

In vivo genetic cell lineage tracing reveals that oviductal secretory cells self-renew and give rise to ciliated cells

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ABSTRACT

The epithelial lining of the fallopian tube is vital for fertility, providing nutrition to gametes and facilitating their transport. It is composed of two major cell types: secretory cells and ciliated cells. Interestingly, human ovarian cancer precursor lesions primarily consist of secretory cells. It is unclear why secretory cells are the dominant cell type in these lesions. Additionally, the underlying mechanisms governing fallopian tube epithelial homeostasis are unknown. In the present study, we showed that across the different developmental stages of mouse oviduct, secretory cells are the most frequently dividing cells of the oviductal epithelium. *In vivo* genetic cell lineage tracing showed that secretory cells not only self-renew, but also give rise to ciliated cells. Analysis of a Wnt reporter mouse model and various Wnt target genes showed that the Wnt signaling pathway is involved in oviductal epithelial homeostasis. By developing two triple-transgenic mouse models, we showed that Wnt/ β -catenin signaling is essential for self-renewal as well as the differentiation of secretory cells. In summary, our results provide mechanistic insight into oviductal epithelial homeostasis.

KEY WORDS: β -Catenin, Oviduct, Ovarian cancer, Fallopian tube, Serous ovarian cancer

INTRODUCTION

Mouse oviduct (analogous to the fallopian tube in humans) is a narrow tubular structure that plays a functional role in the transportation of gametes and acts as a site for fertilization (Stewart and Behringer, 2012). Fertilization occurs at a specific site of the oviduct known as the ampulla (Coy et al., 2012). Oviductal/fallopian tube epithelium is pseudostratified and mainly consists of secretory and ciliated cells (Donnez et al., 1985; Espinasse, 1935). Two other cell types known as basal/reserve cells and peg/intercalated cells were also described in earlier literature (Espinasse, 1935; Ferenczy et al., 1972; Novak and Everett, 1928). However, later studies identified that basal cells are not epithelial cells but T-lymphocytes, and the peg cells are exhausted secretory cells that have lost a considerable amount of apical cytoplasm by secretion into the oviduct lumen (Crow et al., 1994; Odor, 1974; Peters, 1986). Any aberrations in the normal functioning of fallopian tube epithelium may lead to ectopic pregnancy, whereby the embryo is erroneously implanted in the fallopian tube rather than in the uterus (Woodruff and Paurstein, 1969).

Every month, at the time of ovulation, the oviductal epithelium is exposed to inflammatory factor-containing follicular fluid, leading

to tissue damage (King et al., 2011). Therefore, the oviductal epithelium must have regenerative potential to repair and remodel itself in order to restore normal function. The oviductal epithelium has been shown to undergo extensive proliferation during the estrogen-dominant preovulatory phase of the menstrual cycle (George et al., 2012). Therefore, similar to most other epithelia, the oviductal epithelium must be equipped with a population of stem/progenitor cells responsible for tissue homeostasis. Exposure to pro-inflammatory follicular fluid can induce DNA double-strand breaks, as marked by phospho- γ H2AX, in mouse oviductal epithelial cells (King et al., 2011) and in cultured human fimbrial epithelial cells and fimbria tissue (Huang et al., 2015). Using an *ex vivo* culture system, in which the fallopian tube epithelium was exposed to follicular fluid, an increase was observed in the expression of DNA repair pathway components and inflammatory-related genes (Bahar-Shany et al., 2014; Brachova et al., 2017). This could represent a key step in the process of premalignant transformation of fallopian tubal epithelium.

Patients carrying *BRCA1/2* mutations are at high risk of developing serous ovarian carcinoma (SOC), the most prevalent and lethal form of ovarian cancer (Bowtell et al., 2015). Histopathological examination of fallopian tubes from such patients revealed premalignant lesions in the fallopian tubal epithelium (Kindelberger et al., 2007; Kuhn et al., 2012; Lee et al., 2007; Medeiros et al., 2006), suggesting that the tubal epithelium could be the site of origin for SOC. Anti-müllerian hormone receptor type 2 (*Amhr2*)-*Cre*-mediated deletion of *Dicer1*, an essential gene for microRNA synthesis, and *Pten*, a key negative regulator of the phosphoinositide 3-kinase pathway, in the female reproductive tract resulted in high-grade serous carcinomas, providing clear evidence that these carcinomas arise from oviduct in mice (Kim et al., 2012). These findings were further supported by the fact that salpingectomy (surgical removal of the fallopian tubes) significantly reduces the risk of ovarian cancer development in patients at high risk of developing this disease (Falconer et al., 2015). More specifically, oviductal secretory cells were shown to give rise to high-grade serous tumors in mouse models targeting *Brcal*, *Trp53* (*Trp53*) and *Pten* or expressing SV40 large T-antigen (Perets et al., 2013; Sherman-Baust et al., 2014). These tumors are genetically and histopathologically similar to human SOC (Perets et al., 2013; Sherman-Baust et al., 2014). This suggests that the oviductal secretory cell type is likely to be the cell of origin for SOC.

Despite its importance in reproductive function and disease, little is known about the underlying mechanisms governing oviductal epithelial homeostasis and its regenerative potential. Lack of available stem cell markers has further complicated efforts to establish the existence of stem/progenitor cells in the oviduct. Evidence for the existence of these stem/progenitor cells has mostly come from *in vitro* studies. Epithelial cell adhesion molecule (EpCAM)⁺ cells isolated from human fallopian tube were shown to develop into 3D organoids when cultured in medium supplemented

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with growth factors (Kessler et al., 2015; Paik et al., 2012). However, the behavior of these EpCAM⁺ cells under *in vivo* conditions was not studied. To date, no study has provided definitive evidence for the existence of oviductal epithelial stem/progenitor cells. Even though studies have shown increased oviductal Wnt signaling in response to hormonal changes, infection, or ovarian cancer (Kessler et al., 2012; Li et al., 2017; Nagendra et al., 2016), it is still unclear how oviductal epithelial homeostasis is maintained and regulated by Wnt signaling.

In the present study, using genetic cell lineage tracing, we have shown that PAX8⁺ secretory cells act as oviductal epithelial progenitors, which expand over time to repopulate the whole oviductal epithelium and give rise to ciliated cells. Furthermore, by conditional deletion or overactivation of β -catenin (*Cttnb1*) specifically in oviductal secretory cells, we provide evidence that Wnt/ β -catenin signaling is required for the maintenance of secretory cells and their differentiation to ciliated cells. Collectively, our data provide mechanistic insight into oviductal epithelial homeostasis.

RESULTS

Abnormal expansion of secretory cells in the fallopian tube of patients predisposed to ovarian cancer

Normal fallopian tubal epithelium consists of an intermixed population of secretory and ciliated cells (Crow et al., 1994; Nagendra et al., 2016). However, women with genetic predisposition to ovarian cancer frequently have precursor lesions in their fallopian tubal epithelium (Mehra et al., 2011). Unlike normal tubal epithelium, ovarian cancer precursor lesions primarily consist of secretory cells, and have been defined as secretory cell expansions (SCEs), secretory cell outgrowths (SCOUTs) or serous tubal intraepithelial carcinoma (STIC) (Bowtell et al., 2015). It has been shown that SOC is also predominantly composed of secretory cells (Mehra et al., 2011).

In order to determine the distribution pattern of secretory and ciliated cells in the normal and disease states, we examined fallopian tubes from patients ($n=11$, Table S1) at high risk of developing SOC, and compared the tubal lesions with adjacent normal epithelium. These patients had mutations in *BRCA1/2*, which are associated with the pathogenesis of breast and ovarian cancer. Histological and immunofluorescence marker analysis using the well-established secretory cell marker PAX8 (Perets et al., 2013) and the ciliated cell marker acetylated tubulin (Ac-TUB) (Pardo-Saganta et al., 2015) revealed that the normal adjacent epithelium consists of interspersed secretory and ciliated cells (Fig. 1A,A'). By contrast, fallopian tubal lesions were composed of uninterrupted patches of secretory cells (Fig. 1B,B',C,C'). These findings reveal that SOC precursor lesions have altered secretory and ciliated cell population dynamics, and are characterized by abnormal expansion of secretory cells.

Analysis of secretory and ciliated cells in mouse oviducts at different developmental stages

To understand oviductal epithelial cell dynamics during postnatal development, we collected mouse oviducts at different developmental stages (Fig. 1D). The oviductal epithelium at postnatal day (P) 1 is reportedly undifferentiated and, by P28, the epithelium becomes fully differentiated resembling adult oviductal epithelium (Yamanouchi et al., 2010). To investigate the development, and thereby the distribution pattern of the two epithelial cell types, we immunostained mouse oviducts at different developmental stages for secretory cell-specific (PAX8) and ciliated cell-specific (Ac-TUB) markers. At P1, the oviductal epithelium

consisted of only PAX8⁺ cells (88.87 \pm 2.12% in distal oviduct; 95.38 \pm 0.76% in proximal oviduct; Fig. 1Ea,b,Fa,b). Ciliated cells, as marked by Ac-TUB, appeared for the first time at P4 (2.51 \pm 1.30% in distal oviduct; 1.42 \pm 0.69% in proximal oviduct; Fig. 1Ec,d,Fc,d,G,H, Fig. S1A,B). Over time, a gradual decrease in the percentage of PAX8⁺ cells was coupled with a concomitant increase in the percentage of ciliated cells (Fig. 1E-H, Fig. S1). In P28 mouse oviducts, epithelial cells with co-staining for PAX8 and Ac-TUB were also observed (Fig. S1C, $n=3$), suggesting the possibility that PAX8⁺ cells differentiate into Ac-TUB⁺ cells. These results were further confirmed by qPCR analysis of *Pax8* and *Foxj1*, another ciliated cell marker (Pardo-Saganta et al., 2015), which revealed a similar trend to that observed by immunostaining (Fig. 1I,J).

Analysis of Ki67, a marker for cell proliferation, revealed that across the different developmental stages analyzed the majority (>80%) of the secretory cells were Ki67⁺, in contrast to the ciliated cells, which were mostly Ki67⁻ (Fig. 2A,B, Fig. S2A). The percentage of proliferating secretory cells ranged from 100% at P1 to 82.96% at P28. By contrast, the percentage of proliferating ciliated cells was much lower and ranged (in the distal oviduct) from 0.01% at P4 to a maximum of 17.33% at P28, suggesting that the majority of the proliferating cells in the oviductal epithelium are secretory cells.

Collectively, these results show that secretory cells are the most frequently dividing cell type in the developing mouse oviduct, and that the first ciliated cells appear at P4, prior to which the oviductal epithelium consists of only secretory cells.

Genetic cell lineage tracing reveals that secretory cells give rise to ciliated cells

Our results show that secretory cells are the most frequently dividing cell type of the oviductal epithelium, suggesting that they could potentially give rise to the ciliated cell type and thereby maintain oviductal epithelial homeostasis. To investigate the role of secretory cells in oviductal epithelial homeostasis, we performed *in vivo* lineage tracing of these cells using a mouse model, *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}*, in which, upon doxycycline administration, YFP is expressed in cells derived from PAX8-expressing cells. To characterize the specificity of this mouse model, we collected oviducts from doxycycline-treated mice and immunostained them for YFP and PAX8. In both the distal and proximal oviducts, the expression of YFP was exclusively restricted to the PAX8⁺ cells (Fig. 2C). In order to initiate PAX8⁺ cell-driven lineage tracing, doxycycline was administered in drinking water to pregnant females from embryonic day (E) 13.5 to P4, and the fate of any YFP-marked progeny was followed over time (Fig. 2D). We preferred this labeling window as secretory cells are the only cell type present in the mouse oviductal epithelium during this period (Fig. 1, Fig. S1A,B). At P4 (day 0 of chase), YFP expression was exclusively restricted to the PAX8⁺ secretory cells (Fig. 2E). Over the subsequent chase, at P14 (day 10 of chase) and P28 (day 24 of chase), an increase in the number of YFP⁺ cells was observed, suggesting that over time these YFP⁺ cells repopulate the oviductal epithelium. Interestingly, a subset of YFP⁺ cells co-expressed Ac-TUB, a mature ciliated cell marker (Fig. 2E), suggesting that ciliated cells arise from secretory cells. No YFP expression was detected in littermate controls (Fig. 2E). Collectively, these results reveal the contribution of secretory cells to the oviductal epithelial lineage.

To validate our results, and to further assess the contribution of these embryonically labeled PAX8⁺ cells during adult homeostasis, we performed longer-term tracing analyses with another mouse model, *Pax8-rtTA;tetO-Cre;lacZ^{fl/fl}*, in which, instead of YFP, *lacZ*-

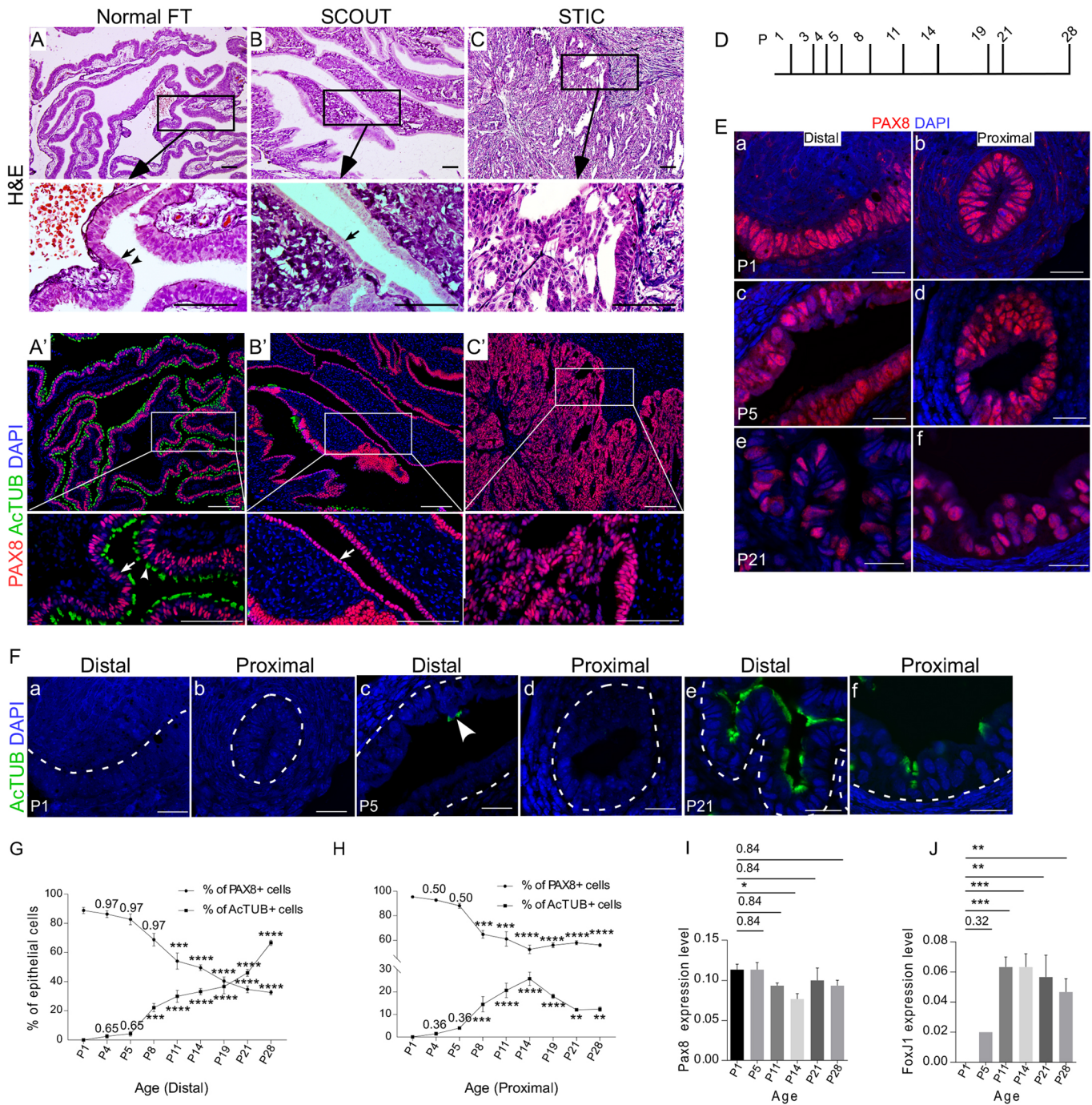


Fig. 1. Secretory cells are the dominant cell type in fallopian tube epithelium of patients at high risk of developing SOC and appear earlier than ciliated cells in mouse oviducts during postnatal development. (A-C') Hematoxylin and Eosin (H&E) (A-C) and PAX8/Ac-TUB co-immunostained (A'-C') sections of fallopian tubes (FT) from normal patients (A,A') and those at high risk of developing SOC (B,B',C,C') revealed interspersed secretory and ciliated cells in normal adjacent epithelium (A,A'), and uninterrupted patches of secretory cells in SCOUT and STIC precursor lesions (B,B',C,C'). Arrows and arrowheads point to secretory and ciliated cells, respectively. Boxed areas are shown in high magnification beneath. (D) Timeline of mouse oviduct collection across developmental stages. (E,F) Immunostaining for PAX8 (Ea-f) and Ac-TUB (Fa-f) of mouse oviductal epithelium at early and late stages of development revealed that the first ciliated cells (arrowhead) appear after P4. Dotted line in F separates oviductal epithelium from underlying stroma. (G,H) Quantification of PAX8⁺ secretory cells and Ac-TUB⁺ ciliated cells in distal (G) and proximal (H) oviducts across different developmental stages, depicting a gradual decrease in the percentage of secretory cells coupled with a concomitant increase in the percentage of ciliated cells. (I,J) qPCR analysis of mouse oviducts for *Pax8* (I) and *Foxj1* (J) revealed a similar trend to that observed by immunostaining. Data are mean±s.e.m., n=3; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared with P1 within respective cell types using one-way ANOVA. Values on the top of bars and lines represent non-significant P values. SOC, serous ovarian cancer; SCOUT, secretory cell outgrowth; STIC, serous tubal intraepithelial carcinoma; Ac-TUB, acetylated tubulin. Scale bars: 20 µm.

encoded β-galactosidase expression is targeted to PAX8-expressing cells. Following induction of labeling by feeding doxycycline-supplemented water to pregnant females from E13.5 to P4, lacZ-

marked progeny were chased over a period of 7 months (Fig. 2F). Initially, at P4 (day 0 of chase), 83.94±1.11% and 91.21±1.68% of secretory cells in distal and proximal oviducts, respectively, were

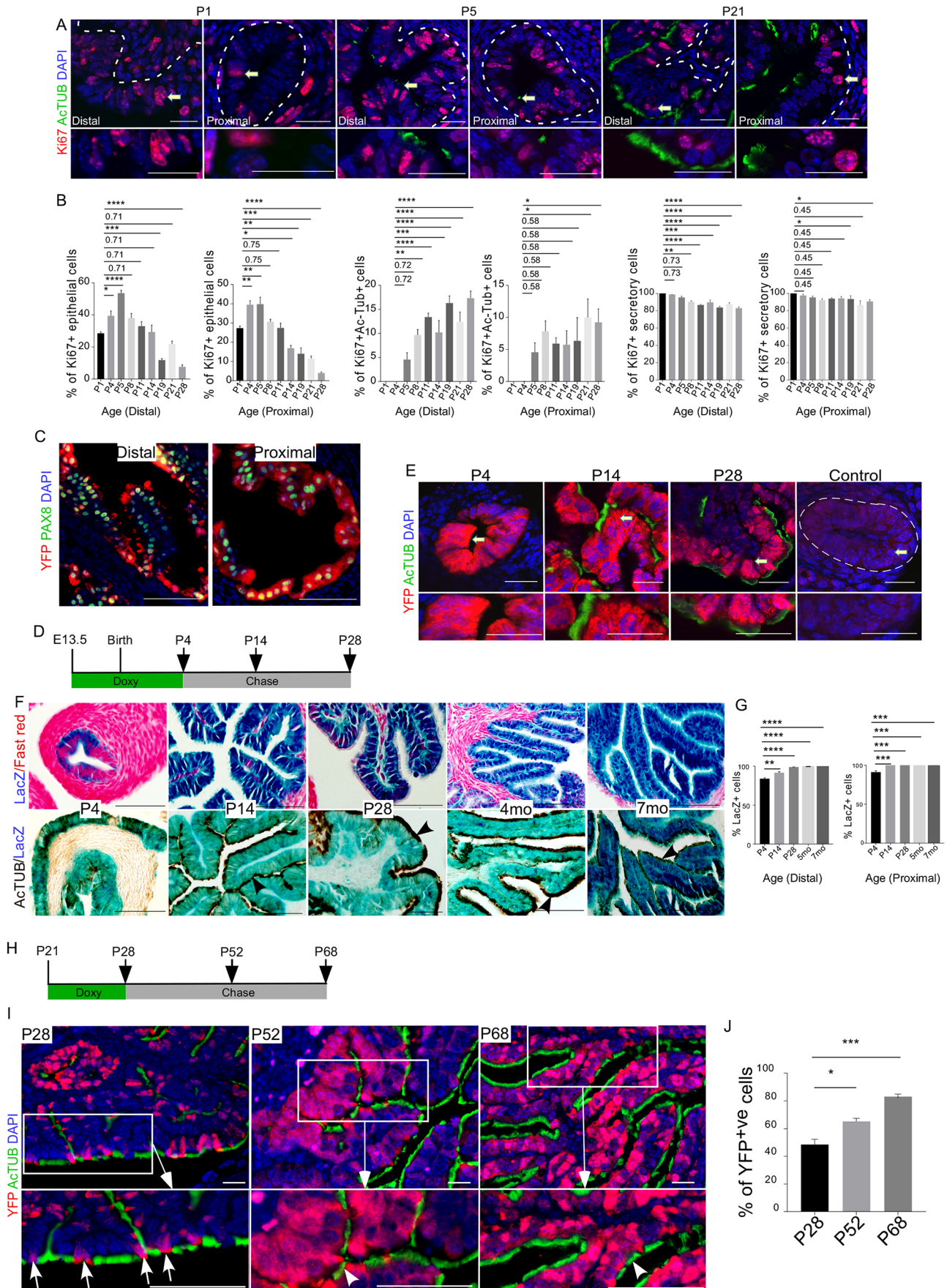


Fig. 2. See next page for legend.

Fig. 2. Highly proliferating PAX8⁺ secretory cells can self-renew and differentiate into ciliated progeny to maintain epithelial cell homeostasis in developing as well as adult mouse oviduct. (A) Immunostaining of oviductal epithelium for Ki67, a proliferation marker, and Ac-TUB across different stages of mouse development. Areas indicated by arrows are presented at higher magnification beneath. (B) Quantification of proliferating epithelial, ciliated and secretory cells across different developmental stages. (C) Immuno-colocalization of PAX8 with YFP in oviducts of *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* mice. (D) Scheme depicting label induction in *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* and *Pax8-rtTA;tetO-Cre;lacZ^{fl/fl}* mice, and subsequent chase across developmental stages. (E) Immunostaining for Ac-TUB and YFP (arrow) in mouse oviductal epithelium at selected chase points P4 to P28. (F) X-Gal (blue) and Nuclear Fast Red (red) stained sections (top) of oviducts from *Pax8-rtTA;tetO-Cre;lacZ^{fl/fl}* mice at the indicated chase points. X-Gal-stained sections immunostained for Ac-TUB (dark brown; arrowhead) are also shown (bottom). mo, month. (G) Quantification of *lacZ⁺* cells in distal and proximal mouse oviductal epithelium. (H) Scheme depicting label induction in *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* mice at adult stages and subsequent chase. (I) Immunostaining for Ac-TUB and YFP of mouse oviductal epithelium at selected chase points P28, P52 and P68. Arrows indicate Ac-TUB- YFP⁺ cells; arrowheads indicate Ac-TUB⁺ YFP⁺ cells. (J) Quantification of YFP⁺ cells across the chase points. $n=3$. Data are mean \pm s.e.m., $n=3$; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$, compared with P1 (in B), P4 (in G) or P28 (in J) using one-way ANOVA. Values on the top of bars represent non-significant P values. Scale bars: 20 μ m in A,E,F,I; 50 μ m in C.

lacZ⁺ (Fig. 2F,G). Subsequently, there was an increase in the percentage of *lacZ*-marked progeny. At P28, 98.99 \pm 0.04% and 100% of the total epithelial cells in distal and proximal oviducts, respectively, were *lacZ⁺*. In agreement with our *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* tracing results, a subset of these *lacZ⁺* cells co-expressed the mature ciliated cell marker Ac-TUB (Fig. 2F). During the ensuing 7-month chase, all of the epithelial cells in distal and proximal oviduct progressively became *lacZ⁺* (Fig. 2F,G). These results show that all epithelial cell lineages of adult oviduct are derived from embryonically labeled PAX8⁺ cells, which act as oviductal epithelial progenitors and give rise to ciliated cells.

To investigate whether the secretory cells of fully differentiated oviduct retain the potential to act as progenitors of ciliated cells, we induced labeling of secretory cells in fully differentiated mouse oviducts by administering doxycycline to *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* mice from P21 to P28, and subsequently chased them into adulthood (Fig. 2H). As expected, YFP expression was initially restricted to secretory cells at P28 (day 0 of chase) (Fig. 2I). Over time, there was a steady increase in the percentage of YFP⁺ cells in the distal oviduct from 48.59 \pm 3.75% at P28 to 65.17 \pm 2.31% at P52, reaching 83.07 \pm 2.97% at P68. More importantly, as with embryonic labeling, there was a subset of YFP⁺ cells that co-expressed Ac-TUB (Fig. 2I,J).

Collectively, these results demonstrated for the first time that PAX8⁺ oviductal secretory cells act as oviductal epithelial progenitors and have the potential to self-renew as well as differentiate to ciliated cell progeny, thereby contributing to oviductal epithelial homeostasis.

Active Wnt signaling in the mouse oviductal epithelium during epithelial maturation and differentiation

Wnt signaling plays a major role in genital tract development (Kobayashi and Behringer, 2003). Many studies have shown that Wnt signaling is associated with pathological conditions of the oviduct, such as endometriosis (Matsuzaki and Darcha, 2013) and ectopic pregnancy (Kodithuwakku et al., 2012; Li et al., 2013). However, the involvement of the Wnt signaling pathway in oviductal epithelial maturation and differentiation during postnatal development is still unexplored. To assess changes in Wnt signaling

activity during postnatal oviduct development, we used a well-established Wnt reporter mouse model, *TCF:GFP*, in which the expression of a GFP-histone H2B fusion protein is under the control of six copies of TCF/Lef elements. This confers nuclear GFP expression in Wnt-responsive cells, allowing us to visualize and track changes in Wnt activity over time.

To analyze Wnt signaling activity in oviductal epithelium, we collected *TCF:GFP* mouse oviducts at crucial postnatal developmental stages between P1 (undifferentiated oviductal epithelium) and P21 (fully differentiated oviductal epithelium) (Komatsu and Fujita, 1978). Immunostaining for GFP revealed higher Wnt activity in proximal than distal oviduct during early stages of postnatal development (Fig. 3A-C). At P1, 37.49 \pm 2.40% of epithelial cells in the proximal oviduct were Wnt responsive as compared with only 4.42 \pm 1.17% of cells in the distal oviduct (Fig. 3A-C). However, with oviductal epithelial maturation and differentiation, there was a gradual increase in Wnt signaling activity in distal oviduct, concomitant with a gradual decrease in the proximal oviduct (Fig. 3A-C). At P21, 33.98 \pm 7.22% of epithelial cells in the distal oviduct were Wnt responsive, as compared with only 16.19 \pm 1.71% of cells in the proximal oviduct (Fig. 3C).

To reveal the cell type-specific activity of Wnt signaling in the oviductal epithelium, we looked for colocalization of GFP with PAX8 and Ac-TUB (Fig. 3A,B, Fig. S2B). At early stages of postnatal development, Wnt signaling activity was mainly present in the secretory cells of both the distal (72.22 \pm 2.78%) and proximal (72.20 \pm 2.81%) oviduct, whereas with epithelial maturation Wnt signaling was prominent in both secretory and ciliary cell types (Fig. 3D,E). Specifically, at P21, in distal oviduct Wnt signaling activity was more prominent in ciliated cells (30.37 \pm 2.51%) than in secretory cells (18.30 \pm 1.68%) (Fig. 3E). By contrast, in proximal oviduct more secretory cells (61.62 \pm 1.95%) than ciliated cells (18.30 \pm 1.68%) were Wnt responsive (Fig. 3E). Collectively, these results highlight the requirement of active Wnt signaling in oviduct epithelial maturation and differentiation during postnatal development.

In order to explore the expression pattern of different members of the Wnt pathway in mouse oviduct, we performed RNA *in situ* hybridization on fully differentiated mouse oviducts (P28, $n=4$) to look at the expression of 19 Wnt ligands, five secreted frizzled-related protein genes (*Sfrp1-5*), *Dkk1-4*, *Axin2* and *Wif1* (Fig. 3F, Fig. S3). We found that *Wnt7a*, *Wnt7b*, *Wnt11* and *Wnt16* were expressed mainly in oviductal epithelial cells (Fig. 3F). Furthermore, *Wnt2b*, *Wnt4*, *Wnt5a*, *Wnt5b*, *Dkk3*, *Dkk4*, *Wif1*, *Axin2* and *Sfrp1* were expressed in the oviductal epithelium and/or stroma of distal and proximal oviducts (Fig. S3). *Sfrp2*, *Sfrp4* and *Dkk2* expression was only present in stromal cells (Fig. S3). *Wnt1*, *Wnt2*, *Wnt3*, *Wnt3a*, *Wnt8a*, *Wnt8b*, *Wnt9a*, *Wnt9b*, *Wnt10a*, *Wnt10b*, *Dkk1*, *Sfrp3* (*Frzb*) and *Sfrp5* were not expressed in mouse oviduct. In summary, these results demonstrate the presence and distribution of active Wnt signaling in adult mouse oviduct.

Conditional ablation of β -catenin (*Ctnnb1*) leads to a reduction in the proportion of secretory cells

Our expression analyses of Wnt pathway members suggest that this signaling pathway might be an important regulator of mature oviductal epithelial homeostasis (Fig. 3F, Fig. S3). To investigate the role of Wnt/ β -catenin signaling in adult oviductal epithelial homeostasis, we developed a mouse model, *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl/fl}*, in which, upon doxycycline administration, *Ctnnb1* is specifically deleted in secretory cells (Fig. 4). For induction of recombination to ablate *Ctnnb1* from secretory cells, 6-week-old mice were given doxycycline-supplemented water for 21 days, and

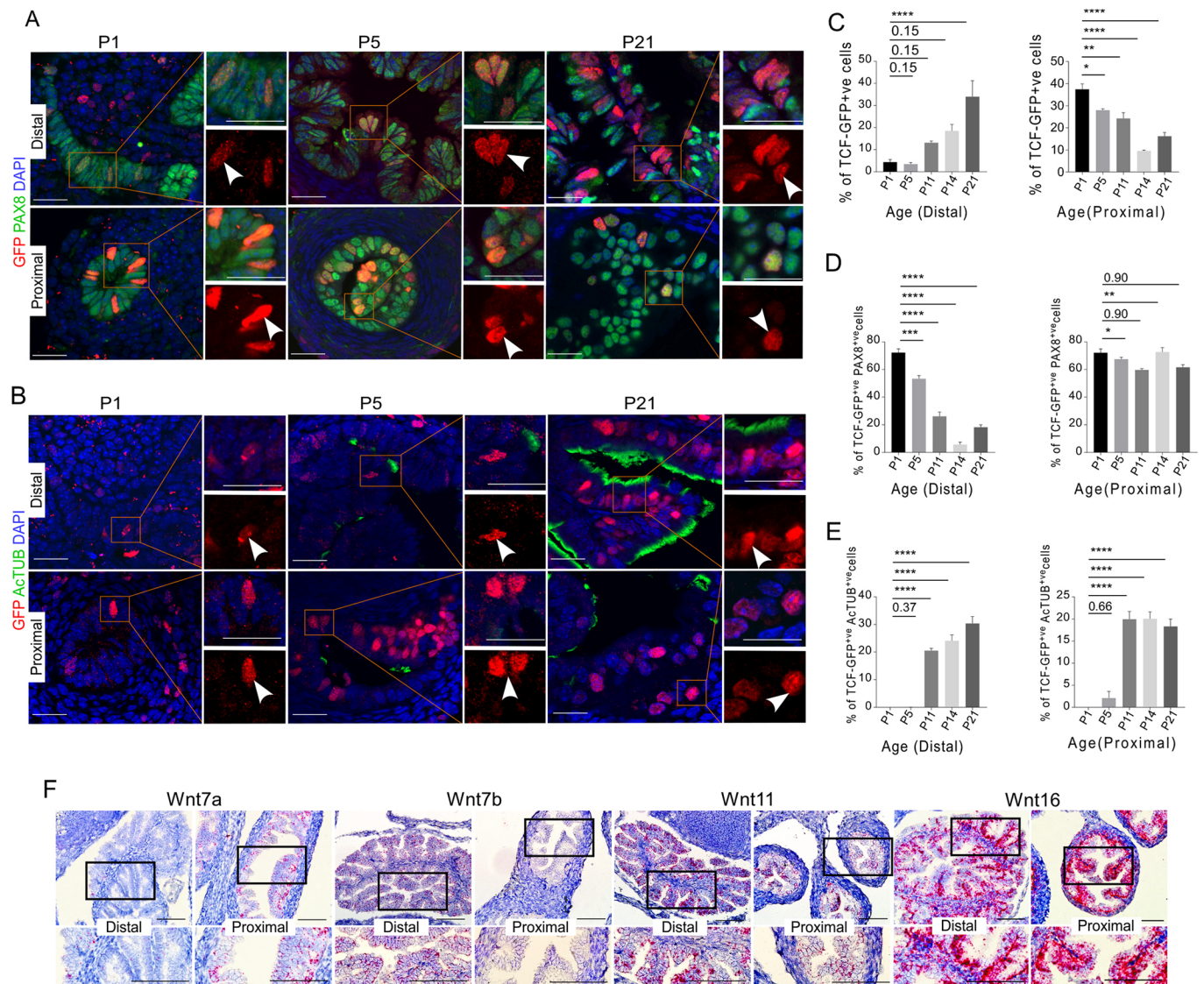


Fig. 3. Active Wnt signaling in mouse oviductal epithelium. (A,B) Immunofluorescence of GFP (arrowhead) with PAX8 (A) or AcTUB (B) in distal and proximal oviductal epithelium at different developmental stages of *TCF:GFP* mice. Boxed areas are shown in higher magnification. (C-E) Quantification of Wnt-responsive total epithelial (C), secretory (D) and ciliated (E) cells in the oviductal epithelium across developmental stages. (F) *In situ* hybridization for selected Wnt ligands *Wnt7a*, *Wnt7b*, *Wnt11* and *Wnt16* (red) in P28 mouse oviductal epithelium. Boxed areas are shown in higher magnification beneath. Data are mean \pm s.e.m., $n=3$ in A-E, $n=4$ in F; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ compared with P1 using one-way ANOVA. Values on the top of bars represent non-significant P values. Scale bars: 20 μ m in A,B; 100 μ m in F.

oviducts were collected at day 0 and at 60 days post induction. *Ctnnb1* deletion from PAX8⁺ secretory cells in mutants, but not controls, was confirmed by immunostaining oviducts for β -catenin (Fig. 4A). This was further confirmed by qPCR analysis, which revealed a 50% reduction of *Ctnnb1* mRNA levels in mutant as compared with control oviducts (Fig. 4D).

At 60 days post induction, the oviducts from mutant mice were smaller and of reduced weight compared with controls (Fig. S4) and histological analyses revealed a significant reduction in the percentage of PAX8⁺ secretory cells in mutants (22.08 \pm 2.78%) compared with controls (53.34 \pm 2.06%) (Fig. 4B,E,F). Furthermore, there was a concomitant decrease in the percentage of mature ciliated cells in mutants (45.00 \pm 0.88%) compared with controls (63.02 \pm 1.17%) (Fig. 4B,E,F). We further confirmed these results by qPCR analysis of oviducts collected 60 days post induction, which revealed a 50% reduction of *Pax8* mRNA levels in mutants

compared with controls (Fig. 4G). Collectively, these results indicate that Wnt/ β -catenin signaling plays an essential role in the self-renewal of mouse oviductal secretory cells, and their differentiation to ciliated progeny.

Overactivation of Wnt/ β -catenin signaling causes expansion of secretory cells

To investigate whether hyperactivation of Wnt/ β -catenin signaling affects self-renewal and differentiation of oviductal secretory cells, we developed a mouse model, *Pax8-rtTA; tetO-Cre; Ctnnb1^{fl(ex3)/fl(ex3)}*, in which doxycycline-induced recombination leads to constitutive activation of β -catenin in PAX8⁺ secretory cells (Fig. 4C). Doxycycline causes deletion of *Ctnnb1* exon 3, which harbors the phosphorylation sites targeted by the adenomatous polyposis coli (Apc) complex for β -catenin destruction. Loss of these sites leads to the accumulation of recombined functional β -catenin protein in cells

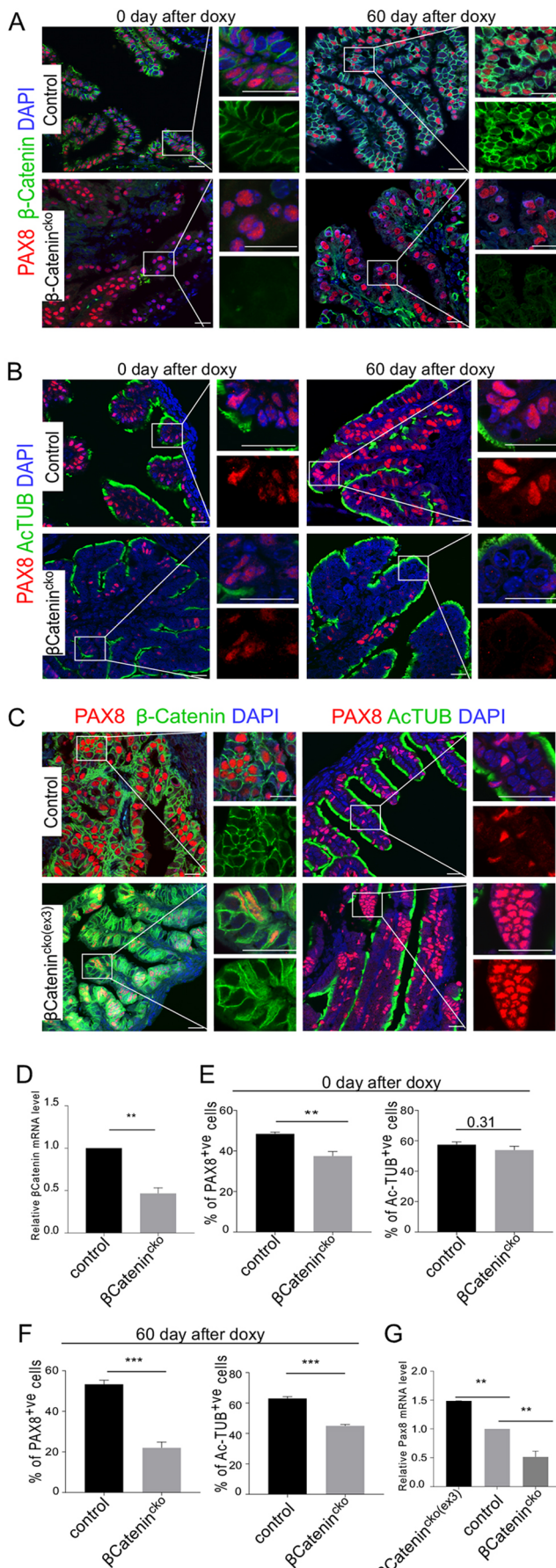


Fig. 4. β -catenin regulates secretory cell self-renewal and differentiation to ciliated progeny. (A,B) Immunocolocalization of PAX8 with β -catenin (A) or Ac-TUB (B) of oviducts collected at day 0 (left) and 60 days (right) post induction of recombination in *Ctnnb1*^{cko} and control mice. (C) Co-immunostaining for PAX8 and β -catenin (left) or PAX8 and Ac-TUB (right) of oviducts collected 76 days post induction of recombination in *Ctnnb1*^{cko(ex3)} and control mice. (D) qRT-PCR analysis of *Ctnnb1* gene expression (mRNA) levels in mutant and control oviductal epithelium. (E) Quantification of PAX8⁺ and Ac-TUB⁺ cells in the distal oviductal epithelium of control and *Ctnnb1*^{cko} mice at day 0 post induction. (F) Quantification of PAX8⁺ and Ac-TUB⁺ cells in the distal oviductal epithelium of control and *Ctnnb1*^{cko} mice 60 days post induction. (G) Relative gene expression (mRNA) levels of *Pax8* in control, *Ctnnb1*^{cko(ex3)} and *Ctnnb1*^{cko} mouse oviducts. *cko*, conditional knockout; *cko* (*ex3*), conditional knockout of exon 3. *n*=3. Values are s.e.m. ***P*<0.01, ****P*<0.001. Scale bars: 20 μ m.

derived from *Pax8*-expressing cells (Harada et al., 1999). To induce recombination, 6-week-old mice were administered doxycycline in drinking water, and oviducts were collected at day 0 and at 76 days post induction.

Histological analyses at 76 days post induction revealed focal and nodular expansions of PAX8⁺ secretory cells in mutant oviducts but not in controls (Fig. 4C). Successful recombination in mutant oviducts was confirmed by immunostaining for β -catenin, which revealed nuclear accumulation of β -catenin in these focal and nodular expansions of PAX8⁺ secretory cells. Compared with controls, in mutants there was an overall expansion of the PAX8⁺ secretory cell population in the distal oviduct (Fig. 4C; 48.94 \pm 2.20% in controls versus 63.20 \pm 3.24% in mutants). This was further confirmed by qPCR analyses, which revealed 1.4-fold upregulation of *Pax8* mRNA levels in mutants as compared with controls (Fig. 4G). Collectively, these results confirm the role of Wnt/ β -catenin signaling in the self-renewal of secretory cells.

DISCUSSION

Among the four histological subtypes of epithelial ovarian cancers (serous, endometrioid, clear cell, and mucinous), SOC is the most common and the most lethal (Bowtell et al., 2015). SOC is usually diagnosed at late stages due to non-specific clinical symptoms, and has a poor prognosis, with a 5-year survival rate of only 30-50% (Bowtell et al., 2015). Historically, it was assumed that SOC originates from the ovary. However, there has been a recent paradigm shift towards extra-ovarian sites of origin (Perets and Drapkin, 2016). This change in direction was prompted by the observation that patients with a predisposition for developing SOC were found to have tubal epithelial dysplastic lesions, such as serous tubal intraepithelial carcinoma (STIC) (Bowtell et al., 2015). Furthermore, studies have shown that surgical removal of both fallopian tubes (bilateral salpingectomy) can reduce the risk of ovarian cancer development in patients with germline *BRCA1/2* mutations (Falconer et al., 2015).

Tissue-resident stem/progenitor cells of various organs such as the colon, intestine and mammary gland have been shown to play a major role in tissue homeostasis and carcinogenesis (Beumer and Clevers, 2016; de Sousa e Melo et al., 2017; Visvader and Stingl, 2014). However, there is a lack of evidence demonstrating the existence of an oviductal epithelial stem/progenitor cell population, which could functionally contribute to oviductal epithelial cell lineages. Secretory cells are the dominant cell type in tubal epithelial dysplastic lesions in patients with a predisposition for SOC development and, as such, they are more likely to acquire cancerous mutations, ultimately leading to the initiation of epithelial ovarian carcinoma (Perets et al., 2013; Sherman-Baust et al., 2014; Yamamoto et al., 2016). Understanding the mechanisms underlying

oviductal epithelial development and differentiation, as necessary to maintain cellular homeostasis, is crucial to understand ovarian epithelial carcinogenesis. PAX8 is a bona fide marker for oviductal secretory cells (Perets et al., 2013). Here, we have used mouse models based on *Pax8*-driven *Cre*-mediated recombination to demonstrate that secretory cells behave as a stem/progenitor cell population of the oviduct. Using an *in vivo* cell lineage-tracing approach, we also showed that PAX8⁺ secretory cells have the potential to self-renew and differentiate to ciliated cells in both the embryonic and adult stages in mice.

Wnt/ β -catenin signaling plays an important role in adult epithelial homeostasis in various organs (Kretzschmar and Clevers, 2017). To investigate the role of this pathway in oviductal epithelial development and differentiation, we examined the expression of different Wnt pathway members in mouse oviduct. Using a Wnt reporter mouse model, we demonstrated that the Wnt/ β -catenin signaling pathway is active during postnatal oviductal epithelial maturation and differentiation. Our expression analyses of different Wnt pathway members in fully differentiated mouse oviducts further confirm that this pathway is likely to be involved in post-pubertal oviductal epithelial homeostasis. To investigate the impact of alterations in the Wnt signaling pathway on oviductal epithelial homeostasis, we used two mouse models to conditionally overactivate and ablate β -catenin in oviductal secretory cells, and showed that overactivation causes expansion, while ablation causes reduction, in the size of secretory cell population. Collectively, we showed that the Wnt/ β -catenin signaling pathway plays a crucial role in the maintenance of oviduct epithelial cell homeostasis during adulthood by regulating the self-renewal and differentiation of secretory cells. Our results are further supported by a recent report that loss of β -catenin in oviductal ciliated cells (*Foxj1^{cre}; Ctnnb1^{fl/fl}*) had no effect on oviduct histology, ciliated cells, or the transport of embryos through the tube (Li et al., 2017). This is in line with our results suggesting that secretory cells continuously replenish ciliated cells.

Despite the importance of oviductal epithelial cell dynamics in homeostasis and disease, the mechanisms underlying the maintenance of secretory and ciliated cell types, and their hierarchical relationship have remained elusive. As early as 1935, while examining histological sections of mouse oviducts, Espinasse made an observation that if secretory and ciliated cells are fully differentiated and distinct cell types then how is oviductal epithelium able to compensate for the loss of epithelial cells, especially non-ciliated cells, which undergo nuclear displacement and are possibly lost through cytoplasmic bulging and nuclear extrusion? (Espinasse, 1935). To explain how the oviductal epithelium is maintained, he proposed that the displaced cells do not detach completely; rather, they move back to their original position to maintain oviductal epithelial homeostasis (Espinasse, 1935). However, many years later, basal/reserve/indifferent cells and peg/intercalated cells, described as two other cell types in the fallopian tube epithelium, were postulated to be responsible for the maintenance of mature epithelial and stromal cells (Clyman, 1966; Ferenczy et al., 1972; Pauerstein and Woodruff, 1967). Subsequent histology and immunohistochemistry-based investigations by many groups firmly established that these basal and peg cells are actually lymphocytes and exhausted secretory cells, respectively. Therefore, tubal epithelium mainly consists of secretory and ciliated cells (Crow et al., 1994; Ferenczy et al., 1972; Fredricsson and Bjorkman, 1973; Odor, 1974; Peters, 1986). In a later study, based on electron microscopy, it was observed that occasional epithelial cells of the fallopian tube have features of both ciliated and secretory cells

(Crow et al., 1994), suggesting that these two cell types are not restricted to ciliated or secretory fate and might be interchangeable. More recently, EpCAM⁺ cells isolated from human fallopian tube and cultured in the presence of epidermal growth factor (EGF), fibroblast growth factor 10 (FGF10), Wnt3a, Rspodin 1, noggin, Rock inhibitor, transforming growth factor β (TGF β) receptor kinase inhibitor, nicotinamide and B27 supplement, developed into 3D organoids consisting of both secretory and ciliated cells (Kessler et al., 2015), suggesting the existence of adult stem cells in the fallopian tube. A subpopulation of these EpCAM⁺ cells was fivefold enriched for cells capable of developing into organoids, further supporting the existence of stem cells (Paik et al., 2012). Later, it was proposed that these stem cells, which develop into functional organoids, are likely to be non-ciliated (Kessler et al., 2015). In a similar study, Yamamoto et al. (2016) cultured fallopian tube epithelial cells on a feeder layer of mouse embryonic fibroblasts with a cocktail of growth factors and regulators of TGF β /bone morphogenetic protein (BMP), Wnt/ β -catenin, EGF, insulin-like growth factor (IGF) and Notch pathways, and demonstrated the existence of tubal epithelial stem cells that were PAX8⁺ and can give rise to both ciliated and secretory cells under *in vitro* conditions (Yamamoto et al., 2016). Collectively, these studies provide evidence in support of secretory cells acting as fallopian tube epithelial stem cells under *in vitro* conditions. However, the exact identity and location of the fallopian tube epithelial stem/progenitor cells within resident niches are still unclear. Our *in vivo* genetic cell lineage-tracing approach provides compelling evidence that PAX8⁺ secretory cells act as oviduct epithelial stem/progenitor cells. As our lineage-tracing approach targeted the majority of secretory cells, it is unclear whether a subset or all secretory cells act as stem progenitor cells. Partial or mosaic labeling of PAX8⁺ cells followed by analysis of clonal evolution is required to answer this question. Alternatively, the identification of novel markers that differentiate subpopulations of PAX8⁺ secretory cells could, combined with subsequent lineage tracing, also address this problem.

In summary, our findings significantly broaden the current understanding of oviductal epithelial homeostasis, and also support the hypothesis of oviductal secretory cell-derived SOC. Furthermore, we identified the role of the Wnt/ β -catenin signaling pathway in oviductal secretory cell fate determination, thereby providing an opportunity to develop molecular therapeutic targets to inhibit uncontrolled self-renewal activity of secretory cells during the early development of SOC. The oviduct is the site of fertilization, and as optimal stem/progenitor cell activity to maintain cellular homeostasis is necessary for the survival of gametes and zygote, our study provides new insight into not only the field of ovarian cancer biology but also the area of fertility. Understanding the role of Wnt/ β -catenin signaling in stem/progenitor cell fate determination might open new avenues for treating patients with subfertility/infertility or predisposition to ectopic pregnancy.

MATERIALS AND METHODS

Human fallopian tube tissue samples

This study was performed under the approval of the Institutional Human Research Ethics Committee at the University of Newcastle. Ovaries and whole fallopian tubes were collected from patients who underwent risk removal bilateral salpingo-oophorectomy at the John Hunter Hospital, Newcastle, New South Wales, Australia. Tissue samples were obtained from 17 patients and validated by a pathologist (Dr Loui Rassam) at the Hunter Cancer Biobank (Table S1). Tissues were sectioned from 10-16 blocks per high-risk patient ($n=17$) and three blocks per normal healthy woman ($n=3$).

Experimental animal models

All mouse experiments performed in this study were approved by the Animal Care and Ethics Committee of the University of Newcastle. Mice were maintained on a mixed genetic background (C57BL/6J;129SvEv) and were kept in standard housing conditions with *ad libitum* access to food and water. The following mouse strains were purchased from Jackson Laboratory: *Pax8-rtTA* (Traykova-Brauch et al., 2008), *tetO-Cre* (Perl et al., 2002), *Ctnnb1^{fl/fl}* (Huelsen et al., 2001), *Ctnnb1^{fl(ex3)/fl(ex3)}* (Harada et al., 1999), *Gt(ROSA)26Sor^{tm1Sor/J} (lacZ^{fl/fl})* (Soriano, 1999) and *Gt(ROSA)26Sor^{tm1(EYFP)Cos}(YFP^{fl/fl})* (Srinivas et al., 2001). *Pax8-rtTA* mice were bred with *tetO-Cre* mice to generate *Pax8-rtTA;tetO-Cre* mice. *Pax8-rtTA;tetO-Cre*, *YFP^{fl/fl}*, *lacZ^{fl/fl}*, *Ctnnb1^{fl/fl}* and *Ctnnb1^{fl(ex3)/fl(ex3)}* mice were mated to produce *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}*, *Pax8-rtTA;tetO-Cre;lacZ^{fl/fl}*, *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl/fl}* and *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl(ex3)/fl(ex3)}*, which are referred to as *Pax8-rtTA;tetO-Cre;Yfp^{ΔΔ}*, *Pax8-rtTA;tetO-Cre;lacZ^{ΔΔ}*, *Ctnnb1^{cko}* and *Ctnnb1^{cko(ex3)}*, respectively, after doxycycline treatment. *TCF/Lef:H2B/GFP* (Ferrer-Vaquero et al., 2010) mice were maintained by crossing with wild-type mice and are referred to as *TCF:GFP* in the text.

Labeling and lineage-tracing experiment

Upon doxycycline administration, *Pax8-rtTA;tetO-Cre;Yfp^{ΔΔ}* and *Pax8-rtTA;tetO-Cre;lacZ^{ΔΔ}* express YFP or lacZ-encoded β-galactosidase, respectively, in all cells derived from *Pax8*-expressing cells. For lineage tracing induced at E13.5, pregnant females of *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* and *Pax8-rtTA;tetO-Cre;lacZ^{fl/fl}* mice were given doxycycline in drinking water (2-5 mg/ml, supplemented with 1% sucrose) from E13.5 to P4 (Fig. 2C). For lineage tracing initiated at P21, *Pax8-rtTA;tetO-Cre;Yfp^{fl/fl}* mice were induced by oral administration of doxycycline in drinking water (2 mg/ml, supplemented with 5% sucrose) for 7 days (Fig. 2H). When doxycycline was administered to *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl/fl}* mice, *Ctnnb1* was deleted from cells derived from *Pax8*-expressing cells. By contrast, when doxycycline was administered to *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl(ex3)/fl(ex3)}* mice, *Ctnnb1* was constitutively activated in cells derived from *Pax8*-expressing cells. For induction of recombination to delete or overactivate *Ctnnb1* in *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl/fl}* and *Pax8-rtTA;tetO-Cre;Ctnnb1^{fl(ex3)/fl(ex3)}* mice, respectively, doxycycline was administered in drinking water (5 mg/ml, supplemented with 5% sucrose) to adult females at 6 weeks of age for 21 days (Fig. 4A,C). Mice were sacrificed at different chase points, and oviducts were collected for analysis. Littermate control mice were used as negative controls for lineage-tracing experiments.

X-Gal staining and Nuclear Fast Red staining

X-Gal staining was performed as described previously (Tanwar et al., 2012). Briefly, oviducts collected from *Pax8-rtTA;tetO-Cre;lacZ^{ΔΔ}* mice were prefixed in 4% paraformaldehyde (PFA) at 4°C for 1 h. Post-fixed tissues were washed three times in rinse buffer [0.1% sodium deoxycholate, 0.2% NP40, 2 mM MgCl₂, 0.1 M sodium phosphate buffer (pH 7.3)] for 30 min each at room temperature. Tissues were incubated in X-Gal solution for 3-4 h at room temperature, fixed in 4% PFA at 4°C overnight, and processed for paraffin embedding. Tissue sections were cut at 6 μm thickness, counterstained with Nuclear Fast Red, and imaged using an Olympus DP72 microscope. Quantification of lacZ⁺ cells was performed using ImageJ (NIH) software.

Immunohistochemistry and immunofluorescence

Oviducts were fixed in 4% (w/v) PFA at 4°C overnight, before processing for paraffin embedding. Immunohistochemistry was performed on deparaffinized and rehydrated 6 μm sections. For antigen retrieval, slides were heated to 98°C for 30 min in 1 mM EDTA buffer pH 8.0. After blocking, tissue sections were incubated with primary antibodies at 4°C overnight. Primary antibodies were mouse anti-β-catenin (1:200; 610153, BD Biosciences), rabbit anti-Ki67 (1:400; Ab15580, Abcam), rabbit anti-Pax8 (1:400; 10336-1-AP, Proteintech), mouse anti-acetylated tubulin (1:1000; T7451, clone 6-11B-1, Sigma-Aldrich) and chicken anti-GFP (1:1000; Ab13970, Abcam). Secondary antibodies were biotin- or HRP-conjugated

anti-mouse IgG (1:250; Jackson ImmunoResearch) for HRP immunostaining or Alexa 488/594-conjugated anti-mouse/rabbit/chicken IgG (1:250; Jackson ImmunoResearch) for immunofluorescence. Images were taken using an Olympus FV10i confocal microscope and an Olympus DP72 microscope. Images were quantified using ImageJ.

RNA in situ hybridization

Oviducts were collected from P28 mice (*n*=4) and fixed in 10% neutral buffered formalin for 24 h at room temperature. Tissues were then paraffin embedded and sectioned at 4 μm thickness. RNA *in situ* hybridization was performed using the RNAscope 2.5 High Definition-Red Kit (Advanced Cell Diagnostics) as described (Goad et al., 2017). *In situ* hybridization probes were: *DapB* (EF191515; negative control), *Ppib* (NM_011149.2), *Wnt2* (NM_023653.5), *Wnt2b* (NM_009520.3), *Wnt3* (NM_009521.1), *Wnt3a* (NM_009522.2), *Wnt4* (NM_009523.2), *Wnt5a* (NM_009524.3), *Wnt5b* (NM_001271757.1), *Wnt6* (NM_009526.3), *Wnt7a* (NM_009527.3), *Wnt7b* (NM_009528.3), *Wnt8a* (NM_009290.2), *Wnt8b* (NM_011720.3), *Wnt9a* (NM_139298.2), *Wnt9b* (NM_011719.4), *Wnt10a* (NM_009518.2), *Wnt10b* (NM_011718.2), *Wnt11* (NM_009519.2), *Wnt16* (NM_053116.4), *Axin2* (NM_015732.4), *Wif1* (NM_011915.2), *Sfrp1* (NM_013834.4), *Sfrp2* (NM_009144.2), *Sfrp3* (*Frzb*) (NM_011356.4), *Sfrp4* (NM_016687.3), *Sfrp5* (NM_018780.3), *Dkk1* (NM_010051.3), *Dkk2* (NM_020265.4), *Dkk3* (NM_015814.2) and *Dkk4* (NM_145592.2). (The Genbank accession numbers for the sequences used to create the probes are given in parentheses.)

RNA isolation and qRT-PCR

RNA was isolated from distal and proximal oviducts separately (in the case of adults) or from the whole oviducts (in case of pups) using the RNeasy Micro Kit (Qiagen) following the manufacturer's instructions. Following RNA quantification, equal amounts of RNA were used for cDNA construction using the RT² First Strand Kit (Qiagen). Quantitative real-time PCR (qRT-PCR) was performed on an ABI 7900 HT FAST using RT² SYBR Green ROX qPCR Mastermix (Qiagen). All Ct values were normalized to the housekeeping gene *Gapdh*. qPCR primer details are provided in Table S2.

Statistical analysis

One-way ANOVA and non-parametric *t*-test statistical analyses were performed using GraphPad Prism 6.0 software. Values are presented as mean±s.e.m. *P*<0.05 was considered statistically significant.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.S.T.; Methodology: A.G., S.M.S., P.S.T.; Validation: A.G., S.M.S., P.S.T.; Formal analysis: A.G., P.S.T.; Investigation: A.G., S.M.S., P.S.T.; Resources: P.S.T.; Writing - original draft: A.G., S.M.S., P.S.T.; Writing - review & editing: S.M.S., P.S.T.; Supervision: P.S.T.; Project administration: P.S.T.; Funding acquisition: P.S.T.

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Supplementary information

Supplementary information available online at <http://dev.biologists.org/lookup/doi/10.1242/dev.149989.supplemental>

References

- Bahar-Shany, K., Brand, H., Sapoznik, S., Jacob-Hirsch, J., Yung, Y., Korach, J., Perri, T., Cohen, Y., Hourvitz, A. and Levanon, K. (2014). Exposure of fallopian tube epithelium to follicular fluid mimics carcinogenic changes in precursor lesions of serous papillary carcinoma. *Gynecol. Oncol.* **132**, 322-327.
- Beumer, J. and Clevers, H. (2016). Regulation and plasticity of intestinal stem cells during homeostasis and regeneration. *Development* **143**, 3639-3649.
- Bowtell, D. D., Böhm, S., Ahmed, A. A., Aspuria, P.-J., Bast, R. C., Jr, Beral, V., Berek, J. S., Birrer, M. J., Blagden, S., Bookman, M. A. et al. (2015). Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat. Rev. Cancer* **15**, 668-679.
- Brachova, P., Alvarez, N. S., Van Voorhis, B. J. and Christenson, L. K. (2017). Cytidine deaminase Apobec3a induction in fallopian epithelium after exposure to follicular fluid. *Gynecol. Oncol.* **145**, 577-583.
- Clyman, M. J. (1966). Electron microscopy of the human fallopian tube. *Fertil. Steril.* **17**, 281-301.
- Coy, P., Garcia-Vazquez, F. A., Visconti, P. E. and Aviles, M. (2012). Roles of the oviduct in mammalian fertilization. *Reproduction* **144**, 649-660.
- Crow, J., Amso, N. N., Lewin, J. and Shaw, R. W. (1994). Morphology and ultrastructure of fallopian tube epithelium at different stages of the menstrual cycle and menopause. *Hum. Reprod.* **9**, 2224-2233.
- de Sousa e Melo, F., Kurtova, A. V., Harnoss, J. M., Klijavin, N., Hoec, J. D., Hung, J., Anderson, J. E., Storm, E. E., Modrusan, Z., Koepfen, H. et al. (2017). A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* **543**, 676-680.
- Donnez, J., Casanas-Roux, F., Caprasse, J., Ferin, J. and Thomas, K. (1985). Cyclic changes in ciliation, cell height, and mitotic activity in human tubal epithelium during reproductive life. *Fertil. Steril.* **43**, 554-559.
- Espinasse, P. G. (1935). The oviducal epithelium of the mouse. *J. Anat.* **69**, 363-368.1.
- Falconer, H., Yin, L., Grönberg, H. and Altman, D. (2015). Ovarian cancer risk after salpingectomy: a nationwide population-based study. *J. Natl. Cancer Inst.* **107**, djv410.
- Ferenczy, A., Richart, R. M., Agate, F. J., Jr, Purkerson, M. L. and Dempsey, E. W. (1972). Scanning electron microscopy of the human fallopian tube. *Science* **175**, 783-784.
- Ferrer-Vaquer, A., Piliszek, A., Tian, G., Aho, R. J., Dufort, D. and Hadjantonakis, A.-K. (2010). A sensitive and bright single-cell resolution live imaging reporter of Wnt/ss-catenin signaling in the mouse. *BMC Dev. Biol.* **10**, 121.
- Fredricsson, B. and Björkman, N. (1973). Morphologic alterations in the human oviduct epithelium induced by contraceptive steroids. *Fertil. Steril.* **24**, 19-30.
- George, S. H. L., Milea, A. and Shaw, P. A. (2012). Proliferation in the normal FTE is a hallmark of the follicular phase, not BRCA mutation status. *Clin. Cancer Res.* **18**, 6199-6207.
- Goad, J., Ko, Y.-A., Kumar, M., Syed, S. M. and Tanwar, P. S. (2017). Differential Wnt signaling activity limits epithelial gland development to the anti-mesometrial side of the mouse uterus. *Dev. Biol.* **423**, 138-151.
- Harada, N., Tamai, Y., Ishikawa, T., Sauer, B., Takaku, K., Oshima, M. and Taketo, M. M. (1999). Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. *EMBO J.* **18**, 5931-5942.
- Huang, H.-S., Chu, S.-C., Hsu, C.-F., Chen, P.-C., Ding, D.-C., Chang, M.-Y. and Chu, T.-Y. (2015). Mutagenic, surviving and tumorigenic effects of follicular fluid in the context of p53 loss: initiation of fimbria carcinogenesis. *Carcinogenesis* **36**, 1419-1428.
- Huelsken, J., Vogel, R., Erdmann, B., Cotsarelis, G. and Birchmeier, W. (2001). beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. *Cell* **105**, 533-545.
- Kessler, M., Zielecki, J., Thieck, O., Mollenkopf, H.-J., Fotopoulou, C. and Meyer, T. F. (2012). Chlamydia trachomatis disturbs epithelial tissue homeostasis in fallopian tubes via paracrine Wnt signaling. *Am. J. Pathol.* **180**, 186-198.
- Kessler, M., Hoffmann, K., Brinkmann, V., Thieck, O., Jackisch, S., Toelle, B., Berger, H., Mollenkopf, H. J., Mangler, M., Sehoul, J. et al. (2015). The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. *Nat. Commun.* **6**, 8989.
- Kim, J., Coffey, D. M., Creighton, C. J., Yu, Z., Hawkins, S. M. and Matzuk, M. M. (2012). High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proc. Natl Acad. Sci. USA* **109**, 3921-3926.
- Kindelberger, D. W., Lee, Y., Miron, A., Hirsch, M. S., Feltmate, C., Medeiros, F., Callahan, M. J., Garner, E. O., Gordon, R. W., Birch, C. et al. (2007). Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am. J. Surg. Pathol.* **31**, 161-169.
- King, S. M., Hilliard, T. S., Wu, L. Y., Jaffe, R. C., Fazleabas, A. T. and Burdette, J. E. (2011). The impact of ovulation on fallopian tube epithelial cells: evaluating three hypotheses connecting ovulation and serous ovarian cancer. *Endocr. Relat. Cancer* **18**, 627-642.
- Kobayashi, A. and Behringer, R. R. (2003). Developmental genetics of the female reproductive tract in mammals. *Nat. Rev. Genet.* **4**, 969-980.
- Kodithuwakku, S. P., Pang, R. T., Ng, E. H., Cheung, A. N., Horne, A. W., Ho, P. C., Yeung, W. S. and Lee, K. F. (2012). Wnt activation downregulates olfactomedin-1 in Fallopian tubal epithelial cells: a microenvironment predisposed to tubal ectopic pregnancy. *Lab. Invest.* **92**, 256-264.
- Komatsu, M. and Fujita, H. (1978). Electron-microscopic studies on the development and aging of the oviduct epithelium of mice. *Anat. Embryol. (Berl)* **152**, 243-259.
- Kretzschmar, K. and Clevers, H. (2017). Wnt/beta-catenin signaling in adult mammalian epithelial stem cells. *Dev. Biol.* **428**, 273-282.
- Kuhn, E., Kurman, R. J., Vang, R., Sehdev, A. S., Han, G., Soslow, R., Wang, T.-L. and Shih, M. (2012). TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma—evidence supporting the clonal relationship of the two lesions. *J. Pathol.* **226**, 421-426.
- Lee, Y., Miron, A., Drapkin, R., Nucci, M. R., Medeiros, F., Saleemuddin, A., Garber, J., Birch, C., Mou, H., Gordon, R. W. et al. (2007). A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J. Pathol.* **211**, 26-35.
- Li, P., Zhu, W.-J., Ma, Z.-L., Wang, G., Peng, H., Chen, Y., Lee, K. K. and Yang, X. (2013). Enhanced beta-catenin expression and inflammation are associated with human ectopic tubal pregnancy. *Hum. Reprod.* **28**, 2363-2371.
- Li, S., O'Neill, S. R., Zhang, Y., Holtzman, M. J., Takemaru, K. I., Korach, K. S. and Winuthayanon, W. (2017). Estrogen receptor alpha is required for oviductal transport of embryos. *FASEB J.* **31**, 1595-1607.
- Matsuzaki, S. and Darcha, C. (2013). In vitro effects of a small-molecule antagonist of the Tcf/ss-catenin complex on endometrial and endometriotic cells of patients with endometriosis. *PLoS ONE* **8**, e61690.
- Medeiros, F., Muto, M. G., Lee, Y., Elvin, J. A., Callahan, M. J., Feltmate, C., Garber, J. E., Cramer, D. W. and Crum, C. P. (2006). The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am. J. Surg. Pathol.* **30**, 230-236.
- Mehra, K., Mehrad, N., Ning, G., Drapkin, R., McKeon, F. D., Xian, W. and Crum, C. P. (2011). STICS, SCOUTs and p53 signatures; a new language for pelvic serous carcinogenesis. *Front. Biosci.* **3**, 625-634.
- Nagendra, P. B., Goad, J., Nielsen, S., Rassam, L., Lombard, J. M., Nahar, P. and Tanwar, P. S. (2016). Ovarian hormones through Wnt signalling regulate the growth of human and mouse ovarian cancer initiating lesions. *Oncotarget* **7**, 64836-64853.
- Novak, E. and Everett, H. S. (1928). Cyclical and other variations in the tubal epithelium. *Am. J. Obstet. Gynecol.* **16**, 499-530.
- Odor, D. L. (1974). The question of "basal" cells in oviductal and endocervical epithelium. *Fertil. Steril.* **25**, 1047-1062.
- Paik, D. Y., Janzen, D. M., Schafenacker, A. M., Velasco, V. S., Shung, M. S., Cheng, D., Huang, J., Witte, O. N. and Memarzadeh, S. (2012). Stem-like epithelial cells are concentrated in the distal end of the fallopian tube: a site for injury and serous cancer initiation. *Stem Cells* **30**, 2487-2497.
- Pardo-Saganta, A., Tata, P. R., Law, B. M., Saez, B., Chow, R. D.-W., Prabhu, M., Gridley, T. and Rajagopal, J. (2015). Parent stem cells can serve as niches for their daughter cells. *Nature* **523**, 597-601.
- Pauerstein, C. J. and Woodruff, J. D. (1967). The role of the "indifferent" cell of the tubal epithelium. *Am. J. Obstet. Gynecol.* **98**, 121-125.
- Perets, R. and Drapkin, R. (2016). It's totally tubular....riding the new wave of ovarian cancer research. *Cancer Res.* **76**, 10-17.
- Perets, R., Wyant, G. A., Muto, K. W., Bijron, J. G., Poole, B. B., Chin, K. T., Chen, J. Y., Ohman, A. W., Stepule, C. D., Kwak, S. et al. (2013). Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca1;Tp53;Pten models. *Cancer Cell* **24**, 751-765.
- Perl, A. K., Wert, S. E., Nagy, A., Lobe, C. G. and Whitsett, J. A. (2002). Early restriction of peripheral and proximal cell lineages during formation of the lung. *Proc. Natl. Acad. Sci. USA* **99**, 10482-10487.
- Peters, W. M. (1986). Nature of "basal" and "reserve" cells in oviductal and cervical epithelium in man. *J. Clin. Pathol.* **39**, 306-312.
- Sherman-Baust, C. A., Kuhn, E., Valle, B. L., Shih, M., Kurman, R. J., Wang, T. L., Amano, T., Ko, M. S., Miyoshi, I., Araki, Y. et al. (2014). A genetically engineered ovarian cancer mouse model based on fallopian tube transformation mimics human high-grade serous carcinoma development. *J. Pathol.* **233**, 228-237.
- Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* **21**, 70-71.
- Srinivas, S., Watanabe, T., Lin, C.-S., Williams, C. M., Tanabe, Y., Jessell, T. M. and Costantini, F. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev. Biol.* **1**, 4.
- Stewart, C. A. and Behringer, R. R. (2012). Mouse oviduct development. *Results Probl. Cell Differ.* **55**, 247-262.
- Tanwar, P. S., Kaneko-Tarui, T., Zhang, L., Tanaka, Y., Crum, C. P. and Teixeira, J. M. (2012). Stromal liver kinase B1 [STK11] signaling loss induces oviductal adenomas and endometrial cancer by activating mammalian Target of Rapamycin Complex 1. *PLoS Genet.* **8**, e1002906.
- Traykova-Brauch, M., Schönig, K., Greiner, O., Miloud, T., Jauch, A., Bode, M., Felsner, D. W., Glick, A. B., Kwiatkowski, D. J., Bujard, H. et al. (2008). An

efficient and versatile system for acute and chronic modulation of renal tubular function in transgenic mice. *Nat. Med.* **14**, 979-984.

Visvader, J. E. and Stingl, J. (2014). Mammary stem cells and the differentiation hierarchy: current status and perspectives. *Genes Dev.* **28**, 1143-1158.

Woodruff, J. D. and Paurstein, C. J. (1969). *The Fallopian Tube: Structure, Function, Pathology, and Management*. Baltimore, MD: Williams and Wilkins.

Yamamoto, Y., Ning, G., Howitt, B. E., Mehra, K., Wu, L., Wang, X., Hong, Y., Kern, F., Wei, T. S., Zhang, T. et al. (2016). In vitro and in vivo correlates of physiological and neoplastic human Fallopian tube stem cells. *J. Pathol.* **238**, 519-530.

Yamanouchi, H., Umezu, T. and Tomooka, Y. (2010). Reconstruction of oviduct and demonstration of epithelial fate determination in mice. *Biol. Reprod.* **82**, 528-533.