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Wnt signaling in ovarian tumorigenesis

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Abstract

Data are emerging implicating Wnt signaling in ovarian tumorigenesis. We sought to review the current literature on the subject and discuss the pathway's potential role as a prognostic marker and therapeutic target. We conducted a systematic literature review of studies investigating the association between Wnt signaling and ovarian cancer. Search strategies included online searching of the MEDLINE database and hand searching of relevant publications and reviews. Additional reports were collected by systematically reviewing all references from retrieved papers. Twenty-nine papers were identified that directly investigate Wnt signaling and ovarian cancer. Mutations in the *CTNNB1* gene that codes for β -catenin, the key effector in the pathway, are directly linked to carcinogenic transformation but are mostly found in ovarian endometrioid adenocarcinomas, a histologic subtype of epithelial ovarian cancer. These mutations, along with others, lead to deregulation of the pathway and transcription of target genes. Differences in various intra- and extracellular components of the Wnt pathway have been demonstrated between normal ovarian and cancer cell lines and between benign tissue and ovarian cancer. These differences implicate Wnt signaling in the molecular events that lead to ovarian cancer development despite the fact that gene mutations are uncommon. The data suggest that Wnt signaling plays a role in ovarian tumorigenesis. The exact mechanisms by which this occurs need to be further elucidated. Wnt signaling is probably involved via multiple, diverse mechanisms. Further research in this area is warranted.

Keywords

ovarian cancer; tumorigenesis; Wnt signaling

Ovarian cancer is the fifth leading cause of death from cancer in women and is the leading cause of death from gynecological cancer. In 2007, it is estimated that there will be 22,430 new ovarian cancer cases with 15,280 deaths in the United States(1). The disease has an insidious onset, often presenting with vague nonspecific symptoms such as increasing abdominal girth, early satiety, and a change in bowel and/or bladder habits(2). Most ovarian cancer presents as stage III or IV disease. Worldwide, less than 30% of those with advanced ovarian cancer survive 5 years after initial diagnosis(3). Epithelial ovarian cancers (EOCs) arise from the ovarian surface epithelium (OSE) and account for greater than 80% of all ovarian

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cancers. They tend to become more poorly differentiated than their tissue of origin and occur as one of the five histologic subtypes: serous, endometrioid, mucinous, clear cell, and undifferentiated, with serous being the most common (.75%). The remaining types of ovarian cancer originate from germ cells and stromal elements in the ovary.

The Wnt pathway participates in many physiologic events in embryogenesis and adult homeostasis including cell fate specification, control of proliferation, and migration. Wnt signaling plays a key role in the embryonic development of the ovary(4,5) and is also involved in normal follicular development and ovarian function(6). The Wnt signaling pathway has been well studied in a number of cancers, most notably colorectal carcinoma. In the majority of colorectal cancers, the mutational inactivation of adenomatous polyposis coli (APC), an important component in the Wnt pathway, is an early event(7). This leads to the stabilization of the cytoplasmic pool of β -catenin leading to its accumulation and translocation to the nucleus where it associates with T-cell factor/lymphoid enhancer factor-1 (TCF/LEF1) and promotes transcription of target genes. Some colorectal cancers exhibit constitutive β -catenin/TCF transcriptional activity despite the lack of an inactivating APC mutation. This has been shown to result from activating β -catenin gene mutations(8). Thus, an inactivating APC gene mutation approximates an activating β -catenin gene mutation: both lesions finally lead to the initiation of constitutive β -catenin/TCF-mediated transcription and colorectal cancer progression.

In the late 1990s, several authors began to detect the presence of β -catenin gene mutations in ovarian cancer, mostly confined to the endometrioid subtype(9–12). These findings led to interest in whether Wnt signaling played a role in ovarian tumorigenesis. Since then, compelling data have emerged implicating components of the Wnt signaling pathway in the molecular events that lead to ovarian cancer development despite the fact that gene mutations are uncommon. Unlike colorectal cancer, a clear-cut, obvious etiologic role such as that involving APC has, to date, not been discovered. Instead, the evidence suggests that Wnt signaling is probably involved via multiple, diverse mechanisms. In this review, we present a brief overview of the pathway's components and the process of Wnt signal transduction. The current literature implicating the Wnt signaling pathway in ovarian tumorigenesis and its potential as a prognostic marker and therapeutic target in ovarian cancer are reviewed and discussed.

Essential components of Wnt signaling

Extracellular and cell membrane components

Wnts are secreted glycoprotein-signaling molecules that bind a transmembrane receptor complex. Wnts exert their effect via one of the three pathways: the “canonical” *Wnt/ β -catenin* pathway, on which the majority of published research focuses, or one of several “noncanonical” pathways. The key effector of the canonical Wnt pathway is β -catenin, a multifunctional protein that also mediates cell–cell adhesion with E-cadherin at the cell surface (13). The common initial step in all three Wnt pathways is the binding of Wnt ligand to a frizzled (FZD) receptor. Nineteen Wnt ligands and ten FZD receptors have been identified in the human genome (<http://www.stanford.edu/~Ernusse/wntwindow.html>). Wnt ligands have been classified into two functional groups. The first group is the *proto-oncogenic* group and activates the canonical β -catenin pathway. It includes Wnt 1, -3a, -8, and -8a. The second group activates the noncanonical pathways and can antagonize the activity of the *proto-oncogenic* Wnts.

In addition to the seven-pass transmembrane FZD receptors, the pathway requires an additional single-pass transmembrane protein, low-density lipoprotein receptor-related protein (LRP). FZD and LRP are generally thought to associate only in the presence of Wnt ligand to form a trimeric complex. Extracellular Wnt antagonists fall into several families, which can be

subdivided into two groups depending on their mode of action. The first subgroup inhibits Wnt signaling by directly binding to Wnt molecules. This group includes Wnt inhibitory factor-1 (WIF-1) and secreted frizzled-related proteins (SFRPs). The second group inhibits Wnt signaling by binding the LRP5 or LRP6 component of the receptor complex and includes the Dickkopf family and sclerostin.

Cytoplasmic components

The tumor suppressors Axin and APC form a structural scaffold that binds β -catenin and presents it to glycogen synthase kinase-3 β (GSK3 β) for phosphorylation. Truncating *Axin1* mutations are found in hepatocellular carcinomas illustrating its relevance to β -catenin regulation in cancer(14). APC, a large (312 kd) protein, has diverse cellular functions that include adhesion, migration, cell-cycle regulation, and chromosome stability. It is mutated in 85% of familial and sporadic colorectal cancers(15). GSK3 β , a normally active kinase in unstimulated resting cells, is a key component in many other cellular pathways(16).

Dishevelled (DVL) is an intracytoplasmic protein that sits at the signaling crossroads of the canonical and noncanonical Wnt pathways(17). Several proteins promote activity in one pathway while inhibiting the other through their interaction with DVL. Casein kinase-1 binds to and phosphorylates DVL promoting its function in the canonical pathway.

Nuclear components

The TCF/LEF family of transcription factors consists of four members: TCF1, LEF1, TCF3, and TCF4. β -catenin is an essential cofactor for the TCF/LEF1 family but does not bind DNA directly. The mechanistic details of this interaction are still unclear. Direct physical interaction of at least two different gene products, Groucho and C-terminal-binding protein, represses TCF/LEF gene expression (<http://www.stanford.edu/~Ernusse/wntwindow.html>). Two other important nuclear players are legless (LGS) and its binding partner pygopus (PYGO)(18). LGS is proposed to regulate TCF/ β -catenin binding by primarily functioning to recruit PYGO to β -catenin(19). Along with PYGO, it is also purported to have a role in the nuclear transport of β -catenin(20). PYGO is thought to act either as a nuclear “escort”(21) or as a critical link in the TCF/LEF complex, recruiting the basal transcription machinery(22).

Wnt pathway signal transduction

In the absence of Wnt signaling, β -catenin localizes to the cytoplasm and is phosphorylated by a multiprotein complex that includes GSK3 β , APC, and Axin. GSK3 β phosphorylates APC and Axin, which form a structural scaffold, increasing their binding affinities for β -catenin, which is also phosphorylated by GSK3 β . Phosphorylated β -catenin is subsequently ubiquitinated by β -transducin repeat-containing protein and degraded, limiting the free intracytoplasmic pool.

In the presence of Wnt signaling, extracellular Wnt ligand binds a receptor complex encompassing a FZD receptor and a LRP coreceptor. This leads to the phosphorylation of DVL by casein kinase-1 inactivating GSK3 β and causing its dissociation from Axin preventing the phosphorylation of β -catenin. Nonphosphorylated β -catenin is not recognized by β -transducin repeat-containing protein and avoids degradation. The intracytoplasmic pool of β -catenin thus increases and it translocates to the nucleus.

In the nucleus, TCF/LEF binds directly to DNA via their high-mobility group domains. However, they are incapable of independently activating gene transcription. In the absence of nuclear β -catenin, TCF/LEF acts as a transcriptional repressor through their interaction with corepressors such as Groucho. In the presence of nuclear β -catenin, LGS recruits PYGO to β -catenin and TCF/LEF is converted from a transcriptional repressor into an activator by complex

formation with β -catenin and coactivators such as CREB (cAMP [cyclic adenosine monophosphate] response element-binding protein)-binding protein. The β -catenin–TCF/LEF complex induces the expression of downstream target genes (Fig. 1).

Alterations of the Wnt pathway in ovarian cancer

β -catenin

CTNNB1 gene mutations—The human *CTNNB1* gene encodes β -catenin and maps to chromosome 3p21. Mutations in the exon 3 domain of *CTNNB1* have been demonstrated in ovarian cancer by a number of studies(9–12,23–25). Activating mutations of *CTNNB1* have been previously identified in colon(26) and melanoma(27) carcinoma cell lines and in primary colon(8) and endometrial(28) tumors. Missense mutations affect the NH₂-terminal regulatory domain of β -catenin (codons 32–45), which overlaps with the consensus sites for GSK3 β phosphorylation and ubiquitin–proteasome degradation. The mutations presumably render the mutant proteins resistant to degradation and occur almost exclusively in the endometrioid subtype. One study reports a mutation in a mucinous tumor(11). In aggregate, the studies in Table 1 suggest that approximately 30% of primary ovarian endometrioid adenocarcinomas (OEs) harbor a *CTNNB1* mutation (range: 16–54%).

In addition to primary tumors, Wu *et al.*(23) analyzed two endometrioid cell lines with elevated constitutive β -catenin/TCF transcriptional activity. TOV-112D cells were shown to harbor mutant β -catenin, and MDHA-2774 cells had a missense Axin1 sequence alteration.

β -catenin subcellular localization and clinicopathologic features—Much has been written about β -catenin expression patterns in ovarian cancer and the reports can be contradicting. However, it appears consistent that aberrant nuclear β -catenin is predominantly expressed in OEs(10,29). In other subtypes, including serous and mucinous, the predominant aberrant expression pattern is cytoplasmic(29,30). Several studies have attempted to find associations between β -catenin expression pattern and pathologic features or clinical outcomes (10,29,31,32). Variations in patient numbers and histologic subtypes make generalization difficult. Gamallo *et al.*(10) found that nuclear β -catenin expression was an indicator of good prognosis mostly seen in OEs. They surmised that OEs with exclusively membranous β -catenin expression may represent a different subgroup with a worse prognosis. Other studies (29,32) reported similar findings, also showing that patients with low-grade differentiation tumors had higher nuclear β -catenin expression than high-grade tumors(29). Lee *et al.*(31) studied serous carcinomas since they are the most common histologic subtype. They found a statistically significant correlation ($P = 0.006$) between nuclear β -catenin expression and high-grade serous carcinomas. They also noted a trend toward decreased survival in the same patients.

Genetics

Other mutations in endometrioid adenocarcinomas—The interaction between β -catenin, APC, and Axin is crucial for β -catenin phosphorylation and subsequent degradation. Deregulation of β -catenin has also been shown to possibly result from mechanisms other than *CTNNB1* mutation(23). One of the two primary tumors with nuclear β -catenin accumulation in the absence of a *CTNNB1* mutation harbored nonsense APC mutations resulting in stop codons. Tumors with wild-type *CTNNB1* alleles were also examined for *Axin1* and *Axin2* mutations. A truncating *Axin1* mutation was found in one tumor, and a frameshift *Axin2* mutation was found in another. In the MDHA-2774 cell line, a missense sequence alteration in Axin1 was identified affecting a residue thought to lie with the β -catenin-binding region. These findings illustrate the likelihood that diverse mechanisms are involved in deregulating β -catenin in OEs (Table 2).

Target genes/gene expression analysis—Several genes have been proposed as targets for β -catenin/TCF regulation in model organisms. Almost all have been proposed based on *in vitro* data or animal studies in colorectal cancer. Zhai *et al.*(33) investigated six putative β -catenin/TCF target genes in a panel of 44 primary OEAs previously characterized for specific defects in β -catenin regulation(23). Comparison between tumors of differing genotype (deregulated versus intact β -catenin) in this single, well-defined histologic subtype was employed as a potentially more robust approach for discerning those genes with a critical role. Using quantitative assays, they found significantly elevated expression for five of the six genes analyzed in tumors with deregulated β -catenin: connexin 43, immunoglobulin transcription factor 2, peroxisome proliferator-activated receptor delta, cyclin D1, and matrix metalloproteinase 7. For one of the genes with the highest transcript levels—namely, *cyclin D1*, immunohistochemical staining was used to evaluate if protein expression correlated with transcription level. Positive staining was observed in 69% of tumors with β -catenin deregulation in contrast to 7% of those with intact β -catenin regulation. While the functional significance of these increased gene expressions remains to be demonstrated, the study provides supporting evidence for the assumption that there is direct regulation of these genes by β -catenin/TCF.

Differential expression analysis of genes in the Wnt pathway has been performed using low-density micro-arrays(34). A number of genes that exhibit either tumor-specific expression when compared to normal OSE or significantly ($P < 0.025$) downregulated expression have been identified. The list includes *Wnt10B*, *Wnt9B*, and *WIF-1*. High-throughput microarray technology has also been used to identify possible target genes of β -catenin/TCF transcription in OEA tumor samples with and without Wnt pathway defects(35). Over 80 potential targets were identified with differential expression of several genes confirmed by reverse transcription–polymerase chain reaction.

In-depth investigation of one such target gene, *fibroblast growth factor-9 (FGF9)*, has shown it to be a key factor contributing to the cancer phenotype in OEA with Wnt pathway defects (36). It was shown to be an indirect downstream transcriptional target of β -catenin/TCF and to promote the neoplastic transformation of epithelial cells. This is relevant since OSE is the origin of most ovarian adenocarcinomas. FGFs are a highly conserved family of secreted, glycosylated proteins. FGF20 is likely a direct β -catenin/TCF target that is upregulated in OEA (37). The receptor FGF R 2-IIIb has been characterized in EOC and implicated in ovarian cancer development(38).

Gene silencing—SFRPs are extracellular inhibitors of the Wnt pathway. The *SFRP1* gene is located on chromosome 8p11.2, which is frequently lost in ovarian cancer(39). *SFRP1* is inactivated by promoter methylation in colon cancer enhancing constitutive Wnt signaling even in the presence of β -catenin or APC mutations(40). Thirteen ovarian cancer cell lines and 17 primary ovarian tumors were analyzed for methylation-associated silencing of the *SFRP1* gene (41). Four cell lines and two tumors had methylated *SFRP1*, while an immortalized ovarian epithelial cell line and seven benign ovarian cyst samples did not. Complete absence of expression in the four cell lines was confirmed by quantitative reverse transcription–polymerase chain reaction. While the sample size was small, *SFRP1* silencing tended to occur in the mucinous subtype. It is possible that *SFRP1* inactivation could play a role in ovarian cancer development perhaps explaining why some ovarian cancers show increased β -catenin expression in the absence of gene mutations(30). Gastric and other cancers with fibroblastoid morphology demonstrate increased invasiveness due to weaker cell–cell adhesion similar to the epithelial-to-mesenchymal transition seen during embryonic development(42). A decrease in *SFRP1* induces epithelial-to-mesenchymal transition in gastric cancer through GSK3 β downregulation. Other factors have been shown to promote epithelial-to-mesenchymal

transition in ovarian cancer(43). However, the role of *SFRP1* and other Wnt inhibitors to promote this transition remains to be analyzed.

Cytoplasmic changes

APC expression was investigated by immunohistochemistry in 113 primary and metastatic serous ovarian tumors(44). APC was found to be absent in a considerable number (41%) of tumors and in all tumors (3%) with nuclear β -catenin staining. Rask *et al.*(30) evaluated the protein expression and localization of GSK3 β in ovarian cancer. They investigated a normal ovarian cell line (IOSE), an ovarian cancer cell line (NIH-OVCAR-3), as well as tissue from normal ovary, borderline-type tumor, adenocarcinoma, and peritoneal metastases. They found statistically significant increases in GSK3 β expression ($P < 0.001$) in the group of adenocarcinomas as compared to the group of normal ovaries and benign adenomas/borderline tumors.

Frequently rearranged in advanced T-cell lymphomas 1 (FRAT1) binds GSK3 β and inhibits the phosphorylation of β -catenin in the presence of Wnt signaling. Thus, it is a positive regulator of the Wnt signaling pathway. Overexpression of FRAT1 leads to β -catenin stabilization and nuclear translocation(45). In serous subtypes, constitutive Wnt throughput and pathway mutations are uncommon and aberrant cytoplasmic staining of β -catenin without nuclear accumulation is predominant. Recently, FRAT1 has been shown to be overexpressed in ovarian serous adenocarcinomas and strongly correlated with the subcellular localization of β -catenin ($P < 0.01$)(46). This raises the possibility that FRAT1 may modulate the Wnt pathway in serous subtypes; however, the mechanism behind the overexpression and its significance are not yet elucidated.

Nuclear changes

The PYGO proteins play a key role in the β -catenin/TCF transcriptional complex. It is proposed that PYGO acts as an activator of the complex(47). The role of *hPygo2*, one of the two PYGO genes in humans, was recently investigated with interesting results(48). Overexpression of *hPygo2* was found in six EOC cell lines and 82% of patient samples compared to control cells and benign disease. When *hPygo2* was depleted using antisense oligonucleotides, growth was halted in both Wnt-active and Wnt-inactive cell lines. Thus, *hPygo2* appears to be widely expressed and most importantly required in the absence of Wnt signaling for the malignant growth of EOC.

Granulosa cell tumors

Sex cord/stromal tumors comprise up to 10% of ovarian neoplasms with granulosa cell tumors (GCTs) as the most prevalent subtype, constituting approximately 5% of all ovarian cancers (49,50). Boerboom *et al.*(51) sought to investigate the role of Wnt signaling in GCT etiology. As GCTs are the most common neoplastic disease of the ovary in domestic species, such as cow, ewe, and mare, they analyzed 6 human and 18 equine GCTs by immunohistochemistry. Nuclear localization of β -catenin was found in 15 of the 24 samples. They generated transgenic mice (*Catnb*^{flox(ex3)/+}; *Amhr2*^{cre/+}) that expressed a dominant stable mutant of β -catenin in granulosa cells. These mice developed follicle-like lesions containing disorganized, pleomorphic granulosa. In older mice, these lesions developed into GCTs in 57% of the mice by 7.5 months of age, bearing many histopathologic similarities to human disease. They postulated that the lesions represent a pretumoral intermediate stage in GCT development.

Subsequently, they undertook gene expression analysis using microarrays comparing ovaries with *Catnb*^{flox(ex3)/+}; *Amhr2*^{cre/+} pretumoral lesions to normal controls(52). They found overexpression of Wnt pathway antagonists WIF-1, naked cuticle-1, and Axin2 in pretumoral lesions. The antagonist expression pattern likely represents the simultaneous induction of

multiple feedback loops. Not surprisingly, this unusual scenario still failed to counteract endogenous signaling that results from mutated β -catenin in these mice since the antagonists act upon or on pathway components upstream of β -catenin. Hence, constitutive activation of Wnt signaling during ovarian development leads to metaplastic cell types that are likely a key intermediate step in GCT development in this model.

Potential therapeutic targets of the Wnt pathway in ovarian cancer

Interest in Wnt signaling inhibitors has been strong for many years given the implication of the Wnt pathway in the pathophysiology of many human diseases. Therapeutic targets of the Wnt pathway are well described in many disease processes. Perhaps, the best known class of these is nonsteroidal antiinflammatory drugs. Aspirin, indomethacin, and sulindac have all been shown to possess anticancer properties(53,54). Nonsteroidal antiinflammatory drugs have been shown to inhibit β -catenin nuclear localization and β -catenin/TCF gene transcription (55). Lithium and valproic acid are established drugs in the treatment of many psychiatric disorders. Both are nonselective GSK3 β inhibitors.(56,57)

Increasingly, research is focusing on the design of targeted small molecule antagonists. Novel strategies for drug design are emerging with promising results. Recently, high-throughput screening of approximately 7000 natural compounds yielded eight compounds displaying reproducible and dose-dependent inhibition of β -catenin and TCF binding in an immunoenzymatic assay(58). Three of the screened compounds, (PF115–584, PKF222–815, and CGP049090) (Fig. 2), all with the same core structure, scored consistently in the secondary *in vitro* assays used to confirm the antagonistic properties of the compounds. This illustrates that high-throughput screening methods using cell-free assays can yield inhibitors that retain their activity when tested in cell-based assays.

Imatinib mesylate or *Gleevec* is the first such rationally designed molecularly targeted drug and was developed as an inhibitor of Bcr–Abl kinase. It was shown to induce almost complete cytogenic remissions in patients with chronic myeloid leukemia(59). In addition to serine/threonine phosphorylation, β -catenin activity is also regulated by tyrosine kinase via growth factors such as hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-1, and -2. It is hypothesized that the tyrosine kinase phosphorylation of β -catenin results in decreased association with E-cadherin causing junctional assembly disruption and increased cancer invasiveness and metastasis(60). *Gleevec* has been shown to effectively inhibit constitutive β -catenin signaling and to suppress proliferation in human colon cancer cell lines(60). Tyrosine phosphorylation of β -catenin may therefore play an important role in Wnt signaling, and inhibition by *Gleevec* should be explored as an alternative/adjuvant treatment of ovarian cancer.

There are two novel classes of Wnt pathway targets that could be further investigated in ovarian cancer based on data from the studies presented: extracellular Wnt antagonists and TCF transcription cofactors. As reviewed previously, SFRP and WIF-1 are extracellular inhibitors that directly bind Wnt ligand. SFRP1, -2, and -5 are inactivated by promoter methylation in colorectal cancer(40,61). Inactivation of *SFRP* genes enhanced constitutive Wnt signaling even in the presence of *APC* or β -catenin mutations(62). Takada *et al.*(41) showed that *SFRP1* is inactivated by promoter methylation in ovarian cancers. They also were able to treat a cell line harboring a silenced *SFRP* gene with a demethylating agent causing reexpression of SFRP1. Two other studies, each in different types of ovarian cancer, have also suggested alterations in Wnt antagonists when compared to normal controls. Steg *et al.*(34) identified tumor-specific WIF-1 expression in OEA tumor specimens with no detectable expression in normal OSE using TaqMan low-density array. In a murine GCT model expressing dominant stable mutant β -catenin, WIF-1 was found to be overexpressed along with another antagonist Dickkopf 4(52).

These studies show clear alterations in Wnt antagonists in ovarian cancer. Manipulation of Wnt signaling via mechanisms involving extracellular antagonists should be explored.

Given the dedicated role of PYGO in canonical Wnt signaling, it would not be expected to have a significant role in nonendometrioid EOC. However, Popadiuk *et al.*(48) have shown otherwise. They showed that hPygo2 is overexpressed in all six of the EOC cell lines they tested relative to normal OSE. Only one cell line (TOV-112D) was endometrioid in histologic subtype. Analysis of 125 archived tumor samples representing all histologic subtypes showed moderate to strong staining for nuclear hPygo2 in 82% of the malignant samples, whereas staining was undetectable or very weak in benign samples. They were able to knockdown hPygo2 using antisense oligonucleotides in two cell lines known to lack TCF-dependent transcription. Surprisingly, this caused growth arrest in these Wnt-inactive ovarian cancer cell lines. When the antisense knockdown was reversed, the significant reduction in cell numbers was restored to the level of controls. These findings suggest that PYGO may have a role outside of Wnt signaling or that it is able to activate downstream effectors of Wnt signaling in EOC in the absence of upstream signaling components. Hence, inhibition of PYGO appears to be a prime therapeutic target requiring further investigation in EOC.

Final comment

Based on the current literature, it appears that Wnt signaling plays a role in ovarian tumorigenesis. It is likely that the pathway is involved in ovarian cancer development via multiple, diverse mechanisms, such as gene mutations, and changes in pathway components such as extracellular inhibitors and intranuclear transcription cofactors. Understanding how the Wnt signaling pathway induces genes involved in tumor growth and invasion is a significant challenge in the study of ovarian carcinogenesis. Further investigation is necessary for the application of targeted therapy.

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Figure 1.
Schema of the Wnt signaling pathways.

Figure 2.
Three of the screened compounds.

Table 1

β-catenin mutations in human ovarian cancer

Histology	D32	S33	G34	S37	T41	S45	%	Frequency	Reference	Year
Endometrioid				2	1		50	3/6	Palacios and Gamallo(9)	1998
Endometrioid				1	2		33	4/12	Sagae <i>et al.</i> (11)	1999
Endometrioid	3	1		2	1		54	7/13	Gamallo <i>et al.</i> (10)	1999
Endometrioid		2	2	6			16	10/63	Wright <i>et al.</i> (12)	1999
Endometrioid	3	4	4	3		1	32	14/45	Wu <i>et al.</i> (23)	2001
Endometrioid	1	1	2	2			38	8/21	Moreno-Bueno <i>et al.</i> (24)	2001
Endometrioid		2	1	2		1	26	6/23	Saegusa(25)	2001
Mucinous							14	1/7	Sagae <i>et al.</i> (11)	1999

Table 2APC and Axin mutations in OEA^a

Mutation	Nucleotide	Amino acid	Codon	Type
APC	CGA—TGA	Arg—Stop	554, 1114	Nonsense
Axin1	AAG—TAG	Lys—Stop	416	Truncating
Axin1	GTA—ATA	Val—Ile	555	Missense
Axin2	G deletion		G665X	Frameshift

^aData from Wu *et al.*(23).