosphate buffered solution (PBS), non-specific binding sites were blocked by incubating wells with 0.2% bovine serum albomin (BSA) for 2h at RT followed by three times washing in PBS, then plate was used for adhesion assay. 20×10^3 SKOV-3 cells were seeded to each well in triplicate with rhWnt11 600ng/ml (R and D Systems, USA) incubated for 4 or 6h at 37°C, then washed for two times with PBS and fixed with 4% paraformaldehyde (Sigma Aldrich, Germany) for 10 min at RT, stained with a solution containing 0.5% crystal violet in 2% ethanol and 40% methanol for 20 min and destained with distilled water. Then, 100 μL sodium dodecyl sulphate (SDS) 1% was added to each well followed by gentle 10 min shaking at RT and A value was measured at 600 nm with Elisa reader, analyzed in quadruplicate against a background of blank wells. Cell adhesion from three independent experiments was expressed as percentage ***(A value of treated cells/control cells×100)***.

Migration and invasion assay

Sub-confluent SKOV-3 cells wereseeded at a density of 2.5×10^4/ml in RPMI-1640 on the upper chamber of an 8 μm pore size transwell insert (Costar, Corning, NY, USA). RPMI-1640 culture solution (650μl) containing 10% FBS was added to the lower chamber of each well, after 3h, rhWnt11 600ng/ml (R and D Systems, USA) was added to upper chamber of treated wells and incubated for 20h at 37°C. Non migratory cells on the upper surface of the membrane were removed and cells were fixed with 4% paraformaldehyde in PBS, stained in 0.5% crystal violet and membranes were mounted on a microscope slide. Migrated cells were counted in ten random fields. Invasion assays were carried out in a similar manner to migration assay except that upper chamber was coated with 1:40 matrigel in ice-cold RPMI-1640, and allowed to gel at 37°C for one hour. Percent of migrated cells was expressed as: ***(number of seeded cells/number of migrated cells×100)***. Percent of invaded cells was expressed as: ***(number of invaded cells/number of migrated cells×100)***. Invasion and migration index were expressed relative to untreated SKOV-3 cells as 100%. All the experiments were carried out three times and results were expressed as mean±SD.

Wound healing assay

Wound healing assay was performed by plating cells (800×10^3) in 6 wells plates. After cells were allowed to attach and reach confluency, starved overnight and a scratch was made through the confluent monolayer using a sterile pipette tip. SKOV-3 cells were treated with rhWnt11 600ng/ml (R and D Systems, USA), 1h before scratching. Photographs of cells invading the scratch were taken at 12, 24 and 48 h after scratching. Migration of untreated (control) and treated cells with rhWnt11 were assayed in three independent experiments.

Statistical analysis

Normality of nominal variables was analyzed by using Kolmogorov Smirnov test. For in vitro studies, results were analyzed by using t-test. Comparative analysis of Wnt5a, Wnt11 immunoexpressions between HGSC and LGSC groups were performed by using Kruskal-Wallis test. The associations between these molecules in each group were analysed by Pearson's Chi-square test. All experimental data were analyzed using a statistical

software package SPSS 19.0 (SPSS Inc., Chicago, IL), p<0.05 was considered statistically significant.

Results

Wnt5a and Wnt11 expression in LGSC and HGSC specimens

In LGSC specimens, moderate Wnt5a immunostaining was observed in cytoplasm and extracellular matrix of all samples (Figure 1A, Figure 2). While, no immunostaining was observed for Wnt11 in LGSC (Figure 1C, Figure 2). In HGSC specimens, all samples showed strong cytoplasmic and extracellular matrix immunostaining for Wnt5a (Figure 1B; Figure 2). However, Wnt11 immunoexpression showed various staining intensity through specimens, from negative to moderate (Figure 1D) and with strong immunostaining (Figure 2). The Kruskal-Wallis analysis revealed a significant difference in Wnt11 expression (p=0.001) between HGSC and LGSC groups.

Relationship between Wnt11 and Wnt5a expression in human ovarian cancer specimens and SKOV-3 cell line

A positive correlation was observed between Wnt5a and Wnt11 expression in HGSC group (r=0.713, p=0.001). In the next step, we sought to determine impact of Wnt5a overexpression or exogenous added rhWnt5a on Wnt11 expression in highly metastatic human ovarian cancer cell line SKOV-3. In SKOV-3/Wnt5a cells, Wnt11 expression was decreased by 35% compared to mock (Figure 3A and 3B). Similarly, in the presence of rhWnt5a, Wnt11 expression was reduced by 47% compared to control (Figure 3A and 3B).

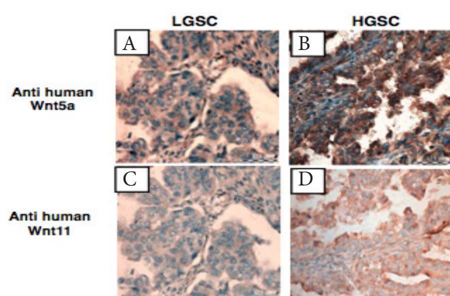

Figure 1. Immunostaining of Wnt5a, and Wnt11 in Human Serous Ovarian Cancer Specimens. A) and B) Wnt5a immunoexpression; C) and D) Wnt11 immunoexpression. LGSC: low-grade serous cancer (n=11); HGSC: high-grade serous cancer (n=49). (Scale bar=62.5µm)

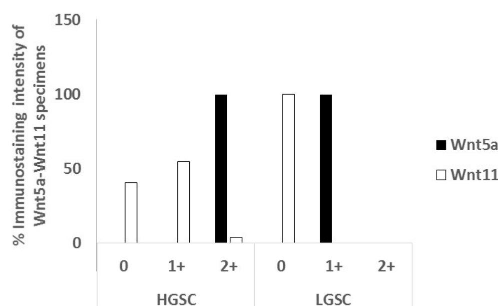

Figure 2. Immunostaining Intensity of Wnt5a and Wnt11 in HGSC and LGSC Specimens. The samples were analyzed semiquantitatively as 0 (0-10% positive tumor cells), 1+ (11-50% positive tumor cells), 2+ (<50% positive tumor cells).(HGSC, n=49 and LGSC, n=11)

Substrate-dependent cell adhesion assay in the presence of rhWnt11

Treatment of SKOV-3 cells with rhWnt11 led to 31% increased viability as revealed by MTT assay (p<0.001) (Figure 4).

Changes in cell adhesion contribute to cancer progression and metastasis, thus, we sought to determine the role of Wnt11 in cell-matrix adhesion. In the presence of rhWnt11, there was 21% increased cell attachment for 4h post seeding (p<0.01) (Figure 5A (a and b) and Figure 5B) and 10% increased cell attachment after 6h post seeding to matrigel-coated wells compared to untreated

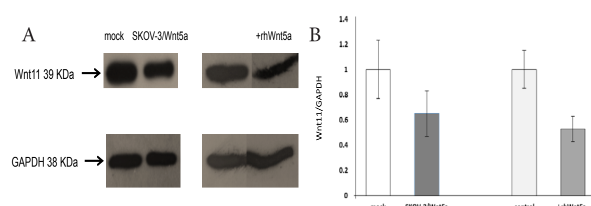

Figure 3. Analysis of Wnt11 Expression in SKOV-3/ Wnt5a and Cells Treated with rhWnt5a. Transfectants with vector pIRES2-EGFP-Wnt5a and pIRES2-EGFP were termed SKOV3/Wnt5a and mock, respectively. **A)** Lower expression of Wnt11 was revealed in SKOV-3/Wnt5a compared to mock. Similar result was obtained in the presence of rhWnt5a (+rhWnt5a) compared to SKOV-3 (control) cells. The western blot shows a representative result of three independent experiments; **B)** Quantification was performed by densitometric analysis using AlphaEaseFC software. GAPDH levels were used as internal control. Data are reported as the mean±SD from at three independent experiments

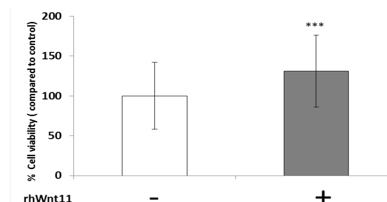

Figure 4. The Effect of rhWnt11 on SKOV-3 Cell Viability. Cells were treated for 48h in serum-free medium in the absence or presence of rhWnt11 and cell viability was assessed by MTT assay. Data are reported as the mean±SD from at least 3 independent experiments. ***p<0.01 compared to SKOV-3 as control

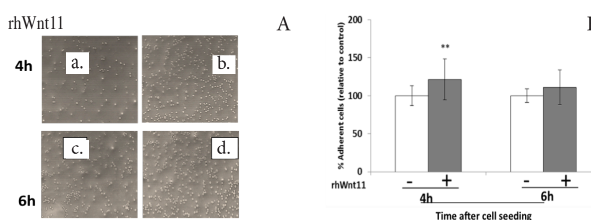

Figure 5. Substrate-dependent Cell Adhesion Assay. SKOV-3 cells were plated on coated microplate with matrigel in the absence or presence of rhWnt11. Cell adhesion was assessed after 4h (a. without and b. with rhWnt11) or 6 h (c. without and d. with rhWnt11). Data (mean±SD; n=3) were presented as percent of remained adherent cells treated with rhWnt11 relative to untreated (control) cells. **p<0.01

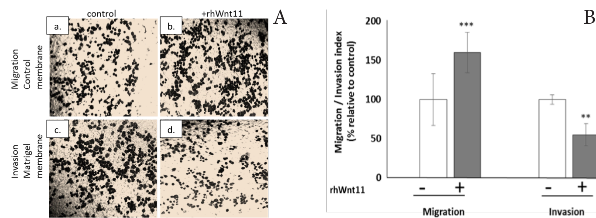

Figure 6. Transwell Migration and Invasion Assay. SKOV-3 cells were seeded on the top of insert in serum free media without or with rhWnt11. The lower chamber contained medium with 10% FBS. Inserts were coated with matrigel for invasion assay. **A)** Quantification was performed by counting cells in ten random views using light microscope. Photographs (50× magnification) shows one of the ten random fields for migration (a. & b.) and invasion (c. & d.); **B)** Increased migration and decreased invasion was observed in the presence of rhWnt11 compared to untreated cells. ***p<0.001; **p<0.01 (mean±SD; n=3)

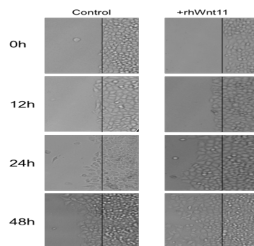

Figure 7. Scratch Wound Healing Assay. Monolayer was wounded by manual scratch with a pipette tip and maintained at 37ºC in medium with 1% FBS. Black line indicates the wound edge. Photographs (100× magnification) were taken at 0, 12, 24 and 48 hours after scratching and were representative of three independent experiments. Cell motility was increased in SKOV-3 cells treated with rhWnt11 compared to control (untreated) cells

cells (control) (Figure 5A (c and d) and Figure 5B).

Cell migration and invasion assay in the presence of rhWnt11

Migration transwell assay showed anincreased cell migration by almost 1.6-fold (p<0.001) (Figure 6A (a and b) and Figure 6B) while, cell invasion was reduced by 0.55-fold (p<0.01) in cells treated with rhWnt11 compared to control (Figure 6A (c and d) and Figure 6B). Moreover, in cells treated with rhWnt11, wound healing assay showed increased cell migration compared to control (Figure 7).

Discussion

Wnt11 and Wnt5a are both non-canonical Wnt molecules which are expressed by ovarian cancer cell lines (Ricken et al., 2002). Although, previous reports showed high Wnt5a expression in epithelial ovarian cancer and its relationship with ovarian cancer progression (Matei et al., 2002; Badiglian Filho et al., 2009). The expression of Wnt11 in ovarian cancer specimens remained unknown. Here, we showed a significant difference in Wnt11 expression between HGSC and LGSC groups which was related to Wnt5a expression only in HGSC specimens. The high expression of Wnt11 has previously been reported in prostate cancer (Zhu et al., 2004), renal and gastric carcinoma cell lines as well as in some primary colorectal adenocarcinoma (Kirikoshi et al., 2001). The positive relationship found between Wnt5a and Wnt11 in HGSC was of particular interest to us, as a physical interaction between Wnt11 and Wnt5a which was reported in xenopus axis formation (Cha et al., 2008). They showed that Wnt11 and Wnt5a interact with each other and that both maternal Wnt11 and Wnt5a are essential for the activation of both canonical and non-canonical Wnt signaling required for Xenopus dorsal axis formation. Another study showed that secreted Xenopus Wnt11/5a complexes have more canonical Wnt signaling activity than secreted Wnt11 or Wnt5a acting alone (Cha et al., 2009). Moreover, it has been demonstrated that activation of canonical Wnt signaling, in xenopus axis formation required the formation of Wnt11 and Wnt5a tyrosine-sulfated oligomers. Bartis et al. (2013) identified Wnt11 and Wnt5a as regulators of cadherin expression and potentiated relocation of β-catenin to the nucleus as an important step in decreased cellular adhesion of squamous cell lung carcinoma. Thus, we sought to determine the role of Wnt11 in SKOV-3 cells and its possible modulation by Wnt5a in SKOV-3/Wnt5a cells. Although, decreased Wnt11 levels were detected in Wnt5a overexpressed cells or in treated cells with rhWnt5a, the underlying mechanism remains to be determined. One possible explanation could be on the basis of the different role of Wnt5a and Wnt11 on canonical pathway. There is two Tcf/lef sites in Wnt11 promotor region mediating Wnt11 up-regulation by canonical pathway (Katoh, 2009).

However, Wnt5a signaling has been shown to inhibit β-catenin pathway in a receptor context dependent manner (Mikels and Nusse, 2006). In hepatocellular carcinoma cells Wnt5a inhibited β-catenin/TCF activation (Yuzugullu et al., 2009). Thus, it is tempting to speculate that increased expression of Wnt5a in SKOV-3 may inhibit β-catenin pathway and subsequent decreased β-catenin-induced Wnt11. Future studies would reveal the underlying mechanism of Wnt5a and Wnt11 relationship in ovarian cancer cells. Moreover, use of Bio ((2'Z,3'E)-6-Bromoindirubin-3'-oxime) a selective inhibitor of GSK3β which could mimic Wnt/β-catenin pathway led to strong cytotoxic effect on SKOV-3 cells (Data not shown). Our observation corroborates with the finding that report activity of GSK3β is required for SKOV-3 cell survival (Cao et al., 2006). Furthermore, it has been demonstrated that LiCl or Wnt3a has no effect on β-catenin/Tcf reporter activity in SKOV-3 cells (Usongo et al., 2013). In addition, we showed recently that cell viability of SKOV-3/Wnt5a was increased by 30% compared to mock. Therefore, we could assume in our model that Wnt11 and Wnt5a may inhibits canonical pathway leading to increased cell survival.

In this study, Wnt11 immunostaining was observed in 94% (46 out of 49 specimens) of HGSC specimens. It would be interesting to study the relationship between Wnt11 expression and progression-free survival or with overall survival of HGSC patients.

Wnt11 has been shown to be important during mouse kidney morphogenesis which maintained the expression of glial-derived growth factor (Pepicelli et al., 1997). One of the characteristic of cancer cells is their self-sufficiency

and down regulation of pro-apoptotic proteins. This is the first report showing increased serum-free survival of cells in the presence of rhWnt11. Further studies of Wnt11 crosstalk with key survival pathways in cancer like PI3K/AKT, EGFR or estrogen receptor pathways would clarify the mechanism of Wnt11 effect on cell survival.

The role of Wnt11 in cell motility was reported related to the directional movements of the cells in the assembling ventricular wall by modulating cell adhesion in connection with changes in the cytoskeleton (Nagy et al., 2010). A common theme is that Wnt11 acts locally to control the turnover of proteins involved in cell-cell and cell-substrate adhesion and thereby facilitate coordinated cell migration (Katoh, 2009; Uysal and Kypta, 2011). Wnt11 increased migration of LNCaP and PC3 prostate cancer cells (Dwyer et al., 2010; Uysal-Onganer et al., 2010), MDA MB 231 breast cancer cells, HCT116 colon cancer cells (Dwyer et al., 2010) and non-transformed rat small intestinal epithelail cells (Ouko et al., 2004). Correpondingly, the present study showed Wnt11-induced increased cell migration of SKOV-3 cells.

Increased cell-matrix adhesion is required for proper cell migration thus, we looked for the effect of Wnt11 on substrate-dependent cell adhesion. Here Wnt11-induced increased cell-matrix adhesion was demonstrated, whether Wnt11 may modulate integrin signaling remains to be determined.Wnt11/Fzd7 was involved in increased invasion of HCT116 colon cancer cells (Ueno et al., 2009). However, here Wnt11 decreased SKOV-3 cell invasion suggesting cell-context dependent functions of Wnt11 on cell invasion.

Taken together, differential expression of Wnt11 compared to Wnt5a in HGSC specimens suggests that this molecule may play an important role in serous ovarian cancer progression. The findings that Wnt11 could influence ovarian cancer cell survival, adhesion, migration and invasion justify further investigation for understanding underlying molecular mechanism. We have previously showed the importance of Wnt5a in ovarian cance thus, it would be of great importance to understand possible interaction between Wnt5a and Wnt11 in ovarian cancer cell lines. To this regard, use of other ovarian cancer cell lines with different mutations in key cancer pathways, compared to normal and cancerous primary human ovarian epithelial cells may help tobetter understand the relationship between Wnt5a and Wnt11 molecules.

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