BJ www.biochemj.org

BJ Disease

Biochem. J. (2011) **437**, 1–12 (Printed in Great Britain) doi:10.1042/BJ20110112

REVIEW ARTICLE

Diverse mechanisms for activation of Wnt signalling in the ovarian tumour microenvironment

Maria V. BARBOLINA*, Rebecca J. BURKHALTER†‡ and M. Sharon STACK†‡[1,2]

*Department of Biopharmaceutical Sciences, University of Illinois, 833 South Wood Street, 335 College of Pharmacy Building, Chicago, IL 60612, U.S.A., †Department of Pathology and Anatomical Sciences, University of Missouri, M263 Med Sci Bldg, Columbia, MO 65212, U.S.A., and ‡Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, One Hospital Drive, MA204, DC018.00, Columbia, MO 65212, U.S.A.

Wnt signalling pathways have been shown to play key roles in both normal development and tumorigenesis. Progression of many human cancers is associated with defined mutations in Wnt pathway components that result in dysregulated β-catenin-mediated gene transcription. Although Wnt pathway mutations are rare in epithelial ovarian cancer (with the exception of the endometrioid histotype), accumulating evidence supports a role for Wnt signalling in ovarian tumorigenesis in the absence of genetic mutations. The present review summarizes evidence in support of activated Wnt signalling in ovarian tumours and discusses alternative mechanisms for Wnt pathway activation in the ovarian tumour microenvironment.

Key words: β-catenin, endothelin, integrin, lysophosphatidic acid, microenvironment, ovarian cancer, T-cell factor/ lipoprotein receptor-related (Tcf/Lef), Wnt.

INTRODUCTION

Overview of canonical Wnt signalling

Wnt signalling regulates a diversity of processes fundamental to embryogenesis, including proliferation, differentiation, polarity, adhesion and motility [1–3]. The highly conserved and complex Wnt pathway transduces signals from the extracellular environment through transmembrane receptors and co-receptors to have an impact on cytoskeletal rearrangements and gene expression changes and thereby modulate cell behaviour. In the adult organism, aberrant activation of these same biological processes can induce neoplasia and promote tumour progression [4]. There are two distinct pathways for transduction of Wnt signals: the canonical Wnt/β-catenin pathway (Figure 1) and the non-canonical β-catenin-independent pathway. The latter can be subdivided further into Wnt/PCP (planar cell polarity) and Wnt/Ca^{2+} signalling pathways. Many excellent reviews have been recently published that detail both the canonical and non–canonical Wnt pathways, thus the present review will provide only a brief summary of the canonical pathway as it relates to EOC (epithelial ovarian carcinoma).

Canonical Wnt signalling is commonly activated by secreted proteins in the Wnt family, which currently consists of 19 members [1–3,5]. Wnts bind to transmembrane Fzd (Frizzled) GPCRs (G-protein-coupled receptors). Interaction of ligated Fzd with co-receptors designated LRP (lipoprotein receptor-related protein)-5 or LRP-6, members of the low-density LRP family, initiates Wnt signalling. In the absence of Wnt signalling, β-catenin functions as a structural component of E-cadherin (epithelial cadherin) junctions and is complexed with the cytoplasmic tail of E-cadherin. In normal epithelial cells, the majority of β-catenin is associated with E-cadherin at cell–cell junctions, and the levels are maintained at low concentrations in the cytoplasm by phosphorylation-dependent degradation of β-catenin. Cytoplasmic β-catenin is targeted to a complex comprised of Axin, APC (adenomatous polyposis coli) and GSK3β (glycogen synthase kinase 3β), resulting in phosphorylation of β-catenin that targets it for degradation through the ubiquitin–proteasome pathway (Figure 1A). Activation of Wnt signalling leads to phosphorylation of LRP-5/6, recruitment of Axin and Dvl (Dishevelled 1) to the plasma membrane, and functional disruption of the β-catenin degradation complex. This in turn enables accumulation of cytoplasmic β-catenin, which can then translocate to the nucleus, bind proteins in the Tcf (T-cell factor)/Lef (lymphoid enhancer factor) family, and activate transcription of Wnt target genes. Wnt signalling can be inhibited by sequestration of Wnt ligands via interaction with SFRP (secreted Fzd-related protein) or WIF (Wnt-inhibitory factor)-1 as well as by blocking co-receptor binding between Kremen and LRP-5/6 via Dkk (Dickkopf).

Accumulation of non-junctional β-catenin, and in particular nuclear translocation, is used as a surrogate marker for activation of the Wnt/β-catenin pathway. This is observed in a number of human cancers, as summarized below, and often results from mutations in Wnt pathway components. With the exception of the endometrioid histotype, Wnt pathway mutations are rare in ovarian cancer [6,7]. However, accumulating evidence suggests a role for Wnt signalling in ovarian tumorigenesis in the absence

Abbreviations used: APC, adenomatous polyposis coli; COX2,cyclo-oxygenase 2; CTGF, connective tissue growth factor; Dkk, Dickkopf; Dvl, Dishevelled 1; E-cadherin, epithelial cadherin; EGF, epidermal growth factor; ET_AR, ET type A receptor; ET_BR, ET type B receptor; EGFR, EGF receptor; EOC, epithelial ovarian carcinoma; ET, endothelin; FRAT1, frequently rearranged in advanced T-cell lymphomas 1; Fzd, Frizzled; GPCR, G-protein-coupled receptor; GSK3β, glycogen synthase kinase 3β; IDAX, inhibitor of the Dvl and Axin complex; IGF-1, insulin-like growth factor-1; IL, interleukin; ILK, integrin-linked kinase; Lef, lymphoid enhancer factor; LPA, lysophosphatidic acid; LPAR, LPA receptor; LRP, lipoprotein receptor-related protein; MCA, multi-cellular aggregate; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SFRP, secreted Fzd-related protein; Tcf, T-cell factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular EGFR; WIF-1, Wnt-inhibitory factor 1.

[1] Present address: Department of Chemistry and Biochemistry, Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN 46556, U.S.A.

[2] To whom correspondence should be addressed (email Sharon.Stack.11@nd.edu).

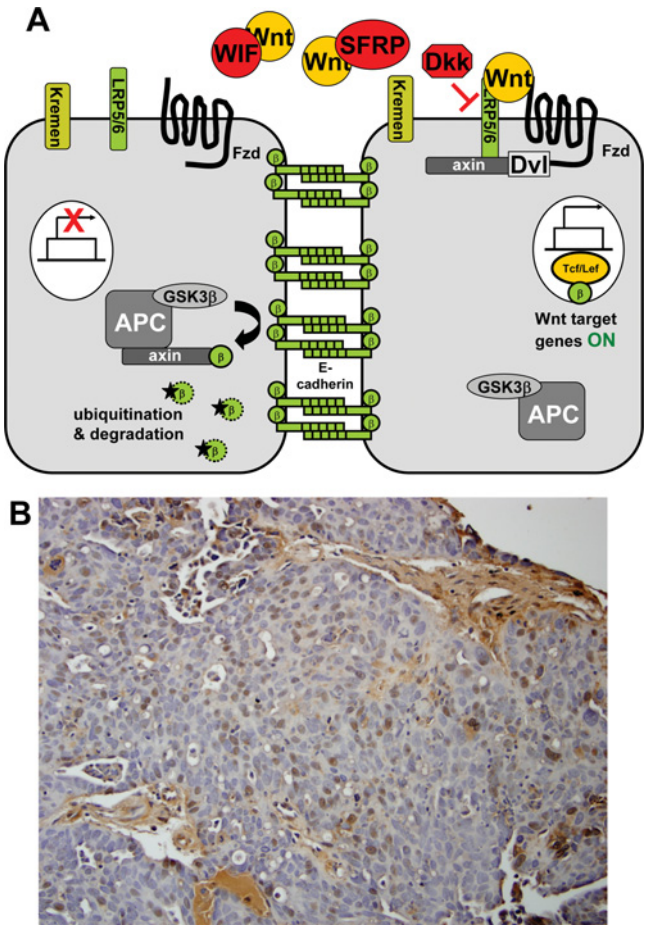

Figure 1 Canonical Wnt signalling and nuclear β-catenin in ovarian tissue

(**A**) In the absence of Wnt pathway activation, β-catenin is either associated with the cytoplasmic tail of E-cadherin or is targeted to an Axin/APC/GSK3β complex whereupon it is phorphorylated and targeted for degradation (left-hand panel). Activation of Wnt signalling, for example by binding of Wnt to the Fzd receptor, results in association of the co-receptors LRP-5/6 with Fzd and recruitment of Dvl to Fzd at the plasma membrane. Binding of Axin to this complex disrupts the β-catenin degradation complex, enabling accumulation of β-Catenin. β-Catenin can then translocate to the nucleus, bind to Tcf/Lef transcription factors and transcriptional co-activators (not shown) to activate transcription of Wnt target genes. Wnt signalling can be blocked by Dkk that binds Kremen and inhibits the interaction between Fzd and LRP-5/6, or by sequestration of Wnt ligands via WIF or SFRP (right-hand panel). (**B**) Example of a serous ovarian tumour exhibiting nuclear β-catenin staining. Magnification $\times$200.

of genetic mutations, suggesting alternative mechanisms for Wnt pathway activation in EOC (Figure 1B) [8–10].

Ovarian tumours inhabit a unique metastatic niche

EOC is a leading cause of death from gynaecologial malignancy. Worldwide, each year approximately 204 000 women are diagnosed with EOC, and 125 000 die due to complications from the disease. Incidence is highest in the United States and Northern Europe and lowest in Asia and Africa. The majority of women are initially diagnosed after the primary tumour has already metastasized, resulting in a 5-year survival of $<$30 %. The normal ovarian surface epithelium is mesenchymally derived and tissue cohesion is provided by the mesenchymal N-cadherin (neural cadherin). In contrast with most carcinomas that lose epithelial characteristics with tumour progression, EOCs undergo a mesenchymal–epithelial transition, acquire a more differentiated phenotype and gain expression of E-cadherin (reviewed in [11]).

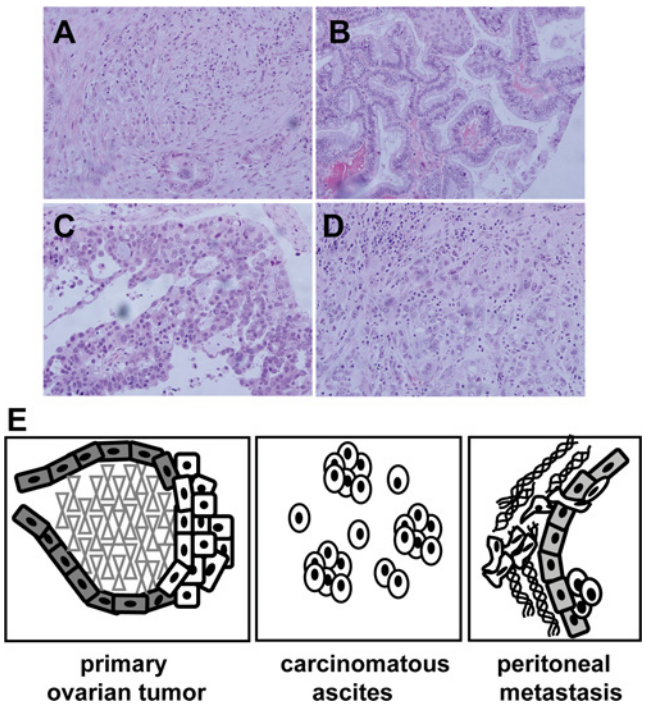

Figure 2 Ovarian tumours and the metastatic niche

Examples of epithelial ovarian carcinoma histotypes after haematoxylin and eosin staining at $\times$200 magnification: serous (**A**), endometrioid (**B**), mucinous (**C**) and clear cell (**D**). (**E**) Model of ovarian cancer metastatic niche. The model depicts a primary ovarian tumour arising from malignant transformation of ovarian surface epithelium. Single cells and MCAs are shed from the primary tumour into the peritoneal cavity. Accumulation of carcinomatous ascites is commonly observed, particularly in women with advanced disease. Metastasis is the result of multiple intraperitoneal adhesive events, whereupon tumour cells attach to peritoneal mesothelium, disrupt mesothelial cell–cell contacts and migrate into the submesothelial matrix to anchor secondary lesions on the bowel, diaphragm, omentum and other sites.

During progression, tumours acquire differentiated characteristics reminiscent of specialized Mullerian duct epithelia, resulting in various histotypes of EOC. Thus serous EOC resemble fallopian tubes, endometrioid EOC have characteristics of the endometrium, mucinous EOC is similar to the endocervix and clear-cell EOC tumours resemble vaginal tissue (Figures 2A–2D).

Clinically, tumours often involve the ovary and omentum, with diffuse multi-focal intraperitoneal metastasis and malignant ascites [12]. Malignant cells are exfoliated as single cells and MCAs (multi-cellular aggregates) from the primary tumour into the peritoneal cavity (Figure 2E), wherein distribution is facilitated by peritoneal fluid. Formation of malignant ascites is believed to result from shed tumour-cell blockage of peritoneal lymphatics and may further facilitate metastatic dissemination. Shed tumour cells interact with mesothelial cells lining the peritoneal cavity, induce mesothelial cell retraction and anchor in the collagen-rich submesothelial matrix to establish secondary lesions [13]. Adhesion of EOC cells to collagen types I and III in the submesothelial matrix is mediated by $\alpha2\beta1$ and $\alpha3\beta1$ integrins [14–16]. This affinity for interstitial collagens is reflective of the mesenchymal origin of the ovarian surface epithelium. $\beta1$ Integrin-mediated adhesion represents an important early event in EOC metastatic dissemination, and adhesion-mediated integrin signalling probably contributes to metastatic success.

As EOC dissemination occurs largely within the peritoneal cavity, a unique microenvironmental niche is established comprised of tumour cells, inflammatory cells and soluble factors

secreted by both the tumour and host cells including bioactive lipids, growth factors, ECM (extracellular matrix) proteins and inflammatory mediators. The contribution of the unique ovarian carcinoma microenvironment to the development and progression of metastatic disease is an area of active investigation. Aberrant activation of numerous signalling pathways has been observed in human EOC that result in stimulated proliferation or dysregulated cell death and most have been reviewed elsewhere. For example, activation of receptor tyrosine kinases such as the EGFR [EGF (epidermal growth factor) receptor] family and the VEGFR (vascular EGFR) family is frequently observed in EOC and regulates cell proliferation, survival, motility and metastasis [17,18]. Moreover, activation of VEGFR also alters vascular permeability, thereby contributing to build-up of ascites in EOC patients [19].

Activation of PI3K (phosphoinositide 3-kinase) signalling is observed in the majority of ovarian cancers as a result of gene amplification, activating mutations or activation downstream of receptor tyrosine kinases [20]. Signalling through the IL (interleukin)-6 receptor is also observed in EOC and results in nuclear translocation of STAT3 (signal transducer and activator of transcription 3) and corresponding stimulation of proliferation and angiogenesis [21]. Cytokine-mediated activation of NF-κB (nuclear factor κB) is also commonly detected in ovarian tumours, leading to up-regulation of anti-apoptotic and antioxidant gene products [22]. Lipid signalling via interaction of LPA (lysophosphatidic acid) with LPA GPCRs is a potent means of microenvironmental regulation, as LPA can be produced by tumour cells as well as stromal components, including mesothelial and inflammatory cells [23]. LPA binds to surface GPCRs, leading to activation of diverse signalling pathways depending on the LPA receptor complement and the specific G-proteins expressed by the cell. For example, LPA stimulation of $G_{\alpha 12/13}$ leads to activation of the small GTPase RhoA, resulting in cytoskeletal changes and cell rounding [24]. In addition, secondary effects of LPA occur as a result of induction of VEGF (vascular endothelial growth factor), TGF (transforming growth factor) α, TGFβ, IL-8, IL-6 and NF-κB expression, thereby contributing to activation of additional signalling pathways [25,26].

WNT SIGNALLING IN EOC

Progression of most cancers has been associated with activation of Wnt signalling acquired through two major routes: mutations in key components of the pathway or through mutation-independent aberrant gene expression. For example, the majority of colon, uterine and bladder tumours are strongly associated with APC, Axin or β-catenin mutations [27–31]. Interestingly, many cancers, including breast, prostate, lung, thyroid and pancreatic cancers (reviewed in [32–37]), do not depend on mutations in APC, Axin or β-catenin for activation of Wnt signalling.

Examples of Wnt signalling activation independent of mutations in key Wnt signalling genes

The presence of nuclear or cytoplasmic β-catenin in human breast cancer specimens is considered to be a strong indicator of activated Wnt signalling in this malignancy [38]. Multiple pathways, such as phosphorylation of β-catenin by EGFR/HER2 (human EGFR) [39], regulation of GSK3β activity by insulin, IGF-1 (insulin-like growth factor-1) [40], ILK (integrin-linked kinase) [41] or PI3K/Akt [42] and loss of PTEN (phosphatase and tensin homologue deleted on chromosome 10) [43] or p53 [44,45], have been associated with Wnt activation in breast cancers. Interestingly, activation of Wnt signalling in prostate cancer also depends on PTEN, PI3K/Akt [42] and IGF-1 [46], and can additionally be regulated by the androgen receptor [47]. In lung carcinoma, activation of Wnt signalling probably occurs through routes involving overexpression of Dvl [48] and repression of Wnt antagonists, such as WIF-1 and Dkk [49,50]. Activation of Wnt signalling in pancreatic adenocarcinoma has been related to overexpression of Wnt-1 and Fzd-2, which promotes stabilization of β-catenin [37].

Histotype-dependent Wnt signalling activation in ovarian carcinoma

Mechanisms for activation of Wnt signalling in ovarian carcinoma exhibit histotype dependence. Thus only endometrioid EOC is strongly associated with activating mutations in β-catenin leading to constitutively active Wnt signalling. The majority of low-grade endometrioid ovarian carcinomas often display nuclear immunoreactivity for β-catenin (70 % of cases), and these cases often harbour mutations in the β-catenin gene at codons that encode for residues phosphorylated by GSK3β (54 % of cases) [51]. Several studies confirmed the predominance of nuclear β-catenin and frequent β-catenin gene mutations in endometrioid EOC as well as in cell lines derived from this histotype [52–54]. Nuclear β-catenin in low-grade endometrioid EOC also associates with squamous differentiation and correlates with good prognosis and lack of relapse [51,55–57]. Moreover, expression of Wnt target genes including *FGF9* (fibroblast growth factor 9) has been described, suggesting that this pathway is active in endometrioid carcinomas [55–57]. Mutations in Axin in cell lines of endometrioid EOC have also been reported [54]; however, a distinct study found no mutations in either APC or Axin in human endometrioid EOC [58]. Furthermore, high-grade endometrioid ovarian carcinomas do not display nuclear β-catenin immunoreactivity and progression is not associated with β-catenin mutations [51]. This evidence supports the existence of two distinct subtypes of endometrioid EOC that may originate from different sources, (reviewed in [59]) based on differences in molecular pathobiology.

In contrast with endometrioid EOC, ovarian carcinomas of serous, clear-cell and mucinous histotypes have only rarely been associated with activating mutations in the key proteins of the Wnt signalling pathway. One report identified cases of clear-cell EOC positive for nuclear β-catenin [60]. Another study identified mucinous EOC positive for mutations in the β-catenin gene in the GSK3β-binding region [61]. Nevertheless, several lines of evidence implicate activation of Wnt/β-catenin signalling in serous EOC in the absence of activating mutations in either APC, Axin or β-catenin. The strongest evidence is the presence of nuclear β-catenin (Figure 1B). A broad range (3–59 %) of serous EOCs have been reported to contain nuclear and cytoplasmic β-catenin [60,62,63]. It is noteworthy that a significantly higher percentage of high-grade (23 %) serous EOC correlated with the presence of nuclear β-catenin compared with low grade (2.1 %) [63], opposite from trends observed for endometrioid EOC [51]. Together, these observations suggest that additional factors initiate Wnt signalling activation in serous EOC progression.

Potential mechanisms of β-catenin stabilization in serous EOC

Nuclear factors

As described above, the results of several studies indicate that ovarian carcinoma cells contain nuclear β-catenin, although the percentage of positive tissues and positive cells within each tested case vary widely. In the transcriptional activation complex, β-catenin partners with Tcf/Lef, Legless and Pygopus to initiate transcription. The presence of cytoplasmic Lef-1 has been

Table 1 RNA expression of *IDAX*, *BCL9* and *RPL19* in EOC and normal ovary human specimens

Real-time reverse transcription–PCR was used to detect the levels of *IDAX* and *BCL9* mRNAs in samples from non-cancerous ovarian tissues and ovarian carcinomas (Origene), according to the manufacturer's suggestions. Expression of *RPL19* was detected as a positive control. Real-time PCR was carried out with the ABI Prizm (Applied Biosystems) and the C_t (threshold cycle) values were obtained according to the manufacturer's instructions. SYBR Green was used for quantitative PCR as a double-stranded DNA-specific fluorophore. The following primers were used for detection of *IDAX*, *BCL9* and *RPL19* mRNAs: IDAX, forward 5′-CAGCCAAGAAGAAGAGGA-3′ and reverse 5′-GGGAACAGGTGTTCTCTCTA3-′; *BCL9*, forward 5′-ACGACCTCAGAGCAGAGTAT-3′ and reverse 5′-GACAAGACAGTGCTGAAGAG-3′; *RPL19*, forward 5′-CAATGAAATCGCCAATGCCAACTC-3′ and reverse 5′-TGGACCGTCACAGGCTTGC-3′. *Information obtained from the manufacturer (OriGene) regarding Ovarian Carcinoma Panel I; †information on stage is related to ovarian carcinoma specimens only; ‡positive expression is defined by C_t value below 35, whereas negative expression (neg) is defined as the absence of a detectible signal or C_t value equal or higher than 35. FIGO, International Federation of Gynecology and Obstetrics; AJCC, American Joint Committee on Cancer.

			C_t values‡		
Diagnosis*	Tumour grade*	Stage†	*BCL9*	*IDAX*	*RPL19*
Adenocarcinoma of endometrium, papillary serous	FIGO G3: poorly differentiated	Non-cancerous	27.7	29.3	20
Carcinoma of cervix, squamous cell	FIGO G3: poorly differentiated	Non-cancerous	28.7	27.4	20
Abscess of tissue	Not reported	Non-cancerous	30.7	30.4	20
Endometriosis	Not reported	Non-cancerous	33.1	30.9	20
Endometriosis	Not reported	Non-cancerous	29.8	28.9	20
Endometriosis	Not reported	Non-cancerous	33.2	32.5	20
Endometriosis	Not reported	Non-cancerous	32.1	31.4	20
Carcinoma of ovary, endometrioid	FIGO G2: moderately differentiated	I	34.4	33.4	20
Adenocarcinoma of ovary, papillary serous	FIGO G2: moderately differentiated	IA	30.4	33.0	20
Tumour of ovary, papillary serous, borderline	AJCC GB: borderline malignancy	IA	31.7	33.0	20
Tumour of ovary, papillary serous, borderline	AJCC GB: borderline malignancy	IA	33.2	30.6	20
Carcinoma of ovary, endometrioid	FIGO G1: well differentiated	IA	28.9	neg	20
Tumour of ovary, serous, borderline	AJCC GB: borderline malignancy	IA	30.3	30.3	20
Tumour of ovary, borderline	AJCC GB: borderline malignancy	IA	32.2	33.0	20
Tumour of ovary, mucinous, borderline	AJCC GB: borderline malignancy	IA	33.3	31.0	20
Adenocarcinoma of ovary, mucinous	FIGO G3: poorly differentiated	IB	30.7	34.0	20
Adenocarcinoma of ovary, endometrioid	FIGO G3: poorly differentiated	IB	33.6	neg	20
Tumour of ovary, borderline	Not reported	IB	31.8	31.4	20
Tumour of ovary, mucinous, borderline	AJCC GB: borderline malignancy	IC	30.7	28.4	20
Tumour of ovary, serous, borderline	AJCC GB: borderline malignancy	IC	31.0	neg	20
Tumour of ovary, serous, borderline	AJCC GB: borderline malignancy	IC	32.2	33.0	20
Adenocarcinoma of ovary, mucinous	FIGO G2: moderately differentiated	IC	32.1	34.9	20
Adenocarcinoma of ovary, endometrioid, sq. feat.	FIGO G2: moderately differentiated	IC	30.0	31.9	20
Adenocarcinoma of ovary, serous	FIGO G3: poorly differentiated	IIB	31.4	31.7	20
Adenocarcinoma of ovary, endometrioid	FIGO G3: poorly differentiated	IIB	30.9	neg	20
Adenocarcinoma of ovary, endometrioid	FIGO G1: well differentiated	IIC	31.4	31.3	20
Adenocarcinoma of ovary, papillary serous	FIGO G2: moderately differentiated	III	29.2	32.0	20
Adenocarcinoma of ovary, serous	FIGO G3: poorly differentiated	III	28.7	neg	20
Carcinoma of ovary, endometrioid	FIGO G2: moderately differentiated	IIIA	33.9	30.3	20
Tumour of ovary, serous, borderline	AJCC GB: borderline malignancy	IIIA	31.6	31.7	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IIIB	30.5	neg	20
Adenocarcinoma of ovary, serous	FIGO G2: moderately differentiated	IIIB	30.5	neg	20
Adenocarcinoma of ovary, endometrioid	FIGO G3: poorly differentiated	IIIB	31.7	neg	20
Adenocarcinoma of ovary, papillary serous	FIGO G2: moderately differentiated	IIIB	31.5	neg	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IIIB	30.9	neg	20
Tumour of ovary, serous, borderline	Not reported	IIIB	31.0	33.3	20
Adenocarcinoma of ovary, metastatic	Not reported	IIIB	32.0	34.2	20
Adenocarcinoma of ovary, papillary serous	FIGO G1: well differentiated	IIIC	29.1	neg	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IIIC	33.2	31.3	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IIIC	32.7	31.1	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IIIC	32.5	32.2	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IIIC	31.0	34.6	20
Carcinoma of ovary	FIGO G3: poorly differentiated	IIIC	31.9	33.6	20
Adenocarcinoma of ovary, papillary serous	Not reported	IIIC	32.3	32.0	20
Adenocarcinoma of ovary, serous	Not reported	IIIC	32.4	neg	20
Adenocarcinoma of ovary, papillary serous	FIGO G2: moderately differentiated	IV	30.6	27.7	20
Adenocarcinoma of ovary, metastatic	Not reported	IV	32.0	30.9	20
Adenocarcinoma of ovary, papillary serous	FIGO G3: poorly differentiated	IV	32.6	neg	20

reported in serous EOC and in NIH:OVCAR-3 (a serous EOC cell line). These authors also demonstrated co-immunoprecipitation of Lef-1 and β-catenin in poorly differentiated stage III serous EOC and NIH:OVCAR-3, but not in the normal ovary [9]. Comparison of Lef-1 expression in ten specimens of normal ovary and 23 specimens of serous EOC revealed no differences between the two groups [9]. The transcriptional co-activator Pygopus2 was found to be widely expressed in ovarian carcinoma tissues and cell lines of all histotypes, and was demonstrated to be required for tumour cell growth [64]. Furthermore, an additional co-activator designated Legless (BCL9) is widely expressed in both human EOC and normal ovary specimens at the RNA level (Table 1). Thus there are no apparent barriers for formation of the transcriptional activation complex, as the key players are expressed in serous EOC. Nevertheless, Wnt signalling is not constitutively activated in the majority of serous EOC cases or in the majority of individual cells in positive cases. However, it is important to note that currently available approaches do not enable detection of transient

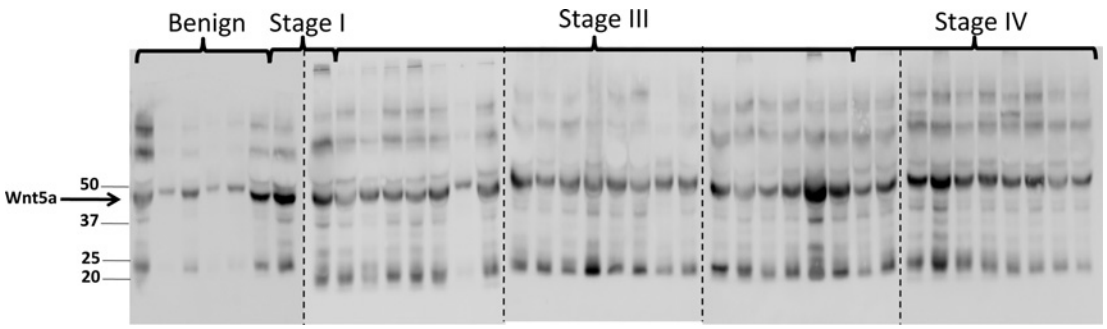

Figure 3 Wnt-5a in malignant EOC ascites

Malignant ascites were collected from women with EOC and non-malignant ascites were collected from women with ovarian hyperstimulation syndrome under an institutional review board-approved protocol at Northwestern University School of Medicine. The protein concentration of each specimen was determined and samples containing equal protein (1 mg) were electrophoresed by SDS/PAGE, electroblotted on to Immobilon membranes and analysed by Western blotting using anti-Wnt-5a primary antibody (1:1000 dilution; R&D systems) and peroxidase-conjugated goat anti-(rat IgG) (1:5000 dilution; Santa Cruz Biotechnology). The blots were developed using enhanced chemiluminescence. Molecular mass in kDa is given on the left-hand side.

activation of Wnt signalling, suggesting that evaluation of Wnt target gene expression may provide potential evidence with which to evaluate signalling pathway activation.

Cytoplasmic factors

There are several proteins that participate in the process of phosphorylation and degradation of β-catenin in the cytoplasm, thus preventing translocation into the nucleus. Expression of GSK3β in 23 cases of primary and metastatic serous EOC was approximately 6-fold higher on average than that in ten normal ovarian tissues. Overexpression of FRAT1 (frequently rearranged in advanced T-cell lymphomas 1), which inhibits phosphorylation of β-catenin by GSK3β, strongly correlated with nuclear β-catenin and its accumulation in the cytoplasm in human serous EOC, suggesting a potential alternative mechanism for β-catenin stabilization [60]. Cytoplasmic immunoreactivity for APC was documented in 67 out of 113 (59 %) serous EOC and was comparable in primary and metastatic tumours [62], suggesting that components of the β-catenin degradation complex are in place. The same study also reported that the absence of cytoplasmic APC only rarely coincided with nuclear β-catenin [62], suggesting that β-catenin degradation did not depend on the presence of APC. Although no data are available regarding the presence of Axin or Dvl, our findings suggest the presence of a Dvl inhibitor, IDAX (inhibitor of the Dvl and Axin complex), in both normal ovary and serous EOC tissues (Table 1). On the basis of the currently available data it is tempting to speculate that the equilibrium between cytoplasmic GSK3β and FRAT1 levels could functionally regulate Wnt signalling activation in serous EOC. In addition, a possible inhibition of Dvl through IDAX could contribute to the liberation of GSK3β and Axin from the complex with Frizzled, thus allowing for β-catenin degradation.

Membranous factors

Expression of Fzd1 and Fzd5 was tested in 26 normal ovary and 38 EOC (including 22 serous) specimens [65]. Interestingly, a higher number of malignant specimens was positive for both receptors relative to normal ovary (97.1 % and 14.3 % of malignant EOC were FZD1- and FZD5-positive, whereas only 54.5 % and 8.7 % of the normal ovarian tissues were positive respectively). Moreover, patients with FZD5-positive tumours had a 6-year probability of survival of 0.2 compared with 0.55 for those with FZD5-negative tumours. In addition, our data suggest that both LRP-6 and Kremen are expressed at the RNA level in the serous ovarian carcinoma cell line DOV13 (M.V. Barbolina and M.S Stack, unpublished work). Furthermore, engagement of collagen-binding β1 integrins, a process that occurs during adhesion to submesothelial interstitial collagens in intraperitoneal EOC metastasis, up-regulates *LRP6* mRNA levels in serous OVCA 433 cells [66].

Secreted factors

Expression of an activator of canonical Wnt signalling, Wnt-1, was found in a larger number of malignant EOC (29.4 % out of a total 38, of which 22 were serous) compared with normal ovary (9.1 % out of 26 total) specimens [65]. However, the same study reported that the increase in Wnt-1 expression in malignant EOC did not lead to stabilization of β-catenin [65]. Expression of Wnt-5a was also highly positive in malignant EOC specimens (80 %) compared with those of the normal ovary (27.3 %). Of note, both Wnt-1- and Wnt-5a-positive EOC patients had a significantly lower chance of survival compared with their Wnt-1-and Wnt-5a-negative counterparts [65]. Similar to the results summarized above for LRP-6, collagen adhesion by serous EOC cells also induces expression of Wnt-5a [66]. Furthermore, Wnt-5a is prevalent in ascites fluid obtained from women with EOC (Figure 3). Although Wnt-5a is most commonly characterized as an inhibitor of canonical Wnt signalling [67–72], emerging evidence suggests that the pathway (canonical, non-canonical or planar cell polarity) activated downstream of Wnt ligand-binding is receptor context-dependent. Using *in vitro* models, Wnt-5a has been shown to inhibit canonical Wnt signalling via an alternate Wnt receptor, mRor2 (mammalian receptor tyrosine kinase-like orphan receptor 2); however, Wnt-5a activates canonical Wnt signalling in the presence of Fzd4 and LRP-5. This activation potentiates nuclear β-catenin accumulation and activates Wnt reporter constructs [73]. This is consistent with data showing that Wnt-5a enhances the proliferation, migration and invasion of pancreatic cancer cell lines via β-catenin-dependent signalling pathways. In that study, Wnt5a treatment promoted nuclear β-catenin localization, which was inhibited by siRNA (small interfering RNA) targeted against Wnt-5a [74,75]. Additional studies are needed to clarify the Wnt receptor/co-receptor profiles of EOC cells of various histotypes to clarify the role of Wnt-5a in EOC pathobiology.

Expression of Wnt target genes in EOC

Indirect evidence for the existence of active Wnt signalling in serous EOC is expression of Wnt/β-catenin target genes in

Table 2 Expression of Wnt target genes in EOC

Overview of published data on Wnt target gene expression in EOC. *BIRC5*, survivin; *CLDN*, claudin 1; *EDN1*, endothelin 1; *FST*, follistatin; *MMP11*, stromelysin 3; *PLAUR*, plasminogen activator, urokinase receptor; *PTTG*, pituitary tumour-transforming 1; *RARG*, retinoic acid receptor γ; *SOX*, sex-determining region box; *JAG*, jagged; *SNAI*, SnaiL.

Gene	Reference(s)
CCND1	[78,79,167,168]
COX2	[111,169]
MMP2	[82,83]
MMP9	[83,170]
VEGF	[171]
CD44	[172]
MET	[173]
c-Myc	[174]
PLAUR	[175,176]
MMP7	[177]
CLDN	[178]
BIRC5	[179]
EON1	[122,123]
JAG	[180]
SOX9	[181]
SOX2	[182]
PTTG	[183]
MMP26	[184]
SNAI	[185]
FST	[186]
RARG	[187]
MMP11	[188]
LEF1	[189]

tumour cells or tissues. Currently over 100 target genes have been identified, the transcription of which is regulated by Wnt signalling (http://www.stanford.edu/~rnusse/pathways/targets.html). It should be noted, however, that many of these genes are also known to be regulated by other pathways, such that target gene expression alone is not sufficient evidence of activated Wnt signalling in serous EOC. Nevertheless, expression of many Wnt target genes, including *CCND1* (cyclin D1) [76–79], *COX2* (cyclo-oxygenase 2) [66,80], various MMPs (matrix metalloproteinases) [13,14,81–85] and *MET* [86–87] is observed in EOC, as summarized in Table 2, providing additional evidence in support of Wnt pathway activation.

MICROENVIRONMENTAL ACTIVATION OF THE WNT PATHWAY

Intraperitoneal dissemination provides an unique microenvironment for ovarian carcinoma metastases. As outlined in Figure 2, progression of EOC is hallmarked by shedding of single and multi-cellular aggregates of malignant epithelial cells from the primary tumour [11,13]. Accumulation of malignant ascites in the peritoneal cavity is common, particularly in women with late-stage EOC. Thus metastasizing EOC cells exist in a milieu rich in inflammatory cells [88] and growth/signalling factors, including VEGF [89–91], EGF [11,92], TGFα, TGFβ [93,94] and LPA [23,95], providing ample opportunity for cross-talk between signalling networks. Ascites accumulation also modifies peritoneal mechanobiology, altering the force environment of both metastatic tumour cells and peritoneal mesothelium [96,97]. Formation of secondary tumours at peritoneal organs (colon, omentum, uterus and liver) is achieved by β1 integrin-mediated anchoring to the mesothelium and submesothelial matrix [14–16,98], representing a significant transition from a free-floating cell or aggregate to a three-dimensional matrix-anchored structure. Within this unique metastatic niche, current evidence suggests multiple opportunities for regulation of Wnt signalling via molecular, mechanical and adhesion-dependent microenvironmental cues.

LPA and LPA GPCRs

LPA, a bioactive lipid signalling molecule, plays a role in numerous cell processes, including proliferation, migration, adhesion and cell survival [23–26,99,100], by acting at a family of GPCRs known as LPARs (LPA receptors) [101,102]. LPA has wide-ranging influence on cell physiology and pathophysiology, including increases in cytokine and growth factor expression, alteration of surface-protein trafficking and modulation of transcription [103,104]. LPA, which is produced by both normal and malignant cells, is present in high concentration (2–80 μM) in ascites and serum from 98 % of ovarian cancer patients, including those with early-stage disease [23,26,104–108]. Furthermore, increasing LPA expression is correlated with poor prognosis, suggesting its potential role as a therapeutic biomarker [109]. Results from our laboratory and others demonstrate LPA regulation of proteases [MMP2, MMP9, MT1-MMP (membrane type 1 MMP) and uPA (urokinase-type plasminogen activator)], inflammatory signalling molecules (COX2), IL-8 and adhesion molecules including E-cadherin and β1 integrin [24,100,104,110–111]. Notably, E-cadherin-based adherens junctions are stabilized by β-catenin [112], and loss of junctional stability may increase the cytoplasmic and/or nuclear pool of β-catenin [66].

LPA induces diverse cellular functions by activating one of five known receptors ($LPA_{1–5}$), which modulate various signalling pathway proteins, including Rho/ROCK (Rho-associated kinase), IP_3 (inositol 1,4,5-triphosphate)/Ca^{2+}, PKC (protein kinase C), PI3K, Ras/MAPK (mitogen-activated protein kinase) and cAMP [101–117]. LPA_1 ($G_{\alpha 12/13}$), LPA_3 ($G_{\alpha q/11}$) and LPA_4 ($G_{\alpha 12/13}$) receptor subtypes are expressed in the ovary, with LPA_4 being the most abundant. LPA_2 and LPA_3 are aberrantly overexpressed in several ovarian carcinoma cell lines [103,104]. This observation has been confirmed *in vivo* by detection of overexpressed *LPAR2* and *LPAR*3 mRNA in human ovarian tumour tissues, compared with normal and benign tissues [105–107]. Furthermore overexpression of LPA_2 and/or LPA_3 potentiates a more proliferative and invasive phenotype in ovarian tumour cells by modulating IL-6, IL-8 and VEGF expression [113]. Interestingly, heterotrimeric G-proteins containing $G_{\alpha 12/13}$ interact with the cytoplasmic domain of E-cadherin, rescuing breast carcinoma cells from E-cadherin-mediated migration suppression, preventing E-cadherin-based cell aggregation and displacing β-catenin from the adherens junction complex [114,115]. In colon cancer cell lines, LPA treatment (1 μM) leads to robust inactivation of GSK3β and nuclear localization of β-catenin [116]. These results correlate with a previous study demonstrating that LPA treatment (0.1–20 μM) or LPA_2/LPA_3 expression induced a deactivating phosphorylation of GSK3β in HEK (human embryonic kidney)-293 cells [117]. Recently, it has been shown that the $G_{\beta\gamma}$ subunit of the G-protein heterotrimer can also mediate Wnt signalling, inhibiting β-catenin degradation and allowing β-catenin-mediated transcriptional activity [118]. In support of these findings, our unpublished work demonstrates that the LPA–LPAR interaction disrupts junctional localization of E-cadherin and induces nuclear translocation of β-catenin, suggesting a mechanism for Wnt ligand-independent activation of Wnt/β-catenin signalling in EOC (R. J. Burkhalter, Y. Liu and M.S. Stack, unpublished work).

Endothelins

The endothelins (ET-1, ET-2 and ET-3) are a family of peptide signalling molecules that play a diverse role in physiology and pathology. Biological function is achieved via autocrine and paracrine signalling through two GPCRs, ET_AR and ET_BR (ET type A and B receptors respectively) [119,120]. ET-1 and ET_AR are overexpressed in ovarian cancer, driving epithelial-to-mesenchymal transition by stimulating tumour cell proliferation, invasive propensity (migration and adhesion), tumour cell escape from apoptosis and angiogenesis through multiple signalling pathways [120–123]. The ET-1/ET_AR axis has been shown to promote the invasive phenotype via inhibition of GSK3β in an ovarian carcinoma cell line [123]. Studies have shown a ET_AR/β-arrestin-1/Src signalling complex activates ILK, leading to GSK3β inhibition [124,125]. Subsequently, this activity leads to down-regulation of E-cadherin and increased Snail and β-catenin levels, promoting epithelial-to-mesenchymal transition [126,127]. Interestingly, studies in colon cancer and prostate cancer suggest reciprocal β-catenin regulation of ET-1 transcription through interaction with Tcf-4 [128,129]. These results suggest a potential signalling feed-forward loop for co-ordinate regulation of β-catenin and ET-1 to sustain an aggressive invasive phenotype necessary for tumour progression to metastasis.

Integrin-mediated matrix engagement activates β-catenin signalling

During the ovarian carcinoma metastatic cascade, disseminating cells anchor to the mesothelium and submesothelial matrix of peritoneal organs [11,13]. Although mechanisms regulating mesothelial receptivity have not been fully investigated, mechanical deformation of the mesothelium caused by accumulated ascites fluid may expose submesothelial matrix and/or modulate surface-expressed proteins on mesothelial cells themselves [130]. Additionally, a fibronectin-rich adhesion-promoting stroma is deposited on the otherwise non-adhesive mesothelium [131,132] Several studies have recently demonstrated a link between integrin signalling and the Wnt/β-catenin pathway activation in physiological and pathological cell signalling processes [66,133–138]. Our laboratory has modelled submesothelial anchoring of metastasizing EOC cells using three-dimensional collagen matrices or microsphere-immobilized anti-(β1 integrin) antibodies to mimic matrix-induced integrin aggregation. Integrin clustering in the serous EOC cell lines OVCA 429 and OVCA 433 results in rapid loss of junctional E-cadherin, dissolution of adherens junctions and loss of surface-localized β-catenin [66]. β-Catenin is translocated to the nucleus wherein it activates transcription of Tcf/Lef target genes, including *LRP6* and *Wnt5a* [66]. Similarly, culturing the serous EOC cell line DOV13 in three-dimensional collagen gels leads to down-regulation of two secreted antagonists of Wnt signalling, CTGF (connective tissue growth factor) [139] and DKK1 [16]. Interestingly, down-regulation of DKK1 and CTGF also occurs in DOV13 cells cultured as spheroids relative to two-dimensional tissue culture (Table 3). On the basis of these results, it is interesting to speculate that activation of Wnt signalling in free-floating MCAs may contribute to the acquired chemoresistance of this cell population.

The mechanical microenvironment may modulate Wnt signalling through β-catenin

More than two-thirds of all cases of EOC simultaneously present with malignant ascites. Ascites development in progressive or recurrent disease is a poor prognostic indicator and is correlated with a lack of response to treatment [140–142]. In comparison with the peritoneal cavity of disease-free women that contains approximately 20 ml of peritoneal fluid, ascitic volumes average 4.9 litres in EOC patients, with a range of 0.8 to 15 litres [143–145]. Although biological components of ascites are increasingly shown to influence progression of ovarian cancer via diverse signalling pathways [74,146,147], potential biomechanical signals activated by increasing intraperitoneal fluid pressure have not been investigated. Tumour cells sense alterations in the force environment via mechanosensing cell-surface-expressed proteins, including integrins, and subsequent 'inside-out' integrin signalling induces cytoskeletal modifications that effect cell behaviour [148–151]. Force modulation in the context of three-dimensional matrix rigidity is under investigation in several other tumour types [140,149,152–154]; however, the potential effects of the complex mix of strain, compression and shear forces conferred by ascites fluid are unknown. Our preliminary results show that static strain results in loss of surface-associated E-cadherin in serous EOC cells (J. Symowicz and M.S. Stack, unpublished work). In colon cancer, laminar shear stress increased DKK1 expression and decreased activated β-catenin [155]. Conversely, transient compression in APC-deficient cell lines facilitated phosphorylation of β-catenin, leading to destabilization of adherens junctions, nuclear translocation of β-catenin and transcriptional activation of the β-catenin target genes *Myc* and *Twist1* [156]. These results highlight the need to consider multiple distinct mechanical stimuli when modelling the force microenvironment of tumour tissues.

Table 3 Changes in expression of genes related to Wnt signalling in ovarian carcinoma MCAs compared with monolayers

Cells were cultured as monolayers or MCAs. To create MCAs, cells were released from the monolayers using trypsin/EDTA solution, resuspended in minimal essential medium containing 2% fetal bovine serum, plated over solidified 0.5% agarose, and allowed to form spheroids overnight at 37°C and 5% CO_2. Total RNA for cDNA microarray experiments was extracted using TRIzol® (Invitrogen), according to the manufacturer's instructions. All DNA microarray gene expression studies used human oligonucleotide arrays custom printed by a dedicated core facility within the Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center (Omaha, N.E., U.S.A.) as described previously [16]. Microarray slides were scanned with a ScanArray 4000 confocal laser system (PerkinElmer). Analysis of microarray gene expression data, accumulated from three independent experiments, was performed as described [16]. n/a, not applicable. *VCAN*, versican.

Gene	Up-regulation (fold)	Down-regulation (fold)	Cell line
DKK1	n/a	3.3	DOV13, ES2
CTGF	n/a	3.4	DOV13
VCAN	2.2	n/a	DOV13
CCND1	2.3	n/a	ES2

FUTURE PERSPECTIVES

As summarized above and in Figure 4, the unique microenvironmental niche inhabited by ovarian tumours provides a number of diverse mechanisms for transcriptional activation of Wnt/β-catenin target gene expression, even in histotypes devoid of activating mutations in Wnt pathway components. As canonical Wnt signalling is known to regulate proliferation, differentiation and 'stemness' [157], clearly additional studies are needed to provide a more detailed understanding of the role of Wnt target gene expression in ovarian tumour progression and metastasis. In particular, the contribution of microenvironmentally regulated Wnt/β-catenin signalling to maintenance of stemness properties in the self-renewing population of ovarian-cancer-initiating cells [158] remains to be explored. As many extracellular proteinases

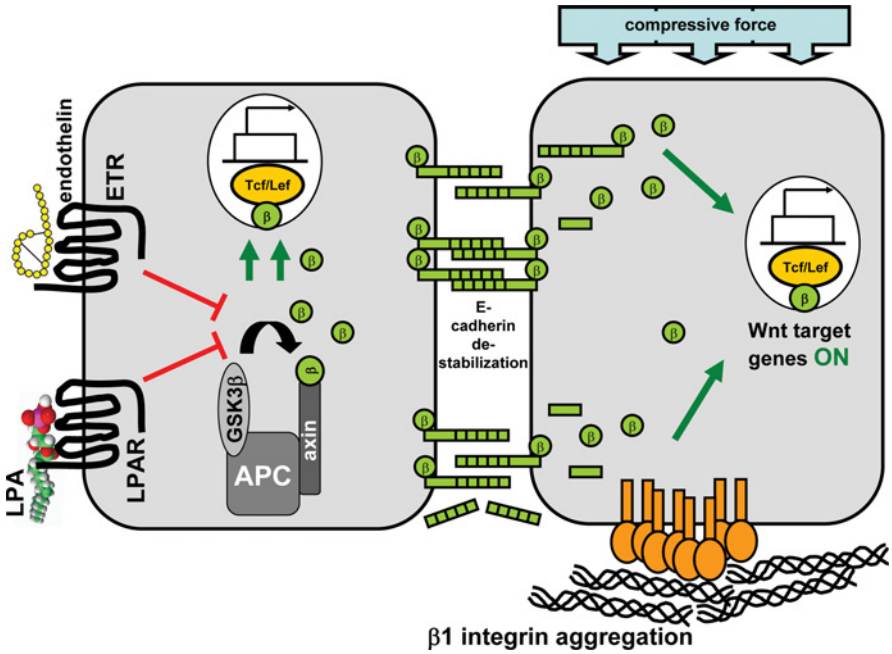

Figure 4 Potential mechanisms for activation of Wnt/β-catenin signalling in the ovarian tumour microenvironment

In the absence of activating mutations in key components of the Wnt signalling pathway, microenvironmental factors may contribute to transcriptional activation of Wnt/β-catenin target genes. For example, interaction of LPA with LPAR or ET with ET receptors (ETR) have been shown to block the activity of GSK3β, thereby resulting in increased nuclear translocation of β-catenin and enhanced β-catenin-mediated transcriptional activity (left-hand panel). Lateral aggregation of collagen-binding β1 integrins leads to dissolution of adherens junctions and enhanced nuclear translocation of β-catenin. In addition, integrin aggregation enhances the expression of LRP-6 and Wnt-5a while down-regulating the Wnt pathway inhibitors DKK and CTGF. Mechanical compression, exerted on MCAs of metastasizing EOC cells in the form of increased intraperitoneal fluid pressure due to accumulated ascitic fluid, may also destabilize adherens junctions and promote nuclear translocation of β-catenin (right-hand panel).

are β-catenin target genes, analysis of the involvement of this pathway in proteinase-mediated intraperitoneal anchoring of metastatic EOC cells may also prove informative.

A number of compounds have been identified that target various components of the Wnt signalling pathway [1,159–161]. For example, non-steroidal anti-inflammatory drugs do not block Wnt signalling themselves, but can induce degradation of Tcf or inhibit Wnt targets such as *COX2* [162]. Various small-molecule inhibitors of β-catenin–Tcf interaction have been identified that disrupt β-catenin–Tcf binding, decrease reporter gene transcription, inhibit cell proliferation *in vitro* and block tumour growth in murine models [163–165]. An alternative approach has been to utilize compounds that stabilize Axin, thereby stimulating β-catenin degradation [166]. As summarized above, with the exception of endometrioid ovarian tumours, progression of EOC has not been commonly associated with activated Wnt signalling. Consequently, there is no available literature reporting the efficacy of Wnt signalling inhibitors in serous EOC, and a search of the clinical trials database (www.clinicaltrials.gov) for trials involving EOC patients and Wnt signalling inhibitors returned no hits. Nevertheless, in light of the accumulating evidence in support of activated Wnt signalling in EOC and increasing evidence for ligand-independent activation of the pathway through microenvironment-regulated cross-talk, preclinical studies evaluating the potential efficacy of small-molecule inhibitors of this signalling pathway are warranted. Following successful preclinical trials, future human clinical trials should be designed to identify the subset of patients most likely to benefit from this biologically targeted therapy (for example, showing nuclear β-catenin staining) and to use validated biomarkers of response.

FUNDING

This work was supported by National Institutes of Health/National Cancer Institute [grant numbers RO1 CA109545, RO1 CA086984 (to M.S.S) and CA086984-11S1 (to R.J.B)], the Illinois Department of Public Health Penny Severns Breast, Ovarian, and Cervical Cancer Fund (to M.V.B), and an Ovarian Cancer Research Foundation Program of Excellence Award (to M.V.B.)

REFERENCES

1 Wend, P., Holland, J. D., Ziebold, U. and Birchmeier, W. (2010) Wnt signalling in stem and cancer stem cells. Seminars Cell Dev. Biol. **21**, 855–863
2 van Amerongen, R. and Nusse, R. (2009) Towards an integrated view of Wnt signalling in development. Development **136**, 3205–3214
3 Barker, N. (2008) The canonical Wnt/β-catenin signalling pathway. Methods Mol. Biol. **468**, 5–15
4 Hanahan, D. and Weinberg, R. A. (2000) The hallmarks of cancer. Cell **100**, 57–70
5 Camilli, T. C. and Weeraratna, A. T. (2010) Striking the target in Wnt-y conditions: intervening in Wnt signalling during cancer progression. Biochem. Pharmacol. **80**, 702–711
6 Wu, R., Hendrix-Lucas, N., Kuick, R., Zhai, Y., Schwartz, D. R., Akyol, A., Hanash, S., Misek, D. E., Katabuchi, H., Williams, B. O. et al. (2007) Mouse model of human ovarian endometrioid adenocarcinoma based on somatic defects in the Wnt/β-catenin and PI3K/PTEN signalling pathways. Cancer Cell **11**, 321–333
7 Zhai, Y., Wu, R., Schwartz, D. R., Darrah, D., Reed, H, Kolligs, F. T., Nieman, M. T., Fearon, E. R. and Cho, K. R. (2002) Role of β-catenin-T-cell factor-regulated genes in ovarian endometrioid adenocarcinomas. Am. J. Path. **160**, 1229–1238
8 Gatcliffe, T. A., Monk, B. J., Planutis, K. and Holcombe, R. F. (2008) Wnt signalling in ovarian tumorigenesis. Int. J. Gynecol. Cancer **18**, 954–962
9 Rask, K., Nilsson, A., Brannstrom, M., Carlsson, P., Hellberg, P., Janson, P. O., Hedin, L. and Sundfeldt, K. (2003) Wnt signalling pathway in ovarian epithelial tumors: increased expression of beta-catenin and GSK3β. Br. J. Caner **89**, 1298–1304

10 Lee, C. M., Shvartsman, H., Deavers, M. T., Wang, S. C., Xia, W., Schmandt, R., Bodurka, D. C., Atkinson, E. N., Malpica, A., Gershenson, D. M. et al. (2003) β-Catenin nuclear localization is associated with grade in ovarian serous carcinoma. Gynecol. Oncol. **88**, 363–368

11 Hudson, L. G., Zeineldin, R. and Stack, M. S. (2008) Phenotypic plasticity of neoplastic ovarian epithelium: unique cadherin profiles in tumor progression. Clin. Exp. Metastasis **25**, 643–655

12 Scully, R. E., Young, R. H. and Clement, P. B. (1998) Tumors of the ovary, maldeveloped gonads, fallopian tube and broad ligament. In Atlas of Tumor Pathology, pp. 51–168, Armed Forces Institute of Pathology, Washington D.C.

13 Barbolina, M. V., Moss, N. M., Westfall, S. D., Liu, Y., Burkhalter, R. J., Marga, F., Forgacs, G., Hudson, L. G. and Stack, M. S. (2009) Microenvironmental regulation of ovarian cancer metastasis. Cancer Treat. Res. **149**, 319–334

14 Ellerbroek, S. M., Fishman, D. A., Kearns, A. S., Bafetti, L. M. and Stack, M. S. (1999) Ovarian carcinoma regulation of matrix metalloproteinase-2 and membrane type 1 matrix metalloproteinase through β1 integrin. Cancer Res. **59**, 1635–1641

15 Ellerbroek, S. M., Wu, Y. I., Overall, C. M. and Stack, M. S. (2001) Functional interplay between type I collagen and cell surface matrix metalloproteinase activity. J. Biol. Chem. **276**, 24833–24842

16 Barbolina, M. V., Adley, B. P., Ariztia, E. V., Liu, Y. and Stack, M. S. (2007) Microenvironmental regulation of membrane type 1 matrix metalloproteinase activity in ovarian carcinoma cells via collagen-induced EGR1 expression. J. Biol. Chem. **282**, 4924–4931

17 Hudson, L. G., Zeineldin, R., Silberberg, M. and Stack, M. S. (2009) Activated epidermal growth factor receptor in ovarian cancer. Cancer Treat. Res. **149**, 203–226

18 Siwak, D. R., Carey, M., Hennessy, B. T., Nguyen, C. T., McGahren Murray, M. J., Nolden, L. and Mills, G. B. (2010) Targeting the epidermal growth factor receptor in epithelial ovarian cancer: current knowledge and future challenges. J. Oncol., doi:10.1155/2010/568938

19 Farley, J. and Birrer, M. J. (2009) Novel therapeutic targets. Cancer Treat. Res. **149**, 63–84

20 Bast, Jr, R. C., Hennessy, B. and Mills, G. B. (2009) The biology of ovarian cancer: new opportunities for translation. Nat. Rev. Cancer **9**, 415–428

21 Rosen, D. G., Mercado-Uribe, I., Yang, G., Bast, Jr, R. C., Amin, H. M., Lai, R. and Liu, J. (2006) The role of constitutively active signal transducer and activator of transcription 3 in ovarian tumorigenesis and prognosis. Cancer **107**, 2730–2740

22 Karin, M. (2006) Nuclear factor κB in cancer development and progression. Nature **441**, 431–436

23 Xu, Y., Wang, D. and Wang, Z. (2009) Lipid generation and signalling in ovarian cancer. Cancer Treat. Res. **149**, 241–268

24 Do, T. V., Symowicz, J. C., Berman, D. M., Liotta, L. A., Petricoin, E. F., Stack, M. S. and Fishman, D. A. (2007) Lysophosphatidic acid down-regulates stress fibers and up-regulates pro-matrix metalloproteinase-2 activation in ovarian cancer cells. Mol. Cancer Res. **5**, 121–131

25 Wang, F. and Fishman, D. A. (2009) Lysophosphatidic acid and invasion. Cancer Treat. Res. **149**, 269–296

26 Mills, G. B., Eder, A., Fang, X., Hasegawa, Y., Mao, M., Lu, Y., Tanyi, J., Tabassam, F. H., Wiener, J., Lapushin, R. et al. (2002) Critical role of lysophospholipids in the pathophysiology, diagnosis, and management of ovarian cancer. Cancer Treat. Res. **107**, 259–284

27 Bienz, M. (2005) β-Catenin: a pivot between cell adhesion and Wnt signalling. Curr. Biol. **15**, R64–R67

28 Kastritis, E., Murray, S., Kyriakou, F., Horti, M., Tamvakis, N., Kavantzas, N., Patsouris, E. S., Noni, A., Legaki, S., Dimopoulos, M. A. and Bamias, A. (2009) Somatic mutations of adenomatous polyposis coli gene and nuclear β-catenin accumulation have prognostic significance in invasive urothelial carcinomas: evidence for Wnt pathway implication. Int. J. Cancer **124**, 103–108

29 Oving, I. M. and Clevers, H. C. (2002) Molecular causes of colon cancer. Eur. J. Clin. Invest. **32**, 448–457

30 Schlosshauer, P. W., Pirog, E. C., Levine, R. L. and Ellenson, L. H. (2000) Mutational analysis of the *CTNNB1* and *APC* genes in uterine endometrioid carcinoma. Mod. Pathol. **13**, 1066–1071

31 Shinohara, A., Yokoyama, Y., Wan, X., Takahashi, Y., Mori, Y., Takami, T., Shimokawa, K. and Tamaya, T. (2001) Cytoplasmic/nuclear expression without mutation of exon 3 of the β-catenin gene is frequent in the development of the neoplasm of the uterine cervix. Gynecol. Oncol. **82**, 450–455

32 Howe, L. R. and Brown, A. M. (2004) Wnt signalling and breast cancer. Cancer Biol. Ther. **3**, 36–41

33 Ishigaki, K., Namba, H., Nakashima, M., Nakayama, T., Mitsutake, N., Hayashi, T., Maeda, S., Ichinose, M., Kanematsu, T. and Yamashita, S. (2002) Aberrant localization of β-catenin correlates with overexpression of its target gene in human papillary thyroid cancer. J. Clin. Endocrinol. Metab. **87**, 3433–3440

34 Mazieres, J., He, B., You, L., Xu, Z. and Jablons, D. M. (2005) Wnt signalling in lung cancer. Cancer Lett. **222**, 1–10

35 Miyake, N., Maeta, H., Horie, S., Kitamura, Y., Nanba, E., Kobayashi, K. and Terada, T. (2001) Absence of mutations in the β-catenin and adenomatous polyposis coli genes in papillary and follicular thyroid carcinomas. Pathol. Int. **51**, 680–685

36 Verras, M. and Sun, Z. (2006) Roles and regulation of Wnt signalling and β-catenin in prostate cancer. Cancer Lett. **237**, 22–32

37 Zeng, G., Germinaro, M., Micsenyi, A., Monga, N. K., Bell, A., Sood, A., Malhotra, V., Sood, N., Midda, V., Monga, D. K. et al. (2006) Aberrant Wnt/β-catenin signalling in pancreatic adenocarcinoma. Neoplasia **8**, 279–289

38 Lin, S. Y., Xia, W., Wang, J. C., Kwong, K. Y., Spohn, B., Wen, Y., Pestell, R. G. and Hung, M. C. (2000) β-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc. Natl. Acad. Sci. U.S.A. **97**, 4262–4266

39 Kanai, Y., Ochiai, A., Shibata, T., Oyama, T., Ushijima, S., Akimoto, S. and Hirohashi, S. (1995) c-erbB-2 gene product directly associates with β-catenin and plakoglobin. Biochem. Biophys. Res. Commun. **208**, 1067–1072

40 Playford, M. P., Bicknell, D., Bodmer, W. F. and Macaulay, V. M. (2000) Insulin-like growth factor 1 regulates the location, stability, and transcriptional activity of β-catenin. Proc. Natl. Acad. Sci. U.S.A. **97**, 12103–12108

41 Novak, A., Hsu, S. C., Leung-Hagesteijn, C., Radeva, G., Papkoff, J., Montesano, R., Roskelley, C., Grosschedl, R. and Dedhar, S. (1998) Cell adhesion and the integrin-linked kinase regulate the LEF-1 and β-catenin signalling pathways. Proc. Natl. Acad. Sci. U.S.A. **95**, 4374–4379

42 Sharma, M., Chuang, W. W. and Sun, Z. (2002) Phosphatidylinositol 3-kinase/Akt stimulates androgen pathway through GSK3β inhibition and nuclear β-catenin accumulation. J Biol. Chem. **277**, 30935–30941

43 Persad, S., Troussard, A. A., McPhee, T. R., Mulholland, D. J. and Dedhar, S. (2001) Tumor suppressor PTEN inhibits nuclear accumulation of β-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation. J. Cell Biol. **153**, 1161–1174

44 Liu, J., Stevens, J., Rote, C. A., Yost, H. J., Hu, Y., Neufeld, K. L., White, R. L. and Matsunami, N. (2001) Siah-1 mediates a novel β-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol. Cell **7**, 927–936

45 Matsuzawa, S. I. and Reed, J. C. (2001) Siah-1, SIP, and Ebi collaborate in a novel pathway for β-catenin degradation linked to p53 responses. Mol. Cell. **7**, 915–926

46 Verras, M. and Sun, Z. (2005) β-Catenin is involved in insulin-like growth factor 1-mediated transactivation of the androgen receptor. Mol. Endocrinol. **19**, 391–398

47 Truica, C. I., Byers, S. and Gelmann, E. P. (2000) β-Catenin affects androgen receptor transcriptional activity and ligand specificity. Cancer Res. **60**, 4709–4713

48 Uematsu, K., He, B., You, L., Xu, Z., McCormick, F. and Jablons, D. M. (2003) Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. Oncogene **22**, 7218–7221

49 Mazieres, J., He, B., You, L., Xu, Z., Lee, A. Y., Mikami, I., Reguart, N., Rosell, R., McCormick, F. and Jablons, D. M. (2004) Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. Cancer Res. **64**, 4717–4720

50 Nozaki, I., Tsuji, T., Iijima, O., Ohmura, Y., Andou, A., Miyazaki, M., Shimizu, N. and Namba, M. (2001) Reduced expression of *REIC/Dkk-3* gene in non-small cell lung cancer. Int. J. Oncol. **19**, 117–121

51 Gamallo, C., Palacios, J., Moreno, G., Calvo de Mora, J., Suarez, A. and Armas, A. (1999) β-catenin expression pattern in stage I and II ovarian carcinomas: relationship with β-catenin gene mutations, clinicopathological features, and clinical outcome. Am. J. Pathol. **155**, 527–536

52 Saegusa, M. and Okayasu, I. (2001) Frequent nuclear β-catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. J. Pathol. **194**, 59–67

53 Schlosshauer, P. W., Ellenson, L. H. and Soslow, R. A. (2002) β-Catenin and E-cadherin expression patterns in high-grade endometrial carcinoma are associated with histological subtype. Mod. Pathol. **15**, 1032–1037

54 Wu, R., Zhai, Y., Fearon, ER. and Cho, K. R. (2001) Diverse mechanisms of β-catenin deregulation in ovarian endometrioid adenocarcinomas. Cancer Res. **61**, 8247–8255

55 Hendrix, N. D., Wu, R., Kuick, R., Schwartz, D. R., Fearon, ER. and Cho, K. R. (2006) Fibroblast growth factor 9 has oncogenic activity and is a downstream target of Wnt signalling in ovarian endometrioid adenocarcinomas. Cancer Res. **66**, 1354–1362

56 Schwartz, D. R., Wu, R., Kardia, S. L., Levin, A. M., Huang, C. C., Shedden, K. A., Kuick, R., Misek, D. E., Hanash, S. M., Taylor, J. M. et al. (2003) Novel candidate targets of β-catenin/T-cell factor signalling identified by gene expression profiling of ovarian endometrioid adenocarcinomas. Cancer Res. **63**, 2913–2922

57 Zhai, Y., Wu, R., Schwartz, D. R., Darrah, D., Reed, H., Kolligs, F. T., Nieman, M. T., Fearon, E. R. and Cho, K. R. (2002) Role of β-catenin/T-cell factor-regulated genes in ovarian endometrioid adenocarcinomas. Am. J. Pathol. **160**, 1229–1238

58 Sarrio, D., Moreno-Bueno, G., Sanchez-Estevez, C., Banon-Rodriguez, I., Hernandez-Cortes, G., Hardisson, D. and Palacios, J. (2006) Expression of cadherins and catenins correlates with distinct histologic types of ovarian carcinomas. Hum. Pathol. **37**, 1042–1049

59 Bell, D. A. (2005) Origins and molecular pathology of ovarian cancer. Mod. Pathol. **18**, S19–S32

60 Wang, Y., Hewitt, S. M., Liu, S., Zhou, X., Zhu, H., Zhou, C., Zhang, G., Quan, L., Bai, J. and Xu, N. (2006) Tissue microarray analysis of human *FRAT1* expression and its correlation with the subcellular localisation of β-catenin in ovarian tumours. Br. J. Cancer **94**, 686–691

61 Sagae, S., Kobayashi, K., Nishioka, Y., Sugimura, M., Ishioka, S., Nagata, M., Terasawa, K., Tokino, T. and Kudo, R. (1999) Mutational analysis of β-catenin gene in Japanese ovarian carcinomas: frequent mutations in endometrioid carcinomas. Jpn. J. Cancer. Res. **90**, 510–515

62 Karbova, E., Davidson, B., Metodiev, K., Trope, C. G. and Nesland, J. M. (2002) Adenomatous polyposis coli (APC) protein expression in primary and metastatic serous ovarian carcinoma. Int. J. Surg. Pathol. **10**, 175–180

63 Lee, C. M., Shvartsman, H., Deavers, M. T., Wang, S. C., Xia, W., Schmandt, R., Bodurka, D. C., Atkinson, E. N., Malpica, A., Gershenson, D. M. et al. (2003) β-Catenin nuclear localization is associated with grade in ovarian serous carcinoma. Gynecol. Oncol. **88**, 363–368

64 Popadiuk, C. M., Xiong, J., Wells, M. G., Andrews, P. G., Dankwa, K., Hirasawa, K., Lake, B. B. and Kao, K. R. (2006) Antisense suppression of pygopus2 results in growth arrest of epithelial ovarian cancer. Clin. Cancer Res. **12**, 2216–2223

65 Badiglian Filho, L., Oshima, C. T., De Oliveira Lima, F., De Oliveira Costa, H., De Sousa Damiao, R., Gomes, T. S. and Goncalves, W. J. (2009) Canonical and noncanonical Wnt pathway: a comparison among normal ovary, benign ovarian tumor and ovarian cancer. Oncol. Rep. **21**, 313–320

66 Burkhalter, R. J., Symowicz, J. S., Hudson, L. G., Gottardi, C. J. and Stack, M. S. (2011) Integrin regulation of β-catenin signalling in ovarian carcinoma. J. Biol. Chem., doi:10.1074/jbc.M110.199539

67 Danielson, K. G., Pillarisetti, J., Cohen, I. R., Sholehvar, B., Huebner, K., Ng, L.-J., Nicholls, J. M., Cheah, K. S. E. and Iozzo, R. V. (1995) Characterization of the complete genomic structure of the human *Wnt-5A* gene, functional analysis of its promoter, chromosomal mapping, and cxpression in early human embryogenesis. J. Biol. Chem. **270**, 31225–31234

68 Topol, L., Juaing, X., Choi, H., Garett-Beal, L., Carolan, P. J. and Yang, Y. (2003) Wnt5a inhibits the canonical Wnt pathway by promoting GSK-3-independent β-catenin. J. Cell Biol. **162**, 899–908

69 Ishtani, T., Kishida, S., Hyodo-Miura, J., Ueno, N., Yasuda, J., Waterman, M., Shibuya, H., Moon, R. T., Ninomiya-Tsuji, J. and Matsumoto, K. (2003) The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca(2+) pathway to antagonize Wnt/ β-catenin signalling, Mol. Cell Biol. **23**, 131–139

70 Holmen, S. L., Salic, A., Zylstra, C. R., Kirschner, M. W. and Williams, B. O. (2002) A novel set of Wnt–Frizzled fusion proteins identifies receptor components that activate β-catenin-dependent signalling. J. Biol. Chem. **277**, 34727–34735

71 Olson, D. J. and Gibo, D. M. (1998) Antisense Wnt-5a mimics Wnt-1-medaited C57MG mammary epithelial cell transformation. Exp. Cell Res. **241**, 134–141

72 Torres, M. A., Yang-Snyder, J. A., Purcell, S. M., DeMarais, A. A., McGrew, L. L. and Moon, R. T. (1996) Activities of the Wnt-1 class of secreted signalling factors are antagonized by the Wnt-5a class and by a dominant negative cadherin in early *Xenopus* development. J. Cell Biol. **133**, 1123–1137

73 McDonald, S. L. and Silver, A. (2009) The opposing roles of Wnt-5a in cancer. Br. J. Cancer **101**, 209–214

74 Mikels, A. J. and Nusse, N. (2006) Purified Wnt5a protein activates or inhibits β-catenin-TCF signalling depending on receptor context. PLoS Biol. **4**, e115

75 Ripka, S., Konig, A., Buchholz, M., Wagner, M., Sipos, B., Kloppel, G., Downward, J., Gress, T. M. and Muchl, P. (2007) Wnt5a-target of CUTL1 and potent modulator of tumor cell migration and invasion in pancreatic cancer. Carcinogenesis **28**, 1178–1187

76 Tetsu, O. and McCormick, F. (1999) β-Catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature **398**, 422–426

77 Rahmani, M., Read, J. T., Carthy, J. M., McDonald, P. C., Wong, B. W., Esfandiarei, M., Si, X., Luo, Z., Luo, H., Rennie, P. S. and McManus, B. M. (2005) Regulation of the versican promoter by the β-catenin-T-cell factor complex in vascular smooth muscle cells. J. Biol. Chem. **280**, 13019–13028

78 Hung, W. C., Chai, C. Y., Huang, J. S. and Chuang, L. Y. (1996) Expression of cyclin D1 and c-Ki-ras gene product in human epithelial ovarian tumors. Hum. Pathol. **27**, 1324–1328

79 Barbieri, F., Cagnoli, M., Ragni, N., Pedulla, F., Foglia, G. and Alama, A. (1997) Expression of cyclin D1 correlates with malignancy in human ovarian tumours. Br. J. Cancer **75**, 1263–1268

80 Howe, L. R., Subbaramaiah, K., Chung, W. J., Dannenberg, A. J. and Brown, A. M. (1999) Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. Cancer Res. **59**, 1572–1577

81 Wu, B., Crampton, S. P. and Hughes, C. C. W. (2007) Wnt signalling induces matrix metalloproteinase expression and regulates T cell transmigration. Immunity **26**, 227–239

82 Fishman, D. A., Bafetti, L. M., Banionis, S., Kearns, A. S., Chilukuri, K. and Stack, M. S. (1997) Production of extracellular matrix-degrading proteinases by primary cultures of human epithelial ovarian carcinoma cells. Cancer **80**, 1457–1463

83 Moser, T. L., Pizzo, S. V., Bafetti, L. M., Fishman, D. A. and Stack, M. S. (1996) Evidence for preferential adhesion of ovarian epithelial carcinoma cells to type I collagen mediated by the alpha2beta1 integrin. Int. J. Cancer **67**, 695–701

84 Ellerbroek, S. M., Hudson, L. G. and Stack, M. S. (1998) Proteinase requirements of epidermal growth factor-induced ovarian cancer cell invasion. Int. J. Cancer **78**, 331–337

85 Ghosh, S., Wu, Y. and Stack, M. S. (2002) Ovarian cancer-associated proteinases. Cancer Treat. Res. **107**, 331–351

86 Boon, E. M., van der Neut, R., van de Wetering, M., Clevers, H. and Pals, S. T. (2002) Wnt signalling regulates expression of the receptor tyrosine kinase met in colorectal cancer. Cancer Res. **62**, 5126–5128

87 Di Renzo, M. F., Olivero, M., Katsaros, D., Crepaldi, T., Gaglia, P., Zola, P., Sismondi, P. and Comoglio, P. M. (1994) Overexpression of the Met/HGF receptor in ovarian cancer. Int. J. Cancer **58**, 658–662

88 Freedman, R. S., Deavers, M., Liu, J. and Wang, E. (2004) Peritoneal inflammation: a microenvironment for epithelial ovarian cancer (EOC). J. Transl. Med. **2**, 23–32

89 Zebrowski, B. K., Liu, W., Ramirez, K., Akgi, Y. and Mills, G. B. (1999) Markedly elevated levels of vascular endothelial growth factor in malignant ascites. Ann. Surg. Oncol. **6**, 373–378

90 Kraft, A., Weindel, K., Ochs, A., Marth, C., Zmija, J., Schumacher, P., Unger, C., Marme, D. and Gastl, G. (1999) Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. Cancer **85**, 178–187

91 Santin, A. D., Hermonat, P. L., Ravaggi, A, Cannon, M. J., Pecorelli, S. and Parham, G. P. (1999) Secretion of vascular endothelial growth factor in ovarian cancer. Eur. J. Gynaecol. Oncol. **20**, 177–181

92 Miyamoto, S., Hirata, M., Yamazaki, A., Kageyama, T., Hasuwa, H., Mizushima, H., Tanaka, Y., Yagi, H., Sonoda, K., Kai, M. et al. (2004) Heparin-binding EGF-like growth factor is a promising target for ovarian cancer therapy. Cancer Res. **64**, 5720–5727

93 Saltzman, A. K., Hartenbach, E. M., Carter, J. R., Contreras, D. N., Twiggs, L. B., Carson, L. F. and Ramakrishnan, S. (1999) Transforming growth factor-alpha levels in the serum and ascites of patients with advanced epithelial ovarian cancer. Gynecol. Obstet. Invest. **47**, 200–204

94 Abendstein, B., Stadlmann, S., Knabbe, C., Buck, M., Muller-Holzner, E., Zeimet, A. G., Marth, C., Obrist, P., Krugmann, J. and Offner, F. A. (2000) Regulation of transforming growth factor-beta secretion by human peritoneal mesothelial and ovarian carcinoma cells. Cytokine **12**, 1115–1119

95 Puiffe, M. L., Le Page, C., Filali-Mouhim, A., Zietarska, M., Ouellet, V., Tonin, P. N., Chevrette, M., Provencherm, D. M. and Mes-Masson, A. M. (2007) Characterization of ovarian cancer ascites on cell invasion, proliferation, spheroid formation, and gene expression in an *in vitro* model of ovarian cancer. Neoplasia **9**, 820–829

96 Esquis, P., Consolo, D., Magnin, G., Pointaire, P., Moretto, P., Ynsa, M. D., Beltrame, J. L., Drogoul, C., Simonet, M., Benoit, M. et al. (2006) High intra-abdominal pressure enhances the penetration and antitumor effect of intraperitoneal cisplatin on experimental peritoneal carcinomatosis. Ann. Surg. **244**, 106–112

97 Henriksen, J. H., Stage, J. G., Schlichting, P. and Winkler, K. (1980) Intraperitoneal pressure: ascetic fluid and splanchnic vascular pressures, and their role in prevention and formation of ascites. Scand. J. Clin. Lab. Invest. **40**, 493–502

98 Lessan, K., Aguiar, D. J., Oegema, T., Siebenson, L. and Skubitz, A. P. N. (1999) CD44 and fl1 integrin mediate ovarian carcinoma cell adhesion to peritoneal mesothelial cells. Am. J. Pathol. **154**, 1525–1537

99 Moolenaar, W., Jalink, K. and Van Corven, E. (1992) Lysophosphatidic acid: a bioactive phospholipid with growth factor-like properties. Rev. Physiol. Biochem. Pharmacol. **119**, 47–65

100 Fishman, D. A., Liu, Y., Ellerbroek, S. M. and Stack, M. S. (2001) Lysophosphatidic acid promotes matrix metalloproteinase (MMP) activation and MMP-dependent invasion in ovarian cancer cells. Cancer Res. **61**, 3194–3199

101 Choi, J. W., Herr, D. R., Noguchi, K., Yung, Y. C., Lee, C.-W., Mutoh, T., Lin, M.-E., Teo, S. T., Park, K. E. et al. (2010) LPA receptors: subtypes and biological actions. Annu. Rev. Pharmacol. Toxicol. **50**, 157–186

102 Fukushima, N. and Chun, J. (2001) The LPA receptors. Prostaglandins Other Lipid Mediators **64**, 21–32

103 Fang, X., Yu, S., Bast, R. C., Liu, S., Xu, H.-J., Hu, S.-X., LaPushin, R., Claret, F. X., Aggarwal, B. B., Lu, Y. and Mills, G. B. (2004) Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. J. Biol. Chem. **279**, 9653–9661

104 Fang, X., Gaudette, D., Furui, T., Mao, M., Estrella, V., Eder, A., Pustilnik, T., Sasagawa, T., Lapushin, R., Yu, S. et al. (2000) Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer. Ann. N. Y. Acad. Sci. **905**, 188–208

105 Xu, Y., Gaudette, D. C. and Boyton, J. D. (1995) Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. Clin. Cancer Res. **1**, 1223–1232

106 Westermann, A. M., Havik, E., Postma, F. R., Neijnen, J. H., Dalesio, O., Moolenaar, W. H. and Rodenhuis, S. (1998) Malignant effusions contain lysophosphatidic acid (LPA)-like activity. Ann. Oncol. **9**, 437–442

107 Fang, X., Schummer, M., Mao, M., Yu, S., Tabassam, F. H., Swaby, R., Hasegawa, Y., Tanyi, J. L., LaPushin, R., Eder, A. et al. (2002) Lysophosphatidic acid is a bioactive mMediator in ovarian cancer. Biochim. Biophys. Acta **1582**, 257–264

108 Xiao, Y. J., Schwartz, B., Washington, M., Kennedy, A., Webster, K., Belinson, J. and Xu, Y. (2001) Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs. nonmalignant ascitic fluids. Anal. Biochem. **290**, 302–313

109 Xu, Y., Shen, Z., Wiper, D. W., Wu, M., Morton, R. E., Elson, P., Kennedy, A. W., Belinson, J., Markman, J. and Casey, G. (1998) Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. JAMA, J. Am. Med. Assoc. **280**, 719–723

110 Gil, O. D., Lee, C., Ariztia, E. V., Wang, F.-Q., Smith, P. J., Hope, J. M. and Fishman, D. A. (2008) Lysophosphatidic acid (LPA) promotes E-cadherin ectodomain shedding and OVCA429 cell invasion in an uPA-dependent manner. Gynecol. Oncol. **108**, 361–369

111 Symowicz, J., Adley, B. P., Woo, M. M., Auersperg, N., Hudson, L. G. and Stack, M. S. (2005) Cyclooxygenase-2 functions as a downstream mediator of lysophosphatidic acid to promote aggressive behavior in ovarian carcinoma cells. Cancer Res. **65**, 2234–2242

112 Nelson, W. J. and Nusse, R. (2004) Convergence of Wnt, β-catenin, and cadherin pathways. Science **303**, 1483–1487

113 Yu, S., Murph, M. M., Lu, Y., Liu, S., Hall, H. S., Liu, J., Stephens, C., Fang, X. and Mills, G. B. (2008) Lysophosphatidic acid receptors determine tumorigenicity and aggressiveness of ovarian cancer cells. J. Natl. Cancer Inst. **20**, 1630–1642

114 Meigs, T. E., Fields, T. A., McKee, D. D. and Casey, P. J. (2001) Interaction of Galpha12 and Galpha13 with the cytoplasmic domain of cadherin provides a mechanism for β-catenin release. Proc. Natl. Acad. Sci. U.S.A. **98**, 519–524

115 Meigs, T. E., Fedor-Chaiken, M., Kaplan, D. D., Brackenbury, R. and Casey, P. J. (2002) Galpha12 and Galpha13 negatively regulate the adhesive functions of cadherin. J. Biol. Chem. **277**, 24594–24600

116 Yang, M., Zhong, W. W., Srivastava, N., Slavin, A., Yang, J., Hoey, T. and An, S. (2005) G protein-coupled lysophosphatidic acid receptors stimulate proliferation of colon cancer cells through the β-catenin pathway. Proc. Natl. Acad. Sci. U.S.A. **102**, 6027–6032

117 Fang, X., Yu, S., Tanyi, J. L., Lu, Y., Woodgett, J. R. and Mills, G. B. (2002) Convergence of multiple signalling cascades at glycogen synthase kinase 3: edg receptor-mediated phosphorylation and inactivation by lysophosphatidic acid through a protein kinase C-dependent intracellular pathway. Mol. Cell. Biol. **22**, 2099–2110

118 Jernigan, K. K., Cselenyi, C. S., Thorne, C. A., Hanson, A. J., Tahinci, E., Hajicek, N., Oldham, W. M., Lee, L. A., Hamm, H. E., Hepler, J. R. et al. (2010) Gbetagamma activates GSK3 to promote LRP6-mediated β-catenin transcriptional activity. Sci. Signaling. **3**, ra37

119 Levin, E. R. (1995) Endothelins. N. Engl. J. Med. **333**, 356–363

120 Bagnato, A., Spinella, F. and Rosano, L. (2008) The endothelin axis in cancer: the promise and the challenges of molecularly targeted therapy. Can. J. Physiol. Pharmacol. **86**, 473–484

121 Nelson, J., Bagnato, A., Battistini, B. and Nisen, P. (2003) The endothelin axis: emerging role in cancer. Nat. Rev. Cancer **3**, 110–116

122 Bagnato, A., Tecce, R., Moretti, C., Di Castro, V., Spergel, D. and Catt, K. J. (1995) Autocrine actions of endothelin-1 as a growth factor in human ovarian carcinoma cells. Clin. Cancer Res. **1**, 1059–1066

123 Bagnato, A., Salani, D., Di Castro, V., Wu-Wong, J. R., Tecce, R., Nicotra, M. R., Venuti, A. and Natali, P. G. (1999) Expression of endothelin 1 and endothelin A receptor in ovarian carcinoma: evidence for an autocrine role in tumor growth. Cancer Res. **59**, 720–727

124 Rosano, L., Cianfrocca, R., Masi, S., Spinella, F., Di Castro, V., Biroccio, A., Salvati, E., Nicotra, M. R., Natali, P. G. and Bagnato, A. (2009) β-Arrestin link endothelin A receptor to β-catenin signalling to induce ovarian cancer cell invasion and metastasis. Proc. Natl. Acad. Sci. U.S.A. **106**, 2806–2811

125 Cianfracca, R., Rosano, L., Spinella, F., Di Castro, V., Natali, P. G. and Bagnato, A. (2010) β-arrestin-1 mediates the endothelin-1-induced activation of Akt and integrin-linked kinase. Can. J. Physiol. Pharmacol. **88**, 796–801

126 Rosano, L., Spinella, F., Di Castro, V., Nicotra, M. R., Dedhar, S., de Herreros, A. G., Natali, P. G. and Bagnato, A. (2005) Endothelin-1 promotes epithelial-to-mesenchymal transition in human ovarian cancer cells. Cancer Res. **65**, 11649–11657

127 Bagnato, A. and Rosano, L. (2007) Epithelial–mesenchymal transition in ovarian cancer progression: a crucial role for the endothelin axis. Cells Tissues Organs **185**, 85–94

128 Kim, T. H., Xiong, H., Zhang, Z. and Ren, B. (2005) β-Catenin activates the growth factor endothelin-1 in colon cancer cells. Oncogene **24**, 597–604

129 Sun, P., Xiong, H., Kim, T. H., Ren, B. and Zhang, Z. (2006) Positive inter-regulation between β-catenin/T Cell factor-4 signalling and endothelin-1 signalling potentiates proliferation and survival of prostate cancer cells. Mol. Pharmacol. **69**, 520–531

130 Zareie, M., Keuning, E. D., ter Wee, P. M., Beelen, R. H. J. and van den Born, J. (2005) Peritoneal dialysis fluid-induced changes of the peritoneal membrane are reversible after peritoneal rest in rats. Nephrol. Dial. Transplant. **20**, 189–193

131 Nagy, J. A., Morgan, E. S., Herzberg, K. T., Manseau, E. J., Dvorak, A. M. and Dvorak, H. F. (1995) Pathogenesis of ascites tumor growth: angiogenesis, vascular remodeling, and stroma formation in the peritoneal lining. Cancer Res. **55**, 376–385

132 Kenny, H. A., Dogan, S., Zillhardt, M., Mitra, A. K., Yamada, S. D., Krausz, T. and Lengyel, E. (2009) Organotypic models of metastasis: a three-dimensional culture mimicking the human peritoneum and omentum for the study of the early steps of ovarian cancer metastasis. Cancer Treat. Res. **149**, 335–351

133 Crampton, S. P., Beibei, W., Park, E. J., Kim, J. H., Solomon, C., Waterman, M. L. and Hughes, C. C. W. (2009) Integration of the β-catenin dependent Wnt pathway with integrin signalling through the adaptor molecule Grb2. PLoS ONE **4**, e7841

134 Maher, M. T., Flozak, A. S., Stocker, A. M., Chenn, A. and Gottardi, C. J. (2009) Activity of the β-catenin phosphodestruction complex at cell–cell contacts is enhanced by cadherin-based adhesion. J. Cell Biol. **186**, 219–228

135 Kim, Y., Kugler, M. C., Wei, Y., Kim, K. K., Li, X., Brumwell, A. N. and Chapman, H. A. (2009) Integrin alpha3beta1-dependent β-catenin phophorylation links epithelial Smad signalling to cell contacts. J. Cell Biol. **184**, 309–322

136 Chandramouly, G., Abad, P. C., Knowles, D. W. and Lelievre, S. A. (2007) The control of tissue architecture over nuclear organization is crucial for epithelial cell fate. J. Cell Sci. **120**, 1596–1606

137 Kam, Y. and Quaranta, V. (2009) Cadherin-bound β-catenin feeds into the Wnt pathway upon adherens junctions dissociation: evidence for an intersection between β-catenin pools. PLoS ONE **4**, e4580

138 Koenig, A., Mueller, C., Hasel, C., Adler, G. and Menke, A. (2006) Collagen type I induces disruption of E-cadherin-mediated cell–cell contacts and promotes proliferation of pancreatic carcinoma cells. Cancer Res. **66**, 4662–4671

139 Barbolina, M. V., Adley, B. P., Kelly, D L., Shepard, J., Fought, A. J., Scholtens, D., Penzes, P., Shea, L. D. and Stack, M. S. (2009) Downregulation of connective tissue growth factor by three-dimensional matrix enhances ovarian carcinoma cell invasion. Int. J. Cancer **125**, 816–825

140 Gotlieb, W. H., Feldman, B., Feldman-Moran, O., Zmira, N., Kreizer, D., Segal, Y., Elran, E. and Ben-Baruch, G. (2002) Intraperitoneal pressures and clinical parameters of total paracentesis for palliation of symptomatic ascites in ovarian cancer. Gynecol. Oncol. **71**, 381–385

141 Dembo, A. J., Davy, M., Stenwig, A. E., Berle, E. J., Bush, R. S. and Kjorstad, K. (1990) Prognostic factors in patients with stage I epithelial ovarian cancer. Obstet. Gynecol. **75**, 263–273

142 Stanojevic, Z., Rancic, G., Radic, S., Potic-Zecevic, N., Dordevic, B., Markovic, M. and Todorovska, I. (2004) Pathogenesis of malignant ascites in ovarian cancer patients. Arch. Oncol. **12**, 115–118

143 Holm, A., Halpern, N. B. and Aldnek, J. S. (1989) Peritoneovenous shunt for intractable ascites of hepatic nephrogenic and malignant causes. Am. J. Surg. **158**, 162–166

144 Yazdi, G. P., Miedema, B. W. and Humphrey, L. J. (1996) High mortality after abdominal operation in patients with large-volume malignant ascites. J. Surg. Oncol. **62**, 93–96

145 McNamara, P. (2000) Paracentesis: an effective method of symptom control in the palliative care setting? Palliative Med. **14**, 62–66

146 Cowden-Dahl, K. D., Symowicz, J., Ning, Y., Gutierrez, E., Fishman, D. A., Adley, B. P., Stack, M. S. and Hudson, L. G. (2008) Matrix metalloproteinase 9 is a mediator of epidermal growth factor-dependent E-cadherin loss in ovarian carcinoma cells. Cancer Res. **68**, 4606–4613

147 Said, N. A., Najwer, I., Socha, M. J., Fulton, D. J., Mok, S. C. and Motamed, K. (2007) SPARC inhibits LPA-mediated mesothelial–ovarian cancer cell crosstalk. Neoplasia **9**, 23–35

148 Ingber, D. E. (2008) Tensegrity-based mechanosensing from macro to micro. Prog. Biophys. Mol. Bio. **97**, 163–179

149 Paszek, M. J., Zahir, N., Johnson, K. R., Lakins, J. N., Rozenberg, G. I., Gefen, A., Reinhart-King, C. A., Margulies, S. S., Dembo, M., Boettiger, D. et al. (2005) Tensional homeostasis and the malignant phenotype. Cancer Cell **8**, 241–254

150 Thamilselvan, V. and Basson, M. D. (2004) Pressure activates colon cancer cell adhesion by inside-out focal adhesion complex and actin cytoskeletal signalling. Gastroenterology **126**, 8–18

151 Ingber, D. E. (2005) Cell tension, matriz mechanics, and cancer development. Cancer Cell **8**, 175–176

152 Butcher, D. T., Alliston, T. and Weaver, V. M. (2009) A tense situation: forcing tumor progression. Nat. Rev. Cancer **9**, 108–122

153 Condeelis, J. and Segall, J. E. (2003) Intravital imaging of cell movement in tumours. Nat. Rev. Cancer **3**, 921–930
154 Wyckoff, J. B., Pinner, S. E., Gschmeissner, S., Condeelis, J. S. and Sahai, E. (2006) ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion *in vivo*. Curr. Biol. **16**, 1515–1523
155 Avvisato, C. L., Yang, X., Shah, S., Hoxter, B., Li, W., Gaynor, R., Pestell, R., Tozeren, A. and Byers, S. W. (2007) Mechanical force modulates global gene expression and β-catenin signalling in colon cancer cells. J. Cell Sci. **120**, 2672–2682
156 Whitehead, J., Vignjevic, D., Futterer, C., Beaurepaire, E., Robine, S. and Farge, E. (2008) Mechanical factors activates β-catenin-dependent oncogene expression in $APC^{1638N/+}$ mouse colon. HFSP J. **2**, 286–294
157 Fodde, R. and Brabletz, T. (2007) Wnt/β-catenin signalling in cancer stemness and malignant behavior. Curr. Opin. Cell Biol. **19**, 150–158
158 Zhang, S., Balch, C., Chan, M. W., Lai, H. C, Matei, D., Schilder, J. M, Yan, P. S., Huang, T. H. and Nephew, K. P. (2008) Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res. **68**, 4311–4320
159 Barker, N. and Clevers, H. (2006) Mining the Wnt pathway for cancer therapeutics. Nat. Rev. Drug Discovery **5**, 997–1014
160 Cho, N. L., Lin, C. I., Whang, E. E., Carothers, A. M., Moore, Jr, F. D. and Ruan, D. T. (2010) Sulindac reverses aberrant expression and localization of beta-catenin in papillary thyroid cancer cells with the BRAFV600E mutation. Thyroid. **20**, 615–622
161 Luo, J., Chen, J., Deng, Z. L., Luo, X., Song, W. X., Sharff, K. A., Tang, N., Haydon, R. C., Luu, H. H. and He, T. C. (2007) Wnt signalling and human diseases: what are the therapeutic implications? Lab. Invest. **87**, 97–103
162 Takahashi-Yanaga, F. and Kahn, M. (2010) Targeting Wnt signalling: can we safely eradicate cancer stem cells? Clin. Cancer Res. **16**, 3153 – 3162
163 Lepourcelet, M., Chen, Y. N. P., France, D. S., Wang, H., Crews, P., Petersen, F., Bruseo, C., Wood, A. W. and Shivdasani, R. A. (2004) Small molecule antagonists of the oncogenic Tcf/β-catenin protein comples. Cancer Cell **5**, 91–102
164 Chen, Z., Venkatesan, A. M., Dehnhardt, C. M., Dos Santos, O., Delos Santos, E., Ayral-Kaloustian, S., Chen, L., Geng, Y., Arndt, K. T., Lucas, J. et al. (2009) 2,4-Diamino-quinazolines as inhibitors of β-catenin/Tcf-4 pathway: potential treatment for colorectal cancer. Bioorg. Med. Chem. Lett. **19**, 4980–4983
165 Dehnhardt, C. M., Venkatesan, A. M., Chen, Z., Ayral-Kaloustian, S., Dos Santos, O., Delos Santos, E., Curran, K., Follettie, M. T., Diesl, V., Lucas, J., Geng, Y. et al. (2010) Design and synthesis of novel diaminoquinazolines with *in vivo* efficacy for β-catenin/T-cell transcriptional factor 4 pathway inhibition. J. Med. Chem. **53**, 897–910
166 Huang, S. M., Mishina, Y. M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G. A., Charlat, O., Wiellette, E., Zhang, Y. et al. (2009) Tankyrase inhibition stabilizes Axin and antagonizes Wnt signalling. Nature **461**, 614–620
167 Barbieri, F., Lorenzi, P., Ragni, N., Schettini, G., Bruzzo, C., Pedulla, F. and Alama, A. (2004) Overexpression of cyclin D1 is associated with poor survival in epithelial ovarian cancer. Oncology **66**, 310–315
168 Dhar, K. K., Branigan, K., Parkes, J., Howells, R. E., Hand, P., Musgrove, C., Strange, R. C., Fryer, A. A., Redman, C. W. and Hoban, P. R. (1999) Expression and subcellular localization of cyclin D1 protein in epithelial ovarian tumour cells. Br. J. Cancer **81**, 1174–1181
169 Denkert, C., Kobel, M., Pest, S., Koch, I., Berger, S., Schwabe, M., Siegert, A., Reles, A., Klosterhalfen, B. and Hauptmann, S. (2002) Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. Am. J. Pathol. **160**, 893–903
170 Cowden-Dahl, K. D., Symowicz, J., Ning, Y., Gutierrez, E., Fishman, D. A., Adley, B. P., Stack, M. S. and Hudson, L. G. (2008) Matrix metalloproteinase 9 is a mediator of epidermal growth factor-dependent E-cadherin loss in ovarian carcinoma cells. Cancer Res. **68**, 4606–4613
171 Boocock, C. A., Charnock-Jones, D. S., Sharkey, A. M., McLaren, J., Barker, P. J., Wright, K., Twentyman, P. R. and Smith, S. K. (1995) Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. J. Natl. Cancer Inst. **87**, 506–516
172 Lee, J. H., Kang, Y. S., Kim, B. G., Park, S. Y., Lee, E. D., Lee, K. H. and Park, K. B. (1995) Expression of the CD44 adhesion molecule in primary and metastatic gynecologic malignancies and their cell lines. Int. J. Gynecol. Cancer **5**, 193–199
173 Di Renzo, M. F., Olivero, M., Katsaros, D., Crepaldi, T., Gaglia, P., Zola, P., Sismondi, P. and Comoglio, P. M. (1994) Overexpression of the Met/HGF receptor in ovarian cancer. Int. J. Cancer **58**, 658–662
174 Watson, J. V., Curling, O. M., Munn, C. F. and Hudson, C. N. (1987) Oncogene expression in ovarian cancer: a pilot study of c-myc oncoprotein in serous papillary ovarian cancer. Gynecol. Oncol. **28**, 137–150
175 Chambers, S. K., Gertz, Jr, R. E., Ivins, C. M. and Kacinski, B. M. (1995) The significance of urokinase-type plasminogen activator, its inhibitors, and its receptor in ascites of patients with epithelial ovarian cancer. Cancer **75**, 1627–1633
176 Sier, C. F., Stephens, R., Bizik, J., Mariani, A., Bassan, M., Pedersen, N., Frigerio, L., Ferrari, A., Dano, K., Brunner, N. and Blasi, F. (1998) The level of urokinase-type plasminogen activator receptor is increased in serum of ovarian cancer patients. Cancer Res. **58**, 1843–1849
177 Tanimoto, H., Underwood, L. J., Shigemasa, K., Parmley, T. H., Wang, Y., Yan, Y., Clarke, J. and O'Brien, T. J. (1999) The matrix metalloprotease pump-1 (MMP-7, matrilysin): a candidate marker/target for ovarian cancer detection and treatment. Tumour Biol. **20**, 88–98
178 Soini, Y. and Talvensaari-Mattila, A. (2006) Expression of claudins 1, 4, 5, and 7 in ovarian tumors of diverse types. Int. J. Gynecol. Pathol. **25**, 330–335
179 Yoshida, H., Ishiko, O., Sumi, T., Matsumoto, Y. and Ogita, S. (2001) Survivin, bcl-2 and matrix metalloproteinase-2 enhance progression of clear cell- and serous-type ovarian carcinomas. Int. J. Oncol. **19**, 537–542
180 Choi, J. H., Park, J. T., Davidson, B., Morin, P. J., Shih Ie, M. and Wang, T. L. (2008) Jagged-1 and Notch3 juxtacrine loop regulates ovarian tumor growth and adhesion. Cancer Res. **68**, 5716–5723
181 Malki, S., Bibeau, F., Notarnicola, C., Roques, S., Berta, P., Poulat, F. and Boizet-Bonhoure, B. (2007) Expression and biological role of the prostaglandin D synthase/SOX9 pathway in human ovarian cancer cells. Cancer Lett. **255**, 182–193
182 Ye, F., Li, Y., Hu, Y., Zhou, C., Hu, Y. and Chen, H. Expression of Sox2 in human ovarian epithelial carcinoma. J. Cancer Res. Clin. Oncol. **137**, 131–136
183 Puri, R., Tousson, A., Chen, L. and Kakar, S. S. (2001) Molecular cloning of pituitary tumor transforming gene 1 from ovarian tumors and its expression in tumors. Cancer Lett. **163**, 131–139
184 Ripley, D., Tunuguntla, R., Susi, L. and Chegini, N. (2006) Expression of matrix metalloproteinase-26 and tissue inhibitors of metalloproteinase-3 and -4 in normal ovary and ovarian carcinoma. Int. J. Gynecol. Cancer **16**, 1794–1800
185 Blechschmidt, K., Sassen, S., Schmalfeldt, B., Schuster, T., Hofler, H. and Becker, K. F. (2008) The E-cadherin repressor Snail is associated with lower overall survival of ovarian cancer patients. Br. J. Cancer **98**, 489–495
186 Welt, C. K., Lambert-Messerlian, G., Zheng, W., Crowley, Jr, W. F. and Schneyer, A. L. (1997) Presence of activin, inhibin, and follistatin in epithelial ovarian carcinoma. J. Clin. Endocrinol. Metab. **82**, 3720–3727
187 Kaiser, P. C., Korner, M., Kappeler, A. and Aebi, S. (2005) Retinoid receptors in ovarian cancer: expression and prognosis. Ann. Oncol. **16**, 1477–1487
188 Mueller, J., Brebeck, B., Schmalfeldt, B., Kuhn, W., Graeff, H. and Hofler, H. (2000) Stromelysin-3 expression in invasive ovarian carcinomas and tumours of low malignant potential. Virchows Arch. **437**, 618–624
189 Rask, K., Nilsson, A., Brannstrom, M., Carlsson, P., Hellberg, P., Janson, P. O., Hedin, L. and Sundfeldt, K. (2003) Wnt-signalling pathway in ovarian epithelial tumours: increased expression of β-catenin and GSK3β. Br. J. Cancer **89**, 1298–1304

Received 18 January 2011/23 February 2011; accepted 1 March 2011
Published on the Internet 14 June 2011, doi:10.1042/BJ20110112