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A mouse model for endometrioid ovarian cancer arising from the distal oviduct

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Ovarian cancer is the deadliest gynecological malignancy in Western countries. Early detection, however, is hampered by the fact that the origin of ovarian cancer remains unclear. Knowing that in a high percentage of endometrioid ovarian cancers Wnt/β-catenin signaling is activated, and in view of the hypothesis that ovarian cancer may originate from the distal oviduct, we studied mice in which Wnt/β-catenin signaling was activated in Müllerian duct-derived tissues. Conditional adenomatous polyposis coli (*Apc*) knockout mice were used to study the activation of Wnt/β-catenin signaling in Müllerian duct-derived organs. These $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice ($n = 44$) were sacrificed at 10, 20, 40 and 80 weeks and uterus, oviduct, ovaries and surrounding fat tissues were assessed using immunohistochemistry. Using nuclear β-catenin staining, Wnt/β-catenin signaling activation was confirmed in the entire epithelium of the adult Müllerian duct (fimbriae, oviduct and endometrium), but was absent in ovarian surface epithelium cells (OSEs). Besides endometrial hyperplasia, in 87.2% of mice intraepithelial lesions of the distal oviduct were found, whereas OSEs remained unaffected. In addition, 62.5% of mice developed tumors in the distal and fimbrial part of the oviduct. In the ovaries, mainly at young age, in 16.3% of mice, simple epithelial cysts were noted, which developed further into endometrioid ovarian tumors, resembling human endometrioid ovarian cancer (27.9% of mice). Next to this, locoregional growth in the utero-ovarian ligament was also shown. Here, for the first time, mutations (activation of Wnt/β-catenin) in the distal oviduct result in precursor lesions that develop into ovarian tumors, resembling human endometrioid ovarian cancer.

Carcinogenesis

Epithelial ovarian cancer is the deadliest gynecological malignancy in Western countries, accounting for more than 140,000 deaths each year worldwide.[1] As ovarian cancer survival is highly dependent on the tumor stage at diagnosis, early detection is key to decreasing death rate. Unfortunately, the origin of ovarian cancer remains unclear, making early discovery very difficult.

For decades, ovarian surface epithelial cells (OSEs) have been appointed as the primary origin of epithelial ovarian cancer despite the fact that precursor lesions were never found.[2] Over recent years, however, researchers have proposed different origins for epithelial ovarian cancer: the secondary Müllerian system,[3] the distal oviduct and fimbriae[4,5] and recently the transitional zone between OSEs and mesothelium at the hilial region of the ovary.[6]

Dubeau[3] and Piek *et al.*[7] were among the first to question OSEs as the only origin of ovarian cancer. In his original article, Dubeau[3] argues that the strong resemblance of ovarian epithelial tumors to tumors arising from organs embryologically derived from the Müllerian ducts and warrants the consideration that components of the secondary Müllerian system play a role in ovarian tumorigenesis. Piek *et al.*[7] described dysplastic lesions in prophylactically removed oviducts of BRCA1 and BRCA2 mutation carriers and further studies characterizing these dysplastic lesions showed serous tubal intraepithelial carcinomas (STICs) to be present in 38% of the oviducts that were prophylactically removed in women at risk for hereditary ovarian cancer.[8] Interestingly, the authors did not observe lesions in OSEs or ovaries of these patients. Furthermore, STICs could be identified in more

Key words: endometrioid ovarian cancer, oviduct, tubal intraepithelial lesions, Wnt/β-catenin signaling, APC

Abbreviations: Apc: adenomatous polyposis coli; Cre: C-recombinase; EMT: epithelial-to-mesenchymal transition; ER: estrogen receptor); Foxl2: forkhead box ligand 2; GFP: green fluorescent protein; IHC: immunohistochemistry; OSE: ovarian surface epithelial cell; Paep: progestagen-associated endometrial protein; PR: progesterone receptor

Additional Supporting Information may be found in the online version of this article.

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What's new?

Early detection of ovarian cancer is expected to significantly improve patient survival, but progress toward that goal has been hampered by a lack of knowledge of the origin of the disease. Here, experiments in conditional *Apc* knockout mice used to investigate Wnt/β-catenin signaling in Müllerian duct-derived tissues indicate that ovarian cancer may originate in the distal oviduct. Affected animals developed intraepithelial lesions of the distal oviduct that evolved into lesions resembling human endometrioid tubal and endometrioid ovarian cancer, with locoregional growth in the utero-ovarian ligament.

than half of the women diagnosed with sporadic serous ovarian cancer and serous pelvic cancer, and in five cases, mutations in TP53 were found to be identical between STICs and carcinoma tissue located in the ovary or pelvis.[9]

Another line of investigation pointing toward the distal oviduct and fimbriae as the possible origin of ovarian cancer is obtained from stem cell research. Using a mouse model, which allowed for the identification and isolation of infrequently dividing cells by green fluorescent protein labeling, our group identified a population of stem-like cells, located in the distal part of the oviduct/fimbriae.[10] Using FAC sorting, these stem-like cells were isolated, could subsequently be cultured and were shown to be able to form spheroids capable of self-renewal. Furthermore, under serum stimulation (differentiation), these spheroids could form glandular structures, which expressed markers of mature Müllerian epithelial cells (estrogen receptor [ER]α, progesterone receptor [PR]ab, progestagen-associated endometrial protein [Paep] and Cd44). Interestingly, the three major ovarian cancer histopathological subtypes (serous [50%], endometrioid [25%] and mucinous [10%]) all resemble Müllerian duct-derived tissues, and no tissues found to be located normally within the ovary.[11]

In endometrioid ovarian cancer, the second most common subtype of epithelial ovarian cancer, the Wnt/β-catenin signaling pathway has been proposed to be an important regulator.[12,13] In 16–54% of the endometrioid ovarian cancers, gene mutations are found in *CTNNB1*, the gene encoding for β-catenin, an essential transcription factor of Wnt-signaling.[14–19] Furthermore, mutations in the adenomatous polyposis coli (Apc) gene were also detected in endometrioid ovarian carcinoma, facilitating nuclear translocation of β-catenin, which indicates activation of the Wnt/β-catenin signaling pathway.[19] These findings are in accordance with the observation of nuclear β-catenin expression in 53% of the endometrioid ovarian cancers.[15,20] Next to these mutations, loss of the Wnt/β-catenin inhibitor SFRP4 was found to correlate with an aggressive phenotype and poor outcome in patients with ovarian cancer and overexpression of WNT7a was reported to facilitate ovarian cancer tumor growth and progression.[21,22]

Knowing that in a high percentage of endometrioid ovarian cancer cases, Wnt/β-catenin signaling is activated and in view of the hypothesis that ovarian cancer may originate from the oviduct, we studied the female reproductive tract from mice in which the Wnt/β-catenin signaling pathway was conditionally activated in the epithelium of the Müllerian duct.

Material and Methods

Generation of mice and genotyping

All experiments conducted with mice were approved by the Dutch Ethics of Animal Experiments Committee (under permit-number DEC106-08-06) in accordance with national and international guidelines and regulations. All animals were bred and kept in the Erasmus MC animal facility under standard conditions. $Pgr^{Cre/+}$ mice were kindly provided by DeMayo.[23] $Apc^{ex15lox}$ mice were developed at our university by Fodde and Smits.[24] Male $Pgr^{Cre/+}$ mice were bred with female $Apc^{ex15lox/lox}$ mice to eventually obtain $Pgr^{Cre/+}$; $Apc^{ex15lox/lox}$ mice. $Pgr^{Cre/+}$ mice were used as controls. Mice used for the experiment were kept on C57BL6/J genetic background. Genotyping was performed by PCR analyses of tail DNA as described earlier.[25]

Tissue collection and storage

Animals were sacrificed at 10, 20, 40 and 80 weeks, and stage of the estrous cycle was determined by vaginal smear before sacrifice. One uterine horn, oviduct and ovary, was snap frozen and stored at −80°C. The other uterine horn, oviduct and ovary, was fixed in 4% paraformaldehyde overnight and paraffin embedded.

Human tubal specimens were obtained from a patient with ovarian cancer at the Erasmus University Medical Centre Rotterdam. Tuba specimens were sectioned according to the SEE-FIM protocol,[8] fixed in 4% formaldehyde overnight and paraffin embedded. Human tissue collection was approved by the Medical Ethical Committee of the Erasmus Medical Centre and the patient gave informed consent for tissue collection for research purposes.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 4 μm (mice) and 5 μm (human) paraffin sections (Supporting Information Table 1, antibody information). Endogenous peroxidase activity was blocked with 30% of H_2O_2/PBS for 5 min and slides were blocked with 5% of milk for 30 min at room temperature (RT) (Friesland Campina, Amersfoort, The Netherlands). Primary antibodies were applied in 5% of milk and incubated overnight at 4°C. Secondary antibody (EnVision-kit, Dako, Glostrup, Denmark) was applied for 30 min at RT and diaminobenzidine (Dako) was used for visualization of antigen–antibody reactivity. After visualization, slides were counterstained with hematoxylin (Klinipath,

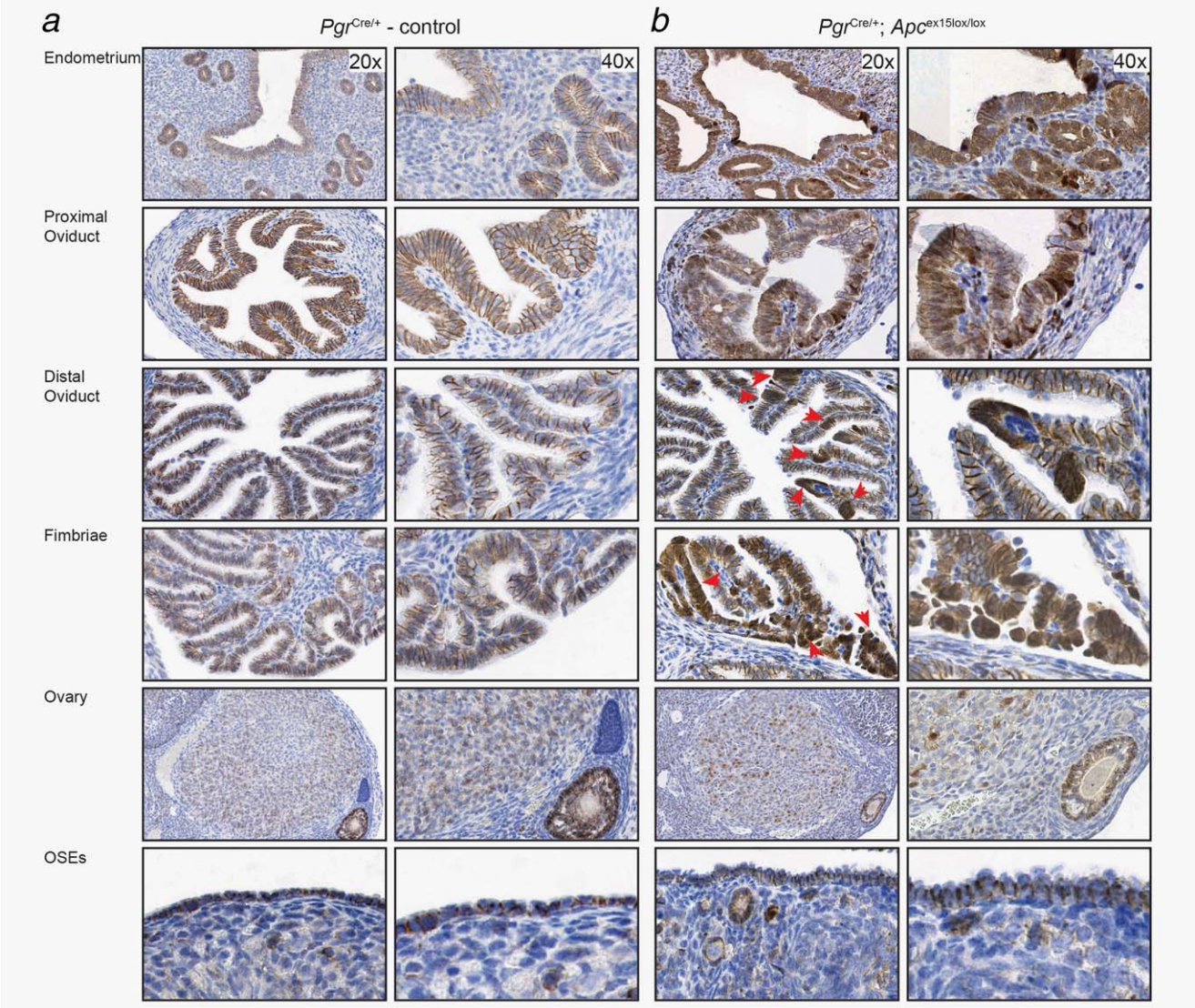

Figure 1. Activation of Wnt/β-catenin signaling by Cre-mediated recombination of APC in the female reproductive tract and ovary. (*a* + *b*) Activation of Wnt/β-catenin shown by β-catenin IHC in the endometrium, proximal oviduct, distal oviduct, fimbriae, ovary and OSE of a 10-week-old $Pgr^{Cre/+}$ control mouse (*a*) and a 10-week-old $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ experimental mouse (*b*). In control mice, Wnt/β-catenin signaling is not activated and β-catenin IHC only shows expression at the membrane. In $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ experimental mice, Wnt/β-catenin activation as indicated by pronounced nuclear β-catenin staining is shown in the endometrium, oviduct, fimbriae and granulosa cells, but not in the ovarian stroma and not at ovarian surface epithelium. In the oviduct and fimbriae, Wnt/β-catenin signaling is activated in fewer cells than (red arrowheads) in the endometrium. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Duiven, The Netherlands) and scanned with the NDP slide scanner (Hamamatsu, Hamamatsu City, Japan). All slides were revised by two pathologists experienced in gynecologic pathology (P.C.E. and C.V.D.).

Results

Activation of Wnt/β-catenin signaling in Müllerian duct-derived organs by C-recombinase (Cre)-mediated recombination of *Apc*

In our study, activation of Wnt/β-catenin signaling in Müllerian duct-derived organs was accomplished as of Day 10 of age onward, by $Pgr^{Cre/+}$-driven recombination of the Apc gene ($Apc^{ex15lox/lox}$) (Fig. 1).[23,24] Apc is part of the so-called destruction complex and as such involved in phosphorylation of β-catenin, which marks this protein for degradation. Cre-mediated recombination of *Apc* destroys the degradation complex, resulting in the stabilization of β-catenin, allowing translocation to the nucleus where it displaces the transcription repressor Groucho (TLE), permitting members of the TCF/LEF transcription factor family to regulate Wnt target gene transcription.[26]

Consequently, in this model, accumulation of β-catenin in the cytoplasm and nucleus indicates the activation of Wnt/β-catenin signaling. Figure 1 shows β-catenin IHC in Müllerian

Table 1. Abnormalities in $Pgr^{Cre/+}$; $Apc^{ex15lox/lox}$ mice[1]

Oviduct	Tubal intraepithelial lesions	No. (%)	34/39 (87.2%)
	Tubal glandular transformation		08/40 (20.0%)
	Endometrioid tubal tumor		25/40 (62.5%)
Ovary	Granulosa cell tumor		01/43 (2.3%)
	Endometrioid ovarian cyst		07/43 (16.3%)
	Endometrioid ovarian tumor		12/43 (27.9%)
Extraovarian lesions			03/32 (9.4%)

[1]Detailed information per mice can be found in Supporting Information Table 3.

duct-derived organs (uterus, oviduct and fimbriae) and in the ovaries of young (age, 10 weeks) $Pgr^{Cre/+}$-control and $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice. It was observed that in $Pgr^{Cre/+}$-control mice β-catenin expression was limited to the cellular membranes of epithelial and some mesenchymal cells, indicating normal cytoskeletal β-catenin activity and reduced activity of the Wnt/β-catenin signaling pathway (Fig. 1*a*). In contrast, in $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, in the endometrium, all epithelial and a number of stromal cells displayed enhanced nuclear and cytoplasmic β-catenin expression next to expression at the cell membrane (Fig. 1*b*). For the endometrium, these results indicate profound and clear activation of the Wnt/β-catenin signaling pathway as it was reported earlier (Fig. 1*b*).[27] For the distal oviduct and fimbriae, the situation is different: in $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, fewer epithelial cells exhibit clear activation of Wnt/β-catenin signaling (Fig. 1*b*, red arrowheads). This reduced level of Wnt/β-catenin signaling in oviduct and fimbriae agrees well with reduced PR expression (and therefore reduced Cre expression) in the distal oviduct and fimbriae when compared to the endometrium.[10]

In the ovary, there is only one cell type expressing PRs and that is the granulosa cell.[28] In accordance with this fact, activation of Wnt/β-catenin signaling, as shown by nuclear β-catenin staining in $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, was found only in some granulosa cells (Fig. 1*b*). Furthermore, cells at the surface of the ovary (OSEs) do not express PRs and consequently do not show any activation of Wnt/β-catenin signaling in $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice (Fig. 1*b*).

Endometrioid tumors in the oviduct

In 87.2% of $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, lesions were found in the epithelium of the distal oviduct and fimbriae (Figs. 2 and 3; Table 1), whereas OSEs remained unaffected. These lesions were in continuity with the normal epithelium and were characterized by enhanced cytoplasmic and nuclear β-catenin expression next to expression at the cell membrane, loss of cilia, bulging of cells into the lumen, layering and suspicious stratification of cells, and rounding and hyperchromatization of the nucleus (Fig. 2*a*). Upon review, the observed lesions were hypothesized to resemble, to a certain extent, the characteristics used to identify human TICs (Figs. 2*a* and 2*b*).[8,29,30] These tubal intraepithelial lesions were located preferentially in the distal oviduct and fimbriae and were estrogen (ERα) and PR (PRab) positive (Fig. 2*c*). Interestingly, these lesions also displayed loss of p63-expression, a finding that, in the previously published literature[31], is associated with increased Wnt/β-catenin signaling (Fig. 2*c*).

Over time, 20.0% of $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice developed glandular transformation of the normal papillary architecture of the oviduct, which seemed to originate from the lesions at the distal oviduct. This glandular transformation was characterized by glandular growth, high nuclear and cytoplasmic accumulation of β-catenin, loss of cilia and a general loss of normal cellular morphology (Table 1, Fig. 3*a*).

As glandular transformation progressed, endometrioid tubal tumors developed in 62.5% of mice, showing severe nuclear atypia, subnuclear vacuolization, pronounced and abnormal proliferation (identified by Ki67), complete loss of normal oviductal architecture and compression of the lumen (Table 1, Figs. 3*b* and 3*c*). As shown in Fig. 3*c*, these tumors were characterized by glandular growth and were PRab, ERα and Paep (low level) positive, resembling human endometrioid tubal cancer.

Endometrioid tumors in the ovary

As explained before, the only cell in the ovary of $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice which shows enhanced nuclear and cytoplasmic β-catenin expression is the granulosa cell (Fig. 4, Table 1, and Supporting Information Fig. 1). As described earlier in the literature, as a result of enhanced Wnt/β-catenin signaling, in 1 out of 43 of $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, a granulosa cell tumor developed.[32] This tumor showed nuclear β-catenin staining and forkhead box ligand 2 (Foxl2) (a specific granulosa cell tumor marker[33]) positivity and was distinct from lesions discussed below (Fig. 4*a*).

In 16.3% of $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, endometrioid cysts were detected (Table 1 and Fig. 4*b*). These cysts were mainly found in younger animals and the endometrioid appearance was characterized by a cyst-shaped gland filled with mucin, which is lined with a well-differentiated single layer of columnar epithelium, resembling those of the endometrium, without any sign of nuclear atypia. Interestingly, columnar epithelium is a hallmark of Müllerian epithelium and was not detected within the ovary or OSEs. The cells exhibited high cytoplasmic and nuclear accumulation of β-catenin, were Foxl2 negative and PRab, ERα and Paep positive and in some cases, cilia were present (Fig. 4*b*, Supporting Information Fig. 1*a*). P63 expression was present in a few cells and IHC showed a similar staining pattern as observed in the distal oviduct (Fig. 2, Supporting Information Fig. 1). As Paep, ERα and PRab are expressed only in Müllerian duct-derived organs, and because OSEs are not affected in this mouse model, an extraovarian origin of these lesions is hypothesized.

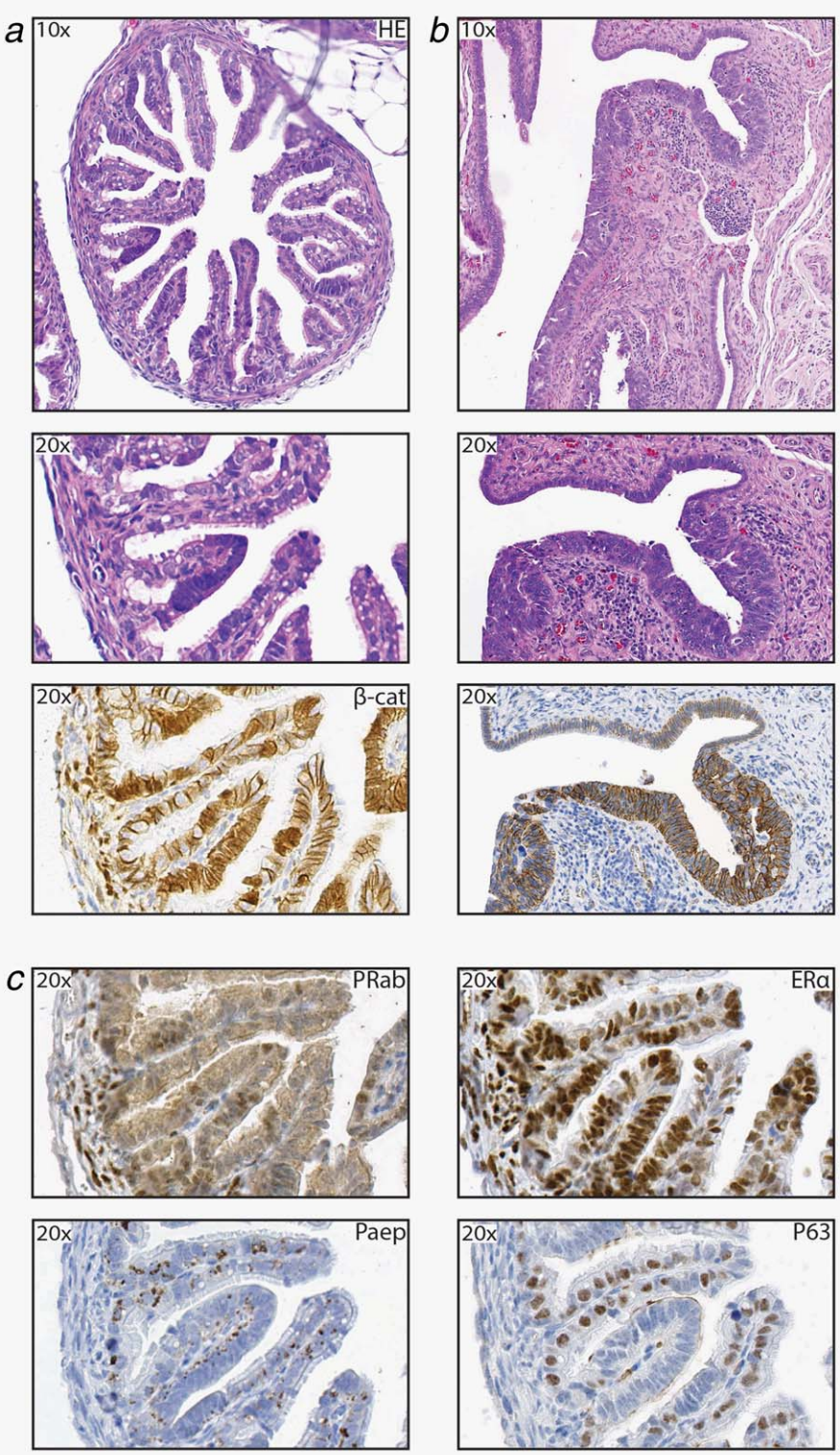

Figure 2. Activation of Wnt/β-catenin signaling causes tubal intraepithelial lesions. (*a*) Tubal intraepithelial lesions can be identified in the oviduct of $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ experimental mice as of 10 weeks of age. Tubal intraepithelial lesions were characterized by pronounced nuclear β-catenin staining, loss of cilia, layering of cells, bulging of cells into the lumen and hyperchromatization of the nucleus. (*b*) Human STICs, although mainly serous in contrast to the endometrioid tubal intraepithelial lesions studied here, also show layering of cells, hyperchomatization of the nucleus, loss of cilia and increased membranous and cytoplasmic β-catenin expression. (*c*) Mouse tubal intraepithelial lesions were further investigated, showing clear expression of Paep and ERα, low expression of PRab and distinct loss of P63 expression. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

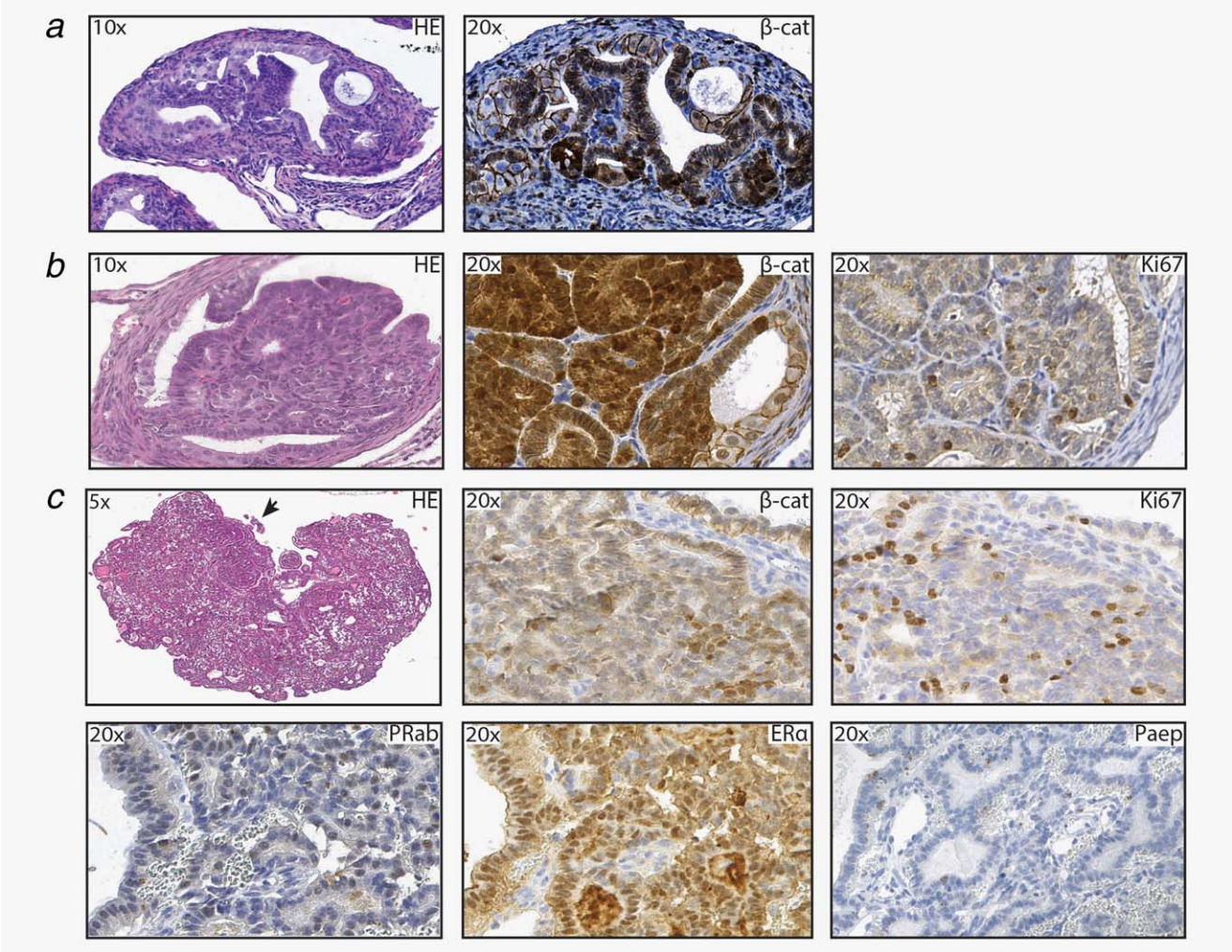

Figure 3. Activation of Wnt/β-catenin signaling causes endometrioid tubal tumors. (*a*) Tubal glandular transformation can be identified in the oviduct starting as of 10 weeks of age. (*b*) Oviductal tumors arise from glandular transformation and show endometrioid tumor growth, enhanced proliferation (Ki67) and suppression of normal oviductal architecture. (*c*) At 40 weeks, the normal oviductal and fimbrial architecture is replaced by an endometrioid tubal tumor, showing high proliferation (Ki67) and PRab, ERα and (low) Paep expression. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In 27.9% of $Pgr^{Cre/+};Apc^{ex15lox/lox}$ mice, endometrioid ovarian tumors developed which could very well originate from the endometrioid ovarian cysts. These tumors were lined with columnar endometrium-like epithelium and were characterized by high accumulation of cytoplasmic and nuclear β-catenin, high proliferation, both solid and back-to-back glandular growth, subnuclear vacuolization and stratification of the epithelium, reminiscent of human endometrioid ovarian cancer (Figs. 4*c*–4*e*). Furthermore, the tumor cells exhibited cilia, nuclear atypia, pronounced nucleoli, mitotic figures and tumoral or malignant morphology (Figs. 4*c*–4*e*). In addition, the glands were spaced in a fibrotic stroma. Interestingly, differentiation of the tumors seemed to decrease over time and less differentiated tumors showed regions with loss of ERα, PRab and nuclear and cytoplasmic β-catenin expression (Figs. 4*c*–4*e*, Supporting Information Fig. 1*b*−1*d*). For ERα and PRab, this is a phenomenon also observed in various dedifferentiated human cancers,[34–36] whereas for nuclear and cytoplasmic β-catenin expression loss of expression has been documented during endometrial carcinogenesis.[27]

In summary, as Wnt/β-catenin signaling is activated in the Müllerian duct and is not activated in OSEs, because of histological similarities between the ovarian and tubal lesions, because of their positivity for ERα, PRab and Paep and because of the absence of staining for the granulosa cell marker Foxl2, it is hypothesized that endometrioid ovarian cysts and endometrioid ovarian tumors originate from the earlier-described tubal intraepithelial lesions located in the fimbriae and distal oviduct.

Activation of Wnt/β-catenin signaling in the Müllerian duct induces extraovarian endometrioid cysts

As ovarian cancer is considered highly invasive, rapidly spreading to organs and tissues in the abdominal cavity, specific attention was paid to the immediate vicinity of the ovary

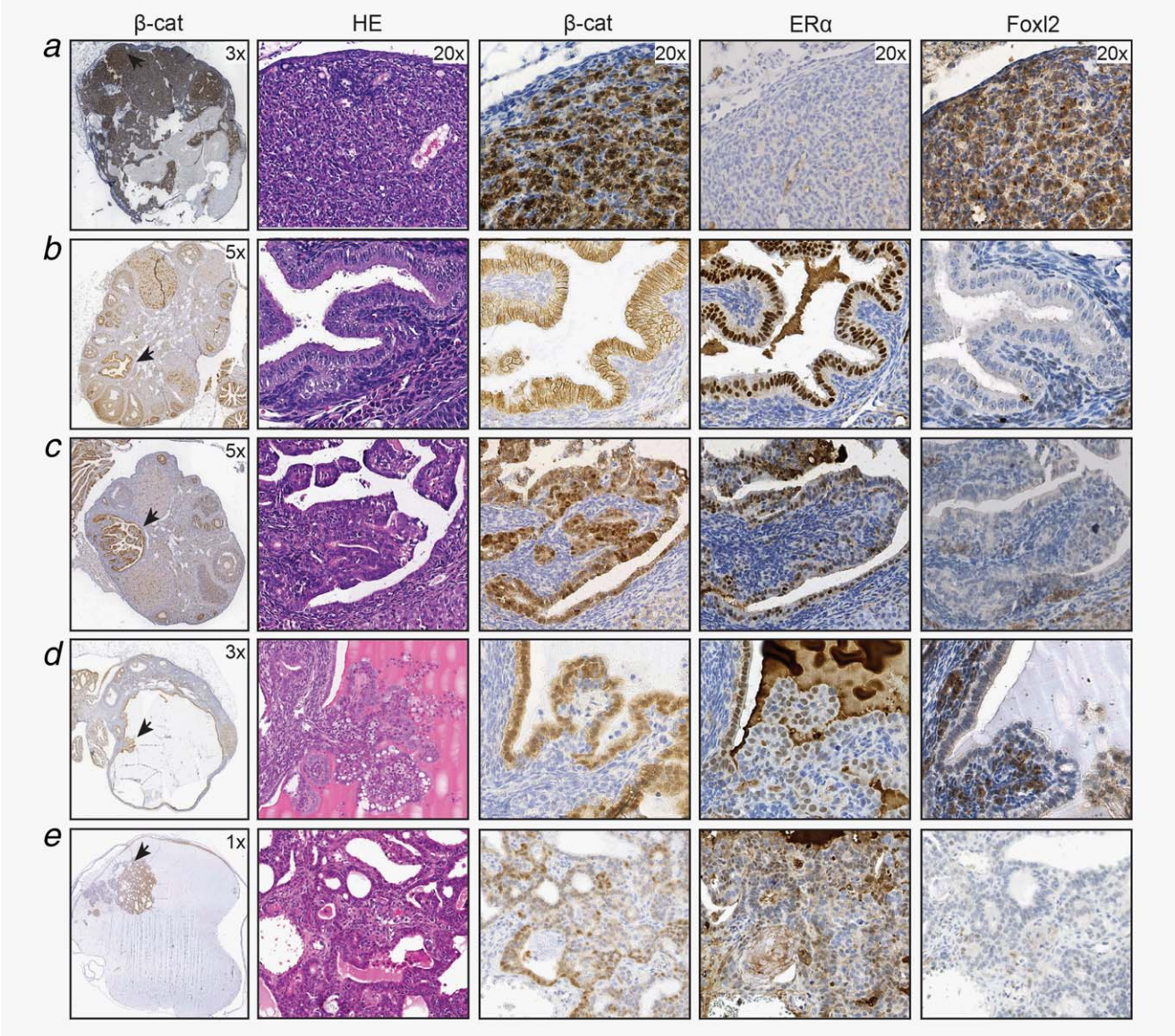

Figure 4. Activation of oviductal Wnt/β-catenin signaling causes endometrioid ovarian tumors. (*a*) Granulosa cell tumors were identified and characterized by high nuclear β-catenin staining, Foxl2 expression and no ERα expression. (*b*) Endometrioid ovarian cysts can be identified as of 10 weeks of age by nuclear accumulation of β-catenin and high expression of ERα. (*c*) Over time, the cysts will develop into endometrioid ovarian tumors as shown in a 20-week-old animal. Here, normal morphology is lost. (*d* + *e*) At 40 (*d*) and 80 weeks (*e*), the endometrioid ovarian tumors developed further with more nuclear atypia, loss of normal architecture and a malignant phenotype. The tumors are positive for ERα and negative for Foxl2 (only present in ovarian stroma), and interestingly, β-catenin and ERα expression decreased over time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(Fig. 5 and Table 1). It should be noted that in mice, in contrast to humans, the ovary and distal oviduct are surrounded by a bursa which is covered with fat pads. In these fat pads, it was noted that in approximately 20% of $Pgr^{Cre/+};Apc^{ex15lox/lox}$ animals simple glandular structures were present which seemed to represent the structures described by Dinulescu *et al.*[37] These structures only showed the expression of β-catenin at the membrane and were in most cases ERα, PRab and $CD10^{+}$, suggesting a Müllerian origin. However, as these structures were also found at similar numbers in control animals ($Pgr^{Cre/+}$), these were not studied further (Fig. 5*a*).

Interestingly, we also observed other structures, which were located outside the ovary, associated with the utero-ovarian ligament, and present only in 9.4% of $Pgr^{Cre/+};Apc^{ex15lox/lox}$ animals (Fig. 5*b* and Table 1). These structures showed enhanced levels of cytoplasmic β-catenin and nuclear β-catenin, and were ERα and PRab positive. Furthermore, pathological review indicated an endometrioid appearance, characterized by Müllerian duct-like glandular columnar epithelium surrounded by stromal cells, similar to the endometrioid cysts and tumors found in the oviduct and ovary. In some cases, cilia were present on the surface of the epithelial cells.

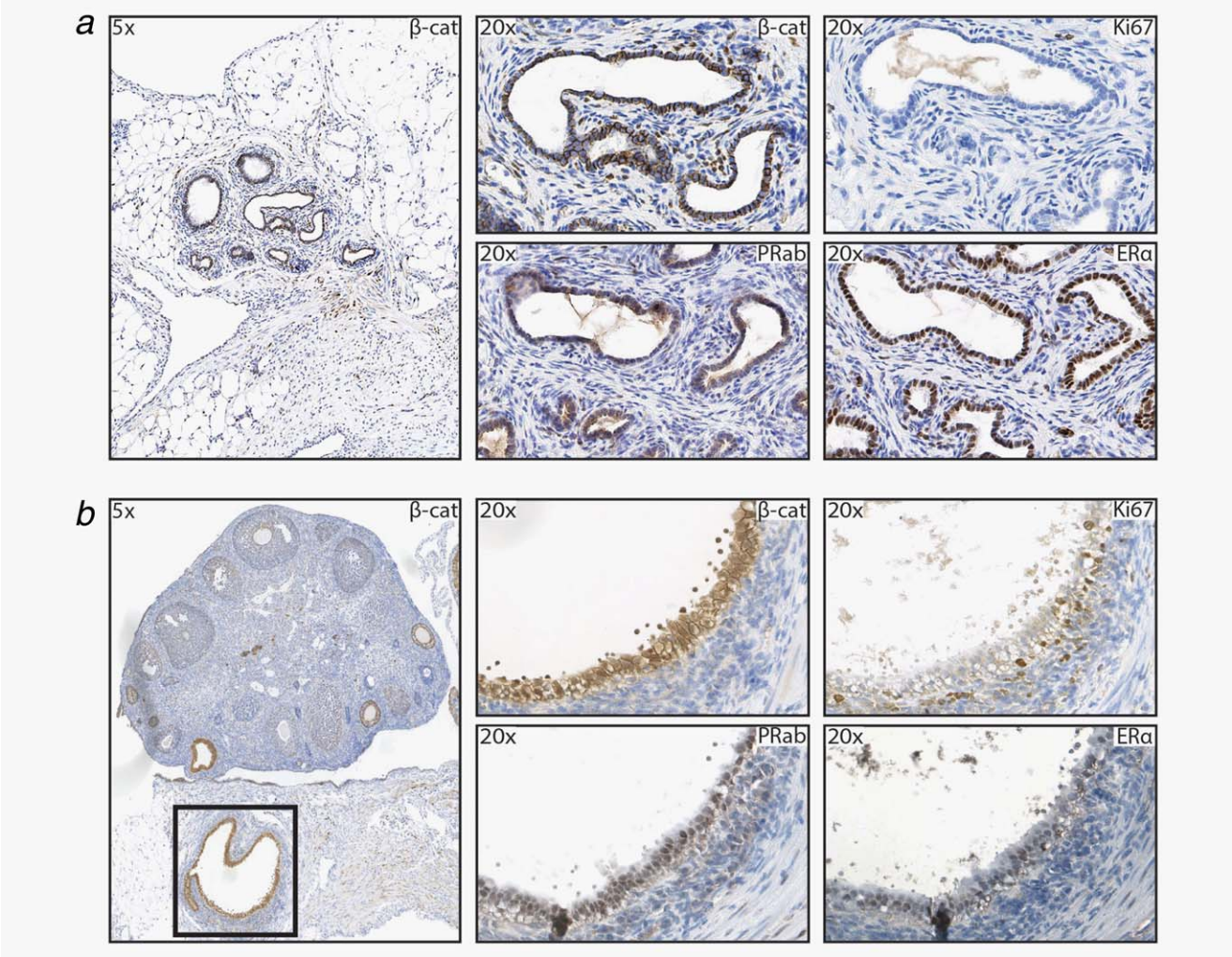

Figure 5. Locoregional growth of endometrioid tumors in the utero-ovarian ligament. (*a*) In the fat pads, simple glandular structures were present which seemed to represent Müllerian structures. These structures showed only membranous staining for β-catenin, showed no increased proliferation and were in most cases ERα, PRab and CD10 positive. Both $Pgr^{Cre/+}$ and $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ animals displayed these lesions in approximately 20% of animals. (*b*) In the utero-ovarian ligament, endometrioid cysts were found which show high resemblance to endometrioid tumors present in the ovary and oviduct. These cysts show nuclear accumulation of β-catenin, increased proliferation (as shown by Ki67 IHC) and expression of PRab and ERα. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Interestingly, in all $Pgr^{Cre/+}$;$Apc^{ex15lox/lox}$ mice, the presence of extr-ovarian endometrioid lesions, coincided with lesions found in both the oviduct and the ovary.

Discussion

In our manuscript, we describe a mouse model in which a conditional mutation in the *Apc* gene results in the activation of Wnt/β-catenin signaling in the Müllerian duct. In the oviduct of these mice, but not in the uterus or OSEs, precursor lesions were found which appear to develop further into endometrioid tubal tumors. Interestingly, the experimental animals also displayed lesions in their ovaries: initially, simple endometrioid ovarian cysts appeared in some animals, and later in older animals endometrioid ovarian tumors developed which bore a high resemblance to human endometrioid ovarian cancer (Supporting Information Table 2). Together with our recent finding of stem-like cells located in the fimbrial region of the distal oviduct,[10] these observations provide further support for the hypothesis that epithelial stem cells from the distal oviduct and fimbriae, owing to ovulation-induced repeated biochemical threats, accumulate DNA modulations and mutations which, over time, may cause transformation into the early malignant precursors of ovarian cancer. Analogous to the OSE hypothesis,[2] cells from these premalignant lesions may become trapped into the ovarian cortical stroma and thus forming cortical inclusion cysts which may develop further into ovarian cancer.

The discussion about the origin of ovarian cancer is not an easy one because the results obtained so far by using several mouse models have been far from conclusive. In general, mouse models induce ovarian carcinogenesis by making use of targeted mutagenesis. For example, adenoviral delivery of

Cre has been used as a tool to induce recombination in tissues inside the bursal pouch surrounding the ovary and distal oviduct in mice.[37,38] Using this technique, however, it is not possible to discriminate between OSE cells and cells located in the fimbrial region of the distal oviduct as the origin of ovarian carcinogenesis. Another approach is to use a specific gene promoter to drive the recombination of a specific gene described to be involved in a specific subtype of ovarian cancer.[39,40] For example, in mice in which AmhR2-Cre was used to drive the recombination of *Pten*$^{lox/lox}$;*lsl-Kras*^{G12D}, low-grade ovarian serous papillary adenocarcinomas were formed in 100% of mice.[41,42] Using *AmhR2*-Cre, *Dicer*, an essential gene for micro-RNA synthesis, and *Pten*, a key tumor suppressor inhibiting the *PI3K* pathway, were conditionally deleted.[43] As a result, high-grade serous carcinomas arising from the fallopian tube with spread to the ovary and metastasis throughout the abdominal cavity were identified in 100% of mice and closely resembled human serous cancer. Tanwar *et al.*[44] combined *AmhR2*-Cre with *Apc*$^{lox/lox}$ and observed the development of epithelial inclusion cysts and, in much older animals, high-grade ovarian endometrioid adenocarcinoma. The finding of endometrioid ovarian cancer is in agreement with the here-presented investigations and with the observations in this particular ovarian cancer subtype, Wnt/β-catenin signaling is often activated.[12] The problem with using the *AmhR2* promoter is that it is expressed in the Müllerian duct as well as in OSE cells. In contrast, for Pgr-Cre it is known that there is activation of the *Pgr* promoter in the Müllerian duct, whereas it is not active in OSE cells.

One of the earliest changes observed in the distal part of the oviduct of the current conditionally Wnt/β-catenin signaling-activated animals was the development of tubal intraepithelial lesions. These lesions are of particular interest, because already in 2001, Piek *et al.*[7] described epithelial dysplasia in the distal oviduct of women predisposed for ovarian cancer. This observation was later confirmed by numerous research groups, leading to the identification and characterization of STICs.[8,45,46] Interestingly, these human STICs show high resemblance to the tubal intraepithelial lesions found in our experimental animals (loss of cilia, bulging of cells into the lumen, layering of cells, and rounding and hyperchromatization of the nucleus). Furthermore, Medeiros *et al.*[8] found that most human STICs were located in the fimbrial part of the human oviduct, a phenomenon reflected in the current mouse model.

Upon characterization of the mouse tubal intraepithelial lesions, it was observed that p63 expression, more specifically its truncated isoform ΔNp63, was reduced in these regions. This is an interesting finding because in the literature, ΔNp63 is described as acting upstream from Wnt/β-catenin signaling, whereas here it seems to act as downstream: activation of Wnt/β-catenin signaling induces loss of p63 expression.[31] Furthermore, the observed downregulation of p63 correlates well with the finding that p63 expression is often lost from human ovarian cancers.[47] Also, ΔNp63 downregulation has been described as stimulating the invasive behavior of human squamous cell carcinoma and knockout of ΔNp63 in breast cancer cell lines induces epithelial-to-mesenchymal transformation (EMT).[47,48] Interestingly, Wnt/β-catenin signaling, which was activated in the tubal intraepithelial lesions from our experimental animals, has also been described to play an important role in the induction of EMT and metastasis in cancer.[49,50]

As ovarian cancer in women is highly invasive, rapidly spreading disease in the abdomen, special attention was paid to tissues in close proximity to the bursa surrounding the ovary. It was observed that in 9.4% of *Pgr*$^{Cre/+}$;*Apc*$^{ex15lox/lox}$ mice, extraovarian endometrioid lesions were present within the utero-ovarian ligament. These lesions may originate from tubal intraepithelial lesions as described earlier or they may represent metastatic spread from oviductal or ovarian tumors. However, given the benign-looking morphological features of these extra-tubal lesions, it is also possible that they have developed from pre-existing extrauterine Müllerian epithelium as it has been described by Dubeau.[50]

Conclusions

In summary, conditional knockout of *Apc* in the oviduct causes tubal intraepithelial lesions. These lesions can evolve and develop into endometrioid tubal tumors or endometrioid ovarian tumors, resembling human endometrioid tubal and ovarian cancer growth. Next to these tubal and ovarian tumors, locoregional growth in the utero-ovarian ligament was shown. In conclusion, the current mouse model can be a valuable tool for further research on ovarian cancer initiation, behavior and therapy.

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