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EXPERT REVIEWS

# Role of canonical Wnt signaling in endometrial carcinogenesis

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While the role of Wnt signaling is well established in colorectal carcinogenesis, its function in gynecologic cancers has not been elucidated. Here, we describe the current state of knowledge of canonical Wnt signaling in endometrial cancer (EC), and its implications for future therapeutic targets. Deregulation of the Wnt/β-catenin signaling pathway in EC occurs by inactivating β-catenin mutations in approximately 10–45% of ECs, and via downregulation of Wnt antagonists by epigenetic silencing. The Wnt pathway is intimately involved with estrogen and progesterone, and emerging data implicate it in other important signaling pathways, such as mTOR and Hedgehog. While no therapeutic agents targeting the Wnt signaling pathway are currently in clinical trials, the preclinical data presented suggest a role for Wnt signaling in uterine carcinogenesis, with further research warranted to elucidate the mechanism of action and to proceed towards targeted cancer drug development.

**Keywords:** β-catenin • canonical Wnt signaling • carcinogenesis • endometrial cancer • novel therapeutic targets • Wnt antagonists

Endometrial cancer (EC) is the most common gynecologic malignancy in the USA, with an estimated 46,470 cases diagnosed in 2011 [1]. It is a heterogeneous disease that can be largely classified into two major types: type I ECs, the most common type, which are usually of endometrioid histology, and are often associated with obesity; versus type II ECs, which are of non-endometrioid histology (e.g., papillary serous or clear cell), are not a result of unopposed estrogen, and usually carry a worse prognosis [2]. Despite a good survival rate for early-stage and Type I ECs, the prognosis for advanced-stage EC has been poor, with survival rates of just 12 months for patients with metastatic EC enrolled in chemotherapy trials [3]. Few effective treatment options are currently available for advanced stage EC, with a limited number of novel biologics showing promise, such as mTOR inhibitors and bevacizumab, both with approximately 14% clinical response rates in Phase II trials [4–6]. There is thus a dire need for a search for further treatment options in advanced EC. Importantly, the molecular pathogenesis of EC is understudied, and research in this field has lagged far behind breast, ovarian, and cervical cancer in terms of grant money allocation and progress. Despite a clinicopathologic model to predict prognosis based on a surgical pathology study carried out by the Gynecologic Oncology Group (GOG) in the 1970s (GOG 33) [7], little is known of the molecular characteristics to predict who will recur, and who should receive what type of treatment (e.g., adjuvant radiation and chemotherapy). Moreover, the response to radiation, cytotoxic or hormonal therapy is difficult to predict. Therefore, identifying novel molecular biomarkers and therapeutic targets is imperative.

The Wingless-type (Wnt) signaling pathways play key roles in embryonic development and maintenance of tissue homeostasis, but additionally regulates diverse developmental processes, such as proliferation, differentiation, motility, and survival and/or apoptosis. Dysregulation of the Wnt pathway has been implicated in a variety of human malignancies, most notably in colorectal cancer (CRC). Greater than 90% of all CRCs carry an activating mutation of the canonical Wnt signaling pathway, most frequently in the form of a mutational inactivation of adenomatous polyposis coli (APC) [8]. This ultimately leads to the stabilization of the cytoplasmic pool of β-catenin, resulting in its accumulation and translocation to the nucleus, where β-catenin associates with T-cell factor (TCF)/lymphoid enhancer factor-1 (LEF1) and

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promotes transcription of target genes. Some CRCs 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 [9]. 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 CRC progression.

Since these key findings in CRC, the role of Wnt signaling in carcinogenesis in many other solid tumors has been explored, including melanoma, osteosarcoma, other gastrointestinal cancers, prostrate, breast, liver, lung and ovarian cancer [10]. In the late 1990s, investigations into the role of Wnt signaling in uterine cancers have primarily focused on findings of β-catenin gene mutations. While activating β-catenin mutations are detected in 50% of CRCs that contain wild-type APC [9], β-catenin gene mutations in EC are less common. Early studies report a β-catenin mutation frequency of 10–45% in ECs [11–21], with frequent associated β-catenin nuclear accumulations in tumors with gene mutations. These findings were more common in endometrioid (type I) ECs than in nonendometrioid ECs. By contrast, APC mutations are less common, with a mutation frequency of 10% or less [22]. More recently, there has been increasing evidence to suggest that altered expression of Wnt antagonists, including members of both the SFRP family [23–27] and Dickkopf family [27–37], may be associated with human cancer development and progression. The Dickkopf proteins are secreted Wnt inhibitors which induce removal of the Wnt coreceptor low-density lipoprotein receptor-related protein (LRP), and thus prevent Wnt signaling. Dkk3 is a member of the Dickkopf family, and has been suggested as a tumor suppressor [31]. Its overexpression suppresses tumor growth *in vitro* in osteosarcoma [27], although Dkk-3 knock-out mice have shown no enhanced tumor formation [29]. The SFRP family members are putative extracellular modulators of the Wnt pathway, which can directly bind Wnt ligands and inhibit Wnt signaling. Several reports have surfaced regarding the downregulation or inactivation of SFRPs in human cancers, suggesting a role for SFRPs as tumor suppressors [26,38]. In prostate cancer, SFRP3 suppresses tumor growth and invasion in prostate cancer cells *in vitro* [26], while in hepatocellular carcinoma (HCC), SFRP1 is significantly downregulated in human HCC specimens, as compared with their adjacent noncancerous tissues; additionally overexpression of SFRP1 *in vitro* significantly inhibits cell growth and colony formation in HCC cells [38]. SFRP expression has been associated not only with carcinogenesis in a number of solid cancers, but also with prognosis and survival; this has been reported in breast cancer, where aberrant methylation of the SFRP1 promoter is associated with a poor overall survival [39]. Furthermore, knockdown of SFRP1 in non-malignant mammary cells showed increased cellular proliferation, increased migration, invasion, and resistance to anoikis (cell death induced by insufficient anchorage to the extracellular matrix) [40]. Inactivation of the SFRP genes appears to occur via epigenetic silencing or promoter hypermethylation in numerous solid tumors, including cervical and ovarian cancers [23,24,41–44]. *SFRP4* was reported to be downregulated in uterine sarcomas, with stable overexpression of this gene inhibiting tumor proliferation *in vitro* [25,45]. The expression pattern of SFRPs in nonsarcomatous uterine cancers has only been explored in the setting of microsatellite instability, where SFRP1 expression was compared between non-matched normal endometrial tissues and microsatellite unstable (MSI) and microsatellite stable (MSS) EC tissues [46].

Unlike in colon cancer, the mechanism of Wnt pathway involvement in EC has not been well elucidated, and does not appear to be as simple as that involving APC and β-catenin mutations. Instead, the evidence suggests that Wnt signaling is probably involved via multiple, diverse mechanisms. In this review, we present a brief overview of the Wnt signaling pathway, the current literature implicating the Wnt/β-catenin pathway in uterine cancer development and progression, and its potential as a prognostic marker and therapeutic target in EC.

## The canonical Wnt signaling pathway

### *Brief overview*

Nuclear β-catenin is the hallmark of an active canonical Wnt pathway. In the absence of Wnt signal, unstimulated cells regulate β-catenin levels through its phosphorylation by a multiprotein complex consisting of APC, axin, and GSK-3β, thus marking it for subsequent ubiquitination and degradation [47]. Upon binding of the Wnt ligand to its frizzled receptor (FZD), a signaling cascade ensues to destabilize this degradation complex, and allows unphosphorylated β-catenin to accumulate and translocate to the nucleus, where it functions as a cofactor for transcription factors of the TCF/LEF family (Figure 1). The result of this process is the transcription of specific genes designed to determine cell fate and regulate proliferation.

### *Extracellular & cell membrane components*

The term 'Wnt' (pronounced 'wint') was introduced in 1982 by Harold and Varmus Roeland Nusse, and fused the names of two orthologous genes: wingless (*Wg*), a *Drosophila* segment polarity gene, and *Int-1*, a mouse proto-oncogene [48,49]. Wnts are secreted glycoprotein-signaling molecules which act as ligands for a transmembrane receptor complex, the FZD, forming a trimeric complex with an additional single-pass transmembrane protein, the LRP. A total of 19 Wnt ligands and ten FZD receptors have been identified in the human genome [201]. Wnts exert their effect either via the 'canonical' Wnt/β-catenin signaling, or the less well-known noncanonical pathways, of which the two best studied are the Wnt/calcium and the Wnt/ras homolog gene family, member A/c-Jun $NH_2$-terminal kinase pathways, of which the latter primarily affects actin cytoskeleton and planar polarity of cells [50–52]. Activation of each signaling pathway depends on the type of ligands and receptors involved, as Wnt ligands exhibit preferential binding to specific receptors [53]. In addition, distinct FZDs appear to exhibit differential activation to the different signaling pathways [54–56]. Wnt ligands which activate the canonical pathway include the proto-oncogenic Wnt1, Wnt3a, Wnt8 and Wnt8a. Wnt ligands that activate the noncanonical pathways and antagonize the proto-oncogenic Wnts are Wnt4, Wnt5a and Wnt11. Wnt5a also has the ability to signal via the canonical

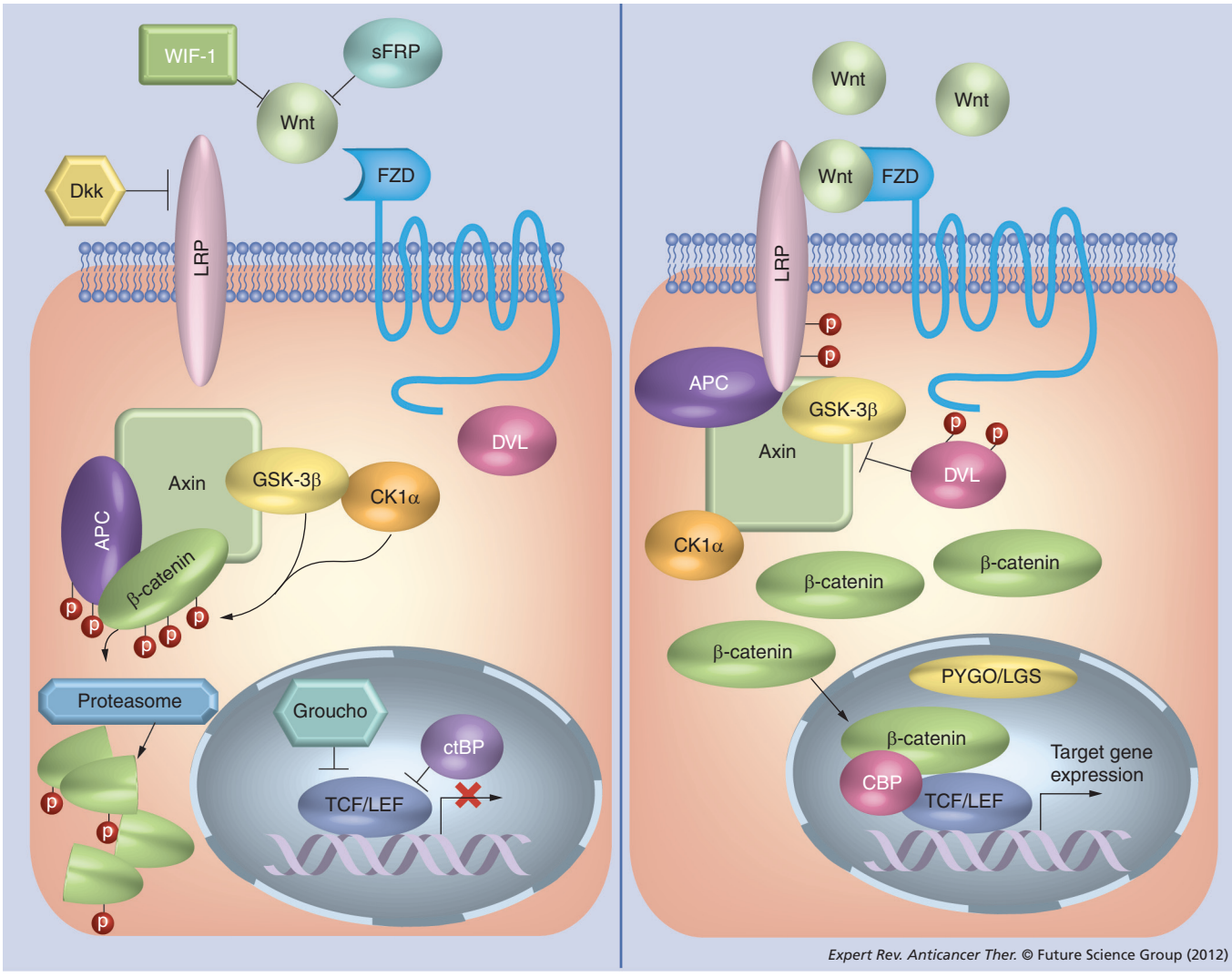


**Figure 1. The canonical Wnt signaling pathway.**
APC: Antigen-presenting cell; CBP: CREB-binding protein; CK1α: Casein kinase α; ctBP: C-terminal binding protein; DVL: Dishevelled; FZD: Frizzled receptor; LRP: Low-density lipoprotein receptor-related protein; PYGO/LGS: Pygopus/legless; TCF/LEF: T-cell factor/lymphoid enhancer factor-1; WIF-1: Wnt inhibitory factor 1.

pathway, dependent on cellular context [57]. The key effector in canonical Wnt signaling is β-catenin, a multifunctional protein that also mediates cell–cell adhesion with E-cadherin.

In its natural state, the canonical Wnt signaling pathway is inhibited by several Wnt antagonists, which can be subdivided into those directly binding to Wnt molecules, which include Wnt inhibitory factor-1, and SFRPs, versus those which indirectly inhibit Wnt ligands by binding to the LRP5 or 6 components of the receptor complex, which include Dickkopfs (Dkks).

### *Cytoplasmic components*

In the absence of the Wnt signal, the tumor suppressors axin and APC form a structural scaffold that interacts with β-catenin and presents it to GSK-3β for phosphorylation. APC is mutated in 85% of familial and sporadic CRCs [58], while truncating Axin1 mutations are found in hepatocellular carcinomas, thus revealing its relevance to β-catenin regulation in cancer [59]. GSK-3β, a normally active kinase in unstimulated resting cells, is a participant of the Wnt pathway and many other cellular signaling pathways [60]. Activation of dishevelled, an intracytoplasmic protein that interacts with both canonical and noncanonical Wnt pathways, is necessary for Wnt signal transduction from the cell surface, and occurs via phosphorylation by casein kinase-1 [10].

### *Nuclear components*

The TCF/LEF family of transcription factors includes LEF1, TCF1, TCF3 and TCF4. β-catenin is a cofactor for the TCF/LEF1 family but does not bind DNA directly; it displaces other proteins, such as Groucho and C-terminal-binding protein, which in turn repress *TCF/LEF* gene expression in the resting state. Two other important nuclear components are legless and its binding partner pygopus (PYGO) [61]. Legless recruits PYGO to β-catenin and, along with PYGO, is involved in the nuclear translocation of β-catenin [62,63]. These proteins may

**Table 1. β-catenin mutations in endometrial cancer.**

| Study (year) | Mutation frequency in endometrial cancer (%) | Ref. |
|---|---|---|
| Fukuchi *et al.* (1998) | 13 (10/76) | [11] |
| Nei *et al.* (1999) | 10 (2/20) | [13] |
| Mirabelli-Primdahl *et al.* (1999) | 45 (13/29) | [14] |
| Ikeda *et al.* (2000) | 11 (5/44) | [15] |
| Moreno-Bueno *et al.* (2002) | 11 (14/128) | [19] |
| Machin *et al.* (2002) | 21 (15/73) | [12] |
| Ashihara *et al.* (2002) | 10 (2/20) | [20] |
| Schlosshauer *et al.* (2000) | 19 (6/32) | [16] |
| Saegusa *et al.* (2001) | 23 (16/70) | [18] |

act as nuclear 'escorts' or as recruiters of the basal transcription machinery [64,65].

## Alterations of the Wnt pathway in EC

### β-*catenin: gene mutations, nuclear localization & interaction with E-cadherin*

The human *CTNNB1* gene encodes β-catenin and maps to chromosome 3p21. β-catenin mutations at its GSK-3β binding consensus site within exon 3 have been demonstrated in EC in a number of studies [11,12–21]. These mutations are frequently missense mutations affecting the $NH_2$-terminal regulatory domain of β-catenin (codons 32–45), which overlaps with the consensus sites for GSK-3β phosphorylation and ubiquitin–proteosome degradation. The mutations presumably render the mutant proteins resistant to degradation and occur almost exclusively in the endometrioid subtype. Mutations in the exon 3 domain of *CTNNB1* have been the most commonly reported alteration in the Wnt pathway in EC [11–21]. The mutation frequency of *CTNNB1* in EC has been reported to be between 10 and 45% (Table 1). Fukuchi *et al.* first reported a single-base missense (serine/threonine) mutation in exon 3 of *CTNNB1*, in ten out of 76 EC tumors (13% mutation frequency), with 90% of mutated specimens showing evidence of nuclear β-catenin accumulation by immunohistochemistry (IHC), in contrast to 30% of nonmutated specimens [11]. Other studies have shown lower mutation frequencies, with Nei *et al.* reporting a 10% mutation frequency for *CTNNB1* in EC (2/20 tumors), with 30% EC specimens showing nuclear β-catenin accumulation [13]. Ikeda *et al.* reported a 11% mutation frequency, with all tumors with mutations showing β-catenin accumulation in the cytoplasm and nucleus [15]. By contrast, Mirabelli-Primdahl *et al.* showed a 45% mutation frequency in 29 EC tumors with or without microsatellite instability, with no IHC staining studies for β-catenin [14]. Several reports have suggested a slightly higher *CTNNB1* mutation frequency in endometrioid ECs; Schlosshauer *et al.* and Saegusa each reported a 18 and 23% frequency in endometrioid ECs, respectively [16,17]. Moreno-Bueno reported an 11% mutation frequency in endometrioid ECs, with no *CTNNB1* mutations detected in nonendometroid ECs [19]. Similarly, Machin *et al.* reported a 20% *CTNNB1* mutation frequency in 59 endometrioid ECs, with no mutations detected in 14 nonendometrioid ECs [12].

It appears that β-catenin mutations are common in EC, and more frequent in type I (endometrioid) ECs. In contrast to CRC, where over 90% of tumors carry either APC or β-catenin mutations, ECs do not usually harbor APC mutations, a finding that is mirrored in other non-CRCs [66,67]. An APC mutation analysis in EC revealed only a 10% mutation frequency in all ECs [22], although that number is increased to 24% in EC tumors with nuclear β-catenin staining [19]. While APC mutations are an infrequent finding in EC, APC gene promoter methylation has been reported to occur in up to 20–45% of ECs, and with higher frequency in MSI tumors [68].

A significant fraction of EC tumors (11–38%) show apparent cytoplasmic or nuclear accumulation of β-catenin protein (Table 2), as analyzed by IHC. While most tumors which carry β-catenin mutations exhibit nuclear β-catenin accumulation by IHC, some tumors do so without any evidence of *CTNNB1* mutations. In Fukuchi's study, nine of the ten EC tumors with *CTNNB1* mutations showed nuclear or cytoplasmic β-catenin accumulation [11], while Nei's study reported a 30% nuclear β-catenin accumulation rate which was not associated with β-catenin mutation status. Membranous β-catenin immunoreactivity appears to decrease in a stepwise fashion from normal endometrium through atypical endometrial hyperplasia, to EC, as shown by Saegusa *et al.* [17], suggesting a role for Wnt signaling in the carcinogenesis of type I ECs.

β-catenin is a multifunctional protein that exerts two important functions in epithelial cells. Besides its role as a transcriptional coactivator in the canonical Wnt pathway, it acts as an adhesion molecule, associated with the protein E-cadherin at the cell–cell junction, and thus connecting it to the actin cytoskeleton [69]. E-cadherin has been shown to have a potential role as a prognostic marker. In a study of 28 patients with stage I EC, absent E-cadherin expression on IHC was predictive of distant metastasis, but not of local recurrence [22]. Recently, a GOG study evaluating stage IV and recurrent ECs treated with tamoxifen and progesterone (GOG 119), confirmed E-cadherin as a prognostic marker, with high E-cadherin expression by IHC resulting in better survival than low expression (adjusted HR: 0.18; 95% CI: 0.05–0.59) [70]. These reports reflect findings in other solid tumors [71], and establish E-cadherin as a potentially clinically relevant tumor biomarker with prognostic value in advanced and recurrent EC.

## Wnt inhibitors

There have been few reports on the role of Wnt inhibitors in gynecologic cancers (Table 3). Yi *et al.* documented that Dkk1, a member of the Dickkopf family, is expressed at reduced levels in ECs compared with benign endometrium [36]. Their study compared Dkk1 expression by IHC in 34 benign endometrial samples versus 30 EC samples. Similarly, in cervical cancers, Dkk3 was found to be frequently downregulated by microarray and real-time PCR, when compared with normal cervical tissue [32]. We

have shown decreased Dkk3 expression in EC compared with normal endometrium [72], which reflects the generally confirmed trend of downregulated Wnt inhibitors in both gynecologic and other malignancies, as evidenced by similar reports in gastrointestinal [73], breast [34], prostate [74] and renal carcinomas [75,76]. By contrast, Jiang *et al.* reported on serum Dkk3 in gynecologic cancers, which revealed higher serum Dkk3 levels in endometrial and cervical cancer patients compared with healthy subjects, with a stage-dependent pattern; however ovarian cancer patients exhibited reduced serum Dkk3 levels compared with their healthy counterparts [77]. Similarly, these authors reported higher Dkk1 serum levels in cervical cancer and EC patients, again in a stage-dependent manner [78]. Why serum Dkk1 and Dkk3 protein levels would be upregulated, in contrast to other reports revealing downregulation of the tissue Dkk genes, is unknown, and requires further study. The mechanism of downregulation of Dkks in EC has not been elucidated, although judging from evidence from the colorectal literature, there is probably a role for epigenetic silencing [28].

**Table 2. Nuclear β-catenin expression in endometrial cancer.†**

| Study (year) | All ECs (%) | Endometrioid ECs (%) | Nonendometrioid ECs (%) | Ref. |
|---|---|---|---|---|
| Fukuchi *et al.* (1998) | 38 (29/76) | | | [11] |
| Nei *et al.* (1999) | 30 (9/30) | | | [13] |
| Ikeda *et al.* (2000) | 11 (5/44) | | | [15] |
| Moreno-Bueno *et al.* (2002) | 23 (30/128) | 31 (29/93) | 3 (1/33) | [19] |
| Schlosshauer *et al.* (2002) | | 47 (8/17) | 0 (0/17) | [16] |
| Ashihara *et al.* (2002) | | 60 (12/20) | 55 (11/20) | [20] |
| Machin *et al.* (2002) | | 73 (11/15) | | [12] |
| Scholten *et al.* (2003) | | 16 (39/233) | | [21] |
| Saegusa *et al.* (2001) | | 28 (55/199) | | [17] |

†Percentage of endometrial cancer samples with β-catenin nuclear expression.
EC: Endometrial cancer.

Only few have reported on the role of SFRPs in ECs. Abu-Jawdeh first reported on the upregulation of SFRP4 (frpHE) mRNA in the stroma of endometrial stroma [79]. By contrast, Carmon *et al.* reported that stable SFRP4 overexpression in Ishikawa EC cells and treatment with recombinant SFRP4 protein inhibits EC growth *in vitro* [25]. In another study, Risinger *et al.* showed that *SFRP1* and *SFRP4* are more downregulated in microsatellite unstable than in microsatellite stable ECs, as identified by microarray in 24 human ECs [46]. Real-time PCR confirmed downregulation of *SFRP4* in MSI-high EC tissues as compared with (unmatched) normal endometrial tissues, with no reduction in MSS ECs. Furthermore, downregulation was accompanied by an increase of nuclear β-catenin and promoter hypermethylation for *SFRP1* in eight out of 12 MSI ECs, compared with only three out of 16 MSS ECs; interestingly the Wnt target FGF 18 was upregulated in MSI cancers, all suggesting an association between MSI and Wnt signaling. Further evidence of *SFRP* gene hypermethylation in ECs was recently reported, with *SFRP1*, *SFRP2* and *SFRP5* revealing promoter methylation status in endometrioid ECs, while SFRP4 showed demethylation [80]. These results reflect similar findings in other solid tumors, which report downregulation of SFRPs via epigenetic silencing [38,81], and correlate these with clinical outcome [24,39,82]. Further research studying the prognostic relevance of these downregulated genes is required.

### *Target genes & gene expression analysis*

A large number of genes relevant for tumor formation and progression have been identified to be transcriptionally activated by the β-catenin/Tcf complex. Some of these are implicated in growth control and cell cycling (c-Myc, c-Jun, cyclin D1 and gastrin), while others are relevant for cell survival (inhibitor of DNA binding-2 and MDR1), or are implicated in tumor invasion and metastasis (matrilysin and VEGF) [83–89]. The best known target gene is axin, which negatively regulates the canonical Wnt pathway; it is the most universally expressed Wnt/β-catenin target gene, making it one of the most essential components of the pathway. Other important target genes related to carcinogenesis are the oncogene myc and the growth-promoting gene cyclin D.

### *Sex hormones*

Type I ECs are associated with various states of unopposed estrogen, such as obesity, polycystic ovary syndrome and tamoxifen use [90,91]. Given the propensity of β-catenin mutations in type I ECs, an association between the sex hormones, progesterone and estrogen, and the Wnt signaling pathway appears likely. A number of published reports have indicated that estrogen can induce the canonical Wnt pathway [92–95]. Estrogens appear to specifically influence the expression level of Wnt ligands; estrogen treatment in a non-malignant mouse uterus was shown to upregulate Wnt4a, Wnt5a and Frizzled-2, thus prompting nuclear β-catenin localization; moreover, estrogen-induced endometrial proliferation was inhibited by the Wnt inhibitor SFRP2 [95]. Another report showed that estrogen treatment in immature female rats resulted in the downregulation of Wnt7a, and upregulation of Wnt4 in the uterus [96]. The Wnt signaling target IGF-I receptor, an important EGF, was strongly upregulated by estrogen in another study, and inhibited after the addition of progesterone [97]. Interestingly, another *in vitro* study showed that estrogens appear to activate the Wnt/β-catenin pathway, but only after initiation by progestogens [98].

Progesterone is a well-known treatment option for recurrent and persistent EC, with a response rate of 15–25% based on a GOG study [99]. It is also an effective fertility-sparing treatment option for women with complex atypical hyperplasia of the endometrium, a preinvasive condition which carries a risk of up to 42% of occult EC [100]. In selected cases, it has been used for early-stage EC in young women who wish to preserve fertility [101,102].

**Table 3. Studies of Wnt inhibitors in endometrial cancer.**

| Wnt inhibitor | Study (year) | Significance | Ref. |
|---|---|---|---|
| SFRPs | Abu-Jawdeh *et al.* (1999) | Upregulation of SFRP4 mRNA in EC | [79] |
| | Risinger *et al.* (2005) | SFRP1 and SFRP4 are more frequently downregulated in MSI than in MSS ECs (microarray of 24 human ECs) | [46] |
| | Carmon *et al.* (2008) | SFRP4 overexpression inhibits EC cell growth *in vitro* | [25] |
| | Di Domenico *et al.* (2011) | SFRP1 downregulation via promoter methylation in 13 EC tissues; SFRP4 upregulation via demethylation | [80] |
| Dkk1 | Yi *et al.* (2009) | Decreased Dkk1 expression in EC tissues compared to benign endometrium, by IHC | [36] |
| | Jiang *et al.* (2010) | Increased serum Dkk1 protein levels in patients with EC compared to healthy women | [78] |
| Dkk3 | Jiang *et al.* (2009) | Increased serum Dkk3 protein levels in patients with EC (n = 28) compared to healthy women | [77] |

EC: Endometrial cancer; IHC: Immunohistochemistry; MSI: Microsatellite unstable; MSS: Microsatellite stable; SFRP: Secreted frizzled-related protein.

As progesterone counteracts the proliferative effects of estrogen in the menstrual cycle, its mechanism of action may be via the Wnt/β-catenin signaling pathway. Various reports have implicated progesterone in the Wnt pathway, including its regulation of β-catenin expression in endometrial tumors [103]. In non-malignant endometrial cells, a knockdown of the progesterone receptor resulted in Wnt activity in human endometrial cells *in vitro* [104], and in pregnant sheep, progesterone induced a transient decline in Wnt signaling [105]. Similarly, in healthy female volunteers, treatment with mifepristone, an antiprogesterone agent, resulted in the upregulation of Wnt5a in the endometrium [106]. Finally, in Wnt-activated Ishikawa cells, progesterone was shown to induce Dkk1 and FOXO1, and to inhibit Wnt signaling; conversely, a knockout of both Dkk1 and FOXO1 circumvented the progesterone inhibition of Wnt activity [107].

Taken together, these findings indicate that estrogen and progesterone play important roles in regulating the canonical Wnt pathway, and that modulation of the Wnt pathway downstream of the estrogen and progesterone regulation, may prove to be a potential target for novel therapeutic agents in EC.

### *Crosstalk with Hedgehog & mTOR pathways*

Both Hedgehog and mTOR pathways are currently being investigated as novel therapeutic targets in gynecologic cancers. Recently, the mTOR inhibitor temsirolimus was shown to have a 14% response rate in persistent and recurrent EC [5], and a Hedghog pathway inhibitor, GDC-0449, has been in clinical trial in recurrent ovarian cancer, with outcome analyses pending. The mTOR pathway regulates cell growth and proliferation in response to multiple upstream factors, including IGFs (e.g., IGF-I and IGF-II), and mitogens, as well as Wnt ligands [108,109]. Not surprisingly, deregulation of mTOR signaling is frequently associated with tumor initiation, growth, invasion, and metastasis [110,111]. As a central regulator of growth, the mTOR pathway interacts with multiple signaling pathways, of which one is the canonical Wnt pathway, which augments mTOR activity [112,113]. Similarly, the Hedgehog family of morphogens are regulators of cell proliferation, differentiation and cell–cell communication, which have important roles in organogenesis, stem cell maintenance and carcinogenesis [114–120]. In mammals, three Hedgehog ligands including Sonic Hedgehog, Indian Hedgehog and Desert Hedgehog have been identified. Through binding of any one of the three ligands to Patched, a transmembrane receptor, the pathway is activated by alleviating Patched-mediated suppression of Smoothened (Smo), thus activating downstream signaling molecules and subsequent Gli-mediated gene transcription [121]. A link between Hedgehog and canonical Wnt signaling has been suggested by the finding of concomitant Gli1 overexpression and nuclear β-catenin immunoreactivity in EC and endometrial atypical hyperplasia [122]. Given the above findings, there probably is crosstalk between these signaling pathways, and the potential for targeting multiple pathways to achieve synergistic drug combinations appears to be an attractive goal in the treatment of EC.

## Potential therapeutic targets of the Wnt pathway in EC

Few effective treatment options are available for women with advanced EC who have failed traditional cytotoxic chemotherapy. Recent Phase II clinical trials have shown promise for novel biologics targeting VEGF and mTOR pathways [5,6]. Given the fact that multiple mutations (*CTNNB1* mutations and epigenetic silencing of Wnt antagonists) can lead to the nuclear translocation of β-catenin, and that these can be targeted at different cellular levels, there is a clear need for drugs which attenuate the transcriptional functions of β-catenin [123,124]. A number of existing drugs and natural compounds already inhibit or modulate the Wnt/β-catenin pathway [125]. Among these are NSAIDs, vitamins and polyphenols. NSAIDs, such as aspirin and sulindac, inhibit cyclooxygenase (COX) activity, and the Wnt signaling pathway is thought to be one of the potential mechanisms of action for their effectiveness in colon cancer, due to evidence that increased COX generated prostaglandin E2 suppresses β-catenin, and thus results in Wnt pathway activation [126,127]. Notably, the COX-2 inhibitor, celecoxib, is approved by the US FDA for the prevention of CRC in patients with familial adenomatous polyposis, after a number of experimental and epidemiological studies suggested that NSAIDs showed chemopreventive effects against colon cancer [128–133]. However, in contrast to CRCs, a significant chemopreventive association between NSAIDs and EC has not been established.

While some studies support a risk reduction in EC with current aspirin or NSAID use [134,135], others have shown no such association [136–138]. *In vitro*, aspirin and NSAIDs have been reported to inhibit proliferation in EC cells [139–144]. Given the critical role of Wnt signaling in the regulation of cell proliferation, an association between the inhibition of endometrial proliferation by NSAIDs and Wnt signaling could be hypothesized, although such association has not been elucidated yet.

Vitamins (retinoids) have been used as cancer therapy in some cancers (such as acute promyelocytic leukemia), but the mechanism of action linking it to the canonical Wnt pathway is not fully understood, and it is suggested that activated nuclear receptors for vitamins interact with β-catenin and compete with TCF [145,146]. Polyphenols are chemicals extracted from plants, characterized by the presence of phenol units; examples include reseveratrol, quercetin, epigallocatechin-3-gallate, and cucumin, which have been implicated in Wnt signaling, although the exact mechanism of action is unknown due to the lack of specificity and effects on multiple pathways [147–151].

Currently, a number of molecular targeted drugs are in preclinical development. Most promising among these are small molecule antagonists, such as PKF115-584, which displayed reproducible and dose-dependent inhibition of β-catenin and TCF binding in an immunoenzymatic assay [152], and XAV939, which targets tankyrase, thus stabilizing axin and antagonizing the Wnt pathway [153]. Various other molecularly targeted agents have been identified via high-throughput screening, including those targeting the β-catenin/TCF interaction [154,155], which have not reached beyond the discovery and preclinical stages, as well as transcriptional coactivator antagonists, of which the β-catenin antagonist ICG-001, was scheduled to enter clinical trials [156]. Among biologics, monoclonal antibodies against Wnt-1 and Wnt-2 have been shown to suppress tumor growth *in vivo* [157–160], and small interfering RNA against Wnt1 and Wnt2, as well as recombinant proteins incorporating SFRP [161] also showed potential therapeutic utility.

## Conclusion

To date, numerous studies have suggested a role for Wnt signaling in endometrial carcinogenesis. Our current understanding is that both β-catenin mutations and Wnt-inhibitor regulation impact EC development, but detailed knowledge of these mechanisms does not exist. Despite the limited literature associating Wnt signaling with endometrial carcinogenesis, this field deserves further study, especially in light of the inadequate treatment options which currently exist for women with advanced and recurrent EC. Further investigation is necessary to elucidate the role of this pathway in EC, and to explore potential applications in targeted novel therapies.

## Expert commentary

The canonical Wnt signaling pathway represents an attractive therapeutic target given its tight regulatory steps at multiple cellular levels, which offer ample targeting points. Its role in the carcinogenesis of gynecologic cancers is rapidly expanding. While still understudied in EC, preclinical data offer convincing evidence for the importance of this pathway in the uterine carcinogenesis. The potential for both biomarker use and cancer drug development is likely to expand with further research. Given the limited treatment options in advanced and recurrent EC, exploring the Wnt signaling pathway for potential therapeutic targeting is imperative.

## Five-year view

Data from other solid tumors, such as breast cancer and prostate cancer, have reported Wnt inhibitors as prognostic markers and tumor suppressors, and novel agents targeting the Wnt signaling pathway have been shown to possess significant anti-tumor activity in mouse models. These studies need to be confirmed in EC in order to establish Wnt pathway components as prognostic and predictive biomarkers, along with the demonstration of preclinical data showing promise for biologic agents targeting this pathway. The study of stem cells in EC would be of particular interest in the near future, given the importance of Wnt signaling in stem cell biology. Crosstalk with other important signaling pathways involved in cellular regulation, such as mTOR, Hedgehog and Notch pathways, may be attractive targets for synergistic drug combinations. Taken together, these milestones would make way for clinical studies leading to personalized molecular therapy for women with advanced and recurrent EC.

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## Key issues

- Advanced and persistent endometrial cancer carries a poor prognosis, and limited treatment options exist.
- The Wnt/β-catenin signaling is a highly conserved signaling pathway, which is regulated at multiple cellular levels, and thus provides an attractive pathway for novel targeted therapeutics in uterine cancer.
- Aberrations in the Wnt pathway which have been linked to endometrial cancer, include a 10–45% mutation frequency of β-catenin, as well as a loss of Wnt antagonists via epigenetic silencing.
- Progesterone and estrogen regulate the Wnt signaling pathway, and modulation of the pathway downstream of this hormonal influence may prove to be a potential therapeutic target.
- Further research is required to expand the current knowledge and move towards clinical trials.

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