Chapter 6

Adult Stem Cell Niches — Stem Cells in the Female Reproductive System

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Additional information is available at the end of the chapter

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1. Introduction

The female genital tract is a complex and physiologically dynamic system of organs which undergo continuous and profound changes during the reproductive years. Each of its components – ovaries, fallopian tubes and uterus - has unique and indispensable roles in reproduction. Each month, under stimulation from the pituitary gland, the ovaries produce and release a mature oocyte, which moves into the neighboring fallopian tube. Conception takes place in the lumen of the tube and the mucosal epithelium lining plays a critical part in the transport of the gametes and the successful transfer of the zygote to the uterus, where it implants 6-12 days after fertilization. Considering the importance of successful reproduction for species survival, there is strong evolutionary pressure to make the process robust and to respond quickly to any cellular damage with effective repair mechanisms. Also, the inner layer of the uterus, the endometrium, is subjected to monthly shedding and regeneration in order to sustain a suitable environment for implantation of a potential embryo. Similar to other tissues like intestine and hair, which continue to undergo rapid cellular turnover throughout adult life, there is an increasing number of studies describing the existence of adult stem cells in the genital tract that ensure tissue renewal throughout life.

Historically, adult stem cells have been described in vivo as rare, slow-cycling cells that maintain self-renewal by asymmetric division and can differentiate into different progenies. The traditional method of identification has been BrdU labeling, and designated stem cells have been described as label retaining cells (LRC), although there has been some doubt concerning the accuracy of the underlying premise that stem cells efficiently incorporate BrdU [1]. Since BrdU is a mutagen and toxic, this prevents successful recovery and in vitro analysis of labeled cells. However, both old and new experimental approaches recently

confirmed the existence of adult stem cells in the female reproductive system using the mouse model system with BrdU pulse labeling [2,3], as well as with transgenic animals with fluorescently labeled histone 2B [4], although it is not yet clear if the two methods do in fact label the same cells.

Deregulation of the adult stem cell niche, which is the main custodian of homeostasis in healthy tissues, is considered a potentially significant step in the etiology of cancer as well as other proliferative disorders, such as endometriosis. Therefore, basic research into the biology of adult stem cells is a new and promising field in the search for novel therapeutic strategies for these diseases. This chapter will provide an overview of the current understanding of adult stem cell niches in the female genital tract, and how new evidence regarding its molecular regulation changes our perspective of analyzing and treating some of its most common pathologies: endometriosis as well as endometrial and ovarian cancer. Based on the available evidence, it is safe to conclude that the female reproductive tract harbors adult stem cells. However, in contrast to an already very comprehensive and detailed insight into the structure and regulation of the adult stem cell niche in the gastro-intestinal tract, hair follicle or hematopoetic tissue, details of the niche organization in the genital tract mucosa remain at best sketchy. Experimental data on adult stem cells from the genital tract almost exclusively originate from in vitro studies of primary culture isolates and so-called "functional assays" describing clonality assays, sphere formation and differentiation capacity for the small population of presumptive stem cell candidates. Still, many questions remain unanswered regarding the molecular mechanisms of epithelial renewal in a system which during the average reproductive period undergoes more than 400 cycles of phenotypical changes in response to shifts in hormonal stimulation. Of particular importance is the relationship between adult stem cells in the healthy tissue and so-called cancer stem cells and we will address the most important developments in this area of research.

We will also briefly review contentious recent evidence for a putative reserve of germline stem cells (GSCs) in the ovary, which would represent a further population of adult stem cells, akin to spermatogonia in men. One of the pillars of reproductive medicine and infertility treatments is the dogma that women are born with all potential oocytes in place, arrested in the first meiotic prophase. Thus, the available "ovarian reserve" is a limiting factor in infertility treatments, and egg donation remains the only option for patients who show diminished parameters for remaining primordial follicles. Following on from the postulation that somatic, mitotically active cells in Drosophila melanogaster can act as GSCs [5] several groups have provided evidence for the existence of GSCs in mouse [6,7] as well as human ovaries [8]. However, they are yet to find final acceptance in the scientific community. If they do indeed exist, they would without doubt revolutionize the field of reproductive medicine.

Before we review the current “state of the art” of adult stem cell research in the female genital tract, it is useful to summarize its basic anatomy and histopathology in order to understand the environment in which stem cells function.

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2. Anatomy and histopathology of cervix, uterine endometrium and fallopian tube and ovary

Distinct portions of the mucosa along the tract are morphologically and functionally specialized to facilitate the successful completion of the reproductive process: from the oocyte maturation (ovary) through the transport of gametes (fallopian tube) to the implantation of the embryo in the endometrium and establishment of a viable pregnancy (uterus). The ovarian surface epithelium (OSE) is built of simple, flat, cuboidal cells, without mature adhesion junctions or prominent polarity (Fig. 1A).

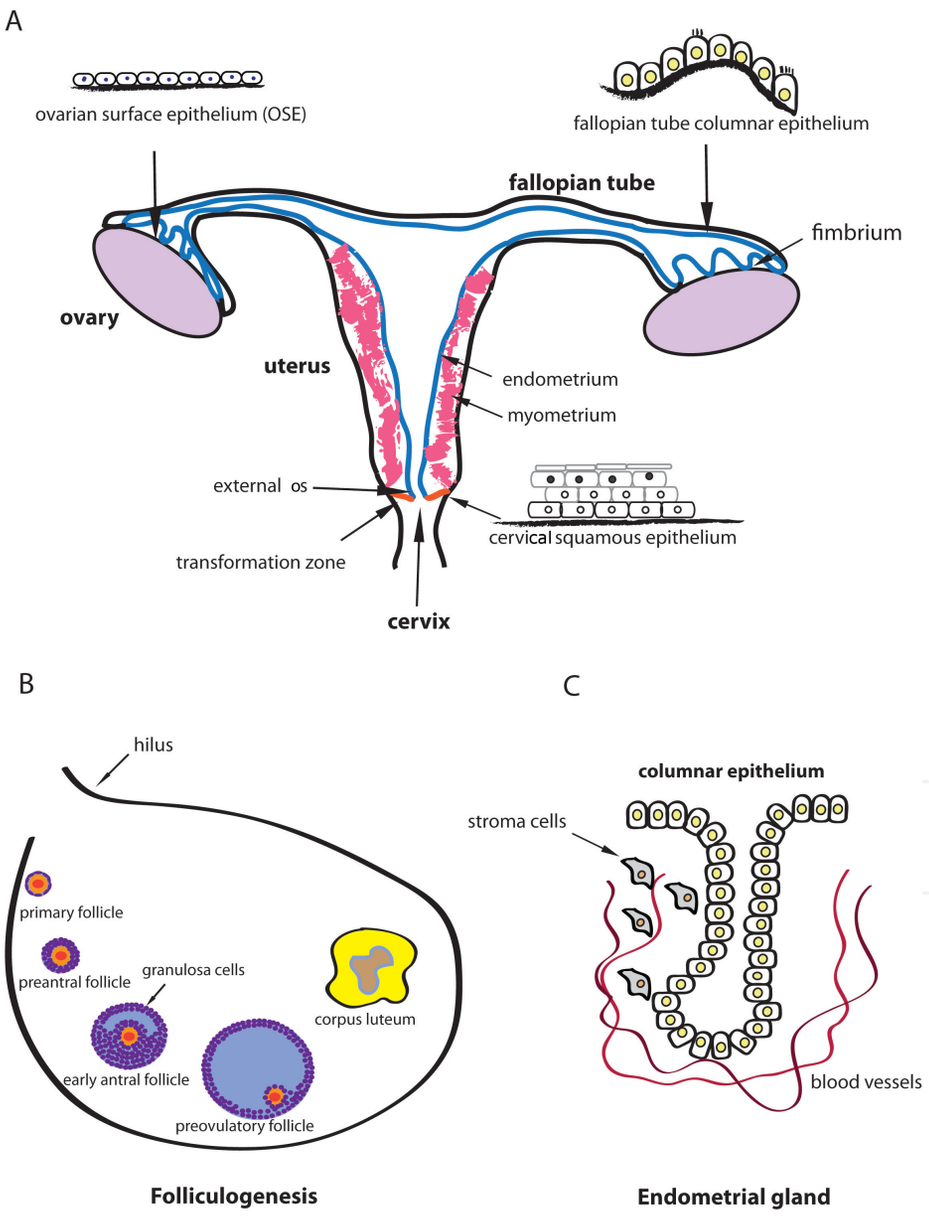

Figure 1. Overview of the histology of the female genital tract

The stroma of the ovary is filled with luteinized stromal cells, decidual cells, neuroendocrine cells, fat and muscle cells and a population of endometrial-like stromal cells. In addition, the ovary contains the pool of primordial follicles, which consist of immature primary oocytes, arrested at birth in the prophase of meiosis 1, surrounded by a densely packed shell of somatic granulosa cells, which are of key importance for successful growth and maturation of the ovum. The developing oocyte and neighboring granulosa cells represent a perfect example of the "niche" where cell-cell interaction and signaling from surrounding tissue compartments determine cell fate and differentiation (Fig. 1B). The process of ovulation, or specifically follicule rupture, creates a pro-inflammatory environment high in reactive oxygen species [9, 10], that requires extensive repair mechanisms and tissue remodeling to avoid permanent damage to the ovary. Indeed OSE cells, though simple in their cellular phenotype, are uniquely adapted to not only respond to stress conditions, but also to actively participate in the tissue breakdown and remodeling that enables rupture of the follicle through the ovarian surface [11]. They express proteolytic enzymes such are metalloprotease 2 and 9 [12], and can undergo epithelial mesenchymal transition (EMT) [13], which may facilitate efficient repair of the injured ovarian surface during the post-ovulatory phase of the menstrual cycle. The ovaries also produce hormones that control the cellular changes associated with the menstrual cycle. The granulosa cells of growing follicles secrete exponentially increasing amounts of estradiol and progesterone. After oocyte release, the remaining follicular cells undergo transformation into the corpus luteum, which immediately starts producing progesterone under the control of pituitary LH pulses. These two main phases of the menstrual cycle – estradiol-driven follicular phase and progesterone-driven luteal phase – determine cyclical homeostasis of all mucosal surfaces in the lower genital tract including fallopian tube, uterus and cervix.

The fallopian tube, or salpinx, is ~ 10 cm long and anatomically divided into three segments: the isthmus, which connects to the uterus, the ampulla, which constitutes the middle part, and the infundibulum, proximal to the ovary. The infundibulum terminates in relatively large opening, the ostium, which has many fine projections, or fimbriae, that capture the oocyte upon follicule rupture and guide it towards the ampulla, where fertilization takes place. The histology of the tube, starkly different from the ovarian surface, is characterized by the presence of an epithelial monolayer of highly differentiated columnar cells with two distinct cell types: secretory – producing tubular fluid – and ciliated – enabling transport along the lumen (Fig. 1A). Numerous mucosal folds in the distal and ampullar regions provide a suitable environment for the early stages of blastocyst development. Contractility of the tube is ensured by the muscular layer surrounding the epithelium. Although, unlike uterine endometrium, fallopian epithelium does not undergo extensive monthly shedding, it responds to the follicular phase hormonal environment by proliferation [14]. Changes in homeostasis are supported by global gene expression data from fallopian tube mucosal samples, which show marked differences between follicular and luteal phase [15]. The tubal epithelium is also exposed to the pro-inflammatory environment associated with ovulation, leading for instance to an increase in double stranded DNA breaks marked by phospho-γH2A.X in a mouse model in vivo [16]. Exposing human fallopian tube isolates to follicular fluid ex vivo increases expression of inflammation-related genes and DNA repair components [17]. In particular, there is a noticeable accumulation of p53 it the nuclei of tubal cells, which is thought to be a

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key step in the development of premalignant lesions. As well as the potentially genotoxic effects of follicular fluid, their connection to the uterus also renders the fallopian tubes vulnerable to ascending infections by sexually transmitted pathogens. Chlamydia trachomatis and Neisseria gonorrhea are major causes of the inflammatory disease salpingitis, which is marked by scarring and tissue injury and dramatically increases the risk for tubal occlusion and infertility. In addition, there is increasing evidence that at least some STDs may also have a pro-malignant effect on host cells [18-20].

The uterus is a muscular organ with great capacity for growth and physiological transformation, which is necessary to support pregnancy. It consists of three layers: the outer serosal layer, or perimetrium, the myometrium, which contributes the most to the volume and mass of the organ, and the inner mucosal layer, or endometrium, which is vital for the initiation of pregnancy. The structure of the endometrial layer has traditionally been divided into the stratum basalis and the stratum functionalis, which is subjected to monthly cyclical renewal, differentiation and shedding. The stratum functionalis changes greatly during each menstrual cycle and is further divided into stratum compactum and stratum spongiosum. The stratum functionalis consists of glandular epithelium (Fig. 1C) residing on the supportive connective tissue and blood vessels which supply nutrients. It is at its thinnest at the beginning of the follicular phase, and proliferates strongly under stimulation by estradiol. The estradiol peak coincides with the maximum follicle diameter prior to ovulation and with maximal thickness of the proliferative endometrium. Following ovulation, rising progesterone levels, trigger differentiation of the stratum functionalis into "secretory" endometrium. At the cellular level, endometrial glands, which resemble narrow straight tubes during the follicular phase, begin to swell as progesterone stimulates the columnar epithelial cells to produce and secrete glycogen granules. It is assumed that this glycogen-rich environment serves as an energy depot for the implanting blastocyst. In addition, stromal cells undergo a profound change, converting from a fibroblast-like phenotype into rounded cells that produce prolactin and insulin growth factor binding protein [21,22]. This complex transformation of the functional layer of the endometrium, called decidualization, is a prerequisite for successful attachment and invasion of the trophoblast and thereby initiation of the pregnancy. Progesterone secretion by the corpus luteum is limited to around two weeks, after which it disintegrates if no chorionic gonadotropin from an implanted embryo is present in the circulation to rescue its function. In the absence of progesterone, the endometrial stratum functionalis, epithelium and supportive connective tissue is shed and expelled by menstrual bleeding.

The uterus is separated from the vaginal canal by the narrow muscular cervix. Due to its physiological elasticity, the cervix tightens under the influence of progesterone, and essentially seals the uterus from the outside environment during the second part of the cycle. This protective barrier is enhanced in the case of pregnancy by the formation of a mucus plug that fills the endocervical canal. The entrance to the cervix is called external orifice of the uterus, or external os. The endocervix is lined by simple columnar epithelium, with a similar structure to the endometrial monolayer, while the outer part is lined by stratified squamous epithelium. The segment in between – the squamo-columnar junction (SCJ) – is a dynamic zone, which does not have a fixed location but migrates under the influence of major hormonal changes,

such as puberty, pregnancy and menopause. Prior to puberty, squamous epithelium covers the outer segment of the cervix and the lower part of the canal. During puberty and first pregnancy, the columnar epithelium of the endocervix expands and covers the outer rim of the external os. Through contact with the low pH of the vagina, the columnar epithelium undergoes metaplasia over time and converts back towards a squamous phenotype (squamous metaplasia). It is widely accepted that over 90% of malignancies of the cervix originate from cellular changes which are initiated in this region of intense tissue remodeling-thus the SCJ is frequently labelled "transformation zone" (TZ).

3. Putative female GSCs

Before devoting our attention to the somatic adult stem cells of the female genital tract, we will review the rather contentions field of presumptive GSCs in the adult ovary. Since the 1950s, the accepted dogma in reproductive biology has been that in mammals primordial germ cell (PGC)-derived oogonia cease proliferation shortly after birth and differentiate into primary oocytes, which arrest in prophase of meiosis I until fertilization triggers the completion of meiosis [23]. As a consequence, the pool of available oocytes is finite – and exhaustion of the pool of resulting follicles is believed to be responsible for menopause. In the last decade, however, this assumption has been challenged by a series of papers reporting the existence of GSCs in the ovaries of mice and humans. First hints came from observations by Tilly's group [6], who tried to assess the dynamics of germ cell loss during adult life. Their estimation that up to a third of immature follicles in mice are degenerating at any given time led them to postulate that this unexpectedly high rate is incompatible with the slow rate of decline observed in the follicle reserve. In trying to resolve this contradiction, they identified cells on the ovarian surface of young mice that express the meiotic entry marker SCP3, as well as BrdU-incorporating cells that simultaneously express the germ cell marker Ddx4, suggesting that these cells may be proliferating GSCs responsible for replenishing the follicle pool.

Further evidence for ovarian GSCs that are capable of proliferation followed by differentiation into oocytes came from the observation that immunomagnetically isolated $Ddx4^+$ cells from both mouse and human ovaries can be expanded in vitro and give rise to oocytes following transplantation into donor ovarian tissue [7,8]. In the mouse, GFP-labelled putative Ddx4+GSCs were able to give rise to offspring following transplantation, with transmission through the germline to subsequent generations [7]. Using transplantation of premeiotic female PGCs Zhang et al [24] further provided proof of principle that the adult ovary is able to support oogenesis. Tilly's group subsequently showed that following extensive doxyrubin-induced loss, follicle numbers recover within 36 h. Reasoning that the small numbers of presumed GSCs they had previously identified in the adult ovary would not be sufficient to support this rapid recovery, they transplanted bone marrow from mice expressing GFP under the Oct 4 promoter into germ cell-deficient $Atm^{-/-}$ mice and identified GFP-labelled primordial follicles [25]. They concluded that a subpopulation of circulating bone marrow stem cells that express PGC marker genes are responsible for the observed oocyte replenishment.

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Putative GSCs have been identified by co-labelling with proliferative and germ cell markers and described either as small cells within the ovarian cortex [6,26-28] or clusters of cells which also include somatic cells [29]. Other studies supported the discovery of ovarian GSCs [26,30] but the considerable differences in marker expression, in vitro phenotype and differentiation potential observed between these studies make the case somewhat controversial. In addition, other groups have failed to repeat these findings and challenged their validity [24,31,32]. Kerr et al described the expulsion of oocytes through the OSE into the peritoneum during the post-natal phase of oocyte reduction, which could potentially be interpreted as GSCs [32]. By using tamoxifen-induced random labelling of cells, Lei and Spradling traced the numbers of follicles over time and argue that the follicle pool is in fact highly stable with a half-life of 10 – 11 months, which would make the follicle pool at birth large enough to support the ~500 ovulations required during the life time of a mouse [33]. They also failed to observe the generation of new follicles even after depletion with busulphan toxin, thus putting into question that there is in fact a need to explain regeneration of follicle numbers, a finding that is further supported by mathematical modelling [34]. Similarly other groups, failed to observe replenishment of the follicle pool by donor bone marrow-derived cells [35] or after chemical depletion [36].

It has been argued that some of these contradicting findings could be explained by the use of Ddx4 –which was previously thought to be a cytoplasmic germ cell marker, but was used by Zou et al [7] and White et al [8] to isolate cells based on their finding that the protein also contains a transmembrane domain in GSCs. They suggest that the reason many other laboratories have failed to identify GSCs in ovaries using Ddx4 [24] is due to the fact that cytoplasmic Ddx4 expression in oocytes masks the presence of rare GSCs. Similarly, SSEA-1, which was used by Johnson's group to identify ovarian GSCs [25], was reported by Bristol-Gould et al [34] to overlap only with cells from the HSC lineage. Widespread acceptance of adult ovarian GSCs will no doubt depend on the identification of a marker signature or more robust methods which allows identification of these cells by all laboratories with the relevant expertise.

The difficulty in pinpointing putative ovarian GSCs or their niche in vivo, while at the same time apparently being expandable and giving rise to the appropriate differentiated tissue in vitro, mirrors the experience for stem cells of somatic adult tissues, as we shall explore below. However, the unique properties and complex embryonic development of germ cells do in fact give rise to some important differences.

In the current model, primordial germ cells (PGCs) derive from a small number of epiblast cells which are specified before differentiation into the different germ layers begins. PGCs subsequently undergo a complex migration through the allantois, along the developing hindgut, finally entering the dorsal mesentery and the developing gonads. Despite the obvious importance of these early developmental processes for future fertility, they remain little understood. Mikedis and Downs have suggested that PGCs temporarily reside in an “allantoic core domain” (ACD) which they propose has similar functions to the Spemann organiser, consisting of a stem cell pool which extends the body axis in a posterior direction – contributing not only to the germ cell lineage but also the three germ layers – effectively creating a strong interface between the future umbilical cord and the developing embryo [37]. The stem cells in the ACD express Oct4, Blimp1, Stella and Fragilis – markers thought to be specific for PGCs –

but appear to contribute also to other tissues [38]. These observations, as well as the fact that hematopoietic stem cells also migrate from the proximal epiblast to the embryonic aorta-gonad-mesonephric region during the same period of development, imply that it is theoretically possible that there may be "intermixing" or indeed that there is a common precursor pool for PGCs and a subpopulation of bone marrow stem cells.

While a lineage tree analysis [39] based on somatic mutations accumulating in microsatellites found that oocytes form a cluster which is entirely distinct from other cell populations – suggesting that there is no intermixing of the germ cell precursor pool with that of any other cell type – it is conceivable that a very rare subpopulation of bone marrow stem cells would be missed in such an analysis. In fact their results also show that the number of mitotic divisions oocytes have undergone increases with age and following unilateral ovarectomy. This may be explained by recruitment of oocytes in the order in which they first differentiated during development – but is also consistent with the notion of continuous oocyte production from cycling stem cells. Many observers have suggested that if GSCs do exist, they are most likely to be derived from the normal developmental precursors of oocytes, i.e. PGCs or oogonia – which have not yet differentiated into oocytes and are still able to undergo mitosis [40-42]. The close relationship of PGCs to pluripotent cells is demonstrated by the fact that following isolation from the embryo, they can be converted back to a pluripotent phenotype termed embryonic germ cells in vitro without genetic manipulation [43-45].

Even if one accepts the existence of GSCs, there are a number of unanswered questions apart from their exact location. Firstly, it is not clear whether they contribute to oocyte production under normal physiological conditions, or only after injury. Secondly, if the follicle pool is replenished by GSCs, why does this replenishment eventually cease, leading to menopause? Niikura et al [27] have suggested that the aging niche environment itself may be responsible – however, the life-long production of spermatozoa in the testis indicates that this is not in itself a sufficient explanation. A large number of germ cells in neonatal mouse ovaries have not yet entered meiosis and can be induced to proliferate, increasing the follicular pool – but by the time animals enter reproductive age the numbers have returned to "normal" levels [46]. This suggests that mechanisms exist within the ovary to actively regulate the number of follicles. Together with the fact that a large proportion of oocytes are eliminated shortly after birth [47] this indicates a highly selective process to ensure the removal of oocytes with reduced meiotic fitness, which runs counter to the idea of continued oocyte replenishment from cycling precursors.

Whether or not GSCs exist in vivo, the presence of cells that can be expanded and differentiated to functioning oocytes in vitro – as suggested by Zou et al [7] and others [28] – would in itself be of huge potential benefit for the treatment of infertility. Nonetheless, the long time required to induce proliferation of these cells in vitro (around 10 weeks), compared to other adult stem cells, suggests that transformation of the cells in vitro may be responsible for the observed phenotype – similar to findings which describe the production of oocytes from other somatic stem cells in vitro – or that indeed the results may be explained by rare primordial oocytes that are carried over during the in vitro period.

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4. Uterine endometrial stem cells

From the volume of work, it is fair to say that human endometrium has been the most intensively studied portion of the female genital tract in the field of adult stem cell research. This is partly due to the fact that the uterus is by far the most accessible portion of the tract, where sample collection and analysis is much less invasive compared to investigating ovary or fallopian tubes, and partly to the logic assumption that such intensely proliferating and renewing tissue should contain a stem cell pool. Since the stratum basalis of the endometrium is necessary for monthly renewal of the functional layer [48], characterized by intense proliferation under stimulation of rising estradiol levels, it is a prime candidate for harboring stem cells. Still, there is no common agreement yet where adult endometrial stem cells reside in vivo. LRCs were first identified by BrdU labeling in the epithelial layer and underlying stroma in postnatal mice [2]. However, by 12 weeks post labeling, no BrdU positive cells were left. Suboptimal timing of the pulse could mean that division of slow-cycling stem cells was missed such that only transitory amplifying progeny was labeled. Nevertheless, the study provided the first in vivo evidence for the existence of long-lived cells in the endometrium.

A significant advance over BrdU labeling for localizing stem cells in the mouse was recently achieved with the development of a histone2B-GFP (H2B-GFP) reporter in a Tet-inducible system, in which a doxyclicline pulse leads to fluorescent labeling of chromosomes, without mutagenic stress. This method allows induction of the construct during embryonic development, which increases the likelihood that stem cells will be labeled. With each cell division the GFP signal is reduced until after ~ 12 weeks it can only be detected in LRCs. In the first study using this system [49], the doxycyclin pulse was administred in adult animals and 12 weeks after pulse withdrawal LRCs were identified only in the distal oviduct segment of fimbrium and ampulla. Their presence was stable even after 47 weeks. In a follow-up study, labeling was extended to the embryonic period, confirming localization of LRCs to the distal oviduct, and identifying an additional population at the endocervical transition region but not within the endometrium. Only a pulse during the pre-pubertal period (post natal day 21-42) resulted in labeling rare cells in the glandular endometrial epithelium [4]. It is perhaps due to these experimental difficulties in identifying the adult stem cells in the uterus of a living organism, despite the undisputedly enormous capacity for endometrial regeneration and plasticity, that alternative models have been proposed to explain regenerative capacity of the endometrium.

An interesting hypothesis that has gained some traction within the scientific community is the possibility that bone marrow could be a source of endometrial stem cells. Previously, bone marrow stem cells were reported to be able to differentiate into a wide variety of cell types [50-52]. Analysis of bone marrow transplant recipients revealed the presence of donor cells in the opening of endometrial glands, raising the possibility that a stem cell pool outside of the reproductive system may contribute to the regeneration processes [53,54], mirroring the findings with female GSCs. Ikoma and colleagues used in situ hybridization to confirm the localization of Y chromosome-positive donor cells in the endometrium of a female patient who had undergone bone marrow transplant from a male donor [55]. However newer studies have questioned the functional importance of such findings, as bone marrow cells residing within

the endometrium do not appear to contribute to the stem cell pool, which exhibits high clonogenic capacity in vitro, but are instead reminiscent of terminally differentiated cells [56], which could be explained by the capacity of bone marrow-derived cells to fuse with differentiated cells from a variety of organs [57].

Regardless of the difficulties in identifying and characterizing the adult stem cell niche in the intact epithelium in vivo, numerous studies in recent years have isolated distinct populations of putative adult stem or progenitor cells from uterine tissue and demonstrated their proliferative capacity and broad differentiation potential in vitro. Two different classes of adult stem cells are found in the endometrium: mesenchymal and epithelial. Mesenchymal stem cells have also been named Endometrial Regenerative Cells (ERC) due to their high proliferative potential. Although the ability to produce a clonal population of cells when seeded at very low dilution is much greater for the stromal mesenchymal subpopulation, the epithelium also contains cells capable of clonal expansion in vitro. Self renewal of endometrial stem cells in vitro was first demonstrated in pioneering work by the group of Caroline Gargett [2,58,59]. In the absence of a widely accepted surface marker for endometrial stem cells, they demonstrated that multipotent cells can be efficiently isolated based on the uptake of Hoechst 33342 [60]. The method of SP cells was originally established in procedures for adult stem cells isolation from the hematoopetic tissue [61]. It made use of the discovery that the small population of adult stem cells in the tissue differs from the differentiated cells by their capacity to efficiently eject Hoechst dye from the cytoplasm, presumably due to the high expression of the ABCG2 transporter [62]. Designated endometrial side population cells (ESP), consisting of both epithelial and mesenchymal SP cells, are able to generate endometrial glandular structures in vivo if transplanted under the kidney capsule of NOD/SCID immunocompromised mice [63]. Interestingly, a fraction of the ESPs migrated and generated blood vessels to supply the endometrium, showing potency to generate different types of tissue in the functional organ. Still, the overall efficiency of the procedure was rather low as endometrium formation occurred in only 2 out of 24 injected animals. Dramatic improvements in the outcome of this type of xenograft was achieved by expanding and cloning lines of SP cells from both epithelial and stromal compartments. Transplantation of individual lines supported by the administration of estrogen (E2) and progesterone (P) to mimic the menstrual cycle gave rise to endometrial tissue in all animals [56]. This is a particularly significant finding since endometrial stem cells do not express E2 and P receptors. Nevertheless, the hormonal environment greatly affects the outcome of the tissue regeneration process. This phenomenon was closely analyzed by Janzen and colleagues [64], who showed that hormonal withdrawal in the xenograft mouse model leads to a pronounced decrease in the size of endometrial tissue while the remaining cells are highly enriched in stem cells. This suggests that the stem cell niche is activated at the beginning of each cycle, when both estradiol and progesterone levels are low, while the subsequent hormonal stimulation drives regeneration and proliferation of transitory amplifying cells up to their final differentiation in response to progesterone stimulation from the corpus luteum in the second phase of menstrual cycle. On the molecular level, endometrial stem cells showed elevated Wnt

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signaling activity and increased expression of the Wnt target genes Axin2, Cyclin D1, ID2, CD44.

As well as studying the role of endometrial stem cells in regeneration of the functional endometrium, a number of methods have been developed to use endometrial progenitors in translational approaches for therapeutic purposes. In vitro differentiation assays suggest that mesenchymal stromal stem cells may be able to differentiate into diverse cell types [58,65,66], and can be efficiently isolated from menstrual blood. A clinical trial of endometrial stem cells as a source of autologous regenerative cardiomyocytes to treat ischemic cardiac injury is underway [67] and promising results were also reported for pancreatic island replacement in a xenograft diabetic mouse model [68].

In parallel to the translational approaches, we need to get a better understanding of the biology of endometrial stem cells in vivo to understand how these cells influence the development of pathologies in the uterus. There are strong indications that deregulation of the stem cell niche plays a role in endometriosis, a disease which affects up to 10% of all women and is present in nearly half of those experiencing fertility problems or pelvic pain after the age of 35 [69]. The hallmark of endometriosis is the presence of ectopic explants of endometrial tissue outside of the uterus, which can affect the fallopian tubes, the ovary but also more distant regions of the pelvic cavity, causing pain and discomfort. Endometriotic lesions have identical responses to hormonal stimuli as the endometrium of the uterus. The model of "retrograde menstruation" is accepted as the most plausible explanation for the spread of endometriosis tissue through the genital tract, but the molecular mechanisms which trigger and control the disease remain obscure. Analysis of menstrual tissue samples from affected women showed that endometriosis patients have significantly more fragments of the basalis layer in the flow than healthy controls [70]. Cells from menstrual blood of patients also have longer than average telomeres, which is in agreement with an enhanced stem cell presence [71]. According to this model, long-lived stem cells from the basalis are disseminated and give rise to endometriotic lesions. In healthy women, on the other hand, only the upper layer of the endometrium is ablated, leaving the basalis layer intact. These data offer hope that characterization of the endometrial stem cell niche and its regulatory mechanisms will lead to a breakthrough in prevention and treatment of endometriosis – a disease that poses an enormous burden for patients' quality of life as well as high costs for the healthcare system.

5. Fallopian tube stem cells

Our understanding of the biology of fallopian tube epithelium is still rudimentary. From the perspective of medical diagnostics, it is the least accessible portion of the female reproductive tract. The fallopian tube is barely visible by ultrasound and even exploratory laparoscopy offers only information about tubal patency, and no insight into the condition of the mucosa. Current knowledge about tubal histology and pathology comes solely from patients of salpingectomy procedures, where tubes are surgically removed, usually as part of a total

hysterectomy. The importance of making progress in this area has been highlighted in recent years, as the potential significance of this tissue in disease initiation has become clear. The distal portion of the tubal fimbrium, and the ampulla with its abundant mucosal folds, are of critical importance for oocyte capture and fertilization. Intimate contact of the fimbrium with the surface of the ovary exposes the epithelial layer of the tube to the inflammatory signals associated with ovulation which are present in follicular fluid. Thus, there is an increased requirement for robust renewal mechanisms. Indeed, in vitro experiments with cell isolates from the fallopian tube have defined a population that is positive for CD44 and integrin $\alpha 6$ and has the capacity for clonal growth, self renewal and differentiation into the secretory and ciliated cells found in the tubal epithelial monolayer-specifically in the distal region of the tube [72]. Strong clonogenic potential of a H2B-GFP-retaining subset of epithelial cells in the distal oviduct and their capacity to form and maintain spheroids in vitro long-term has been demonstrated in a mouse model [49]. These spheroids are able to differentiate into more complex structures of the mucosa, strongly suggesting that they do indeed contain progenitor cells of the tube with the potential to proliferate and differentiate in vitro. However, the exact organization of the fallopian tube stem cell niche in vivo remains unknown apart from the positive identification of candidate cells in label-retaining experiments in vivo mentioned above. Moreover, the exact turnover rate of the epithelium and how it responds to different physiological and environmental stimuli or stresses is yet to be established.

Beyond the requirement for Wnt signaling for normal development of the genital tract, several studies have reported changes in Wnt signaling associated with pathology of the fallopian tube. For example, activation of β-catenin signaling is implicated as a contributing factor in ectopic pregnancy [73,74] and endometriosis [75]. Increased Wnt signaling is a mucosal response to infection [76], confirming that this paracrine pathway plays a role in homeostasis inside the fallopian tube. More studies are needed, however, to illuminate all aspects of paracrine signaling in the fallopian tube epithelium in health and disease. As mentioned above, Wnt signaling alterations are one of the hallmarks of ovarian cancer. Since there is now consensus in the medical community, based on cumulative molecular and clinical evidence, that a significant portion of high grade serous ovarian carcinoma originates from the fallopian tube fimbrium rather than the ovarian surface epithelium, it has become imperative to illuminate the regulatory mechanisms involved in fallopian tube epithelium homeostasis. Different models of ovarian carcinogenesis and the potential role of adult stem cells in this process will be reviewed fully later in this chapter.

6. Ovarian and cervical stem cells

In contrast to the uterus, direct evidence for adult stem cells in the ovary is sparse. However, the tissue remodeling processes involved in ovulation require a considerable regeneration potential within this organ that has to be maintained for several decades. Beyond the healing of the ovulatory wound created in the epithelial surface during follicle rupture, ovarian epithelial cells also undergo proliferation at the beginning of the menstrual cycle [77].

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Label-retaining cells on the ovarian surface were identified in vivo by comparing a histone-GFP inducible transgenic model and BrdU labeling of animals [78]. It is important to note that both methods resulted in identification of positive cells in the OSE epithelium of the ovary but the two populations overlapped only in a minority of cases. This further underscores the complexity and difficulty of identifying adult stem cells solely on the basis of cell division rate. Recently, the ovarian surface was subjected to a more detailed analysis, and the highest proportion of LRCs was detected in the hilum region close to the fallopian tube. These cells expressed high levels of aldehyde dehydrogenase 1 (ALDH1), and were able to proliferate long-term in vitro [3], forming spheres. Long-term pulse experiments and staining with the proliferation marker Ki67 also suggested that slow-cycling cells are activated in a cyclical fashion during the estrus phase, which supports a repair mechanism for the damaged OSE monolayer. Finally, genetic lineage tracing of Lgr5 expression in the ovary was performed [3], by using the $Lgr5^{tm1(cre/ERT2)Cle}$/J mouse which harbors a genetic construct that enables GFP labeling of Lgr5 expressing cells and tamoxifen-inducible expression of Cre recombinase. When crossed with a strain harboring a stop codon in a dTomato sequence that can be excised by Cre recombinase, offspring mice are produced in which easy tracing of the progeny of Lgr5-expressing cells is possible by a simple tamoxifen pulse at the desired time point [79]. This experiment revealed an $Lgr5^+$ population at the hilum, which contributes to the whole OSE monolayer during renewal of the epithelium in the course of 1 month [3]. Notably, this study was focused on the ovary and no lineage tracing was analyzed in other portions of the genital tract. A similar approach, with the SOX2 gene promoter controlling expression of CRE recombinase in a tamoxifen-inducible fashion, revealed the existence of long-term lineage labeling in the cervical epithelium of mice (Arnold et al 2012). It remains unclear, however, if other parts of the female genital system contain epithelial cells originating from $SOX2^+$ progenitors and it will be interesting to find out how these populations of putative stem cell candidates identified by independent methods correlate with each other. It is of course feasible that different tissue compartments harbor stem cells which are defined by different molecular markers. Although these two studies represent a methodological breakthrough in the detection of adult stem cells, by demonstrating the in vivo capacity of these cells to give rise to differentiated progeny in the tissue through tracing cellular markers in physiological conditions, they are not entirely comprehensive. This approach is hypothesis-driven by selecting candidate genes; however, the list of potential stemness regulators that could be tested is much longer. For example, Lgr5 is only one member of the Lgr receptor family, and other members are also involved in regulating tissue regeneration, e.g. Lgr6 in the hair follicle [80] and Lgr4 in the prostate [81]. More detailed studies are needed to confirm whether the $LGR5^+$ cells detected in the hilum represent the pool of true stem cells or a more dynamic population of amplifying cells that have already undergone a degree of lineage commitment. The $SOX2^+$ cells identified in the cervix are a good starting point to define the molecular characteristics of the long-lived basal cells which have so far proven to be elusive using other tracing methods, and this genetic system will provide a valuable tool for a more detailed analysis of the adult stem cell niche in the future.

7. Adult stem cells between development and disease — Role of the Wnt pathway

The precise signaling pathways controlling the renewal processes of the fallopian tube epithelium, endometrium and ovary remain unknown, but there are several reasons to assume that they depend on paracrine Wnt, Notch and BMP signaling. Wnt signaling controls crucial developmental processes during all phases of embryogenesis and during adult life. Wnt ligands interact with a family of receptors, inducing a variety of responses depending on the cellular context. The main transducer of Wnt signalling within the cell is β-catenin, which translocates to the nucleus and induces expression of target genes via several transcription factors (Figure 2).

Although a detailed overview of Wnt signaling is beyond the scope of this chapter, we will briefly outline its involvement in the control of the cell behavior within a tissue, affecting, among other processes, proliferation, establishment of polarity, differentiation, morphogenetic movements and apoptosis. With respect to tissue homeostasis, Wnt signaling acts as a key cell-cell communication network during tissue formation in development, as well as for maintaining tissue function. The embryological development of the female genital tract relies on active Wnt signaling, as evidenced by Müllerian aplasia. This autosomal mutation in the Wnt4 gene leads to a severely underdeveloped or absent uterus. Mouse models have further revealed a strong dependence of developmental processes on Wnt 5a, 7a, and 9b, since mutant animals showed severe malformations of different parts of the genital tract raging from defective coiling of oviducts to absence of the upper vagina or uterine glands [82-84]. The importance of the Wnt pathway for maintenance of homeostasis is well-documented by molecular and genetic analysis of numerous human malignancies. Perhaps the most startling example of the tight relationship between Wnt signaling and control of proliferation in mucosal epithelium is provided by the relationship between Adenomatous polyposis coli (APC) mutations and colon cancer. APC protein in complex with Axin1 promotes degradation of β-catenin and thereby inhibits Wnt signaling transduction (see Fig 2). In familial adenomatous polyposis patients, who carry an APC functional deletion mutation, the risk of developing colon cancer is almost 100% and 5% of sporadic colon cancer patients harbor either APC loss-of-function or β-catenin activating mutations [85]. Mutations in components of the Wnt pathway are frequently found in numerous other malignancies as well, e.g. pancreas, liver, kidney, pituitary, and notably also in the ovary and endometrium [86].

Although the significance of Wnt signaling for tissue maintenance has been known for the last couple of decades, it is the discovery of adult tissue stem cells which led to a breakthrough in our understanding of the regulatory mechanisms at the molecular level. Lgr5-expressing stem cells of the intestine ensure renewal of the mucosa every 3-5 days. Although $Lgr5^+$ cells at the base of intestinal crypts are sufficient to recreate the complete epithelial layer in vitro, an alternative model attributes true “stemness” to rare, more quiescent cells in the wall of the crypt, which express Bmi1, HOPX and mTERT [87-89]. Ablation of $Lgr5^+$ cells does not disrupt homeostasis, as $Bmi1^+$ cells compensate for the loss [90]. This illustrates the complexity of the regulatory stem cell niche, where different cells can be recruited and even reprogrammed,

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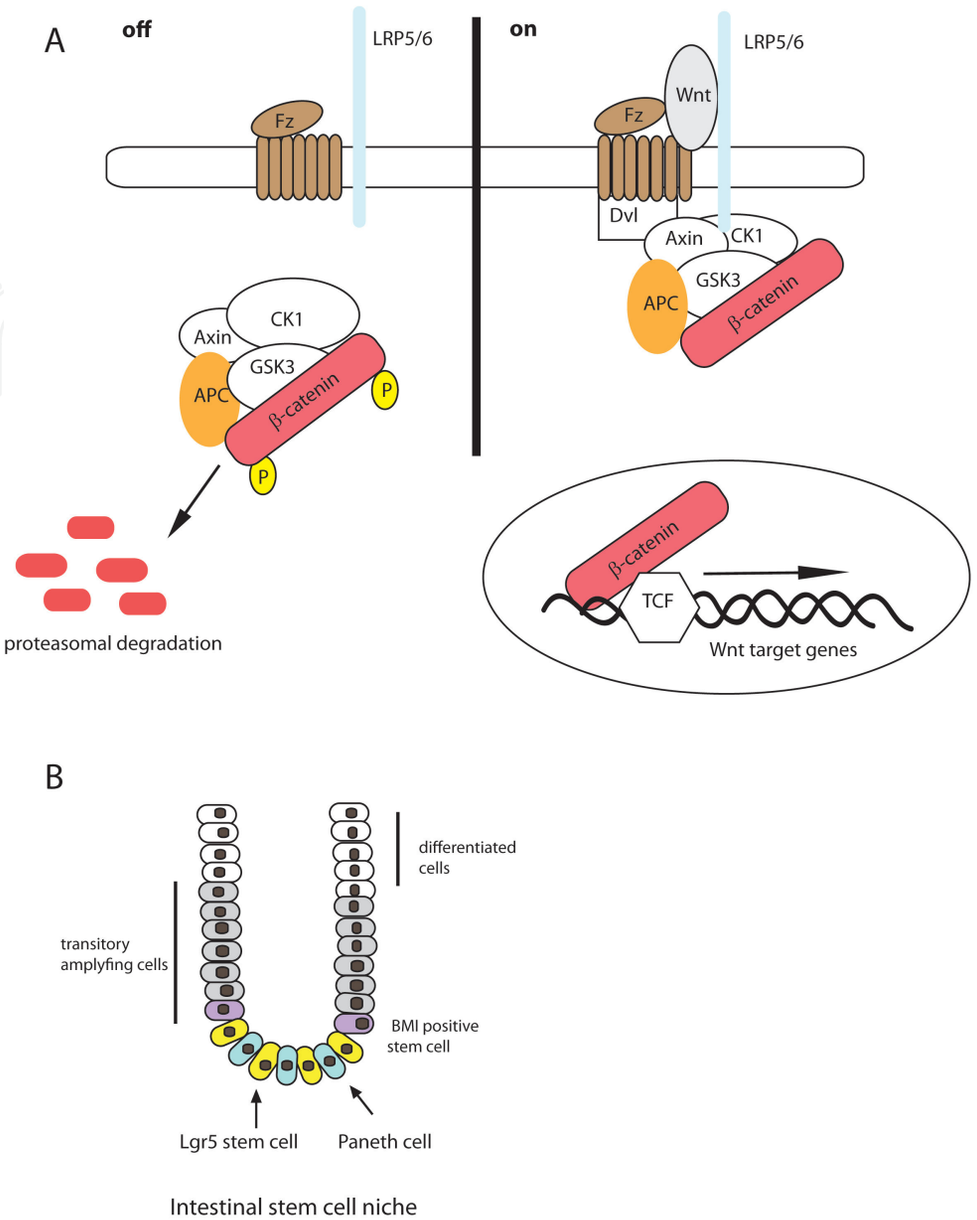

Figure 2. Paracrine regulation of the adult stem cell niche. A) Schematic representation of the cellular mechanism of β-catenin turnover in the cytoplasm. After the Wnt signal triggers dimerization of receptors, β-catenin is released from the degradation complex and translocates to the nucleus, where it activates Wnt target genes; B) Hierarchical organization of the intestinal crypt, with Lgr5+stem cells localizing to the bottom off the crypt between nurturing Paneth cells. Above the stem cells, there is a zone of intense proliferation (transitory amplifying cells), followed by terminally differentiated epithelium, which has short life span (1-2 days)

depending on the conditions during epithelial renewal. Although Lgr5 is a Wnt signaling target, crypt organization, cell fate determination and differentiation are also dependent on integration of signals from the Notch [91] and TGF-β pathways. Such paracrine signaling pathways have emerged as a common principle of functioning stem cell niches in other organs [92-95]. The inherent longevity of adult stem is a potent mechanism for dissemination of accumulated mutations within the tissue.

8. Cancer stem cells and the putative link to adult stem cells

Parallel to the discovery of adult stem cells in healthy tissues, cancer research has produced a bulk of evidence showing that most malignancies are not homogenous cell masses but rather heterogeneous tissues whose progression depends on the fitness of a distinct cellular fraction: cancer stem cells (CSCs). CSCs can confer resistance to chemotherapeutic agents or exhibit other characteristics that provide a competitive advantage to the cancer and ensure its progression [96-99]. In many cases, long-term prognosis and patient survival can be correlated with the frequency of cancer stem cells in the tumor at the time of diagnosis [100]. CSCs exhibit the characteristics of adult stem cells: self renewal and differentiation capacity. They can be distinguished from the bulk of the tumor tissue as the only fraction that is able to generate new tumors when transplanted into immunocompromised mice [101,102]. For this reason, they are sometimes referred to as tumor initiating cells. This property of cancer stem cells has been demonstrated for numerous malignancies, including ovarian and endometrial cancer. As with markers of adult stem cells, markers specifically associated with cancer stem cells are also proving difficult to identify. It is of course possible that there is no unique cancer stem cell for ovarian, endometrial or other genital malignancies and that stemness can be achieved by alternative routes in individual cases, thereby resulting in different combinations of surface markers such as CD44, EpCAM, ALDH1, CD117, CD133 etc, which have been identified by different studies [81,103,104]. Cancer stem cells are intermixed with the bulk of the tumor tissue, but are found enriched in advanced stages of metastasis, in spheres present in effusions recovered from ascites [105]. Spheres exhibit anchorage-independent growth but can efficiently adhere to mesothelial cells in the peritoneum via integrin 1, which may play a role in metastatic spread. It is not yet known what triggers formation of spheres, although the physiological implications for disease progression and response to treatment are immense. The compact organization of the cells in a cluster makes drug delivery ineffective, either due to the difficulty of drugs to penetrate, or induction of a quiescent state in cells at the core through tight cell-cell contacts, making them insensitive to agents targeting actively replicating cells. Either way, it is clear that 3D organization represents another layer of protection for cancer cells from chemotherapy. This effect has been demonstrated by comparing the response to the standard therapeutic drugs cisplatin and paclitaxel for 11 different ovarian cancer cell lines in 2D in 3D [106].

Importantly, however, it remains unknown if and how cancer stem cells are related to normal adult stem cells. Cancer stem cells may develop from adult stem cells by losing dependence on niche regulatory factors, or they may simply be the progeny of differentiated cells that have acquired "stemness" characteristics during the accumulation of mutations and cellular transformation. It is conceivable that both mechanisms could occur in all or some types of cancer. If there is indeed a causal relationship between dysregulation of the adult stem cell niche and carcinogenesis, this may open up new possibilities for early diagnosis and timely intervention. Since the research field of cancer stem cells is currently offering a variety of sometimes competing models of what defines this population in endometrial, cervical and serous ovarian cancer, we focus our attention on the current understanding of carcinogenesis in the genital tract. Understanding of the cellular events that lead to initial transformation

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could prove to be of pivotal importance for resolving remaining questions about tumor spread and the role of cancer stem cells. In this light, we will address changes in tissue architecture and physiology of the female reproductive tract that could favor malignant transformation and bring it into context of therapeutic implications, particularly in light of recent evidence that dysregulation of tissue homeostasis could be the result of infection with certain sexually transmitted pathogens.

Malignant tumors of the genital tract are the third leading cause of cancer related deaths after breast cancer and lung cancer [107]. Based on the primary organ, affected cancers are classified as ovarian, uterine, cervical, vulval and vaginal. The high prevalence of these cancers is likely to be related to the great plasticity of these tissues, their regenerative potential and thus probably their high cell turnover as well. On top of this, openness to the external environment and exposure to pathogens makes them potentially vulnerable to the transformation. The latter has been demonstrated by the dramatic correlation between HPV infection cervical cancers, since nearly 100% of patients are HPV positive [108].

9. Serous ovarian cancer and stem cells of the fallopian tube

From the perspective of patient care and long-term prognosis, one of the biggest challenges for medicine represents high grade serous ovarian cancer, an aggressive form of epithelial ovarian cancer which has a survival rate of under 40 % after 10 years [109]. This deadly malignancy takes more than 14,000 lives per year in the US alone and no improvement can be expected in the foreseeable future, due to the absence of early screening methods and the aggressive nature of the cancer. The origin of high-grade serous ovarian cancer (HG-SOC) has puzzled medical doctors and pathologists for decades. Naturally, the site of carcinogenesis was initially attributed to OSE cells [110]. Numerous examples of developmental genetics show that relatively complex changes in phenotype are frequently induced by expression of only one master regulator gene. In the case of OSE cells, ectopic expression of homeobox transcription factor HOX9 in the xenograft mouse model causes Müllerean metaplasia-transformation of cuboidal to papillary columnar epithelium resembling fallopian tube mucosa and serous ovarian cancer [111]. However, there is as yet no clinical evidence that this conversion also occurs in vivo. The scarcity of precancerous lesions or early stage carcinoma in situ detected by pathologies [112], raises questions whether this hypothesis is supported by clinical data. In parallel, the hypothesis that the etiology of HG-SOC could be explained by malignant transformation in neighboring tubal epithelium has gained increasing support. The fallopian tube develops from the Müllerian duct tissue, encompassing a columnar epithelial monolayer, which continues to express PAX8, the main cellular marker of “Müllerian differentiation" – a phenotype which is reminiscent of cancer tissue from HG-SOC patients. A breakthrough came from a cohort of clinical studies in BRCA mutation carriers, who have a very high hereditary risk of developing ovarian and breast cancer later in life. Small malignancies within the tubal epithelium (so-called serous tubal intraepithelial carcinoma – STIC) were discovered in up to 10% of patients who underwent prophylactic surgery to remove both fallopian tubes and ovaries [113]. These apparently healthy patients thus already had cancer in their fallopian

tubes, which had not yet metastasized to the ovary. Samples from HG-SOC patients subsequently confirmed that STICS were present in up to 60% of cases [114]. Although not definitive proof, these findings strongly support the hypothesis that the fallopian tube epithelium is the tissue of origin of serous ovarian cancer. Recently, malignant transformation of the fallopian epithelium into full-blown ovarian cancer has been successfully triggered in a transgenic mouse model [115]. Here, the Cre recombinase system was used under control of the PAX8 promoter to introduce mutations into Brca1/2, Tp53 and Pten, which are frequently found mutated in HG-SOC. The animals developed malignant disease with a HG-SOC phenotype, which metastasized to the ovary, peritoneum or liver – modes of spread that are also found in patients, and were for a long time considered as evidence for separate origins of the disease. Regardless of the important function that BRCA1/2-mediated DNA repair mechanisms have in fallopian tube cells, in order to solve the complex problem of HGSC etiology it is necessary to understand the role of the p53 tumor suppressor. p53 is mutated in nearly all HG-SOC patients, but somatic mutations of p53 on their own do not increase the likelihood of HGSC development [116]. Nevertheless, nuclear accumulation of p53 is much more frequent in tubes where STICs are also found, arguing that this phenotype is perhaps characteristic of a cellular state which is prone to transformation upon further "hits". However, a valid causal relationship between these cellular atypias and the appearance of fully transformed malignant serous tubal intraepithelial carcinoma (STICs) is not yet conclusively proven. Therefore, true premalignant lesions in the fallopian tube as precursors of HG-SOC are yet to be defined.

An understanding of the molecular mechanisms of epithelial homeostasis and renewal in healthy fallopian tube tissue is thus likely to be essential for successful resolution of the molecular events that lead to serous ovarian cancer. Of particular interest in this context is how slow-cycling adult stem cells in the distal fallopian tube respond to long-term changes in the tissue microenvironment from numerous different stimuli such as inflammation, genotoxic stress changes in extracellular matrix, or the presence of pathogens. The role of the microenviroment via cooperation of different cellular compartments and extracellular matrix not only provides conditions for physiologically regulated responses, such are repair and healing, but can also decisively influence the progression of disease. As discussed in the section on cancer stem cells, cancer tissue is heterogeneous and keeping in mind the monoclonal origin of cancers, it is unclear whether differentiated cells are subject to transformation followed by expansion, whether some of them are reprogrammed into cancer stem cells, or whether deregulated stem cells are the source of the malignancy after additional somatic mutations. The latter hypothesis is somewhat more likely, given the presence of cells with stem cell characteristics in tumors, and that the alternative would mean acquisition of pluripotency. However, at this stage reverse reprogramming of differentiated cells cannot be excluded as the underlying mechanism.

The same is true for endometrial cancer. In contrast to the complete absence of diagnostic tools for early detection of ovarian cancer, there is a detailed classification of neoplastic changes in the endometrium – known as endometrial intraepithelial neoplasia (EIN), although there is a lot of controversy among pathologists how reliable the existing methodology is as a prognostic factor. Moreover, there is still no consensus regarding the cellular origin of the malignant

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tissue, or indeed the role played by adult stem cells. Approximately 75-80% of endometrial cancers are classified as type 1-endometrioid endometrial cancer (EEC) – with the remaining cases belonging to papillary, mucinous or clear cell types. Histologically, they consist of malignant endometrial cells in the columnar monolayer, although squamous metaplasia is sometimes observed [117]. They frequently harbor alterations in PTEN and Wnt signaling pathways, and a prevalent staining pattern of nuclear catenin localization is found in up to 60% of endometrial hyperplasias and 30% of endometrial cancers [118]. In a mouse model with constitutively active Wnt signaling due to APC deletion in the genital tract, loss of PTEN function was found to be the rate-limiting step of carcinogenesis inside the uterus, while activation of β-catenin signaling increased the severity of the disease [119]. Strikingly, a follow-up study of the mice that were initially declared tumor free by inspection of the uterus revealed that 62% did get sendometrioid cancer, but not in the uterus, as would be expected, but rather in the neighboring distal oviduct [120]. Thus, this recent study argues that the fallopian tube may participate not only in the carcinogenesis HG-SOC but also in a subset of endometrial cancers. Notably, in human patients loss of PTEN associates with better prognosis and reduced risk of metastasis, since the tumor appears more differentiated [121]. These at first sight contradictory facts might be explained by the different requirements of cancer cells in the early and late stages of progression. While PTEN is a tumor suppressor, its major downstream effector, p-Akt, has dual functions in tumor cells. In mammary carcinoma, activated Akt enhances carcinogenesis via the ErbB-2 pathway, but inhibits invasion of the tumor and its metastasis [122]. Of course, mutations in the PTEN-PI3 kinase pathway components represent only one of the important signaling routes that have been found to be altered in endometrial cancer. Similarly, as in ovarian cancer, it remains to be seen how discrete cytological changes progress into full-blown cancer and which cells give rise to the cancer stem cells that are detected in the later stages of malignant disease. A recent study, based on a detailed analysis of 113 cases of endometrial cancer, postulated an important role for SALL4 protein which is known as a strong determinator of pluripotency in human embryonic cells [123]. The authors found SALL4 expressed in 47% of cancer samples while no expression was detected either in healthy controls or in the hyperplastic tissue, and increased levels of expression negatively influenced prognosis and patient survival. SALL4 increases c-Myc transcriptional activity and reduces the response of affected cells to carboplatin treatment. While it cannot be excluded that SALL4 expression in precancerous stages is limited to very low levels in sparse stem cells below detection limit of whole tissue sample, these results strongly indicate that pluripotent capacity of the tumor is acquired at later stages of disease development. The existence of cells with stem cell characteristics has recently also been demonstrated for cervical cancer. Sox2-expressing cells constitute a small percentage of cells in cervical cancer cell lines, but they show much greater tumor forming capacity in the xenograft mouse model than the remaining cells [124]. This correlates well with findings of tracing experiments from the Sox2 mouse model mentioned above, which identified stem cell lineages in the cervix [125]. It is becoming increasingly clear that the basal layer of cervical epithelium plays a decisive role in the initiation of cervical cancer, as these are the only dividing cells in squamous stratified epithelium. Active progression of the cell cycle is a prerequisite for HPV-driven cellular transformation, as host cells have to pass through the prophase of mitosis for transcription of viral genes to be initiated

and infection established [126]. Cervical cancer develops from premalignant lesions called cervical intraepithelial neoplasia (CIN), which are routinely detected by regular Pap smears. Importantly, CINs occur almost exclusively within the transformation zone of the cervix, a region of metaplastic conversion of columnar to squamous epithelium. Regular screening has greatly improved the long-term prognosis and survival rates of cervical cancer [127].

The mounting evidence for an involvement of the fallopian tube in development of ovarian and potentially other cancers thus highlights the importance of developing methodologies to improve diagnostic sampling and visualization of this organ. In particular, there is an imperative to improve diagnostics of the tube in patients. Ideally, some kind of endoscopy would enable detailed exploration of the mucosa in vivo, including the taking of biopsies. This would improve our understanding of phenotypical changes that take place in the epithelium, better define and categorize alterations and discover real premalignant lesions or early malignancies. The result would hopefully be comparable to the advancement that routine colonoscopy brought to early diagnosis and proper management of colon cancer.

10. Conclusion

We have presented in detail the current "state of the art" in the field of adult stem cells of the female reproductive system, their role in maintaining healthy mucosal tissue and changes that occur during disease. Moreover, we have focused on the phenomenon of cancer stem cells, which appears to be very important for the progression of malignancies by giving tumor tissue a competitive advantage. Still, the question remains how adult stem cells relate to cancer stem cells, and the models which have been postulated so far, as in the case of cervical cancer, have yet to be experimentally proven.

Regardless of the final outcome, it is almost certain that adult stem cells play an important role in the process of cancer initiation. Carcinogenesis is considered to be a long, stepwise process of accumulation of mutations. Therefore, differentiated cells with short life spans are unlikely to pass on mutations unless the initial acquired change leads to immortalization. Adult stem cells on the other hand, are long-lived and thereby inevitably accumulate mutations over decades. With each asymmetric division, mutations are passed to the differentiating progeny. The final "transformation step" may occur afterwards in the differentiated cell, or the stem cell itself may reach the stage where it becomes cancerous. The latter model presumes that as tumor tissue grows, the stem cell continues to give rise to differentiated progeny, while becoming itself a cancer stem cell. In both cases, a definitive premalignant molecular fingerprint should be found in the original adult stem cell. Therefore, it will be of great importance to further characterize adult stem cells in the ovary, fallopian tube and uterus and elucidate the molecular mechanisms of epithelial renewal in healthy tissue, but also to determine the genomic changes which occur over time in response to altered hormonal stimulation, tissue injury or infection.

Recent studies with novel genetic lineage tracing tools in the mouse give a promising outlook that concrete molecular pathways controlling stemness will be defined in the near future. Only when this goal has been achieved is it conceivable that a major breakthrough in the early

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treatment and diagnosis of female reproductive cancers and endometriosis can be achieved. As we have outlined, the high activity of adult stem cells in the female reproductive tract that is required to maintain cyclical changes in tissue architecture put these tissues at a particularly high risk for accumulating mutations with the potential for transformation. This is likely to be exacerbated by the fact that the genital system is exposed to a variety of sexually transmitted pathogens. There is mounting evidence that these infections may play a role in initiation of malignancies in the ovary, uterus or as co-factors to HPV in the cervix [18-20]. Although deciphering the behavior of adult stem cells in disease remains a very challenging research area, a dynamic field of translational approaches has emerged, using stem cells as a source of healthy tissue in different models of in vitro differentiation and transplantation. There is justified optimism that such models will help to elucidate not only the events involved in de-regulation of the adult stem cell niche, but also the interaction of human pathogens with this niche, as well as the somatic tissue derived from it. A more complete understanding of these events will provide a basis for the development of more effective preventative and therapeutic measures for diseases of the female reproductive tract.

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