Am J Transl Res 2021;13(11):12168-12180
www.ajtr.org /ISSN:1943-8141/AJTR0135522

Review Article

Addressing activation of WNT beta-catenin pathway in diverse landscape of endometrial carcinogenesis

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Received May 21, 2021; Accepted August 24, 2021; Epub November 15, 2021; Published November 30, 2021

Abstract: The WNT-beta-catenin pathway (WP) is one of the major oncogenic pathways in solid tumors. Wnt beta-catenin pathway plays a unique role in a wide range of endometrial dysfunctions, from embryo implantation failure to severe pathogenic changes like endometriosis and endometrial cancer. Although abnormal activation of the pathway has long been known to be associated with endometrial tumorigenesis, the pathway's exact mode of involvement remains to be understood. As more evidence has been presented in favor of a crucial role of the WP in solid tumors, including endometrial cancer, anti-WP drugs are currently being tested to manage the disease. Aggressive tumor cells are nurtured by the tumor microenvironment (TME). The genetic alterations within tumor cells are the primary driving force to activate the extra-tumoral micro-environment. TME (a) provides metabolic support for the proliferation of tumor cells, (b) orchestrates immune-evasion, (c) initiates mechanistic signaling for several metastasis-associated phenotypes, and (d) supports cellular events for the development of drug resistance. To get metabolic as well as immune support from the tumor microenvironment, tumor cells cross-talk with components of the TME, most critically to the cancer-associated fibroblasts. Thus it is expected that the tumor-TME cross-talk throughout the process of tumorigenesis and metastasis is one of the characteristic features of an aggressive tumor. Here we review the WP's mechanistic involvement as a common culprit (*Un Colpevole Comune*) in endometrial tumor cells and endometrial cancer-associated fibroblast (CAF). In this review, we have attempted to discuss the activation of the WP in the genesis and progression of endometrial cancers, including endometrial tumor biology, tumor microenvironment, cancer-associated fibroblasts, and wnt-beta catenin genetic alteration. We interrogated the available literature on the various aspects of endometrial carcinogenesis leading to the pathway's activation. We examined how genetic alterations in WP directly influence tumor cell signaling to bring out different tumor cell phenotypes, and present palpable evidence to envision a role of WP inhibitors in the future management of the disease.

Keywords: Wnt beta-catenin pathway, cell signaling, tumor cells, cancer-associated fibroblasts

Prologue

Metastasis is a clinically divergent phenomenon in endometrial cancers where both tumor and tumor microenvironment (TME) plays a complex and individually distinct role. The WNT-beta-catenin pathway is critically important in metastasis. Activation of the WNT-beta-catenin pathway frequently occurs in endometrial cancers in addition to the co-activation of other oncogenic pathways like the PI3K pathway, p53-VEGF-mediated angiogenic pathway, or DNA-damage pathway. In the era of precision medicine, the response to a single-pathway targeted drug is limited, especially in advanced diseases.

The WP is a *bona fide* cancer-causing pathway. The role of the WNT-beta-catenin pathway (WP) (https://web.stanford.edu/group/nusselab/cgi-bin/lab/) in embryogenesis and organogenesis is preserved throughout the evolutionary tree [1-3]. Functional deregulation of the pathway has been associated with tumorigenesis of the colorectal, breast, and gynecological tumors, including endometrial cancers [4-6]. Dysfunction of the WP initiates tumorigenesis and participates in metastasis-associated phenotypes in several organ-type cancers [7, 8]. In endometrial cancer, WP is upregulated at different nodes involving both alterations of genes of the pathway as well as by activating signals from other oncogenic pathways like the PI3K path-

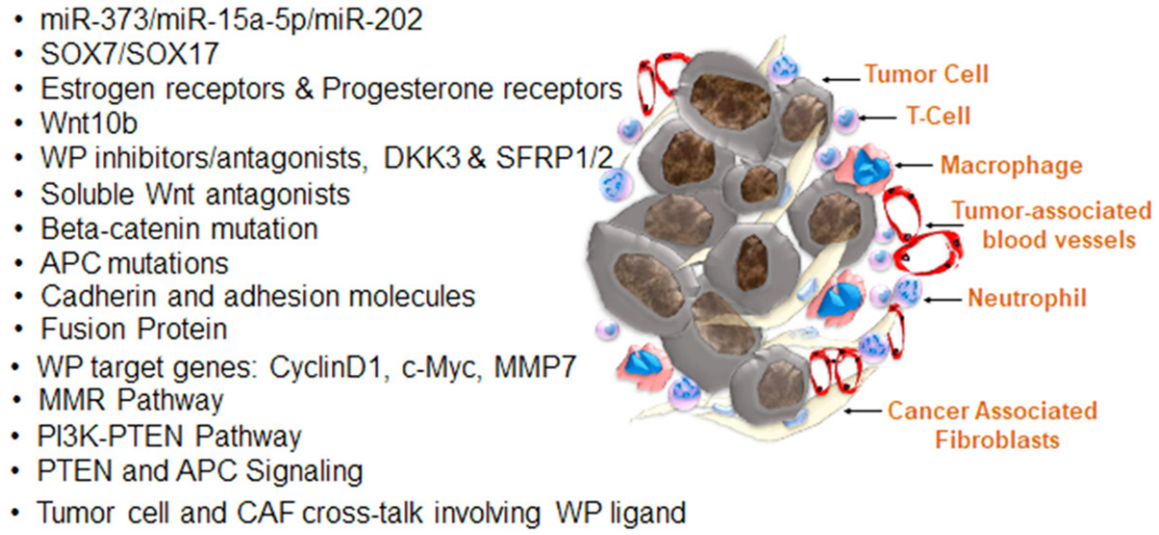

Figure 1. Modes and mediators of WP activation of beta-catenin in endometrial cancers: The WP pathway has multiple identified regulatory mechanisms in addition to the phosphorylation and ubiquitination of beta-catenin protein. Thus several mechanisms of dysregulation-mediated WP hyperactivation have been identified, which are responsible for the oncogenesis of various cancers, including endometrial cancers. The dysregulation of the WP pathway is mediated through different modes involving alterations of different genes, miRNAs, transcription factors, proteins, inhibitors, and antagonists.

way in the tumor cells. In endometrial cancer, studies have shown cross-talk between tumor cells and cancer-associated fibroblast (CAF) within the TME. Activation of WP either by the activating mutations of the WP genes or inactivating mutations of inhibitors of WNT in the CAF results in the WNT-activated CAF with TME. Since the WNT-beta-catenin pathway is a targetable entity, we undertook a critical evaluation of the pathway in endometrial cancers. The review presents a systemic examination of the role of the WNT-beta-catenin pathway in endometrial cancer. We critically interrogated the pathway's importance in the tumor compartment of endometrial cancers and tried to weigh the tumor compartment's interaction with the stromal micro-environment.

Activation of WP in endometrial cancers

WP pathway activation in tumor cells of several solid tumors has been reported [9, 10], which has ignited interest in targeting the pathway to design anti-WP therapies in managing WNT-related diseases. There are several mediators of WP activation in endometrial cancer, both in tumor cells and CAF of the TME, including miRNAs, SOX family of transcription factors, hormone receptors, WP ligands, WP receptors, WP antagonists, WP signaling nodes, adhesion molecules, fusion proteins, MMR pathway, and PI3K-PTEN pathway (**Figure 1**). There are a number of modes by which these mediators cause activation of the WP: (1) receptor-mediated activation of WP signaling, (2) blockade of degradation of beta-catenin, (3) upregulation of WP target genes, (4) initiating cross-talk from other oncogenic pathways, and (5) initiating cross-talk with CAF.

Both estrogen and progesterone signalings are critical in the growth and progression of gynecologic cancers. The premise for the thought that WP and sex hormone signaling in endometrial homeostasis and cancer are functionally connected came from the proposal that WP plays a critical role in the monthly re- and degenerative phases of the female reproductive tract. Estradiol potentiates WP during the proliferative phase of the cycle, which is then regulated by progesterone that attenuates WP signal during the secretory phase. In addition to female steroid hormones, the steroid receptor activator, a long non-coding RNA, is a critical regulator of gynecologic cancers, which promotes the progression of endometrial cancer through WP, and its expression levels were found upregulated in endometrial cancers [11]. An estradiol-mediated constitutive and sustained activation of WP is associated with tumori-

genesis [12]. In line with this hypothesis, several studies have found cross-talk of WP and hormone receptors in endometrial cancers. In recent years, serum levels of DKK1 (Dickkopf1) protein were found to be higher in patients with endometrial cancers than their age-matched controls [13]. Protein expression levels of estrogen receptor alpha were correlated with the expression levels of DKK1 and cytoplasmic beta-catenin. More importantly, their study revealed that the association between estrogen receptor alpha expression and overall survival was not significant when tested with DKK1- and cytoplasmic beta-catenin expression levels. The result indicated that signals arising from WP and from activation of estrogen receptors are functionally connected. Hyperplastic lesions of the endometrium due to dysregulated and heightened estrogen signals have been shown to overactivate the WP [14]. Progression of hyperplastic lesions to cancer is associated with additional activation of the WP. The study brings forward an oncogenic relationship between estrogen signaling and WP activation, explaining that (a) estrogen hyper-signal is responsible for approximately 80% of all endometrial cancer, and (b) 30% of the endometrial cancer patients carry mutations in the WP.

Progesterone, on the other hand, has a role in protecting against and preventing endometrial cancer. One way progesterone has been shown to exert its suppressive action on endometrial cancer progression is by regulation of lncRNA NEAT1/miR-146b-5p-mediated WNT/beta-catenin signaling pathway. Progesterone has been shown to inhibit cell cycle and viability by regulating the NEAT1/miR-146b-5p axis by WP [15]. Progesterone has been reported to inhibit WNT/beta-catenin signaling in both normal endometrium and endometrial cancers [16]. The functional relationship between progesterone and WP is more intriguing, considering WP's role in maintaining a delicate balance between stemness and differentiation. The inhibition of WP by progesterone is mediated via induction of DKK1 and FOXO1 in WNT-activated Ishikawa cells. In the study of Wang et al., progesterone acted as an inhibitor of WNT signaling in hyperplasia and in well-differentiated endometrial cancer [16]. Progesterone's inhibitory effect through induction of DKK1 and FOXO1 on WP may be critical for tumorigenesis and metastatic progression in endometrial cancer.

A SOX17-beta-catenin-EMT axis has been reported to control the migration of endometrial tumor cells. The expression of SOX17 was increased in higher stages of the disease and correlated to better outcomes. Mechanistically, SOX17, in an N-terminus-dependent way, was found to inhibit cell migration by inactivating the WP-mediated EMT [17]. Another SOX family member of transcriptional factors, SOX7, has been downregulated with WP activation in endometrial cancers [18]. Frequently underexpressed in endometrial cancers, SOX7 was able to inhibit TCF/LEF-1-dependent luciferase activity induced by WNT-1 in the *in vitro* model, which subsequently suppressed the expressions of WP targets like cyclin D1 and c-Myc in endometrial cells.

The involvement of MicroRNA in the oncogenesis of endometrial cancers has been studied. Li et al. [19] reported that miR-373 inhibited large tumor suppressor kinase 2 (LATS2), a direct target gene of miR-373. Overexpression of miR-373 promoted EMT and upregulated WP. However, the causal relation between WP and the upregulation of miR-373, which was identified in endometrial cancers that predicted poor prognosis needs to be established. In a different report, miR-202 inhibited migration/invasion and inactivated WP by suppressing beta-catenin expression in endometrial carcinoma. Chen et al. demonstrated that miR-202 was down-regulated in endometrial cancer tissues as tested by qRT-PCR and was associated with poor prognosis in patients [20]. The role of another miR, miR-15a-5p, was identified from the result that the miR-15a-5p was decreased in endometrial cancer cells and tissues. A putative binding site in the 3'-UTR of the Wnt3a gene was targeted by MiR-15a-5p, leading to the decreased Wnt3a protein expression under experimental conditions of miR-15a-5p overexpression [21].

A fusion protein-mediated activation of the WP has been observed in low-grade endometrial stromal sarcoma, which harbors chromosomal rearrangements that affect proteins associated with chromatin remodeling Polycomb Repressive Complex 2 (PRC2), including SUZ12, PHF1, and EPC1. Przybyl et al. reported that one of the targets affected by the fusion proteins is WP which is associated with a nuclear accumulation of beta-catenin in a significant proportion of low-grade endometrial stromal

sarcomas. The study demonstrated that a concordant nuclear expression of beta-catenin, as well as LEF1 in 7/16 low-grade endometrial stromal sarcomas and fusion proteins in low-grade endometrial stromal sarcomas, contribute to overexpression of Wnt ligands with subsequent activation of WP as well as the formation of an active beta-catenin/LEF1 transcriptional complex [22].

Modes of activation of WP involves nodes of WNT signaling

The activation of WP can occur at several nodal points of the WP, including (a) Wnt-ligands, (b) Wnt-receptors, (c) secreted Wnt-inhibitors, (d) beta-catenin, and (e) inter-pathway activation of WP. In endometrial cancers, the deregulation of the canonical WP observed is caused by activating beta-catenin mutations in approximately 10-45% of cases and by downregulation of WP antagonists by epigenetic silencing [10].

A role for Wnt-ligand, Wnt10b, in the oncogenesis of the endometrium has been proposed to promote proliferation and inhibit apoptosis through the activation of the WP [23]. Among many Wnt-ligands, Wnt-receptors, and signaling nodal points of the WNT-beta-catenin axis including Wnt1, Frizzled-1 (FZD1), Wnt5a, Frizzled-5 (FZD5), and beta-catenin tested in endometrial cancer type I as compared to atrophic endometrium, FZD5 expression was downregulated in endometrial adenocarcinoma [24]. An inverse correlation was reported between secreted frizzled-related protein 4 and beta-catenin expression in endometrial stromal sarcomas [25]. Similarly, FZD2 has been associated with EMT responses in metastasis of endometrial cancers [26], and secreted frizzled-related protein 4 has been shown to regulate Wnt7a signaling [27].

Soluble Wnt antagonists keep WP under tight control in normal tissues, and hence a strong association has been found between the tumorigenesis and expression of WP antagonists in several solid tumors. Expression of the WP antagonist, DKK3 gene, is frequently downregulated in endometrial cancers and is associated with prognostic clinicopathologic characteristics of the disease. Dellinger et al. demonstrated a biomarker role of the tumor suppressor, DKK3, in endometrial cancer, which controls both proliferation and invasiveness of tumor cells [28]. In a report from the NRG Oncology/Gynecologic Oncology Group, expression patterns of the WP inhibitors DKK3 and secreted Frizzled-Related Proteins 1 and 4 were evaluated in endometrioid adenocarcinoma [29]. The mRNA and protein levels of DKK3, SFRP1, and SFRP4 were assessed by a real-time reverse transcription-polymerase chain reaction and western blot analysis in 87 tissue specimens. Downregulation of mRNA was observed in patients with the high-grade disease and was associated with locoregional and distant recurrence.

Beta-catenin defined WP status

Beta-catenin is a physiological readout of the WP, and thus, it is tightly controlled by its "death box" (beta-catenin destruction complex) and secreted WP inhibitors. The upregulation of WP involves activation of beta-catenin signals leading to the transcriptional activation of several oncogenes associated with proliferation, migration, invasion, stemness, and EMT. The known mechanisms of the activation of the WP includes (a) blockade of degradation of beta-catenin at the "death box" due to mutation and/or phosphorylation-dependent inhibition of the signalosome, which is primarily comprised of adenomatous polyposis coli (APC), GSK3beta, axin, and casein kinase 1, (b) activation of the WP following downregulation of secreted WP inhibitors, (c) upregulation of WP ligands, and (d) mutation of beta-catenin [30]. Since beta-catenin is the physiological readout of WP's activation status, several modes of upregulation of the cellular levels of beta-catenin have been identified. The two most relevant modes are (1) hotspot mutation of beta-catenin, and (2) downregulation of beta-catenin degradation (**Figure 2**, Upper panel and Lower panel, respectively). Most of the mutations of beta-catenin identified so far were single missense mutations at exon 3 on serine/threonine residues (residues 33, 37, 41, and 45 as well as 32 and 34). Of these, 33, 37, and 41 are the cognate phosphorylation sites for GSK3beta altering the GSK3beta phosphorylation at its consensus motif, while 44 is the phosphorylation site for casein kinase 1. The phosphorylation(s) at the above positions are necessary for the initiation of degradation of beta-catenin. Sites 32 and 34 are the essential site for the interaction of beta-catenin with FBW1. FBW1

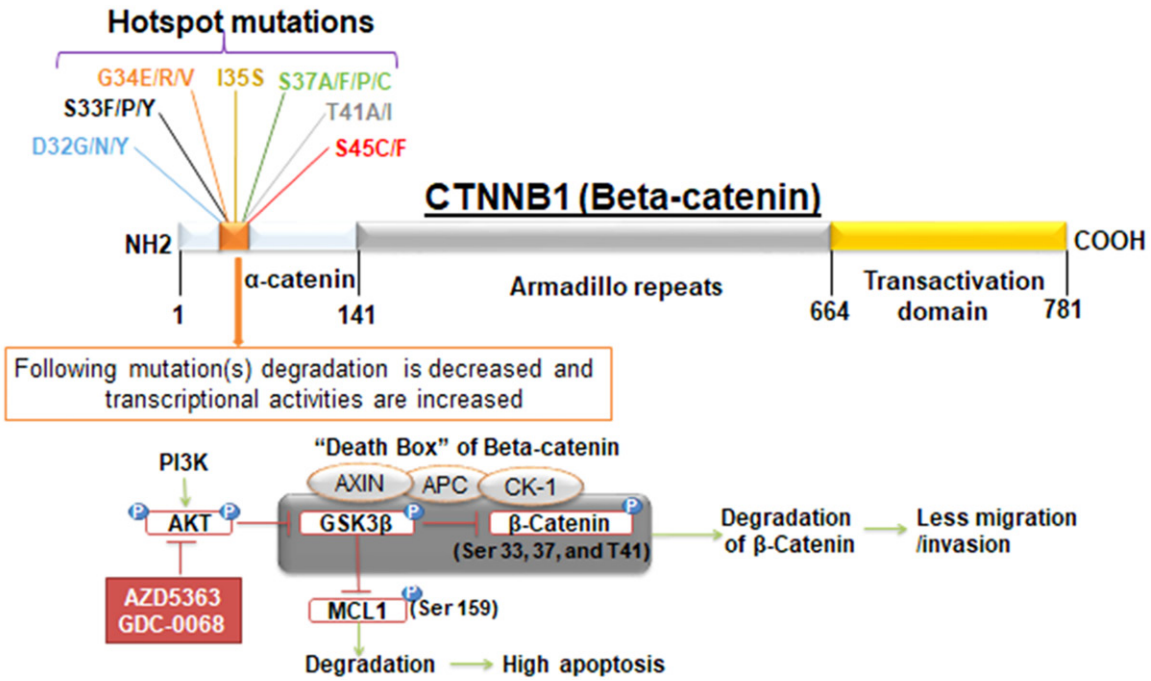

Figure 2. Hotspot mutations of Beta-catenin and events at the "death box" of beta-catenin in Endometrial Cancers: Mutations of beta-catenin were so far identified as a single missense mutation(s) on serine/threonine residues (residues 33, 37, 41, and 45 as well as 32 and 34) within exon 3. The sites at 33, 37, and 41 are the consensus phosphorylation sites for GSK3beta binding, altering the GSK3beta mediated phosphorylation of the beta-catenin. The site at 44 is the phosphorylation site for casein kinase 1. The phosphorylation(s) at the above positions are necessary for the initiation of degradation of beta-catenin within its "death box". Sites 32 and 34 are the essential site for the interaction of beta-catenin with *FBW1*. *FBW1* interacts with p300 to enhance the transcriptional activity of beta-catenin in the nucleus. Mutation sites (hotspot) of the *CTNNB1* gene and the events at the "death box" of beta-catenin are presented (upper panel). Activated AKT phosphorylates GSK3beta and thus inactivates it. Phosphorylated/inactivated GSK3beta fails to initiate ubiquitin-mediated degradation of beta-catenin in the "death box" involving AXIN1 and APC. Casein kinase-1 (CK1) initially phosphorylates beta-catenin at Ser 45, which induces the sequential phosphorylation of beta-catenin at Ser 33, 37, and T41 by GSK3beta. Following serine/threonine phosphorylation, beta-catenin is recognized and degraded by E3 ubiquitin ligase. The resulting increase in beta-catenin levels in the nucleus causes the transcriptional activation of beta-catenin target genes. Drugs that blocked the activation of AKT perturbs WP (lower panel).

interacts with p300 to enhance transcriptional activity in the nucleus [31]. It has been reported earlier that the undegraded beta-catenin initiates transcriptional activity of a plethora of genes, including *cMYC, CCND1,* and *MMP7* in the nucleus leading to the activation of the WP [30, 32].

The stabilization of beta-catenin due to mutations in exon 3 of the gene and other mechanisms plays a vital role in the development of endometrial carcinomas [32]. In endometrial cancers, the *CTNNB1* gene gets somatically mutated at codon 34, causing amino acid alterations at residues next to Ser 33 (one of the targets for phosphorylation of glycogen synthase kinase GSK-3beta), leading to the accumulation of its protein, more specifically in the nucleus [33]. Activation of the WP is conventionally evaluated in clinical samples by nuclear IHC for beta-catenin. Scholten et al. tested the hypothesis that the WP abnormality resulting in nuclear beta-catenin immunopositivity is a molecular feature of type I endometrial carcinoma [34].

We analyzed the expression pattern of different genes in 82 tumors from patients with endometrial cancers who were seen in our Avera Cancer Institute from 2014 to 2020 (**Figures 3**, **4**). The tumor samples were subjected to comprehensive genomic profiling (FoundationOne). We recorded alterations of 4 WP genes in 82 patients with endometrial cancer, including *CTNNB1, APC, LRP1B, and SOX9*. We recorded alterations of the beta-catenin gene in 20

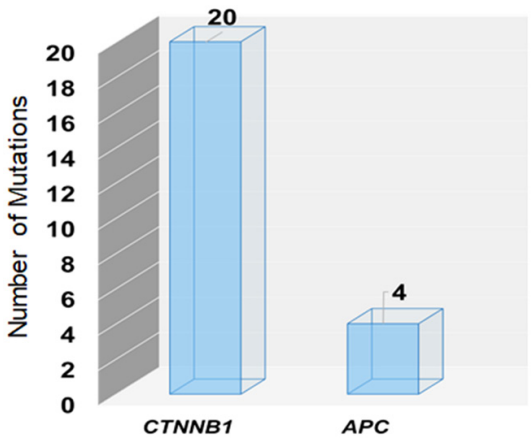

Figure 3. Alterations of the WP genes in patients with endometrial cancers: The Avera Experience: Alterations of the WP genes in patients with endometrial cancers from Avera Cancer Institute are presented. The data (FoundationOne reports) were obtained from 82 tumor samples from patients with endometrial cancers. The figure shows the number of alterations of the genes of the "death box" of beta-catenin.

patients. *APC* alterations were observed in 4 patients, while alterations in each of LRP1B and SOX9 genes were recorded in 1 patient. The alterations of the "beta-catenin death-box" genes of the WP in endometrial cancer are presented (**Figure 3**). Hotspot mutations observed at different locations in tumors from patients with endometrial cancer at Avera Cancer Institute are presented. Different locations on the beta-catenin gene are depicted as numbers. We observed alterations at seven sites within the hotspot of beta-catenin. The alterations included deletions and point mutations. Maximum alterations were recorded at positions 32, 33, and 34 within the hotspot (**Figure 4**). We observed two types of alterations of the beta-catenin gene, missense mutations and deletion mutation (as represented by numbers in the figure). All the above alterations occurred within the hotspot of the gene. While alterations at positions from 33 to 45 have been previously identified and reported earlier [35], the deletion mutations at Q28_S37 del have been observed for the first time (cohort of endometrial patients from Avera). Hyperactivations of WNT-beta catenin signaling in tumor cells containing these mutations have been implicated in tumorigenesis, metastasis, and drug resistance in several cancers, including gynecological malignancies [8, 36, 37].

Recently a new mode of activation of WP through beta-catenin has been reported in several solid tumors, including endometrial cancers. Chen et al. [38] studied a functional relationship between a potential target in cancer chemotherapy, multi-drug resistance protein 4 (MRP4), and WP upregulation in human endometrial cells *in vitro* and in a mouse embryo-implantation model *in vivo*. MRP4 and beta-catenin were found to be co-localized and co-immunoprecipitated in both mouse and human endometrial cells, and MRP4-knockdown accelerated beta-catenin degradation in human-endometrial cells. Although the mode of action of MRP4 in stabilizing beta-catenin towards sustaining an upregulated WP signaling was not elucidated in their study, a positive correlation between MRP4 and beta-catenin in endometrial cancer was found to associate to WNT-beta-catenin target genes.

Although beta-catenin mutation has been known to be a mediator of WP activation and has been implicated in the development of endometrial carcinoma, a beta-catenin mutation independent activation of WP has also been reported in endometrial cancer. Up to 25% of the disease has increased beta-catenin in the nucleus without evidence of beta-catenin mutations, indicating that alterations of *APC*, gamma-catenin, *AXIN1* and *AXIN2* are independent of mutations of beta-catenin, and are involved in the process [39]. In another study, a differential role of beta-catenin mutation was recorded as compared to *APC* mutations responsible for the development of uterine endometrioid carcinoma [40]. Future studies will identify whether different subtypes of endometrial cancer bear different signatures of activation of the WP.

Beta-catenin levels in cells reflect how much beta-catenin is degraded. WP is upregulated when mutations occur in the tumor suppressor gene, *APC* leading to the loss of beta-catenin-degradation. Interestingly, *APC* gene alteration plays a critical role in both sporadic and germline endometrial cancer. In studying the involvement of *APC* and beta-catenin in hereditary (nonpolyposis colorectal cancer)-related endometrial cancers, Kariola et al. reported a frequent overactivation of the WP in hereditary endometrial cancer with abnormal accumulation of beta-catenin protein in nuclei [41].

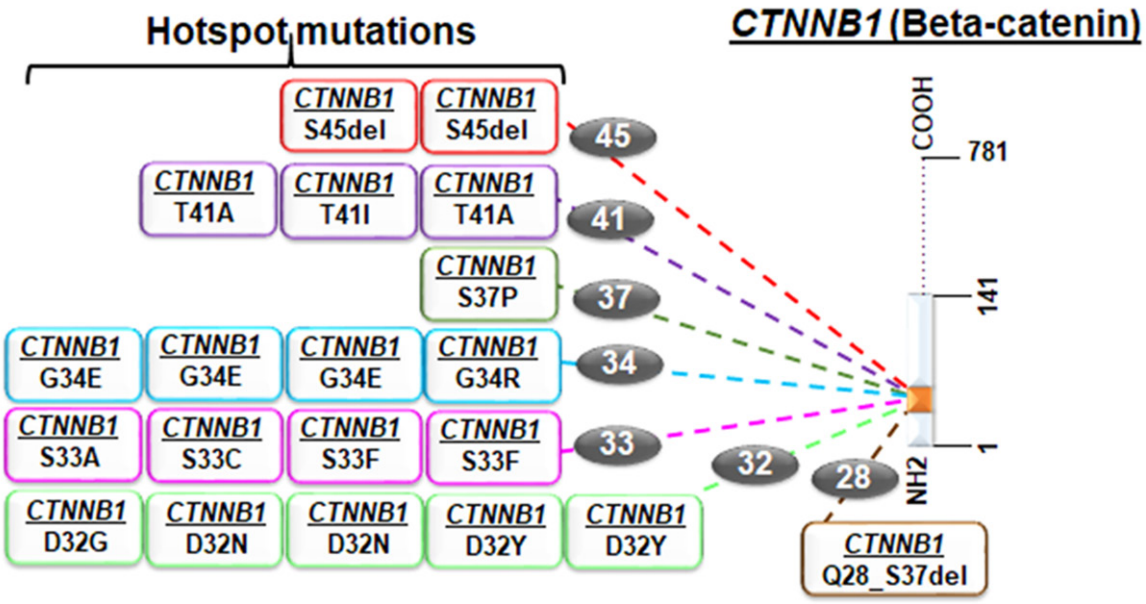

Figure 4. Hotspot mutations of *CTNNB1* gene in patients with endometrial cancers: The avera experience: hotspot mutations were observed at different locations of the beta-catenin gene in tumors from patients with endometrial cancers. Different locations on the beta-catenin gene are depicted as numbers. Two types of alterations of the beta-catenin gene, missense mutations and deletion mutations, were observed in patients' tumors with our cohort. Numbers in the figure represent the positions of the alterations. All the above alterations occurred within the hotspot of the beta-catenin gene. While alterations at positions from 33 to 45 have been previously identified and reported earlier, the deletion mutations at Q28_S37 del has been observed for the first time in the cohort of endometrial patients from Avera.

Clinical significance of *CTNNB1* mutation-driven WP activation was reported in the seminal work of Liu et al. in tumors from 271 patients with endometrioid endometrial carcinoma, the most common form of endometrial carcinoma [42]. High expression levels of CTNNB1, MYC, and CCND1 were associated with poorer overall survival in patients with low-grade endometrioid endometrial carcinomas. Liu et al. performed an integrated analysis on the multiple-dimensional data types, including whole-exome and RNA sequencing, RPPA profiling, and clinical data in The Cancer Genome Atlas (TCGA) to identify molecular fingerprints: four transcriptome subtypes (clusters) with distinct clinicopathologic characteristics. *CTNNB1* exon 3 mutations were found in 87.0% of Cluster II comprising younger, obese patients with lowgrade endometrioid endometrial carcinoma with diminished survival that exhibited a low overall mutation rate which was associated with activation of the WP signaling. Interestingly, the WP was not enriched in cases where the mutation of *CTNNB1* was found outside exon 3, and the poorer outcome was observed in Cluster II patients with *CTNNB1* mutation-associated activation of the WP. Thus *CTNNB1 mutations defined a subtype of low-grade or low-stage endometrioid endometrial carcinoma with poor outcome,* demanding a more aggressive clinical management.

WP target genes

WP's involvement in endometrial tumors is evidenced by (1) the observed increase in nuclear accumulation of beta-catenin and (2) upregulation of WP target genes. Kurihara et al. evaluated overexpression of cyclin D1 and MMP-7, which are direct transcriptional target genes of beta-catenin in endometrial stromal tumors and related high-grade sarcomas wherein the nuclear accumulation of beta-catenin was observed [43]. Undifferentiated endometrial sarcomas featuring uniform nuclei exhibited frequent coincident expression of beta-catenin and cyclin D1, indicating the possibility that cyclin D1 is upregulated by beta-catenin in these high-grade endometrial stromal sarcomas.

Activation of WP in the context of other oncogenic pathways

WP is a well-connected pathway to influence a plethora of cellular events in health and disease in various organs, including the endometrium. Thus it is expected that WP will be regulated by a number of signals of many pathways. In endometrial cancer, WP signals meet the crossroads of (1) PI3K-PTEN pathway, (2) TP53 signals, (3) MSI, and other pathways associated with tumorigenesis, including TGFbeta signaling [44, 45]. Pathway analysis in endometrial cell lines by Wang et al. showed that genes in the PI3K and WNT pathways are commonly affected [46]. Tumor samples of patients with endometrioid type endometrial cancer FIGO-stage 1 were obtained from the randomized PORTEC-2 trial. *TP53* status and MSI-H were found to be the critical genetic prognostic factors for decreased DFS, whereas high PI3K-AKT pathway activation showed only a trend. Beta-catenin was not prognostic [47].

Both beta-catenin and E-cadherin are epithelial cell adhesion molecules. WP's role has been revealed in the disease in the context of intercellular adhesion molecules and attachment proteins. Protocadherin10 has been reported as a novel WP regulatory element in endometrioid endometrial carcinoma, wherein it was downregulated following aberrant methylation of its promoter, providing a piece of evidence for a novel role of the protocadherin10-WNT-beta-catenin-MALAT1 regulatory axis in the development of endometrioid endometrial carcinoma [48]. In the development of recurrent endometrial carcinoma, the role of APC, beta-catenin, and E-cadherin was tested by Pijnenborg et al. [49]. In contrast to epigenetic and genetic aberrations in APC and beta-catenin genes to develop local recurrences and distant metastases, E-cadherin expression was predictive for developing distant metastases in endometrial carcinoma in a limited number of patients. In testing the hypothesis that these adhesion molecules' expression patterns characterize the histological subtypes of endometrial carcinoma, Schlosshauer et al. identified that the expression patterns in high-grade endometrial carcinoma are associated with two histological subtypes; beta-catenin^{High}/E-cadherin^{Low} endometrioid adenocarcinoma and beta-catenin^{Low}/E-cadherin^{High} serous carcinoma [50].

The PI3K-PTEN pathway activation upregulates WP via beta-catenin. The PI3K-PTEN pathway activation may occur following (a) growth factor stimulation, (b) activating mutation of PIK3CA, and/or (c) inactivating mutation of PTEN. Upregulation of the pathway leads to the activation of AKT, which in turn inhibits GSK3beta mediated cytoplasmic degradation of beta-catenin via phosphorylation at serine residue leading to the activation of WP [30]. Not degraded at the "beta-catenin-death-box", beta-catenin is thus increased in the cytoplasm and reaches the nucleus to activate WP-target genes transcriptionally. The degradation of beta-catenin can also be blocked following the mutation of APC, a constituent member of the "beta-catenin-death-box". Activation of the WP and loss of PTEN activity are frequently observed in endometrioid endometrial cancers. Zee et al. interrogated the loss of PTEN and APC gene function in the endometrium [51]. Functional loss of APC led to a cytoplasmic and nuclear accumulation of beta-catenin. Beta-catenin's nuclear accumulation mediated activation of WP alone in mice led to uterine hyperplasia and squamous cell metaplasia, but without malignant transformation. Similarly, loss of PTEN function led to the development of squamous metaplasia. In contrast to the effect of loss of APC function, PTEN loss initiates endometrial cancer indicating that a somatic loss of the wild-type Pten allele represents the rate-limiting initiation step in endometrial cancer. Thus, both PTEN and APC's simultaneous loss led to an earlier onset and an aggressive form of tumor in mice, demonstrating a synergistic action of the WP and PTEN signaling. At present, the impact of specific mutations of PTEN on the activation of WP remains debatable, even though PTEN and beta-catenin mutations constitute the predominant genetic alterations in endometrioid carcinomas of the endometrium. In contrast to prostate cancer, mutations in the PTEN gene do not affect the beta-catenin protein's subcellular distribution in endometrial carcinoma [52].

Mismatch repair deficiency (dMMR) cancers exhibit characteristic predictors of drug response. One of the reasons for such an event is the functional relationship between WP and T-cell infiltration in different solid tumors and the status of the PD-1-PD-L1 axis. In a recent study, Rowe et al. examined the relationship between the activation of WP and the PD-L1

axis by evaluating the nuclear beta-catenin expression in a well-characterized cohort of endometrial cancer by mismatch repair status and PD-L1 expression. In their study, 83.3% of the tumors with nuclear beta-catenin expression demonstrated concomitant tumoral PD-L1, and both tumoral and immune cell expression of PD-L1 was significantly associated with dMMR tumors [53]. Their study inferred that nuclear beta-catenin status might be considered to be a predictive biomarker for non-response to immune checkpoint inhibition in mismatch repair-deficient tumors.

Connecting tumor to tumor micro-environment: WP signaling in endometrial CAF

The tumor microenvironment (TME) has the capability to define a tumor, its progression, its response to a drug, its immune response, and its fate. The role of CAF as the most versatile and diverse component of TME influencing all of the above functions is well-documented in solid tumors [54, 55]. The abnormal/activated form of stromal fibroblasts is one of the determinants of clinical outcome. Replacement of abnormal/activated stromal CAF (Normalization of activated CAFs/Stromal reprogramming) is a possible avenue for novel CAF-directed anticancer therapy in the management of solid tumors [55-57]. Understanding of the cellular signaling in CAF and its cross-talk with tumor cells [58] has been the basis of tumor-stroma-oriented management of the disease. The involvement of CAF in tumorigenesis and determining the fate of the tumor progression has been recorded in several solid tumors, including gynecologic tumors [58-62].

The role of stromal CAF in tumorigenesis and a direct role of the WP in the activation of these stromal CAF are documented in solid tumors, including endometrial cancer [63]. Activation of *CTNNB1* was found essential for the expression of progesterone receptors that mediated the final differentiation step of abnormal endometrial stromal fibroblasts before implantation [64]. In endometrial cancer, the evidence for activated CAF involvement and its cross-talk has been somewhat limited. However, in this case, the *absence of evidence is not evidence of absence*. Aprelikova et al. reported the cross-talk between CAF and tumor cells involving miR-148a in mediating tumor cell's metastasis-associated phenotypes in endometrial cancer [65]. miR-148a is downregulated in 94% of CAF by DNA methylation compared with matched normal tissue fibroblasts established from patients in their studies. Target genes of miR-148a are Wnt1 and Wnt10B. Wnt10B stimulated migration and matrigel-invasion of tumor cells. Elevated levels of Wnt10B protein in CAFs were observed, which decreased following lentiviral re-introduction of miR-148a. The cross-talk between CAF and tumor cells was tested using conditioned media from CAF overexpressing miR-148a, which impaired migration in endometrial cell lines in co-cultures. Their study demonstrated a cross-talk between tumor and stromal CAF in endometrial cancer, which regulated tumor cells' phenotypes involving the WP. Future mechanistic studies to test how CAF and tumor cells signal synergistically in bringing tumor cell phenotypes involving the WP will be required to design stroma-based treatment strategies in endometrial cancer. Recent studies provide evidence that canonical WNT-beta-catenin signaling (1) promotes T cell lymphopoiesis and regulates peripheral T cell activation and differentiation, (2) regulates differentiation of NK cell and dendritic cells, (3) participates in the immune escape through the expression of checkpoint inhibitors, PD-L1 and CD47, transcriptionally controlled by WP target gene, MYC, (4) influences cancer immunosurveillance and (5) controls survival of Tregs [66-68].

Epilogue

WNT-beta-catenin signaling is highly activated in certain solid tumors, which has led to the development of several WP signaling inhibitors for cancer therapy. To suppress the signals from WP ligands or receptors for the management of the disease, inhibitors/antagonists of PORCN, WP ligand, and FZD have been examined in clinical trials. Ipafricept (OMP-54F28) is a recombinant fusion protein containing a combination of the extracellular ligand-binding domain of the human FZD8 receptor and human IgG1 Fc fragment [69, 70]. Ipafricept blocks the WNT-beta-catenin signaling pathway by playing as a decoy receptor while binding and confiscating Wnt ligands. Since it can bind all Wnt proteins, it functions as a broad-spectrum Wnt antagonist. Wnt antagonists have been used in clinical trials in various solid tumors and different chemotherapy regimens. Recent-

ly, encouraging clinical efficacy was recorded in gynecological cancer, especially in recurrent platinum-sensitive ovarian cancer, along with paclitaxel and carboplatin. Overall, 28 patients of the ITT (intention to treat) had a complete or partial response (CR or PR). CR was reported in 29.7% of patients. Median PFS was 10.3 months, and OS was 33 months (*NCT02092363*) [71]. This data is highly encouraging to initiate a new clinical trial in endometrial cancer patients, and especially those who have beta-catenin pathway upregulation. The future mechanistic studies on the tumor-CAF cross-talk involving the WP will pave the way to the novel strategies intercepting the pathway in endometrial cancer.

Much effort has been funneled towards molecular profiling of the tumor/biopsy towards the clinical management of patients empowered by sequencing-based drug matching in today's world of precision medicine. Like most other solid tumors, the WP's alteration(s) rarely occur without a simultaneous co-mutation(s) of different oncogenic pathways. Such a co-existence of activating mutation(s)/alteration(s) upregulates more than one oncogenic pathway and the presence of clonal heterogeneity within the tumor landscape. Although it challenges sequencing-based drug matching, yet it provides the most fail-safe option for the treatment. As the tumor presented with a set of molecular features (genomic alterations and sub-clonal characters) responds to the drug combination, it evolves. Real-time monitoring of the drug's effect and tumor evolution through treatment and post-treatment follow-up by liquid biopsy, including ctDNA and CTC, provides the best option to know the tumor and manage it clinically. Such real-time knowledge about the evolution of cancer in question offers a unique opportunity to apprehend the sometimes inevitable development of drug resistance and provides a scope to counter a resistance preemptively.

Acknowledgements

Authors acknowledge Avera Research Institute, Sioux Falls, SD, and Department of Internal Medicine, SSOM, USD, Sioux Falls, SD. Authors acknowledge FoundationOne. Authors acknowledge Avera Research Institute, Sioux Falls, SD, for funding. No external funding was received.

Disclosure of conflict of interest

None.

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