

## Wnt/ $\beta$ -catenin/Lef-1 signaling in the uterus and its implications in uterine gland formation and cancer development

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### Abstract:

*Wnt/b-catenin signaling appears to be important in a multitude of cancers. This review article details current knowledge of the Wnt/b-catenin/Lef1 pathway in generalized gland regulation and formation in addition to endometrial cancer. Wnt signaling is critical for the development of the female urogenital system, in particular mullerian duct formation. Dysregulation of this signaling pathway at multiple nodes has been observed in numerous tumor types, including endometrial cancer. In particular, nuclear accumulation of b-catenin correlates with tumor severity. As such, therapeutic modulation of Wnt signaling represents an emerging avenue for the treatment of cancers that rely on this pathway.*

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### Macro Overview of the Uterus

The mammalian uterus is a complex reproductive organ whose primary purpose is to support the implanted embryo/fetus throughout gestation. It undergoes four main developmental programs over the course of a lifetime. The first program establishes the simple uterine tube. In mice this tube forms in the absence of estrogen receptors (ER)<sup>1</sup> or progesterone receptors (PR),<sup>2</sup> indicating that the early program acts independently of the steroid hormones estradiol or progesterone. The second program begins with the onset of puberty, as the endometrium undergoes cyclical waves of proliferation, differentiation, and, barring implantation, regression; this cyclic wave is termed the menstrual cycle in primates and estrous cycle in non-primate species. In preparation for implantation of the embryo into the uterine lining, the third program begins with decidualization induced by progesterone signaling.<sup>3</sup> This process causes increased glandular cell secretions, vascular remodeling,

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and stromal storage of glycogen. These changes support the developing embryo until the placenta matures as well as modulate maternal-fetal communications. Finally, as the ovaries stop producing estradiol, testosterone, and progesterone, the uterus regresses into a state of atrophy and a woman enters menopause. While the roles of estrogen, progesterone, and testosterone in these programs have been widely studied, the molecular mechanisms determining initial development, adenogenesis, and regeneration of the endometrium are still unclear.

#### **Molecular/histological description of the uterus and stages of development**

The uterus consists of a highly regenerative endometrium and a muscular outer myometrium.<sup>4</sup> The endometrium is made up of the luminal epithelium and tree-like endometrial glands, which are embedded within a fibroblast-like stroma. In humans the endometrium is further divided into the basalis layer of tissue and epithelial glands. The Basalis layer consists of a dense stroma near the myometrium and is interspersed with epithelial glands, in which reside the putative adult stem cells needed to regenerate the endometrium following menses.<sup>5,6,7</sup> These stem cells reepithelialize the lumen, and then expand the stromal and glandular portions to reform the functionalis layer. Though histologically distinct in humans, these layers appear less well defined

in mice. However, studies examining label-retaining cells (LRCs) in the murine endometrium following decidualization and endometrial breakdown during the estrous cycle indicate the existence of putative stem cells near the myometrial layer in the remnants of endometrial glands.<sup>8</sup>

The uterus is derived from the sexually undistinguished bilateral embryonic Mullerian ducts, which are formed during invagination of the coelomic epithelium into tubular stoma.<sup>9</sup> Expression of the male-determining factor, SRY, stimulates expression of antiMullerian hormone (AMH), which induces regression of the Mullerian duct in males. Without SRY and AMH, the Mullerian ducts continue developing and eventually become the female internal reproductive organs.<sup>10</sup> As the ducts proliferate and expand caudally, the Mullerian ducts fuse at the midline, thus forming the uterus. In primates this fusion is complete and yields a single uterus, whereas in most non-primate mammals incomplete fusion occurs and leads to the presence of two uterine horns. While development of the uterus is well-understood at the anatomical level, we are still unraveling the cellular pathways that mediate these distinct stages of development. However, several pieces of data have demonstrated a critical role for the Wnt signaling pathway in formation of the uterus.

### **Indications that the Wnt pathway is critical to uterine development**

Mouse knockout studies demonstrate the importance of the Wnt signaling pathway in reproductive organ development. Mullerian duct formation requires Wnt4 and Wnt9b expression, as deficiency of either of these genes prevents formation of the female urogenital system.<sup>11,12</sup> At the cellular level, Wnt7a is expressed within the luminal cells of the early developing uterus and is required for gland formation.<sup>13,14</sup> Wnt5a is expressed within the uterine stroma and also required for adenogenesis.<sup>15</sup> Mice engineered to express activated beta-catenin in the endometrium develop proliferative hyperplasias,<sup>16</sup> while expression in the myometrium leads to uterine fibroids.<sup>17</sup> Wnt4 is also required for the decidualization process following implantation, as demonstrated by conditional knockout of Wnt4 in PR-expressing endometrial cells.<sup>18</sup> Aberrations in Wnt signaling are associated with infertility, endometriosis, endometrial cancer and gestational diseases,<sup>19</sup> underscoring the importance of this pathway. Further, there are indications that the Wnt pathway is required to mediate the actions of estrogen signaling<sup>20</sup> whereas progesterone inhibits the Wnt pathway,<sup>21</sup> exemplifying the intrinsic dance of steroid hormonal signaling and Wnt-mediated proliferation and differentiation.

### **Wnt pathway overview**

The Wnt pathway was initially discovered via two parallel areas of research, early development and tumorigenesis. In *Drosophila*, mutations in the *Wingless (Wg)* gene led to wingless flies and other early developmental abnormalities.<sup>22</sup> Studies involving the *int* gene in mice revealed the role of this protein in tumorigenesis.<sup>23</sup> Many years later homology studies indicated that the *Wg* and *int* genes belong to the same evolutionary family, and the canonical Wnt pathway was born.<sup>24</sup> Wnt is now known to be a secreted ligand that binds to the Frizzled (Fz) receptor family of nearby cells, often in a gradient fashion, to regulate cell differentiation and proliferation in early development.<sup>25,26,27</sup> Fz then activates the Dishevelled (Dsh) protein, which causes the disassociation of the “β-catenin destruction complex.”<sup>28, 29, 30, 31, 32</sup> This complex of proteins consists of the adenomatous polyposis coli gene (APC), axin, and GSK3, which induce degradation of β-catenin through proteolysis. In response to a Wnt activating signal, β-catenin levels rise, and then β-catenin translocates to the cell nucleus and binds to transcription factors of the TCF/LEF family.<sup>33,34,35</sup> Thus, β-catenin serves as transcriptional activator for downstream target genes. The list of Wnt signaling targets include cyclin D, a regulator of cell cycle progression,<sup>36,37</sup> c-myc, a strong oncogene that recruits histone acetyltransferases (HATs), which remodel the chromatin and allow for increased transcription,<sup>38</sup>

and several matrix metalloproteins (MMPs), which are involved in cell migration and differentiation.<sup>39,40,41</sup> By controlling expression of multiple genes that mediate in a plethora of cellular events, the Wnt pathway is a central regulator of basic cellular processes.

In addition to the canonical Wnt pathway, several studies have indicated Wnt signaling can be propagated through alternate, non-canonical pathways, including Wnts 4, 5a and Wnt 11. For example, in the planar cell polarity pathway (PCP), Wnts maintain signals through Fz5 to activate Dsh, but Dsh then binds to other proteins of the Rac/Rho/Rock/JNK pathway that regulate actin remodeling of the cytoskeleton and cell migration. Yet another non-canonical pathway is the Wnt/calcium pathway, where Wnts interact with Fz5 and activate Dsh in combination with coupled G-proteins. Downstream signaling through the G-proteins eventually leads to the release of intracellular calcium from the endoplasmic reticulum. Wnt/Ca<sup>2+</sup> signaling is believed to counter classical Wnt signaling, but further research is needed to understand the precise mechanism.

### **Wnt pathway and cancer**

Since the initial finding that the mutations in the *int* gene results in formation of mammary tumors in mice, mutations in the various components of the Wnt pathway have been documented in many human cancers. One of the best-

studied mutations is in the APC gene, which was originally identified by linkage studies performed on patients with hereditary adenomatous polyposis coli. Patients with mutations in APC develop numerous intestinal and colonic polyps very early in life and have a near 100% penetrance of colorectal cancer by their early 40s.<sup>42</sup> Hereditary mutations in APC account for only ~1% of all colorectal cancers, but, when combined with sporadic mutations, account for >80%, indicating the importance of the Wnt pathway in colorectal cancer. Further, mutations or alterations in the Wnt pathway, most specifically  $\beta$ -catenin, have been found in cancers of the breast, colon, ovaries, uterus, blood, liver, pancreas, and skin, among others.<sup>43,44</sup>

### **Wnts in endometrial cancer**

Endometrial carcinoma is the most common gynecologic malignancy and ranks second as a cause of gynecologic cancer mortality in the United States; in 2010, the American Cancer Society predicted 43,470 new cases and 7,950 deaths in that year alone.<sup>45</sup> Most endometrial carcinomas arise from the glands of the endometrium (adenocarcinomas), and the remainder are derived from the supporting stroma (sarcomas). Endometrial carcinomas are divided into two broad categories of tumors based on their etiology.<sup>46</sup> Type I endometrioid endometrial carcinomas (EEC) are typified by a hyperestrogenic state, general low

grade, and low stage. They are typically responsive to progesterone treatment and express estrogen and progesterone receptors. They also occur in pre- and perimenopausal women with a generally high BMI and account for roughly 80% of endometrial cancers. Luckily, with surgical, hormone, and/or chemotherapeutic treatments, they are roughly 85% curable at the 5 year survival mark. Type II non-endometrioid endometrial carcinomas (NEEC) do not correlate with a hyperestrogenic state and occur in postmenopausal women with atrophic endometrium. These tumors are generally high grade, poorly differentiated, and very aggressive with poor outcomes. They generally do not express ER or PR and are not responsive to hormonal treatment. While it is useful to divide the tumors into broad categories, it should be noted that there is overlap between the two types. For example, some subsets of endometrioid tumors progress to high grade and poor prognosis, while others exhibit mixed characteristics of both. Therefore, an understanding of the molecular profile of the tumors may be more informative in terms of guiding treatment for individual endometrial tumors, regardless of segregation into Type I or II status.

As expected, the two types of endometrial cancer also have differing molecular dysregulations. The two predominant alternations noted in most Type I endometrioid cancers are changes in the tumor suppressor PTEN, a protein and lipid phosphatase, and elevated levels of

$\beta$ -catenin. Loss of PTEN results in abrogation of cell cycle checkpoint control and up-regulation of anti-apoptotic pathways. PTEN mutations are reported in 25-83% of endometrioid tumors, whereas these occur in less than 10% of Type II NEECs.<sup>47</sup> Conditional knockout of PTEN in ER-expressing cells results in endometrial hyperplasia as early as 10 days postnatally, with progression to carcinoma by one month.<sup>48</sup> These data indicate that loss of PTEN specifically results in endometrial carcinoma with 100% penetrance. In contrast to Type I tumors with mutant PTEN, the most common alterations noted in Type II tumors are mutations in the tumor suppressor P53.<sup>49</sup> Occasionally mutations in P53 are observed in grade 3 Type I endometrioid tumors, while 90% of NEECs exhibit P53 mutations. Of particular interest, P53 mutations often occur in the absence of hyperplasias again indicating hormone indifference.<sup>50</sup>

The other predominant alteration noted in Type I endometrial tumors is nuclear accumulation of  $\beta$ -catenin (e.g., 13-69% of cases of endometrioid endometrial carcinoma), but only a few studies have examined the role of Wnt signaling in its etiology. Among these, one study by Fukuchi et al.<sup>51</sup> assessed endometrial tumors for  $\beta$ -catenin mutations in exon 3, the site of regulatory phosphorylation by GSK3 $\beta$ . Mutations in this domain result in constitutive  $\beta$ -catenin nuclear accumulation and corresponding transcriptional activity and have been associated with many

solid tumors. Of the 76 tumors examined, approximately 13% had mutations within this domain, and 38% expressed  $\beta$ -catenin at high levels.<sup>51</sup> Similar to studies of ovarian and colorectal cancers, a relationship between nuclear  $\beta$ -catenin expression and stage, grade, and prognosis have been found in endometrial cancer.<sup>52,53,54,55,56</sup> Other studies have shown that, whereas overall  $\beta$ -catenin levels correlate negatively with cancer grade, nuclear accumulation in cells at the invasive front of the tumor correlates positively with tumor stage, grade, and poor prognoses.<sup>57,58,59</sup> In one study, transgenic mice in which activated  $\beta$ -catenin was expressed specifically in the uterus exhibited high levels of nuclear  $\beta$ -catenin accumulation, increased expression of c-myc and cyclin D1, increased glandular proliferation, and extensive hyperplasia – but, strikingly, no cancer within the first year of life.<sup>16</sup> Thus, these data suggest that elevated nuclear  $\beta$ -catenin alone is not tumorigenic.

Another study examined the role of Lef1 in endometrial cancer, a downstream target of  $\beta$ -catenin signaling (Shelton and Goodheart, submitted Clinical Cancer Research). Aberrant overexpression of the Wnt-pathway target gene LEF1 has been detected in several cancers, principally colon, leukemia, melanoma, and pancreatic. In unpublished data, we found that expression of Lef1 is also elevated in human endometrial tumors regardless of histological subtype and in non-cancerous proliferative

endometrium but not in inactive endometrium. Lef1 mRNA was expressed at higher levels in EEC than in NEEC, and was expressed at higher levels in tumors of grades 1 and 2 than in those of grade 3. Importantly, Lef1 expression was higher in cohorts with good prognosis. Collectively, these data suggest a role for Lef1 in tumorigenesis but not metastasis.

In addition to Lef1 and  $\beta$ -catenin, we have observed significant dysregulation of several components of the Wnt pathway (Goodheart, et al, unpublished observations). Taken together with the current understanding of  $\beta$ -catenin and Lef1 in endometrial cancer, these data underscore the importance of Wnt signaling in not only endometrial gland formation, but also in endometrial cancer formation.

## **Conclusions**

Wnt/ $\beta$ -catenin signaling appears to be important in a multitude of cancers, including endometrial cancer. Our data, detailed above, supports a role for the Wnt/ $\beta$ -catenin/Lef1 pathway involvement in endometrial cancer in addition to generalized gland regulation and formation (submitted Neoplasia). Many commercial pharmaceutical companies are developing targeted inhibitors of Wnt signaling, and currently there are compounds available for use in cell culture and animal experiments (e.g., XAV939 [www.reagentsdirect.com](http://www.reagentsdirect.com)). Further investigation into the complex interactions between Wnt/ $\beta$ -catenin

signaling and endometrial gland formation and cancer appears to be warranted in order to selectively inhibit pathways that are integral for endometrial cancer development.

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