Ovary and fimbrial stem cells: biology, niche and cancer origins

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Abstract | The mammalian ovary is covered by a single-layered epithelium that undergoes rupture and remodelling following each ovulation. Although resident stem cells are presumed to be crucial for this cyclic regeneration, their identity and mode of action have been elusive. Surrogate stemness assays and *in vivo* fate-mapping studies using recently discovered stem cell markers have identified stem cell pools in the ovary and fimbria that ensure epithelial homeostasis. Recent findings provide insights into intrinsic mechanisms and local extrinsic cues that govern the function of ovarian and fimbrial stem cells. These discoveries have advanced our understanding of stem cell biology in the ovary and fimbria, and lay the foundations for evaluating the contribution of resident stem cells to the initiation and progression of human epithelial ovarian cancer.

Fimbria
The most distal part of the fallopian tube (or oviduct in non-primates). The fimbria comprises finger-like fringes that collect the released oocyte from the surface of the ovary and transport it into the uterus for fertilization.

Ovary
A reproductive organ that is responsible for the production and cyclic release of oocytes. In mammals, the ovary is present as a pair of glands that are attached to the uterus by the ovary ligaments.

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The ovarian surface epithelium (OSE) participates in cyclic ovulatory rupture and repair throughout reproductive life[1] (BOX 1). During each ovulation, the OSE at the apex of the ovulatory follicle ruptures to form a stigma wound that facilitates oocyte release and is then rapidly repaired. Ovulatory stigmas reach diameters of 170–180 μm in mice[2] and up to several millimetres in non-human primates[3]. The repair mechanism of the OSE is highly robust and efficient, with complete closure of the stigma wound achieved within 12 hours to 3 days following rupture in mice[2,4]. This remarkable cyclic regenerative capacity of the OSE suggests the existence of resident stem cells. During the ovulation process, the fimbria sweeps along the surface of the ovary to collect the released oocyte for fertilization[1]. Moreover, repeated exposure of the fimbria to the pro-inflammatory follicular fluid that is released during follicular rupture[5–7] (BOX 1) may impose an obligate requirement for resident stem cells in the underlying epithelium for cellular replenishment.

Somatic stem cells have been identified in many adult epithelial systems, including the gastrointestinal tract[8,9], the mammary gland[10,11], the prostate[12] and the oesophagus[13,14], as well as in skin and its appendages[15–20]. In these tissues, resident stem cells drive epithelial homeostasis and repair throughout life. To carry out this function, stem cells have the unique ability to indefinitely self-renew and to give rise to one or more specialized cells[21]. Such 'stemness' has long been considered to be a largely cell-intrinsic property. However, recent evidence of injury-driven stem cell plasticity in several organs has highlighted the instrumental role of the local niche in defining stem cell identity and function *in vivo*[22]. Indeed, local niche influences can rapidly convert committed progenitors or even fully differentiated epithelial cells into functional stem cells to facilitate tissue repair following injury[22–26].

Understanding the biology of stem cells of the OSE and of fimbrial epithelia has been hampered by the lack of molecular markers for prospective identification and characterization of stem cell populations. This has been compounded by the inherent difficulties of isolating sufficient epithelia for detailed analyses and by the lack of *ex vivo* methods for long-term culture of OSE and fimbrial epithelia (for recent reviews, see REFS 23,27,28). Surrogate stemness assays, including long-term DNA label retention and side-population enrichment, have been employed to identify candidate subsets of epithelial cells that display stem-like activities[29–33]. More recently, novel marker genes that were functionally validated by fate-mapping studies were used to document the existence of stem cells that establish the epithelial cell lineages of the ovary and fimbria during development[34], as well as contributing to lifelong homeostasis and post-ovulatory repair of the ovary[34,35].

An understanding of the normal biology of stem cells in the ovary and fimbria is crucial for deciphering the mechanisms underlying ovary dysfunction and tumorigenesis in humans. Long-lived stem cells are prime candidates for the gradual accumulation of the mutation cohort that is required to drive initiation of cancer in many adult epithelia[24], and the same may be true for epithelial ovarian cancer (EOC). The cellular origins

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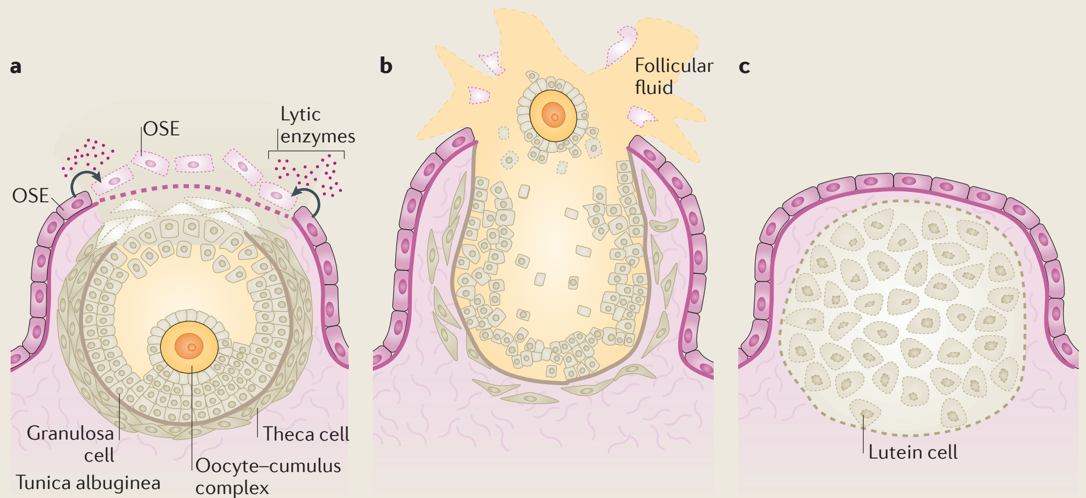
Box 1 | **Cyclic epithelial remodelling in the ovary and fibria**



Cyclic ovarian surface epithelium (OSE) rupture and repair can be broadly divided into three phases: an ovulatory phase; a rupture phase; and a repair phase. In the ovulatory phase (see the figure, part **a**), with impending ovulation, the ovulatory follicle becomes hyperaemic as it protrudes above the ovary surface[3,34]. Sequential actions of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) trigger the onset of ovulation. The OSE cells and the underlying basal lamina (pink dashed line), together with the tunica albuginea and cell layers of the ovulatory follicle (which include theca cells, granulosa cells and their basement membranes) undergo proteolysis and degradation[156]. Tissue degradation is spatially restricted at the follicular apex. Cultured OSE cells have the autonomous ability to release matrix-digesting proteases, lysosomal enzymes and plasminogen activators and may contribute to follicular disintegration *in vivo*[156–159]. In the rupture phase (see the figure, part **b**), follicular wall disintegration and desquamation of OSE cells at the follicular apex create a wound stigma in the epithelial barrier, through which the cumulus–oocyte complex is released into the periovarian space[160]. In the repair phase (see the figure, part **c**), wound stigma closure post-ovulation is achieved by cellular proliferation and migration. Increased OSE proliferation has been observed tandem to large follicles[4,161], the stigma wound periphery[34,161] and post-ovulatory corpora lutea[4], and at the hilum[35]. Intriguingly, proliferative repair in response to ovulation has not been observed in the primate OSE[3,162,163]. This has led to the notion that the primate OSE is dispensable for post-ovulatory repair[162]. Nevertheless, mechanical OSE ablation studies have shown that the primate OSE is capable of regenerative proliferation[163]. OSE cells may also contribute to the deposition of a new basal lamina and stromal matrix post-ovulation, as exemplified by their intrinsic capacity for *de novo* synthesis of laminin, collagens (types I, III and IV) and extracellular matrix components in culture[159,164].

The fimbria also undergoes cyclic remodelling. In humans, there is evidence to suggest that fimbrial epithelium proliferates[165] and becomes pseudo-stratified at the follicular phase (pre-ovulation) before reverting to a single layer at the luteal phase (post-ovulation)[166].

of EOC are controversial[36–41]. Traditionally, EOC was believed to arise from the ovary itself[36,42]. Accumulating recent evidence, however, has led to a fundamental paradigm shift, according to which EOC may be derived from extra-ovarian sites, notably the fimbria[38,43,44]. These new insights are altering our views of the aetiology of EOC and have important implications for future research and preventive therapeutic approaches.

In this Review, we summarize the current knowledge on epithelial stem cell biology in the ovary and fimbria, and the nature of the local stem cell niche components *in vivo*, and speculate on the cell(s) of origin of EOC. Finally, we discuss current challenges and research directions that may accelerate progress in this relatively immature field.

Structure of the OSE and the fimbrial epithelium

The adult mammalian ovary and fallopian tube (including the fimbria) are lined by a single-layered epithelium that is derived from a common embryonic origin in the pluripotent coelomic epithelium[45] (FIG. 1). Divergent differentiation during development results in the ovaries and fallopian tubes having distinct histology and phenotypes. In adulthood, the cycling OSE is a simple epithelium that remains incompletely committed and transitions between a squamous and a cuboidal cell shape, depending on positional and oestrus cues (FIG. 1a). By contrast, adult fimbrial epithelia are highly differentiated, assuming specialized columnar shapes comprising ciliated and secretory cell types that are intercalated with rare, basally located cells[31] (FIG. 1b). OSE and fimbrial epithelia express common epithelial (for example, keratins) and mesenchymal (for example, vimentin) markers[1]; however, their divergence is associated with the selective expression of several epithelial differentiation-specific proteins[31,37,46–49] (FIG. 1).

Despite being generally considered to be anatomically separate, the ovary and fimbria are in fact contiguous at a narrow isthmus. This ovary–fimbria connection is evident in humans (in which it is termed the ovarian fimbriae[37,40]), non-human primates[46] and mice[34,35] (FIG. 1). In mice, the ovary–fimbria connection is located at the

Follicular fluid
A growth factor-enriched liquid that fills the ovarian follicle. The composition of the follicular fluid dramatically changes during folliculogenesis, as well as during the various stages of the oestrus cycle.

Niche
The supportive local environment in which stem cells reside. The stem cell niche supplies all the extrinsic signalling cues that dictate stem cell maintenance and proliferative activity.

DNA label retention
Introduction of a nucleotide analogue (for example, 5-bromo-2′-deoxyuridine (BrdU) or 5-iodo-2′-deoxyuridine (IdU)) or labelling by genetic means (for example, using histone 2B–GFP) for a short period, followed by a prolonged period in the absence of the labelling reagent. After several rounds of cell division, fast-cycling cells rapidly dilute the label, whereas quiescent cells retain most of the original label. These cells are termed label-retaining cells.

Side-population enrichment
A flow-cytometry assay that discriminates for and enriches cell populations that efflux fluorescent dyes (for example, Hoechst 33342) at a higher pace, owing to the expression of ATP-binding cassette transporter proteins within the cell membrane.

Fate-mapping
Permanent labelling of a cell type with a reporter marker (LacZ or a fluorescent protein) that is inherited by its progeny upon cellular division, thus facilitating analyses of its cell-fate decisions and behaviour. Also known as lineage tracing.

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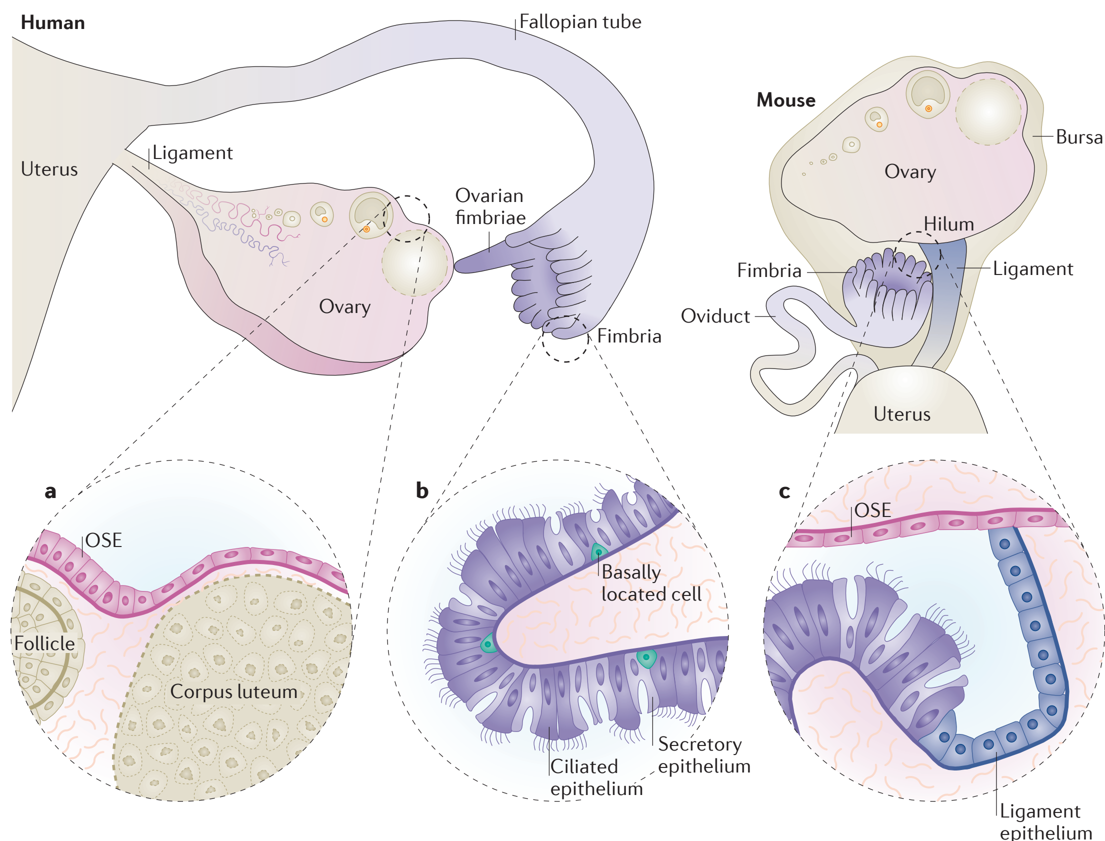

Marker	OSE	Fimbrial epithelium	Refs
Keratins (K7, K8, K18 and K19)	+	+	37
Vimentin	+	+	37
Calretinin	+	–	37,46
Cancer antigen 125 (CA125)	–	+	47
Epithelial cell adhesion molecule (EPCAM)	–	+	31,37
Epithelial membrane antigen (EMA)	–	+	37
Oviduct-specific glycoprotein (OVGP1)	–	+	48
Paired box 8 (PAX8)	–	+ (secretory type only)	31,49
Tubulin β4 (TUBB4)	–	+ (ciliated type only)	31,49
CD44	+	+ (basally located cells only)	31

Figure 1 | **The adult human and mouse ovary and associated tissues.** The diagrams show the organization and anatomy of the adult human and mouse ovary; the fallopian tube (also known as the oviduct), including finger-like fimbria; the ovary ligament; and the uterus. A thin bursa membrane encapsulates the mouse ovary but is absent in humans. The placement of the ovary ligament differs between humans and mice. In humans, the ovary and the fimbria are anatomically contiguous at the ovarian fimbriae, whereas the ovary ligament abridges the ovary–fimbria connection in mice. A single-layered epithelium lines the ovary, fimbria and ovary ligament. **a** | The ovary is entirely encapsulated by the ovarian surface epithelium (OSE), which is a single-layered simple epithelium. Compared with other types of epithelial cell, the cells of the OSE are uncommitted and express both epithelial and mesenchymal markers. Cells of the OSE adopt cuboidal or squamous cell shapes, depending on oestrus cues and on their proximity to a follicle or to a corpus luteum, respectively. **b** | The adult fimbrial epithelium comprises highly differentiated columnar ciliated and secretory epithelial cells, and basally located cells that contact the basement membrane. **c** | In mice, an OSE–ligament–fimbria epithelial continuum exists at the ovary hilum; whether this continuum is also present in other mammalian species is unknown. The table lists epithelial differentiation-specific markers that are expressed in the OSE[37,46], in fimbria[31,37,46–49] or in both[31,37].

hilum region and is abridged by the ovary ligament, which connects the ovary to the uterus and contains blood vessels and nerves entering the ovary[34]. This ovary ligament is also lined by a single-layered epithelium, which establishes an OSE–ligament–fimbria epithelial continuum (FIG. 1c). Whether a ligament continuum also exists in other species remains unclear.

Identity of stem cells in the ovary and fimbria

The adult ovary is composed of germ cells (oocytes or oogonia) and somatic cells (OSE, granulosa and theca cells) that are supported on a stromal mesh (tunica albuginea and interstitial cells). Although the existence of stem cell pools that establish and maintain germline and ovarian somatic cell lineages has been described,

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their identity and precise locations are still the subject of much debate.

Germline stem cells. A long-standing dogma posits that female mammals are born with a fixed ovarian follicle reserve. Several studies[50–55] have challenged this dogma by finding that the OSE monolayer in adult mice and humans contains putative germline stem cells. These cells expressed the oocyte-specific mouse VASA homologue MVH (also known as DDX4), could self-propagate *in vitro* and generated new primordial follicles following their xenograft onto donor ovarian tissue *in vivo*. Independent studies have failed to reproduce these seminal findings[56–59], and the concept of postnatal oogenesis has yet to gain universal acceptance. However, the study of potentially persisting oogenesis in adult female mammals is an exciting area of reproductive research that holds tremendous promise for the treatment of infertility pathologies.

Granulosa and theca stem cells. No stem cell activity has been detected within adult granulosa or theca cell lineages. However, early work has described a small proportion of adult granulosa cells that display stem-like characteristics in culture, including the ability to divide and form colonies without substrate anchorage[60]. More recently, *in vivo* fate-mapping studies have revealed two distinct somatic subpopulations within embryonic mouse ovaries that establish the granulosa lineages of the adult medullary and cortical follicles[61,62]. Putative theca precursors have also been identified in the ovaries of newborn mice; these cells differentiated into mature steroidogenic cells *in vitro* and rapidly invaded the theca layers of follicles within the host ovary post-transplantation[63].

OSE stem cells. Microarray profiling and immunohistochemical studies have shown that human OSE expresses several classical stem cell markers, including NANOG, CD44, aldehyde dehydrogenase 1 family member A1 (ALDH1A1; also known as retinal dehydrogenase 1), ALDH1A2, secreted Frizzled-related protein 1 (SFRP1), LIM homeobox protein LHX2 and LHX9 (REFS 64,65).

The first evidence of putative stem cells on the ovary surface came in 2008, when 5-bromo-2′-deoxyuridine (BrdU) or 5-iodo-2′-deoxyuridine (IdU) and doxycycline-inducible histone 2B–GFP pulse-chase surrogate stemness assays were used to identify a subset of stem-like cells in the adult mouse OSE[29] (FIG. 2). These OSE cells displayed cellular quiescence, DNA label retention and enhanced *in vitro* colony formation[29]. The label-retaining cells also displayed Hoechst 33342 dye-effluxing cytoprotective capacity, a trait that is believed to be specific to stem cells, owing to their intrinsic ability to have efflux pumps on their membrane. The location of these label-retaining OSE cells, juxtaposed at ovulatory follicles, suggests that they may participate in post-ovulatory wound closure. Subsequently, a subset of verapamil-sensitive OSE cells expressing lymphocyte antigen 6A2–6E1 (LY6A; also known as SCA1), which is a common haematopoietic stem cell marker, with Hoechst-effluxing capability was identified in adult mouse ovaries[32] (FIG. 2). In culture, *Ly6a*⁺ cells had enhanced sphere-forming abilities and were responsive to transforming growth factor-β1 (TGFβ1) and leukaemia-inhibitory factor (LIF), two growth factors that are abundant in follicular fluid[32]. A role for these label-retaining and *Ly6a*⁺ OSE cells in epithelial regeneration *in vivo*, however, has not been formally established.

More recently, two studies[34,35] used *in vivo* fate-mapping methodologies to provide direct evidence for the existence and locations of self-renewing epithelial stem cells in the adult mouse ovary. Fluorescence-activated cell sorting was used to isolate a subpopulation of OSE cells located at the ovary hilum that displayed high ALDH activity[35] (FIG. 2). ALDH activity, which confers drug resistance and cell protection, has been widely used to identify potential stem cells in various tissues[66]. In culture, *Aldh1*⁺ hilum OSE cells generated large epithelial spheres at higher frequency and exhibited enhanced colony formation compared to their *Aldh1*⁻ counterparts. Expression analyses showed that *Aldh1*⁺ cells expressed Leu-rich repeat-containing G protein-coupled receptor 5 (LGR5), a seven-transmembrane receptor that is a facultative component of the WNT receptor complex and is expressed by stem cells in various epithelial tissues[8,9,16,67–69]. Lineage tracing with an *Lgr5–Cre*-knock-in mouse model expressing enhanced GFP (*Lgr5–egfp–ires–CreER^{T2}*) led to the conclusion that hilum cells expressing ALDH1 and LGR5 are the major OSE stem cell reservoir that supplies epithelial cells to the entire ovary surface *in vivo* (BOX 2).

Subsequent experiments using the same *Lgr5*–Cre-knock-in allele[34] challenged the existence of an exclusive *Aldh1*⁺*Lgr5*⁺ stem cell compartment in the ovary hilum. By combining *Lgr5* reporter gene profiling and endogenous single-molecule mRNA fluorescence *in situ* hybridization (FISH) expression analyses, these studies reported multiple clusters of *Lgr5*⁺ cells located at interfollicular cleft regions throughout the ovary surface, in addition to the population at the hilum (FIG. 2). Long-term lineage marking analyses indicated that both hilum and extrahilum *Lgr5*⁺ cell populations were capable of generating phenotypically distinct epithelial lineages over the 16-month reproductive lifetime of the mouse, thus formally establishing the entire *Lgr5*⁺ cell pool as OSE-resident stem cells (BOX 2).

Whether the ovary epithelium contains additional stem cell subsets with distinct lineage features remains an open question. Definitive answers to such questions would require the discovery of specific genes that mark putative stem cell pools for isolation and downstream characterization, using clonal marking techniques and/or *in vivo* conditional gene ablation strategies to establish stem cell identity.

Interestingly, human OSE cells express LGR5 (REF. 34), suggesting that putative *LGR5*⁺ stem cells are present in the ovaries of other mammalian species. Future experimentation relies on a validated antibody against human LGR5 that can be used to purify and characterize *LGR5*⁺ stem cell activity using functional assays, such as *in vivo* regeneration following transplantation and *in vitro* sphere-forming assays.

Cellular quiescence
A reversible, non-dividing cell state. Some stem cells are quiescent under steady state, a property that is believed to sustain lifelong tissue maintenance and preservation of the stem cell compartment.

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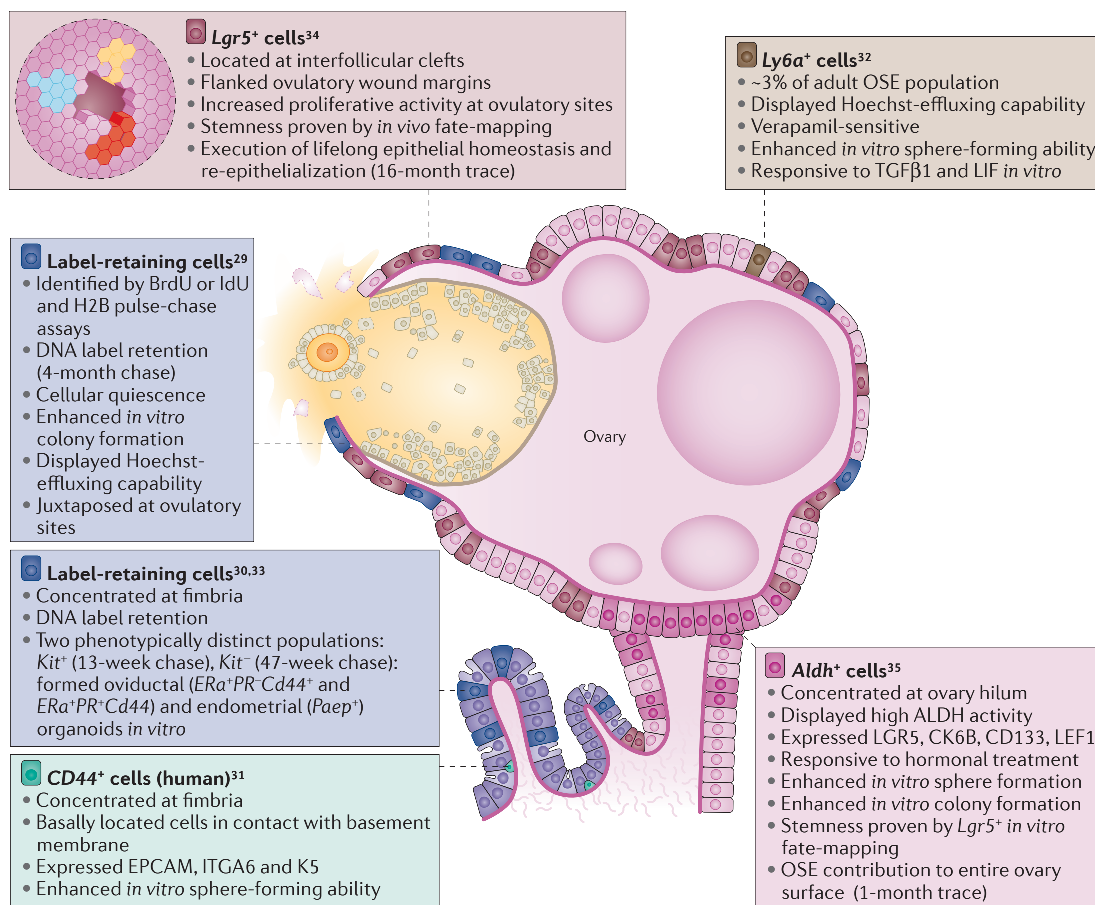

Figure 2 | **Locations and characteristics of epithelial stem and stem-like cells in the ovarian surface epithelium (OSE) and the fimbria.** Surrogate stemness assays and *in vivo* fate-mapping strategies were used to identify populations of epithelial stem and stem-like cells on the ovary surface and fimbrial lining. All putative epithelial stem cells were identified in the mouse unless indicated otherwise. The figure lists the characteristics of stem and stem-like cells identified by either label retention or expression of a specific marker (Leu-rich repeat-containing G protein-coupled receptor 5 (LGR5), lymphocyte antigen 6A2–6E1 (LY6A), CD44 and aldehyde dehydrogenase (ALDH) family members). Only the *Lgr5*+ populations located at the hilum and extrahilum regions have been formally established to contribute to *in vivo* OSE homeostasis and post-ovulatory regeneration[34]. The inset in the *Lgr5*+ box is a schematic representation of clonal fate-mapping using the ROSA4 colour lineage reporter[34], which demonstrated the involvement of several *Lgr5*+ stem cells in post-ovulatory wound repair. Each coloured (red, RFP; yellow, YFP; cyan, CFP, membranous) clone represents the progeny of a single *Lgr5*+ OSE stem cell. BrdU, 5-bromo-2′-deoxyuridine; CK6B, cytokeratin 6B; EPCAM, epithelial cell adhesion molecule; *ERa*, oestrogen receptor α; H2B, histone 2B; IdU, 5-iodo-2′-deoxyuridine; ITGA6, integrin α6; K5, keratin 5; LEF1, lymphoid enhancer-binding factor 1; LIF, leukaemia inhibitory factor; *Paep*, progestogen-associated endometrial protein; *PR*, progesterone receptor; TGFβ1, transforming growth factor-β1.

Fimbrial stem cells. Fate-mapping studies that formally document somatic stem cells in the adult mouse fimbria are currently lacking. LGR5 is not expressed in the adult mouse fimbria (although human fimbria highly expresses LGR5)[34]. Furthermore, fate-mapping of *Lgr5*+ epithelial cells at the ovary–ligament–fimbria junction showed that these cells did not contribute to *in vivo* homeostasis of the fimbria[34], suggesting the existence of as-yet undiscovered stem cell populations in this tissue.

Several studies have described stem-like epithelial cells in the fallopian tube in humans[31] and in the oviduct in mice[30,33], which were concentrated at the fimbriated end (FIG. 2). In mice, two phenotypically distinct populations of stem-like fimbrial epithelial cells, which were distinguished by positive[33] and negative[30] expression of mast–stem cell growth factor receptor KIT, were identified on the basis of long-term label-retention assays. In culture, *Kit*− label-retaining cells, which did not express oestrogen or progesterone receptors (*ERa*−*PR*−) formed self-renewing organoids that contained mature epithelia that are normally present in the native distal (*ERa*+*PR*−) and proximal (*ERa*+*PR*+) oviduct[30]. Remarkably, the organoids also expressed progestogen-associated endometrial protein (PAEP; also known as glycodelin), which is a marker of mature endometrium[30]. This suggests that the fimbrial label-retaining cells had considerable plasticity *ex vivo*. In humans, fimbrial stem-like cells were identified as tubulin β4 (*TUBB4*)−, paired box 8 (*PAX8*)−, *CD44*+ basally-located cells[31] that were capable of generating *in vitro* spheres that contained differentiated

Organoids
Three-dimensional cell culture structures that recapitulate the multipotent cellular differentiation and functional complexity of the native tissue of origin.

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Box 2 | **Ovulatory re-epithelialization by *Lgr5*$^{+}$ ovarian surface epithelium (OSE) stem cells in mice**

In mice, follicular rupture can occur anywhere on the ovary surface (whether this is also true in mono-ovulating species such as humans or primates remains unexplored). An aldehyde dehydrogenase 1 (*Aldh1*)$^{+}$ Leu-rich repeat-containing G protein-coupled receptor 5 (*Lgr5*)$^{+}$ stem cell pool restricted to the hilum would require massive cell proliferation and extensive migration to repair distant ovulatory stigma wounds. This would be no easy feat, given that surface barrier continuity is typically restored within days following ovulation in the mouse[2,4]. Increased proliferation in OSE cells around the ovary hilum, which is suggestive of participation in post-ovulatory wound repair, has been reported[2,167], although this was not confirmed by other studies[168,169]. There was also no significant difference in the proliferative activity of *Lgr5*$^{+}$ stem cells at the hilum compared to those at other regions of the ovary[34].

Epithelial restoration occurring in a fast and spatially localized manner intuitively seems to favour a model of post-ovulatory repair by local, wound-adjacent stem cells. The widespread distribution of *Lgr5*$^{+}$ stem cells throughout the mouse ovary surface is consistent with a model of rapid and effective re-epithelialization by these cells at the wound perimeter. Supporting this notion is the observation that *Lgr5*$^{+}$ OSE stem cells at wound stigmas were highly proliferative[34]. During regular tissue homeostasis (that is, in the absence of ovulation), *Lgr5*$^{+}$ stem cells were relatively quiescent and had a slow cellular turnover. This is consistent with previous reports of negligible levels of proliferation within non-ovulating regions in various species[2,3,159,163,168]. In this context, widespread proliferation of the OSE does not seem to be required during homeostatic expansion of the ovary surface associated with folliculogenesis. In response to local ovulatory damage, however, *Lgr5*$^{+}$ stem cells at OSE sites flanking ovulatory follicles were activated to contribute progeny to repair the subsequent stigma wound[34]. Clonal fate-mapping using the ROSA4 colour lineage reporter has documented the recruitment of multiple *Lgr5*$^{+}$ OSE stem cells during the re-epithelialization process (FIG. 2). Comprehensive clonal lineage-tracing studies carried out in conjunction with real-time cell tracking should help to establish the population kinetics and migration patterns of *Lgr5*$^{+}$ stem cells onto the rupture wound during homeostatic repair.

ciliated (*TUBB4*$^{+}$), secretory (*PAX8*$^{+}$) and basally located (*CD44*$^{+}$) epithelia[31].

Early* Lgr5^{+} *stem cells in somatic lineage specification. Expression of LGR5 was first detected in surface and subsurface somatic cells in the embryonic day E12.5–E13.5 ovary anlagen, coincident with female sex determination[34,61]. *In vivo* lineage tracing during this embryonic period showed that early *Lgr5*$^{+}$ ovarian cells possessed lineage-differentiation capabilities, contributing to both the future OSE[34] and granulosa cell lineages[61]. Granulosa contribution by early *Lgr5*$^{+}$ cells was restricted to the pre-granulosa lineage of adult cortical follicles, which is responsible for folliculogenesis throughout life[61]. A distinct forkhead box L2 (*Foxl2*)$^{+}$ *Lgr5*$^{-}$ pool established the adult medullary follicles[62]. In the developing Müllerian duct, expression of LGR5 was restricted to the anterior-most region (that is, the primordia of the oviduct, including the fimbria). These early *Lgr5*$^{+}$ cells contributed to the epithelia of the adult oviduct, including the fimbria, as well as the ovary–ligament–fimbria junction at the ovary hilum[34]. Intriguingly, examination of *Lgr5*-knockout female gonads has shown that OSE and granulosa cell lineage differentiation proceeds normally in the absence of LGR5 (REF. 61). Instead, *Lgr5*-mutant ovaries had variable degrees of defects in the differentiation of germ cells, which do not normally express LGR5 (REF. 61). It is plausible that the closely related LGR4, which is also expressed in ovarian somatic cells[61,70], may provide functional redundancy with LGR5 during ovary organogenesis.

OSE and fimbrial stem cell regulation

The behaviour of epithelial stem cells is governed by a combination of intrinsic programmes and extrinsic stimuli derived from the local niche. Perturbation of these regulatory cues is instrumental in driving formation of epithelial cancer in many tissues, including the intestine[8], stomach[9] and skin[71].

Currently, little is known about the extrinsic regulation of stem cell fate (for *Lgr5*$^{+}$ cells and other potentially undiscovered cells) in the ovary and fimbria. However, a large body of work has characterized *Lgr5*$^{+}$ stem cell-driven epithelial systems in other tissues. Insights gained from these studies may facilitate the identification of stem cell niche components and regulatory signals in the ovary and fimbria and uncover similarities between different epithelia.

The WNT-enriched* Lgr5^{+} *stem cell transcriptome. Comparative microarray analyses have shown that *Lgr5*$^{+}$ and *Lgr5*$^{-}$ cells have distinct transcriptional profiles[34]. Consistent with the role of WNT signalling in the maintenance and renewal of adult epithelial stem cells[20,68,72–74], *Lgr5*$^{+}$ OSE stem cells are enriched in expression of several WNT signalling components, including the WNT ligand WNT4, as well as WNT target genes axis inhibition 2 (*Axin2*) and the gene encoding tumour necrosis factor receptor family member Troy (*Tnfrsf19*)[34] (FIG. 3a). Activation of WNT signalling relies on the binding of secreted WNT ligands to their Frizzled receptors and to low-density lipoprotein receptor-related protein 5 (LRP5)–LRP6 co-receptor complexes on the cell surface. This leads to stabilization and entry of β-catenin into the nucleus to induce transcription of target genes through interactions with the T-cell factor (TCF) and lymphoid enhancer factor (LEF) transcription factors. Thus, the ability to produce their own WNT4 ligands indicates that *Lgr5*$^{+}$ OSE stem cells have an intrinsic ability to regulate their own fate through an autocrine WNT signalling loop, as has been recently demonstrated for the interfollicular epidermis[20].

A finely balanced WNT signal strength is crucial to achieving optimal stem cell function. For instance, overactive WNT signalling in mice leads to adenoma and tumour formation in the gastrointestinal tract and

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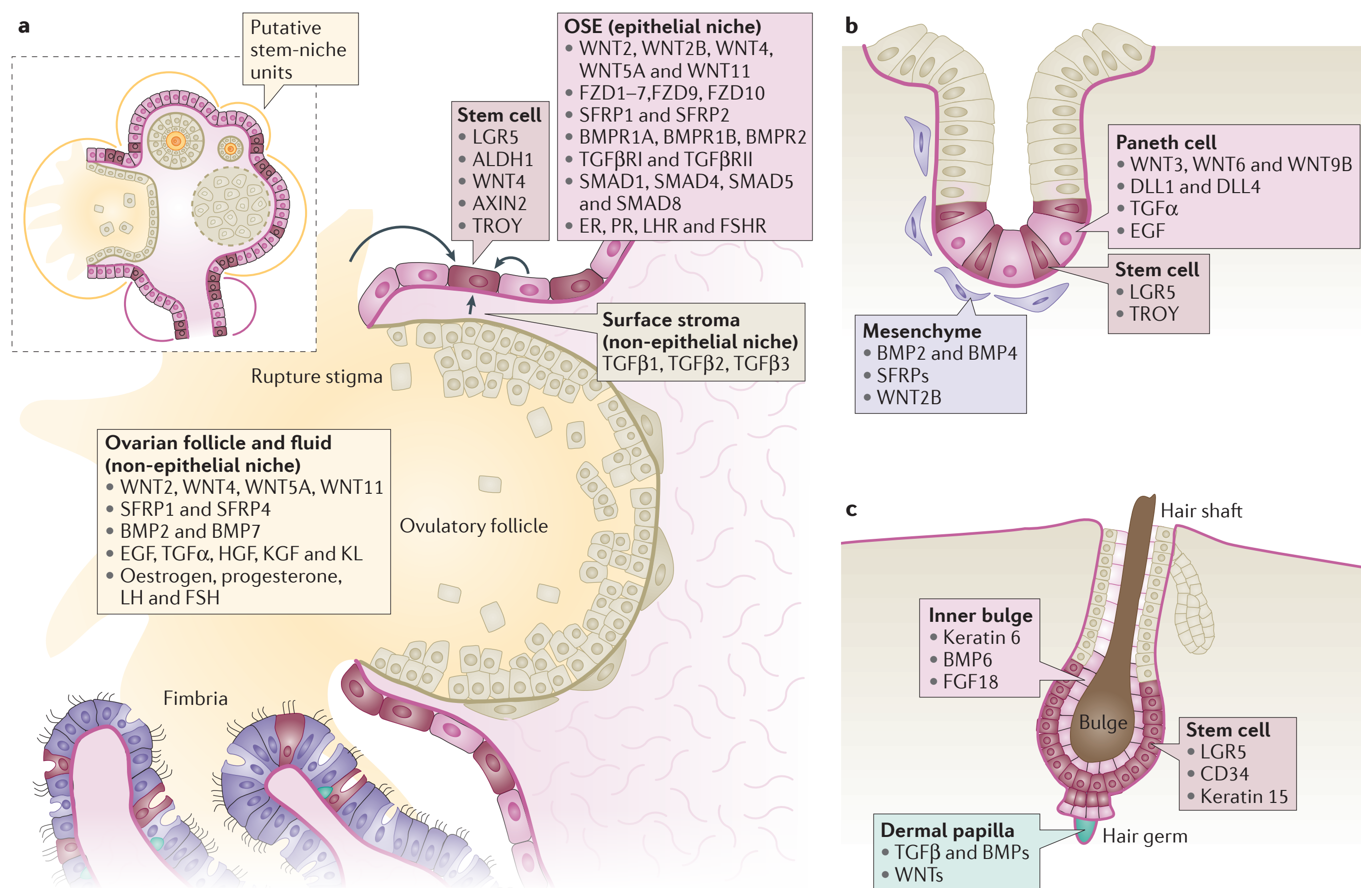

Figure 3 | **Putative ovarian surface epithelium (OSE) and fimbrial niche locations and signals.** **a** | A schematic of the putative OSE and fimbrial stem cell niche is shown. OSE and fimbrial stem cells (dark pink) are in close contact with their mature epithelial progeny (pink and purple) and with the underlying follicle. Expulsion of follicular fluid during ovulation provides direct contact between follicular milieu and stem cells in the vicinity of the wound. Candidate regulatory niche signals emitted from the mature epithelial progeny (epithelial niche) are depicted, as well as the ovulatory follicle with its follicular fluid, and surface stroma (non-epithelial niches). In the inset, presumptive stem-niche units comprising OSE cell clusters and the underlying follicular structure (follicle or corpus luteum) on the ovary surface are indicated by orange arcs; potential stem cell niches at the ovary hilum are indicated by magenta arcs. **b** | The small intestinal stem cell niche is defined by geometrical arrangement of stem cells (dark pink) and their differentiated Paneth cell progeny surrounded by mesenchyme at the base of the crypts. Paneth cells and mesenchyme function as intestinal stem cell niches. **c** | The hair follicle stem cell niche is composed of stem cells (dark pink) residing at the outer bulge and hair germ, supported by niche signal-producing secondary hair germ, dermal papilla and stem cell progeny in the inner bulge. ALDH1, aldehyde dehydrogenase 1; AXIN2, axis inhibition 2; BMP, bone morphogenetic protein; DLL, delta-like protein; EGF, epidermal growth factor; ER, oestrogen receptor; FGF, fibroblast growth factor; FSH, follicle-stimulating hormone; FZD, Frizzled; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; KL, KIT ligand; LGR, Leu-rich repeat-containing G protein-coupled receptor; LH, luteinizing hormone; PR, progesterone receptor; SFRP, secreted Frizzled-related protein; TGFβ, transforming growth factor-β.

the stomach[8,9], and *de novo* hair follicle morphogenesis and formation of hair follicle tumours[71], whereas inhibition of WNT causes depletion of intestinal stem cell and crypt compartments[74]. Although it remains unclear how internal WNT signals within *Lgr5*⁺ OSE stem cells are regulated, *Lgr5* (which is a WNT target gene) is likely to bind to its secreted R-spondin ligands (RSPO1 to RSPO4) to augment the OSE WNT signals that are initiated by WNT ligands[75]. At the same time, Troy, which is a negative regulator of WNT signalling within *Lgr5*⁺ stem cells in the intestine and the gastric corpus[76,77], might fine-tune the signal strength of WNT and RSPO signalling to restrict the OSE stem cell zone.

Robust WNT–RSPO signalling has been shown to be important during embryonic development of the ovary anlagen. Females lacking *Wnt4* or *Rspo1* were born with oocyte-depleted masculinized ovaries, indicative of an aberrant male phenotypic pathway during ovarian somatic cell differentiation[78–80]. The physiological role of Troy could not be ascertained from loss-of-function studies, as mice deficient for Troy were viable and fertile, displaying no abnormalities in their ovaries[81,82].

Whether a similar WNT-intrinsic programme regulates adult fimbrial stem cell function remains largely unexplored. Active canonical WNT signalling has been reported in the adult mouse oviduct[83].

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Moreover, human pathological studies have suggested a correlation between aberrant WNT signalling activation and altered fallopian tubal homeostasis[84]. Together, these findings support a role for WNT in fimbrial stem cell specification. As in the ovary, WNT–RSPO signalling promotes embryonic Müllerian duct development. Mouse *Wnt4*-mutants lacked a Müllerian duct at birth, in addition to having ovary defects[78], whereas inactivation of *Wnt9b*[85] or *Wnt7a*[86] led to absence of the duct or partial elongation of the Müllerian duct.

Putative niche components. Niche signals can be supplied by epithelial (that is, the differentiated stem cell progeny) and/or non-epithelial sources[87]. As niche signals typically function over short distances[88], resident stem cells are often located close to their niche cells for optimal niche-to-stem-cell signal transmission.

The base of the intestinal crypts of Lieberkühn, for instance, comprises discrete niche units containing multipotent *Lgr5*$^+$ stem cells that are intercalated with their antimicrobial peptide-secreting Paneth cell progeny and surrounded by mesenchyme (for a recent review, see REF. 24). Paneth cells and mesenchyme represent crucial intestinal niche sources, secreting essential stemness signals to balance stem cell maintenance and differentiation *ex vivo* and *in vivo*[75,89–93] (FIG. 3b). Similarly, each hair follicle represents a discrete stem cell niche entity. Multipotent *Cd34*$^+$*K15*$^+$*Lgr5*$^+$ stem cells[16,18,94] reside within the lower bulge and the hair germ and rely on a range of stem cell activation and quiescence signals from the inner bulge, hair germ and dermal papilla to achieve homeostatic hair follicle regeneration (for recent reviews, see REFS 95,96) (FIG. 3c).

Using close proximity as a criterion to define stem cell niches, candidate niche components of the ovary are the mature epithelial progeny of stem cells (epithelial niche), as well as the underlying follicular structure (the follicle or ovulatory by-product, known as the corpus luteum, and its follicular fluid) and the surface stroma at the OSE interface (non-epithelial niches) (FIG. 3a). The fimbria probably shares some of the OSE niche components, given its proximity to the ovary surface during ovulation. Although this is speculative, it is thought that the ovary surface may contain several interspersed niche units comprising clusters of OSE cells and their underlying follicular structures (FIG. 3a). Within each unit, OSE stem cells respond to the unique set of paracrine cues derived from their epithelial and non-epithelial niche components, to achieve the appropriate self-renewal and differentiation. A potential *Aldh1*$^+$*Lgr5*$^+$ stem cell niche at the mouse ovary hilum has also recently been identified[35] (FIG. 3a).

WNT niche signals. Secreted WNT molecules are putative paracrine OSE niche factors. In mice, follicular granulosa cells and theca cells, as well as lutein cells of corpora lutea, express several WNT ligands (WNT2, WNT4, WNT5A and WNT11) and WNT antagonists (secreted Frizzled-related protein 1 (SFRP1) and SFRP4)[97] (FIG. 3a). Of note, WNT2 (REFS 98,99) and WNT4 (REF. 98) were regulated according to the oestrus cycle and showed preferential expression in large follicles and corpora lutea, respectively, and high expression of SFRP4 occurred in large follicles and in corpora lutea[100]. Likewise, the adult human and mouse OSE express the entire repertoire of functional WNT signalling components, including ligands (WNT2, WNT2B, WNT4, WNT5A and WNT11), receptors (Frizzled 1 (FZD1), FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD9 and FZD10) and WNT antagonists (SFRP1 and SFRP2) to achieve robust OSE autocrine and paracrine WNT signalling[64,101] (FIG. 3a).

Exactly which WNT or WNTs are the endogenous WNT-modulating niche signals for OSE stem cell activity is currently unknown. Loss-of-function mouse models have failed to provide direct evidence of this: *Wnt2*-null mice have placental defects, and surviving females do not show an ovary defect[102], probably because of the compensatory function of other WNTs. Furthermore, conditional ablation of WNT4 in adult granulosa cells (using an *Amhr2–Cre* driver) efficiently blocked folliculogenesis, but a detailed analysis of the OSE was lacking[97].

TGFβ and bone morphogenetic protein (BMP) niche signals. Secreted TGFβ and BMP is another candidate paracrine niche signal. The biological effects of TGFβ and BMP signalling are mediated by ligand–receptor interactions at the cell surface, leading to nuclear translocation of SMAD transcription factors to regulate stemness-promoting genes. TGFβ signalling within the niche has been implicated in epithelial stem cell quiescence and differentiation, as well as induction of epithelial–mesenchymal transition (EMT)[103]. EMT has also been reported to increase expression of stemness markers in human mammary epithelial cells [104]. Interestingly, EMT involvement in post-ovulatory wound repair is supported by evidence that artificially generated scratch wounds were healed by migratory OSE cells in the presence of the EMT inducer pro-epidermal growth factor (EGF)[105].

BMP4 or TGFβ1 has been shown to induce EMT of OSE cells *in vitro*[106,107]. Adult OSE cells, including *Lgr5*$^+$ OSE stem cells, express all known TGFβ and BMP receptors (BMPR1A, BMPR1B, BMPR2, TGFβRI and TGFβRII) and downstream signalling effectors (SMAD1, SMAD5 and SMAD8, and the co-SMAD SMAD4), indicating that the ovary epithelium is a target of TGFβ and BMP signalling[34,108–110] (FIG. 3a). Furthermore, disruption of SMAD3 leads to OSE hyperproliferation[111]. Candidate paracrine BMP signals emitted from the follicles include BMP2 and BMP7, the expression of which in granulosa cells and theca cells, respectively, peaked at ovulation, before rapidly diminishing within lutein cells post-ovulation[112] (FIG. 3a). Also of potential importance is the expression of all three TGFβ isoforms (TGFβ1, TGFβ2 and TGFβ3) at the surface stroma at the OSE interface[108,109] (FIG. 3a). These secreted molecules might gain direct access to the adjacent OSE stem cells following breakdown of OSE basement membranes during ovulation.

Corpus luteum
The vascularized by-product of a recently ruptured follicle. Over time, the corpus luteum regresses into the centre of the ovary to become stromal or interstitial tissue.

Epithelial–mesenchymal transition
(EMT). A biological process by which epithelial cells dissolve their tight junctions with one another and convert into a free, migratory form.

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Follicular fluid milieu. The follicular fluid is a rich source of growth factors, steroid hormones and gonadotropins, which regulate growth and differentiation (FIG. 3a). Expulsion of follicular fluid at the stigma wound that is created during ovulation potentially brings the regulatory milieu within signal-receiving range of OSE stem cells at wound margins. A centripetal morphogenetic gradient of follicular fluid milieu arising from the wound epicentre probably establishes the boundaries to facilitate spatially targeted re-epithelialization at the follicular apex, whereas wound-distant regions remain unperturbed and maintain stem cell quiescence. Expulsed follicular fluid may also bathe the fimbrial fringes at the ovulatory rupture site (FIG. 3a).

During follicular maturation, the composition of the follicular fluid milieu, and hence the nature and strength of potential niche signals, dramatically changes. Among the growth factors that are present in high concentrations in ovulatory follicular fluid, EGF and TGFα[105,113–115], hepatocyte growth factor[116,117], keratinocyte growth factor[116,118] and KIT ligand[116] exerted the most potent mitogenic effects on OSE cells *in vitro*. Oestrogen, which is an OSE mitogen, also reaches peak levels in the follicular fluid of ovulatory follicles. A dramatic shift from oestrogen to progesterone production occurs post-ovulation in the corpus luteum[119]. As progesterone has been shown to exert a growth-inhibitory effect on cultured OSE cells[119], this steroid hormone may be an important endogenous niche signal to prevent OSE overproliferation. Large amounts of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are deposited into the ovulatory follicular fluid by the blood circulation; these long-range signals are known to stimulate OSE proliferation *in vitro* and *in vivo*[4,120,121]. Importantly, the cell-surface receptors for all of these putative growth-stimulating and growth-inhibitory regulatory factors and hormones are present in the OSE and in the fimbrial epithelium[3,117,122–125]. In sheep, expression of FSH and LH receptors was highest in OSE overlying large follicles[123], suggesting that gonadotropins probably exert the strongest growth-stimulating effects in receptor-enriched OSE cells on top of ovulatory follicles.

***Dissection of niche regulatory mechanisms* in vivo *and* in vitro.** Definitive proof of the existence of the OSE and fimbrial stem cell niche, and its characterization, awaits genetic studies to decipher the mechanisms that control the OSE and *Lgr5*+ stem cells. *In vivo* ablation strategies targeting the removal of candidate niche cells will also provide direct evidence of a role for the niche in maintaining *Lgr5*+ stem cell activities. However, the lack of Cre lines to precisely modulate these signalling pathways in the ovary remains a major technical barrier to such studies.

Recently, advances have been made in growing ovary fragments, whole ovaries or oviductal tissues with intact basal lamina and follicular structures in an alginate hydrogel matrix[126–128] or as multicellular spheroids[129,130]. Although the use of these models has facilitated analyses of the function of stem cells within their native microenvironments, the main limitation of current 3D culture systems is their inability to sustain long-term ovary growth and follicle maturation, which precludes the study of ovulatory re-epithelialization. An alternative approach is the development of near-physiological Matrigel-based 3D *ex vivo* culture systems that are capable of sustaining the long-term growth of functional OSE and fimbrial epithelia, for studying the regulation of stem cell behaviour in real time. Such culture systems have achieved considerable success for the intestine[75,89], the stomach[9], the colon[131,132] and the mammary gland[133]. 3D-based culture studies are particularly useful for real-time tracking of *Lgr5*–*egfp*+ OSE and fimbrial stem cells, combined with clonal fate-mapping strategies in a supportive *in vitro* environment. Aspects of *Lgr5*+ stem cell behaviour — mode and rate of cell division, self-renewal and migration properties — during regular tissue homeostasis versus in response to ovulatory wound repair could easily be studied, as could the effects of specific regulatory growth factors and hormonal influences on *Lgr5*+ stem cell characteristics and function. It is therefore of paramount importance to optimize culture conditions for the long-term growth of near-physiological OSE and fimbrial epithelium *in vitro*.

Stem cells as putative EOC cell(s) of origin

EOC comprises four phenotypically distinct subtypes, including serous, endometrioid, mucinous and clear cell carcinomas. Depending on their clinicopathology and molecular profile, these EOC subtypes can be classified as either type I or type II tumours. Slow-growing type I tumours, which encompass low-grade serous, low-grade endometrioid, clear cell and mucinous carcinomas[41], are often preceded by a well-recognized OSE precursor intermediary in benign or borderline tumours[42]. They are defined by several somatic mutations, including mutations in the genes encoding β-catenin, PTEN, PIK3CA, AT-rich interactive domain-containing protein 1A (ARID1A), GTPase KRAS, Ser/Thr protein kinase BRAF and Ser/Thr protein phosphatase PPP2R1A[41]. By contrast, type II tumours, comprising high-grade serous, high-grade endometrioid, undifferentiated and malignant-mixed carcinomas[41], are aggressive and are invariably detected at advanced stages when the disease has spread beyond the ovaries. High-grade serous carcinomas (HGSCs), which are the most prevalent type II tumours, have a unique genetic fingerprint involving mutations in the tumour suppressor gene *p53*, as well as alterations in components of the breast cancer type 1 susceptibility protein (BRCA1)–BRCA2, RB1, RAS–PI3K and NOTCH signalling pathways and the forkhead box protein M1 (FOXM1) transcription factor network[134].

The identity of the transformed EOC precursor(s) remains controversial. The central question is whether a single precursor cell generates the entire EOC spectrum, or whether each EOC subtype is derived from a distinct cell and tissue source. Although the long-held paradigm posits that EOC is the result of the transformation of an OSE cell, recent compelling evidence has suggested that a mutant fimbrial cell may be the cancer-initiating culprit for some EOCs, notably HGSCs. This OSE versus fimbrial cancer cell of origin controversy (BOX 3) has been extensively

Cancer cell of origin
The cell that sustains the first cancer-promoting mutation or mutations that initiate tumour development.

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Box 3 | **Ovary versus fimbria as epithelial ovarian cancer (EOC) source**

Traditionally, the ovarian surface epithelium (OSE), or its cortical inclusion cyst, is regarded as the sole EOC source (FIG. 4a). The incessant ovulation hypothesis posits that the cyclic rupture and repair trauma that is endured by OSE increases cell proliferation and consequently accumulation of deleterious somatic mutations[170]. Cortical inclusion cysts form in the superficial ovarian cortex as a result, and exposure of the entrapped cyst-lined OSE cells to the activated stromal milieu[42] and OSE-secreted milieu within the cystic lumen[171] causes their transformation (FIG. 4a). Unlike many epithelial cancers, in which carcinogenesis is accompanied by a block in differentiation, the ovary epithelium undergoes metaplasia to acquire highly complex histology that resembles Müllerian duct-derived fallopian tube (serous EOC), endometrium (mucinous EOC), endocervix (endometrioid EOC) or vagina (clear cell EOC)[1] (FIG. 4a). Consistent with the capability of OSE to give rise to the wide EOC histological spectrum, ectopic expression of the Müllerian-expressed homeobox genes *Hoxa9*, *Hoxa10* and *Hoxa11* induced immortalized OSE cells to differentiate along Müllerian lineages *in vitro*[172], and subsequent intraperitoneal inoculation of these transformed cells generated tumours resembling serous, endometrioid and mucinous EOCs, respectively[172]. Excessive gonadotropin stimulation and repeated exposure to the inflammatory factors within follicular fluid were subsequently identified as factors contributing to EOC development[135].

Failure to identify convincing EOC precursor lesions in the OSE has led to speculation that these carcinomas either arise *de novo* from epithelial inclusion cysts without an intermediary lesion[42] or are derived from an extra-ovarian source or sources.

An alternative hypothesis posits that many EOCs, notably high-grade serous carcinomas (HGSCs), are derived from the fimbria. Pre-neoplastic p53 signature-containing lesions termed serous tubal intraepithelial carcinomas (STICs) have been identified in the fimbria of carriers of breast cancer 1 (*BRCA1*) and *BRCA2* mutations predisposed to ovarian cancer and in patients with sporadic pelvic–ovarian HGSC, whose ovary histology was otherwise normal[44,173–177]. Secretory fimbrial cells have an intrinsic delayed response to DNA damage compared to their ciliated counterparts[178]. This trait might make secretory fimbrial cells vulnerable to the accumulation of deleterious mutations in their genomes following repeated exposure to the pro-inflammatory follicular fluid milieu. The current model of fimbrial involvement in HGSC genesis (FIG. 4b) proposes ectopic implantation of abnormal STIC fimbrial cells into the ovary stroma as cortical inclusion cysts through the ovulatory rupture stigma site[39]; analogous to the entrapped-OSE model, exposure of the implanted fimbrial epithelia to an unfavourable ovary microenvironment leads to HGSC. As a subset of HGSC cases showed no fimbrial STIC involvement[173,179], the entrapped-OSE model might still apply.

reviewed elsewhere[36–41,43,135]. Genetically engineered mouse models for the *in vivo* transformation of OSE[35,136–144,145] or fimbria[48,146–148] support a paradigm in which both tissues are potential sources of ovarian carcinogenesis.

Epithelial stem cells have been shown to be cells of origin in many human epithelial cancers. Their longevity allows for the accumulation of the range of genetic mutations that are needed for perturbation of growth controls leading to metaplasia and malignant transformation. The plasticity of transformed stem cells may also account for the phenotypic heterogeneity present in EOC.

Several epithelial cancers have been shown to originate from normal stem cells. For example, constitutive activation of the WNT pathway through conditional deletion of the adenomatous polyposis coli tumour suppressor in *Lgr5*⁺ stem cells (but not in their transit-amplifying and differentiated progeny), or expression of the oncogenic β-catenin in Polycomb complex protein BMI1- and prominin 1-expressing stem cells, resulted in epithelial cell transformation and rapid formation of adenomas in the mouse small intestine[149–151] and stomach[9].

Recently, meta-analysis of human ovarian cancer expression microarrays has revealed an EOC tumour cluster, designated stem-A (proliferative) and stem-B tumours, that expressed markers typical of epithelial stem cells, notably LGR5 and CD133 (also known as prominin 1)[152]. Thus, EOC may have stem-like components. Of note, stem-A tumours were linked with poor clinical prognosis and with high expression of proliferation-related genes[152] and several WNT–planar cell polarity (PCP) pathway genes, including the WNT signalling receptor gene *FZD7*, the knockdown of which decreased cell proliferation and migration[153].

From this perspective, the recent identification of *LGR5*⁺ cells in healthy OSE and fimbrial epithelium in humans has led to speculation regarding their potential role as the EOC cell of origin[34]. A stem-driven cancer-initiating scenario may be proposed, by which an initiating mutation that is sustained by a stem cell (*LGR5*⁺ and/or others) leads to its neoplastic transformation and differentiation along several Müllerian tumour lineages (FIG. 4).

Also of potential importance in EOC genesis is the identification of an enriched *Aldh1*⁺ and *Lgr5*⁺ stem cell population at the ovary–fimbrial border in the mouse ovary hilum[35]. Compared to their differentiated progeny, OSE cells of the ovary hilum show enhanced proliferation *in vitro* and increased transformation potential *in vivo* following inactivation of tumour suppressor genes transformation related protein 53 (*Trp53*) and *Rb1*, which are often lost in human HGSCs[35].

In humans, the ovary–fimbrial border (termed the ovarian fimbriae) represents an epithelial transitional zone with overlapping OSE and fimbrial marker expression[36,37,40] and a robust stem cell programme[65]. It is plausible that regulation of stem cell fate may be less defined in this region, making the cells more susceptible to carcinogenesis. Furthermore, we speculate that combined niche signals emitted by the OSE and fimbrial niches enable resident stem cells to differentiate into OSE and Müllerian tumour lineages. Epithelial transitional zones have been implicated as major sources of many epithelial cancers. For example, a small population (~40 cells) of cuboidal epithelial cells at the squamocolumnar junction of the uterine cervix is believed to be the target precursor for cervical carcinogenesis[154], whereas Barrett's oesophagus, which is a precursor of oesophageal adenocarcinoma, is thought to originate from the embryonic-like squamous epithelium at the oesophagus–stomach border[155].

The availability of the *Lgr5–Cre* mouse model should make it an ideal *in vivo* platform for evaluating the behaviour of ovary-resident *Lgr5*⁺ OSE and ovary–fimbrial transitional epithelia following conditional targeting of key EOC-associated oncogenes and/or tumour suppressor genes. Such a model will directly assess whether transformation of an *Lgr5*⁺ stem cell is the initial transformation event that leads to epithelial precancerous lesions and ultimately to EOC.

Future directions and outlook

In contrast to many adult epithelia, our understanding of stem cell biology in the ovary and fimbria has remained frustratingly poor. Until recently, we could only

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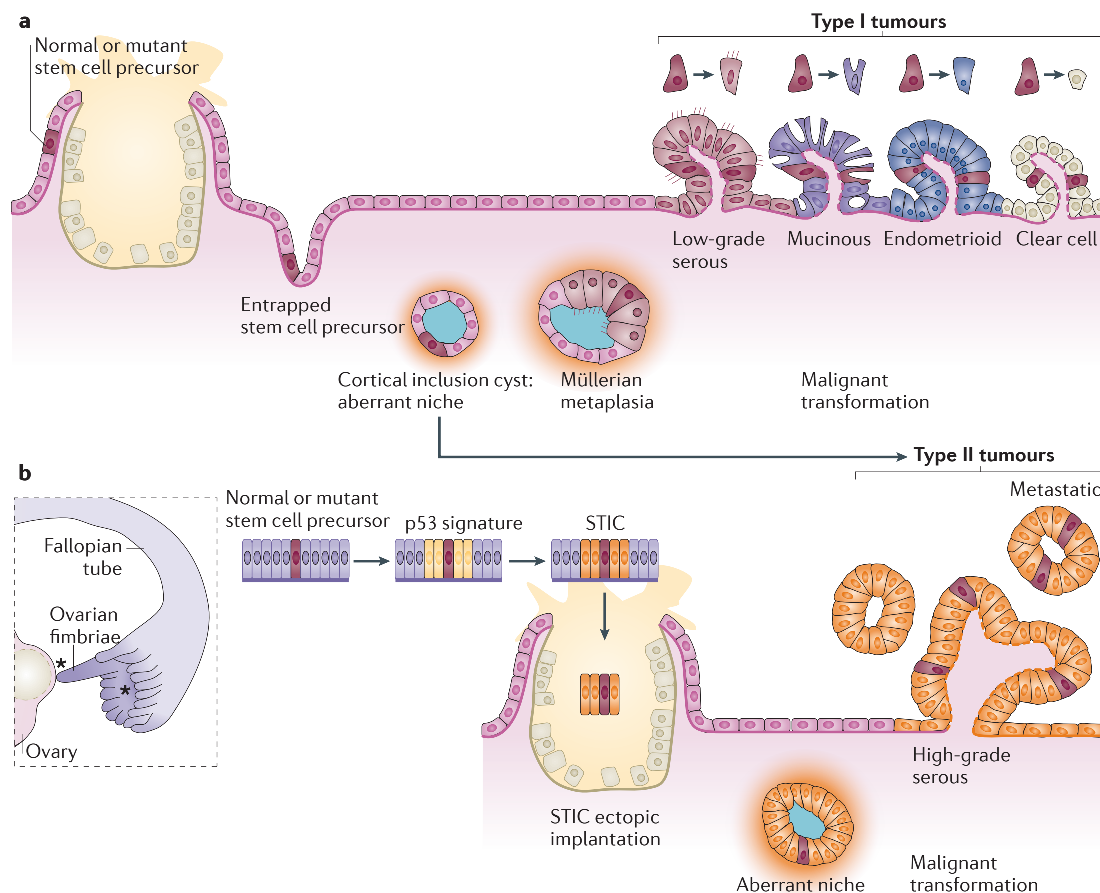

Figure 4 | **Proposed model of stem cell-driven epithelial ovarian cancer (EOC) carcinogenesis.** **a** | A putative ovarian surface epithelium (OSE) stem cell as EOC cell of origin is shown. During incessant ovulation, a normal or mutated OSE stem cell (dark pink) at ovulatory wound margins becomes entrapped within the ovary cortex to form a cortical inclusion cyst. The entrapped stem cell is exposed to aberrant paracrine signals from the activated stromal niche (orange area) and/or autocrine signals within the cystic lumen (grey area). Over time, the entrapped stem cell undergoes Müllerian metaplasia, followed by malignant transformation. Depending on the nature of the niche signals and acquisition of critical mutations, the transformed OSE stem cell differentiates along different Müllerian lineages to generate the different type I EOC cell types. Type II tumours, conversely, arise *de novo* from cortical inclusion cysts (or from fimbria, see part **b**). **b** | A putative fimbrial stem cell as high-grade serous carcinoma (HGSC) cell of origin is shown. A stem cell (dark pink) in the fimbria or ovarian fimbriae (marked with *) develops a p53 signature, followed by development of serous tubal intraepithelial carcinoma (STIC). During ovulation, the STIC cell (or cells) dislodges and becomes entrapped within the ovary cortex to form a cortical inclusion cyst. Exposure to the activated stromal niche (orange area) and/or autocrine signals within the cystic lumen (grey area) induces HGSC carcinogenesis and metastasis. No Müllerian metaplasia is involved.

speculate on the existence of somatic stem cells involved in post-ovulatory wound repair and remodelling in these tissues. With the discovery of LGR5-marked stem cells in the ovary, it is now possible to investigate how these stem cells execute wound repair and maintain ovary homeostasis. Future efforts should focus on characterizing the OSE stem cell niche components and signals, as well as identifying stem cells that are responsible for lifelong homeostasis of the adult fimbrial epithelium.

A more comprehensive understanding of normal stem cell biology in the ovary and fimbria is also expected to shed light on the genesis of EOC, which is one of the most fatal, yet least understood, reproductive malignancies. Of paramount importance will be investigating the role of *LGR5*$^{+}$ cells in EOC initiation. The discovery of a transformation-susceptible *Aldh1*$^{+}$*Lgr5*$^{+}$ stem cell niche at the mouse ovary hilum is particularly promising. Whether a similar cancer stem cell niche exists in humans is unknown, and this warrants future investigation. Armed with these new stem cell (and potentially cancer stem cell) markers, and recently developed mouse ovarian cancer models, we are now well placed to make important advances in our understanding of ovary and fimbrial epithelial stem cell biology and cancer biology in the next decade

The development of near-physiological 3D human ovary and fimbrial culture systems and gene-editing technologies will also provide essential tools for studying ovary and fimbrial stem cell biology and for disease modelling. Given the immense heterogeneity and complexity of EOC, elucidating how stem cells execute tissue repair during normal homeostasis, and identifying OSE and fimbrial niche components and signals, are crucial steps towards understanding how tumours develop in these tissues.

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Competing interests statement
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