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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

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, prostate, 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-3 β , 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 NH₂-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; CK1 α : 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 Dickkops (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-3 β 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-3 β ,

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 <i>et al.</i> (1998)	13 (10/76)	[11]
Nei <i>et al.</i> (1999)	10 (2/20)	[13]
Mirabelli-Primdahl <i>et al.</i> (1999)	45 (13/29)	[14]
Ikeda <i>et al.</i> (2000)	11 (5/44)	[15]
Moreno-Bueno <i>et al.</i> (2002)	11 (14/128)	[19]
Machin <i>et al.</i> (2002)	21 (15/73)	[12]
Ashihara <i>et al.</i> (2002)	10 (2/20)	[20]
Schlosshauer <i>et al.</i> (2000)	19 (6/32)	[16]
Saegusa <i>et al.</i> (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-3 β 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 NH₂-terminal regulatory domain of β -catenin (codons 32–45), which overlaps with the consensus sites for GSK-3 β phosphorylation and ubiquitin–proteasome 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 nonendometrioid 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].

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 2. Nuclear β -catenin expression in endometrial cancer.[†]

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

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

Table 3. Studies of Wnt inhibitors in endometrial cancer.

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

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1 Jemal A, Siegel R, Xu J, Ward E. Cancer statistics 2010. *CA Cancer J. Clin.* 60(5), 277–300 (2010).
- 2 Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol. Oncol.* 15(1), 10–17 (1983).
- 3 Obel JC, Friberg G, Fleming GF. Chemotherapy in endometrial cancer. *Clin. Adv. Hematol. Oncol.* 4(6), 459–468 (2006).
- 4 Dellinger TH, Monk BJ. Systemic therapy for recurrent endometrial cancer: a review of North American trials. *Expert Rev. Anticancer Ther.* 9(7), 905–916 (2009).
- 5 Oza AM, Elit L, Tsao MS *et al.* Phase II Study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the NCIC Clinical Trials Group. *J. Clin. Oncol.* (2011).
- 6 Aghajanian C, Sill MW, Darcy KM *et al.* Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: a Gynecologic Oncology Group study. *J. Clin. Oncol.* 29(16), 2259–2265 (2011).
- 7 Creasman WT, Boronow RC, Morrow CP, DiSaia PJ, Blessing J. Adenocarcinoma of the endometrium: its metastatic lymph node potential. A preliminary report. *Gynecol. Oncol.* 4(3), 239–243 (1976).
- 8 Vogelstein B, Fearon ER, Hamilton SR *et al.* Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.* 319(9), 525–532 (1988).
- **Landmark article associating adenomatous polyposis coli mutations with colorectal cancer.**
- 9 Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. *Cancer Res.* 58(6), 1130–1134 (1998).
- 10 Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim. Biophys. Acta* 1653(1), 1–24 (2003).
- **Review article summarizing the role of Wnt signaling in cancer.**
- 11 Fukuchi T, Sakamoto M, Tsuda H, Maruyama K, Nozawa S, Hirohashi S. Beta-catenin mutation in carcinoma of the uterine endometrium. *Cancer Res.* 58(16), 3526–3528 (1998).
- **First study to report the role of β -catenin mutations in endometrial cancer.**
- 12 Machin P, Catusas L, Pons C, Munoz J, Matias-Guiu X, Prat J. *CTNNB1* mutations and beta-catenin expression in endometrial carcinomas. *Hum. Pathol.* 33(2), 206–212 (2002).
- 13 Nei H, Saito T, Yamasaki H, Mizumoto H, Ito E, Kudo R. Nuclear localization of beta-catenin in normal and carcinogenic endometrium. *Mol. Carcinog.* 25(3), 207–218 (1999).
- 14 Mirabelli-Primdahl L, Gryfe R, Kim H *et al.* Beta-catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. *Cancer Res.* 59(14), 3346–3351 (1999).
- 15 Ikeda T, Yoshinaga K, Semba S, Kondo E, Ohmori H, Horii A. Mutational analysis of the *CTNNB1* (beta-catenin) gene in human endometrial cancer: frequent mutations at codon 34 that cause nuclear accumulation. *Oncol. Rep.* 7(2), 323–326 (2000).
- 16 Schlosshauer PW, Pirog EC, Levine RL, Ellenson LH. Mutational analysis of the *CTNNB1* and APC genes in uterine endometrioid carcinoma. *Mod. Pathol.* 13(10), 1066–1071 (2000).
- 17 Saegusa M, Hashimura M, Yoshida T, Okayasu I. Beta-Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. *Br. J. Cancer* 84(2), 209–217 (2001).
- 18 Saegusa M, Okayasu I. Frequent nuclear beta-catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. *J. Pathol.* 194(1), 59–67 (2001).
- 19 Moreno-Bueno G, Hardisson D, Sanchez C *et al.* Abnormalities of the APC/beta-catenin pathway in endometrial cancer. *Oncogene* 21(52), 7981–7990 (2002).
- 20 Ashihara K, Saito T, Mizumoto H, Nishimura M, Tanaka R, Kudo R. Mutation of beta-catenin gene in endometrial cancer but not in associated hyperplasia. *Med. Electron Microsc.* 35(1), 9–15 (2002).
- 21 Scholten AN, Creutzberg CL, van den Broek LJ, Noordijk EM, Smit VT. Nuclear beta-catenin is a molecular feature of type I endometrial carcinoma. *J. Pathol.* 201(3), 460–465 (2003).
- 22 Pijnenborg JM, Kisters N, van Engeland M *et al.* APC, beta-catenin, and E-cadherin and the development of recurrent endometrial carcinoma. *Int. J. Gynecol. Cancer* 14(5), 947–956 (2004).
- 23 Su HY, Lai HC, Lin YW, Chou YC, Liu CY, Yu MH. An epigenetic marker panel for screening and prognostic prediction of ovarian cancer. *Int. J. Cancer* 124(2), 387–393 (2009).
- 24 Su HY, Lai HC, Lin YW *et al.* Epigenetic silencing of SFRP5 is related to malignant phenotype and chemoresistance of ovarian cancer through Wnt signaling pathway. *Int. J. Cancer* 127(3), 555–567 (2009).
- 25 Carmon KS, Loose DS. Secreted frizzled-related protein 4 regulates two Wnt7a signaling pathways and inhibits proliferation in endometrial cancer cells. *Mol. Cancer Res.* 6(6), 1017–1028 (2008).
- 26 Zi X, Guo Y, Simoneau AR *et al.* Expression of Frzb/secreted Frizzled-related protein 3, a secreted Wnt antagonist, in human androgen-independent prostate cancer PC-3 cells suppresses tumor growth and cellular invasiveness. *Cancer Res.* 65(21), 9762–9770 (2005).
- 27 Hoang BH, Kubo T, Healey JH *et al.* Dickkopf 3 inhibits invasion and motility of Saos-2 osteosarcoma cells by modulating the Wnt-beta-catenin pathway. *Cancer Res.* 64(8), 2734–2739 (2004).
- 28 Aguilera O, Fraga MF, Ballestar E *et al.* Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. *Oncogene* 25(29), 4116–4121 (2006).
- 29 Barrantes Idel B, Montero-Pedrazuela A, Guadano-Ferraz A *et al.* Generation and characterization of Dickkopf3 mutant mice. *Mol. Cell Biol.* 26(6), 2317–2326 (2006).
- 30 Fong D, Hermann M, Untergasser G *et al.* Dkk-3 expression in the tumor endothelium: a novel prognostic marker of pancreatic adenocarcinomas. *Cancer Sci.* 100(8), 1414–1420 (2009).
- 31 Kawasaki K, Watanabe M, Sakaguchi M *et al.* REIC/Dkk-3 overexpression downregulates P-glycoprotein in multidrug-resistant MCF7/ADR cells and induces apoptosis in breast cancer. *Cancer Gene Ther.* 16(1), 65–72 (2009).
- 32 Lee EJ, Jo M, Rho SB *et al.* Dkk3, downregulated in cervical cancer, functions as a negative regulator of beta-catenin. *Int. J. Cancer* 124(2), 287–297 (2009).
- 33 Tanimoto R, Abarzua F, Sakaguchi M *et al.* REIC/Dkk-3 as a potential gene therapeutic agent against human testicular cancer. *Int. J. Mol. Med.* 19(3), 363–368 (2007).
- 34 Veeck J, Bektas N, Hartmann A *et al.* Wnt signalling in human breast cancer:

- expression of the putative Wnt inhibitor Dickkopf-3 (DKK3) is frequently suppressed by promoter hypermethylation in mammary tumours. *Breast Cancer Res.* 10(5), R82 (2008).
- 35 Voorzanger-Rousselot N, Goehrig D, Journe F *et al.* Increased Dickkopf-1 expression in breast cancer bone metastases. *Br. J. Cancer* 97(7), 964–970 (2007).
  - 36 Yi N, Liao QP, Li T, Xiong Y. Novel expression profiles and invasiveness-related biology function of Dkk1 in endometrial carcinoma. *Oncol. Rep.* 21(6), 1421–1427 (2009).
  - 37 Yue W, Sun Q, Dacic S *et al.* Downregulation of Dkk3 activates beta-catenin/TCF-4 signaling in lung cancer. *Carcinogenesis* 29(1), 84–92 (2008).
  - 38 Huang J, Zhang YL, Teng XM *et al.* Down-regulation of SFRP1 as a putative tumor suppressor gene can contribute to human hepatocellular carcinoma. *BMC Cancer* 7, 126 (2007).
  - 39 Veeck J, Niederacher D, An H *et al.* Aberrant methylation of the Wnt antagonist SFRP1 in breast cancer is associated with unfavourable prognosis. *Oncogene* 25(24), 3479–3488 (2006).
  - 40 Gauger KJ, Hugh JM, Troester MA, Schneider SS. Down-regulation of sfrp1 in a mammary epithelial cell line promotes the development of a cd44^{high}/cd24^{low} population which is invasive and resistant to anoikis. *Cancer Cell Int.* 9, 11 (2009).
  - 41 Chung MT, Lai HC, Sytwu HK *et al.* SFRP1 and SFRP2 suppress the transformation and invasion abilities of cervical cancer cells through Wnt signal pathway. *Gynecol. Oncol.* 112(3), 646–653 (2009).
  - 42 Chung MT, Sytwu HK, Yan MD *et al.* Promoter methylation of SFRPs gene family in cervical cancer. *Gynecol. Oncol.* 112(2), 301–306 (2009).
  - 43 Fukui T, Kondo M, Ito G *et al.* Transcriptional silencing of secreted frizzled related protein 1 (SFRP 1) by promoter hypermethylation in non-small-cell lung cancer. *Oncogene* 24(41), 6323–6327 (2005).
  - 44 Kongkham PN, Northcott PA, Croul SE, Smith CA, Taylor MD, Rutka JT. The SFRP family of WNT inhibitors function as novel tumor suppressor genes epigenetically silenced in medulloblastoma. *Oncogene* 29(20), 3017–3024 (2010).
  - 45 Hrzenjak A, Tippl M, Kremser ML *et al.* Inverse correlation of secreted frizzled-related protein 4 and beta-catenin expression in endometrial stromal sarcomas. *J. Pathol.* 204(1), 19–27 (2004).
  - 46 Risinger JJ, Maxwell GL, Chandramouli GV *et al.* Gene expression profiling of microsatellite unstable and microsatellite stable endometrial cancers indicates distinct pathways of aberrant signaling. *Cancer Res.* 65(12), 5031–5037 (2005).
  - **Reports downregulation of SFRP1 and SFRP4 in microsatellite unstable endometrial carcinomas.**
  - 47 Orford K, Crockett C, Jensen JP, Weissman AM, Byers SW. Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. *J. Biol. Chem.* 272(40), 24735–24738 (1997).
  - 48 Nusse R, van Ooyen A, Cox D, Fung YK, Varmus H. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature* 307(5947), 131–136 (1984).
  - 49 Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 31(1), 99–109 (1982).
  - 50 Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT. The Wnt/Ca²⁺ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet.* 16(7), 279–283 (2000).
  - 51 Pandur P, Lasche M, Eisenberg LM, Kuhl M. Wnt-11 activation of a non-canonical Wnt signalling pathway is required for cardiogenesis. *Nature* 418(6898), 636–641 (2002).
  - 52 Huelsen J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates. *Curr. Opin. Genet. Dev.* 11(5), 547–553 (2001).
  - 53 He X, Saint-Jeannet JP, Wang Y, Nathans J, Dawid I, Varmus H. A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* 275(5306), 1652–1654 (1997).
  - 54 Slusarski DC, Corces VG, Moon RT. Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature* 390(6658), 410–413 (1997).
  - 55 Hartmann C, Tabin CJ. Dual roles of Wnt signaling during chondrogenesis in the chicken limb. *Development* 127(14), 3141–3159 (2000).
  - 56 Medina A, Reintsch W, Steinbeisser H. Xenopus frizzled 7 can act in canonical and non-canonical Wnt signaling pathways: implications on early patterning and morphogenesis. *Mech. Dev.* 92(2), 227–237 (2000).
  - 57 Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol.* 4(4), e115 (2006).
  - 58 Gryfe R, Swallow C, Bapat B, Redston M, Gallinger S, Couture J. Molecular biology of colorectal cancer. *Curr. Probl. Cancer* 21(5), 233–300 (1997).
  - 59 Satoh S, Daigo Y, Furukawa Y *et al.* AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat. Genet.* 24(3), 245–250 (2000).
  - 60 Cao Q, Lu X, Feng YJ. Glycogen synthase kinase-3beta positively regulates the proliferation of human ovarian cancer cells. *Cell Res.* 16(7), 671–677 (2006).
  - 61 Thompson B, Townsley F, Rosin-Arbesfeld R, Musisi H, Bienz M. A new nuclear component of the Wnt signalling pathway. *Nat. Cell Biol.* 4(5), 367–373 (2002).
  - 62 Parker DS, Jemison J, Cadigan KM. Pygopus, a nuclear PHD-finger protein required for Wingless signaling in *Drosophila*. *Development* 129(11), 2565–2576 (2002).
  - 63 Kramps T, Peter O, Brunner E *et al.* Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear beta-catenin-TCF complex. *Cell* 109(1), 47–60 (2002).
  - 64 Townsley FM, Cliffe A, Bienz M. Pygopus and Legless target Armadillo/beta-catenin to the nucleus to enable its transcriptional co-activator function. *Nat. Cell Biol.* 6(7), 626–633 (2004).
  - 65 Townsley FM, Thompson B, Bienz M. Pygopus residues required for its binding to Legless are critical for transcription and development. *J. Biol. Chem.* 279(7), 5177–5183 (2004).
  - 66 Morin PJ. Beta-catenin signaling and cancer. *Bioessays* 21(12), 1021–1030 (1999).
  - 67 Polakis P. The oncogenic activation of beta-catenin. *Curr. Opin. Genet. Dev.* 9(1), 15–21 (1999).
  - 68 Tao MH, Freudenheim JL. DNA methylation in endometrial cancer. *Epigenetics* 5(6), 491–498 (2010).
  - 69 Mareel M, Boterberg T, Noe V *et al.* E-cadherin/catenin/cytoskeleton complex: a regulator of cancer invasion. *J. Cell Physiol.* 173(2), 271–274 (1997).
  - 70 Singh M, Darcy KM, Brady WE *et al.* Cadherins, catenins and cell cycle regulators: Impact on survival in a Gynecologic Oncology Group Phase II endometrial cancer trial. *Gynecol Oncol* 123(2), 320–328 (2011).

- 71 Shiozaki H, Tahara H, Oka H *et al.* Expression of immunoreactive E-cadherin adhesion molecules in human cancers. *Am. J. Pathol.* 139(1), 17–23 (1991).
- 72 Dellinger T, Planutis K, Gatliffe T, DiSaia P, Monk B, Holcombe R. Wnt inhibitor expression as a predictor of progression in patients with endometrial carcinoma: a potential role as prognostic biomarker. *Gynecol. Oncol.* 116 3(Suppl. 1), S86 (2010).
- 73 Hsieh SY, Hsieh PS, Chiu CT, Chen WY. Dickkopf-3/REIC functions as a suppressor gene of tumor growth. *Oncogene* 23(57), 9183–9189 (2004).
- 74 Abarzua F, Sakaguchi M, Takaishi M *et al.* Adenovirus-mediated overexpression of REIC/Dkk-3 selectively induces apoptosis in human prostate cancer cells through activation of c-Jun-NH2-kinase. *Cancer Res.* 65(21), 9617–9622 (2005).
- 75 Kawakami K, Hirata H, Yamamura S *et al.* Functional significance of Wnt inhibitory factor-1 gene in kidney cancer. *Cancer Res.* 69(22), 8603–8610 (2009).
- 76 Kurose K, Sakaguchi M, Nasu Y *et al.* Decreased expression of REIC/Dkk-3 in human renal clear cell carcinoma. *J. Urol.* 171(3), 1314–1318 (2004).
- 77 Jiang T, Huang L, Wang S, Zhang S. Clinical significance of serum Dkk-3 in patients with gynecological cancer. *J. Obstet. Gynaecol. Res.* 36(4), 769–773 (2010).
- 78 Jiang T, Wang S, Huang L, Zhang S. Clinical significance of serum DKK-1 in patients with gynecological cancer. *Int. J. Gynecol. Cancer* 19(7), 1177–1181 (2009).
- 79 Abu-Jawdeh G, Comella N, Tomita Y *et al.* Differential expression of frpHE: a novel human stromal protein of the secreted frizzled gene family, during the endometrial cycle and malignancy. *Lab. Invest.* 79(4), 439–447 (1999).
- 80 Di Domenico M, Santoro A, Ricciardi C *et al.* Epigenetic fingerprint in endometrial carcinogenesis: the hypothesis of a uterine field cancerization. *Cancer Biol. Ther.* 12(5), 447–457 (2011).
- 81 He B, Lee AY, Dadfarmay S *et al.* Secreted frizzled-related protein 4 is silenced by hypermethylation and induces apoptosis in beta-catenin-deficient human mesothelioma cells. *Cancer Res.* 65(3), 743–748 (2005).
- 82 Urakami S, Shiina H, Enokida H *et al.* Wnt antagonist family genes as biomarkers for diagnosis, staging, and prognosis of renal cell carcinoma using tumor and serum DNA. *Clin. Cancer Res.* 12(23), 6989–6997 (2006).
- 83 Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398(6726), 422–426 (1999).
- 84 He TC, Sparks AB, Rago C *et al.* Identification of c-MYC as a target of the APC pathway. *Science* 281(5382), 1509–1512 (1998).
- 85 Yamada T, Takaoka AS, Naishiro Y *et al.* Transactivation of the multidrug resistance 1 gene by T-cell factor 4/ beta-catenin complex in early colorectal carcinogenesis. *Cancer Res.* 60(17), 4761–4766 (2000).
- 86 Crawford HC, Fingleton BM, Rudolph-Owen LA *et al.* The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. *Oncogene* 18(18), 2883–2891 (1999).
- 87 Kolligs FT, Nieman MT, Winer I *et al.* ITF-2, a downstream target of the Wnt/TCF pathway, is activated in human cancers with beta-catenin defects and promotes neoplastic transformation. *Cancer Cell* 1(2), 145–155 (2002).
- 88 Koh TJ, Bulitta CJ, Fleming JV, Dockray GJ, Varro A, Wang TC. Gastrin is a target of the beta-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. *J. Clin. Invest.* 106(4), 533–539 (2000).
- 89 Mann B, Gelos M, Siedow A *et al.* Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc. Natl Acad. Sci. USA* 96(4), 1603–1608 (1999).
- 90 Bergman L, Beelen ML, Gallee MP, Hollema H, Benraadt J, van Leeuwen FE. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of liver and endometrial cancer risk following tamoxifen. *Lancet* 356(9233), 881–887 (2000).
- 91 Webb PM. Commentary: weight gain, weight loss, and endometrial cancer. *Int. J. Epidemiol.* 35(1), 166–168 (2006).
- 92 Cardona-Gomez P, Perez M, Avila J, Garcia-Segura LM, Wadosell F. Estradiol inhibits GSK3 and regulates interaction of estrogen receptors, GSK3, and beta-catenin in the hippocampus. *Mol. Cell Neurosci.* 25(3), 363–373 (2004).
- 93 Varea O, Arevalo MA, Garrido JJ, Garcia-Segura LM, Wadosell F, Mendez P. Interaction of estrogen receptors with insulin-like growth factor-I and Wnt signaling in the nervous system. *Steroids* 75(8–9), 565–569 (2009).
- 94 Wagner S, Weber S, Kleinschmidt MA, Nagata K, Bauer UM. SET-mediated promoter hypoacetylation is a prerequisite for coactivation of the estrogen-responsive pS2 gene by PRMT1. *J. Biol. Chem.* 281(37), 27242–27250 (2006).
- 95 Hou X, Tan Y, Li M, Dey SK, Das SK. Canonical Wnt signaling is critical to estrogen-mediated uterine growth. *Mol. Endocrinol.* 18(12), 3035–3049 (2004).
- 96 Katayama S, Ashizawa K, Fukuhara T *et al.* Differential expression patterns of Wnt and beta-catenin/TCF target genes in the uterus of immature female rats exposed to 17alpha-ethynyl estradiol. *Toxicol. Sci.* 91(2), 419–430 (2006).
- 97 McCampbell AS, Broaddus RR, Loose DS, Davies PJ. Overexpression of the insulin-like growth factor I receptor and activation of the AKT pathway in hyperplastic endometrium. *Clin. Cancer Res.* 12(21), 6373–6378 (2006).
- 98 Rider V, Isuzugawa K, Twarog M *et al.* Progesterone initiates Wnt-beta-catenin signaling but estradiol is required for nuclear activation and synchronous proliferation of rat uterine stromal cells. *J. Endocrinol.* 191(3), 537–548 (2006).
- 99 Thigpen JT, Brady MF, Alvarez RD *et al.* Oral medroxyprogesterone acetate in the treatment of advanced or recurrent endometrial carcinoma: a dose-response study by the Gynecologic Oncology Group. *J. Clin. Oncol.* 17(6), 1736–1744 (1999).
- 100 Zaino RJ, Kauderer J, Trimble CL *et al.* Reproducibility of the diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. *Cancer* 106(4), 804–811 (2006).
- 101 Yahata T, Fujita K, Aoki Y, Tanaka K. Long-term conservative therapy for endometrial adenocarcinoma in young women. *Hum. Reprod.* 21(4), 1070–1075 (2006).
- 102 Kim YB, Holschneider CH, Ghosh K, Nieberg RK, Montz FJ. Progesterone alone as primary treatment of endometrial carcinoma in premenopausal women. Report of seven cases and review of the literature. *Cancer* 79(2), 320–327 (1997).
- 103 Saegusa M, Hamano M, Kuwata T *et al.* Upregulation and nuclear localization of beta-catenin in endometrial carcinoma in response to progesterone therapy. *Cancer Sci.* 94(1), 103–111 (2003).
- 104 Cloke B, Huhtinen K, Fusi L *et al.* The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. *Endocrinology* 149(9), 4462–4474 (2008).

- 105 Satterfield MC, Song G, Hayashi K, Bazer FW, Spencer TE. Progesterone regulation of the endometrial WNT system in the ovine uterus. *Reprod. Fertil. Dev.* 20(8), 935–946 (2008).
- 106 Catalano RD, Critchley HO, Heikinheimo O *et al.* Mifepristone induced progesterone withdrawal reveals novel regulatory pathways in human endometrium. *Mol. Hum. Reprod.* 13(9), 641–654 (2007).
- 107 Wang Y, Hanifi-Moghaddam P, Hanekamp EE *et al.* Progesterone inhibition of Wnt/beta-catenin signaling in normal endometrium and endometrial cancer. *Clin. Cancer Res.* 15(18), 5784–5793 (2009).
- 108 Laplante M, Sabatini DM. mTOR signaling at a glance. *J. Cell Sci.* 122(Pt 20), 3589–3594 (2009).
- 109 Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 78(1), 35–43 (1994).
- 110 Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell* 12(1), 9–22 (2007).
- 111 Menon S, Manning BD. Common corruption of the mTOR signaling network in human tumors. *Oncogene* 27(Suppl. 2), S43–S51 (2008).
- 112 Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat. Rev. Mol. Cell Biol.* 10(5), 307–318 (2009).
- 113 MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell* 17(1), 9–26 (2009).
- 114 Ingham PW, Taylor AM, Nakano Y. Role of the *Drosophila* patched gene in positional signalling. *Nature* 353(6340), 184–187 (1991).
- 115 Lee JJ, von Kessler DP, Parks S, Beachy PA. Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene Hedgehog. *Cell* 71(1), 33–50 (1992).
- 116 Lanske B, Karaplis AC, Lee K *et al.* PTH/PTHrP receptor in early development and Indian Hedgehog-regulated bone growth. *Science* 273(5275), 663–666 (1996).
- 117 Wijgerde M, Ooms M, Hoogerbrugge JW, Grootegoed JA. Hedgehog signaling in mouse ovary: Indian Hedgehog and desert Hedgehog from granulosa cells induce target gene expression in developing theca cells. *Endocrinology* 146(8), 3558–3566 (2005).
- 118 Hebrok M, Kim SK, St Jacques B, McMahon AP, Melton DA. Regulation of pancreas development by Hedgehog signaling. *Development* 127(22), 4905–4913 (2000).
- 119 Niemann C, Uden AB, Lyle S, Zouboulis Ch C, Toftgard R, Watt FM. Indian Hedgehog and beta-catenin signaling: role in the sebaceous lineage of normal and neoplastic mammalian epidermis. *Proc. Natl Acad. Sci. USA* 100(Suppl. 1), 11873–11880 (2003).
- 120 Perron M, Boy S, Amato MA *et al.* A novel function for Hedgehog signalling in retinal pigment epithelium differentiation. *Development* 130(8), 1565–1577 (2003).
- 121 Evangelista M, Tian H, de Sauvage FJ. The Hedgehog signaling pathway in cancer. *Clin. Cancer Res.* 12(20 Pt 1), 5924–5928 (2006).
- 122 Liao X, Siu MK, Au CW *et al.* Aberrant activation of Hedgehog signaling pathway contributes to endometrial carcinogenesis through beta-catenin. *Mod. Pathol.* 22(6), 839–847 (2009).
- 123 Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. *Nat. Rev. Drug Discov.* 5(12), 997–1014 (2006).
- 124 Kim JS, Crooks H, Foxworth A, Waldman T. Proof-of-principle: oncogenic beta-catenin is a valid molecular target for the development of pharmacological inhibitors. *Mol. Cancer Ther.* 1(14), 1355–1359 (2002).
- 125 Takahashi-Yanaga F, Sasaguri T. The Wnt/beta-catenin signaling pathway as a target in drug discovery. *J. Pharmacol. Sci.* 104(4), 293–302 (2007).
- 126 Maier TJ, Janssen A, Schmidt R, Geisslinger G, Grosch S. Targeting the beta-catenin/APC pathway: a novel mechanism to explain the cyclooxygenase-2-independent anticarcinogenic effects of celecoxib in human colon carcinoma cells. *FASEB J.* 19(10), 1353–1355 (2005).
- 127 Dihlmann S, Siermann A, von Knebel Doeberitz M. The nonsteroidal anti-inflammatory drugs aspirin and indomethacin attenuate beta-catenin/TCF-4 signaling. *Oncogene* 20(5), 645–653 (2001).
- 128 Baron JA, Cole BF, Sandler RS *et al.* A randomized trial of aspirin to prevent colorectal adenomas. *N. Engl. J. Med.* 348(10), 891–899 (2003).
- 129 Sandler RS, Halabi S, Baron JA *et al.* A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N. Engl. J. Med.* 348(10), 883–890 (2003).
- 130 Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J. Natl. Cancer Inst.* 94(4), 252–266 (2002).
- 131 Thun MJ, Namboodiri MM, Heath CW Jr. Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.* 325(23), 1593–1596 (1991).
- 132 Chan TA. Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention. *Lancet Oncol.* 3(3), 166–174 (2002).
- 133 Giardiello FM, Hamilton SR, Krush AJ *et al.* Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N. Engl. J. Med.* 328(18), 1313–1316 (1993).
- 134 Viswanathan AN, Feskanich D, Schernhammer ES, Hankinson SE. Aspirin, NSAID, and acetaminophen use and the risk of endometrial cancer. *Cancer Res.* 68(7), 2507–2513 (2008).
- 135 Bardia A, Ebbert JO, Vierkant RA *et al.* Association of aspirin and nonaspirin nonsteroidal anti-inflammatory drugs with cancer incidence and mortality. *J. Natl. Cancer Inst.* 99(11), 881–889 (2007).
- 136 Danforth KN, Gierach GL, Brinton LA *et al.* Nonsteroidal anti-inflammatory drug use and endometrial cancer risk in the NIH-AARP Diet and Health Study. *Cancer Prev. Res. (Phila.)* 2(5), 466–472 (2009).
- 137 Bodelon C, Doherty JA, Chen C, Rossing MA, Weiss NS. Use of nonsteroidal antiinflammatory drugs and risk of endometrial cancer. *Am. J. Epidemiol.* 170(12), 1512–1517 (2009).
- 138 Prizment AE, Folsom AR, Anderson KE. Nonsteroidal anti-inflammatory drugs and risk for ovarian and endometrial cancers in the Iowa Women's Health Study. *Cancer Epidemiol. Biomarkers Prev.* 19(2), 435–442 (2010).
- 139 Xiaoxin M, Yingnan J, Yanxia L, Shu L, Yuanqi H, Hongwei L. Experimental research on the depressant effect of aspirin on Ishikawa adenocarcinoma endometrium cell growth. *Int. J. Gynecol. Cancer* 19(7), 1182–1185 (2009).
- 140 Wood NJ, Quinton NA, Burdall S, Sheridan E, Duffy SR. Exploring the potential chemopreventative effect of aspirin and rofecoxib on hereditary nonpolyposis colorectal cancer-like endometrial cancer cells *in vitro* through mechanisms involving apoptosis, the cell cycle, and mismatch repair gene expression. *Int. J. Gynecol. Cancer* 17(2), 447–454 (2007).

- 141 Hasegawa K, Ohashi Y, Ishikawa K *et al.* Expression of cyclooxygenase-2 in uterine endometrial cancer and anti-tumor effects of a selective COX-2 inhibitor. *Int. J. Oncol.* 26(5), 1419–1428 (2005).
- 142 Gao J, Niwa K, Takemura M *et al.* Significant anti-proliferation of human endometrial cancer cells by combined treatment with a selective COX-2 inhibitor NS398 and specific MEK inhibitor U0126. *Int. J. Oncol.* 26(3), 737–744 (2005).
- 143 Gao J, Niwa K, Sun W *et al.* Non-steroidal anti-inflammatory drugs inhibit cellular proliferation and upregulate cyclooxygenase-2 protein expression in endometrial cancer cells. *Cancer Sci.* 95(11), 901–907 (2004).
- 144 Arango HA, Icely S, Roberts WS, Cavanagh D, Becker JL. Aspirin effects on endometrial cancer cell growth. *Obstet. Gynecol.* 97(3), 423–427 (2001).
- 145 Shah S, Hecht A, Pestell R, Byers SW. Trans-repression of beta-catenin activity by nuclear receptors. *J. Biol. Chem.* 278(48), 48137–48145 (2003).
- 146 Palmer HG, Gonzalez-Sancho JM, Espada J *et al.* Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J. Cell Biol.* 154(2), 369–387 (2001).
- 147 Park CH, Chang JY, Hahm ER, Park S, Kim HK, Yang CH. Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells. *Biochem. Biophys. Res. Commun.* 328(1), 227–234 (2005).
- 148 Kim J, Zhang X, Rieger-Christ KM *et al.* Suppression of Wnt signaling by the green tea compound (-)-epigallocatechin 3-gallate (EGCG) in invasive breast cancer cells. Requirement of the transcriptional repressor HBP1. *J. Biol. Chem.* 281(16), 10865–10875 (2006).
- 149 Rao CV, Desai D, Rivenson A, Simi B, Amin S, Reddy BS. Chemoprevention of colon carcinogenesis by phenylethyl-3-methylcaffeate. *Cancer Res.* 55(11), 2310–2315 (1995).
- 150 Jaiswal AS, Marlow BP, Gupta N, Narayan S. Beta-catenin-mediated transactivation and cell–cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene* 21(55), 8414–8427 (2002).
- 151 Roccaro AM, Leleu X, Sacco A *et al.* Resveratrol exerts antiproliferative activity and induces apoptosis in Waldenstrom's macroglobulinemia. *Clin. Cancer Res.* 14(6), 1849–1858 (2008).
- 152 Gandhirajan RK, Staib PA, Minke K *et al.* Small molecule inhibitors of Wnt/beta-catenin/lef-1 signaling induces apoptosis in chronic lymphocytic leukemia cells *in vitro* and *in vivo*. *Neoplasia* 12(4), 326–335 (2010).
- 153 Huang SM, Mishina YM, Liu S *et al.* Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 461(7264), 614–620 (2009).
- 154 Chen Z, Venkatesan AM, Dehnhardt CM *et al.* 2,4-diamino-quinazolines as inhibitors of beta-catenin/Tcf-4 pathway: potential treatment for colorectal cancer. *Bioorg. Med. Chem. Lett.* 19(17), 4980–4983 (2009).
- 155 Trosset JY, Dalvit C, Knapp S *et al.* Inhibition of protein-protein interactions: the discovery of druglike beta-catenin inhibitors by combining virtual and biophysical screening. *Proteins* 64(1), 60–67 (2006).
- 156 Takahashi-Yanaga F, Kahn M. Targeting Wnt signaling: can we safely eradicate cancer stem cells? *Clin. Cancer Res.* 16(12), 3153–3162 (2010).
- Review article exploring potential therapeutic targets involving the Wnt signaling pathway.
- 157 You L, He B, Xu Z *et al.* An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumor growth. *Cancer Res.* 64(15), 5385–5389 (2004).
- 158 Wei W, Chua MS, Grepper S, So SK. Blockade of Wnt-1 signaling leads to anti-tumor effects in hepatocellular carcinoma cells. *Mol. Cancer* 8, 76 (2009).
- 159 Tang Y, Simoneau AR, Liao WX *et al.* WIF1, a Wnt pathway inhibitor, regulates SKP2 and c-myc expression leading to G1 arrest and growth inhibition of human invasive urinary bladder cancer cells. *Mol. Cancer Ther.* 8(2), 458–468 (2009).
- 160 He B, You L, Uematsu K *et al.* A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells. *Neoplasia* 6(1), 7–14 (2004).
- 161 DeAlmeida VI, Miao L, Ernst JA, Koeppen H, Polakis P, Rubinfeld B. The soluble wnt receptor Frizzled8CRD-hFc inhibits the growth of teratocarcinomas *in vivo*. *Cancer Res.* 67(11), 5371–5379 (2007).

Website

- 201 The Wnt homepage.
www.stanford.edu/group/nusselab/cgi-bin/wnt