Stem cell potential of the mammalian gonad

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1. ABSTRACT

Stem cells have enormous potential for therapeutic application because of their ability to self-renew and differentiate into different cell types. Gonads, which consist of somatic cells and germ cells, are the only organs capable of transmitting genetic materials to the offspring. Germ-line stem cells and somatic stem cells have been found in the testis; however, the presence of stem cells in the ovary remains controversial. In this review, we discuss studies focusing on whether stem cell properties are present in the different cell types of male and female gonads and their implications on stem cell research.

2. INTRODUCTION

Stem cells are characterized by their self-renewal potential and ability to differentiate into various cell lineages (pluripotency or multipotency) or one specific cell lineage (unipotency). Stem cells can be obtained directly from organs or induced to exhibit stem cell properties in culture. Based on the origins of the stem cells, at least three major types of stem cells have been categorized: embryonic stem (ES) cells, germ-line-derived stem cells, and adult somatic stem cells. ES cells, which are derived from the inner cell mass of blastocysts, are innately pluripotent with the capacity to differentiate into cells of all three germ
layers. Germ-line-derived stem cells can be induced either from embryonic primordial germ cells or from spermatogonial stem cells (SSCs) of the neonate, juvenile or adult testes. While SSCs are normally unipotent when they reside within the seminiferous epithelium, they can be induced to pluripotency in vitro or after transplantation into a different niche microenvironment (1). Adult somatic stem cells, also known as non-embryonic somatic stem cells, are obtained from adult tissues such as bone marrow and their differentiation ability (multipotency) is more restricted as compared to ES cells. Recently, a novel source of pluripotent stem cells (iPS) was established by reprogramming fibroblasts or other somatic cells to pluripotency through ectopic expression of defined transcription factors (2-6). In this review, we focus specifically on whether different cell types of the gonads have stem cell properties and the implication of these findings for stem cell research.

The gonadal primordium (or genital ridge) is the only primordium that has the potential to differentiate into two distinct organs (testis or ovary). In mammals, presence or absence of the Sry gene (Sex-determining gene of the Y chromosome) activates and/or inhibits molecular programs in the gonadal primordium, directing the differentiation program toward testis organization (7). The sex-determining programs operate mainly in the somatic cell lineages in the gonadal primordium and the somatic environment eventually decides the developmental fate of primordial germ cells (PGCs). In mouse embryos, epiblast-derived PGCs migrate through the hindgut into the gonadal primordium between embryonic day 9-11.5 or E9-11.5 (8, 9). Meanwhile, the coelomic epithelium surrounding the gonadal primordium starts to thicken and, under the control of a network of transcription factors, precursors of somatic cell lineages arise (10). In the male gonad, under the control of Sry, somatic cell precursors differentiate into Sertoli cells, Leydig cells, and peritubular myoid cells. On the other hand in the ovary, granulosa and theca cell lineages appear as the ovarian follicles start to assemble. PGCs and spermatogonia in testes have stem cell properties and are able to differentiate into other cell types under certain conditions (11-18). We will discuss whether other cell types in the gonads also possess such ability.

3. STEM CELL POTENTIAL OF GERM CELLS

Three types of germ-line derived stem cells have been identified and isolated: embryonal carcinoma (EC) cells, embryonic germ (EG) cells, and spermatogonial stem cells (SSCs) from neonate and adult testes (19). EC cells are derived from adult testicular teratocarcinomas (or mixed germ cell tumors), which are gonadal tumors containing tissues from the three germ layers. Information of EC can be found in other reviews (20) and is not the focus of this review. The following discussion is focused on EG cells and spermatogonial stem cells.

3.1. Primordial germ cells

Primordial germ cells (PGCs), the progenitors of the germ cell lineage in both ovary and testis, are the only cell type capable of transmitting genetic materials from generation to generation. In mouse embryos as early as E6.25, PGC precursors with germ-line competence can be identified by the expression of B-lymphocytes-induced maturation protein 1 (Blimp1) in a founder population of epiblast cells (21). Via interaction of several transforming growth factor beta (TGF-beta) family members such as bone morphogenetic protein 4 (BMP4) and BMP8b from the extra-embryonic ectoderm, a cluster of PGC precursors arise from the proximal epiblast adjacent to the extra-embryonic ectoderm around E7 in mouse embryos (22-25).

By E7.2, a cluster of ~50 PGCs are found posterior to the primitive streak at the base of the allantois (26). PGCs then migrate through the hindgut and dorsal mesentery by E8.5 and eventually enter the gonadal primordium by E10.5. On their way to the gonads, PGCs undergo proliferation and the number of germ cells increases from about 50 cells on E7 to more than 25,000 cells by E13.5 (27). PGCs in male and female gonads are indistinguishable until days after they settle in the gonadal primordium (are now called gonocytes). In the mouse fetal ovary, gonocytes enter meiosis I between E13.5-14.5 regulated by retinoic acid, Star8, and Dazl (28, 29) and then arrest at the prophase of the first meiotic division. In the fetal testis, however, gonocytes do not enter meiosis as a result of lack of retinoic acid signaling. Gonocytes in the fetal testis continue proliferating until around E15.5 and then arrest in the G0 phase of cell cycle until shortly after birth (Figure 1A).

PGCs can be isolated from developmental stages such as before and during migration (E8-9.5) and after entry into the gonadal primordium (E11.5 and 12.5). In the presence of feeder cell layer and certain growth factors such as bFGF (basic fibroblast growth factor), LIF (leukemia inhibitory factor), and Steel factor, isolated PGCs can be maintained in culture and proliferate indefinitely (11, 30). Eventually these cells develop into colonies of EG cells with characteristics similar to those found in ES cells. Like ES cells, EG cells are pluripotent and immortal and can differentiate into various cell types (11, 30). EG cells also can differentiate into the germ line (31). Because of their germline origin, EG cells may undergo epigenetic changes similar to those seen in the germ cell lineage, such as demethylation of maternal or paternal alleles, making them unique and different from the ES cells (32).

3.2. Spermatogonial stem cells in prepubertal and adult testes

After the PGCs have reached the gonadal primordium of the developing male embryo, they stop proliferating and remain mitotically quiescent within the testis cords. Shortly after birth they resume mitosis and begin migrating toward the basement membrane, contributing to the reorganization of the seminiferous epithelium (33). In the mouse, the gonocytes reach the basement membrane around day 4-5 after birth, and are then called A single spermatogonia (34). Upon division, the A single Spermatogonia produce either identical daughter cells (self-renewal) or daughter cells that are linked by an intercellular bridge. The latter are called A paired spermatogonia and have entered the germ cell
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differentiation process that ultimately leads to the formation of mature sperm. A paired spermatogonia divide further to form chains of A single spermatogonia, which further divide to form A1 to A4 spermatogonia, type B spermatogonia, and, finally spermatocytes that will complete meiosis and further become haploid spermatids and sperm. A single spermatogonia have been regarded as the putative spermatogonial stem cells (SSCs) for decades and have been studied in whole mount preparations and by electron microscopy (35-37). However, distinguishing them from the other undifferentiated spermatogonia only by their morphology has been challenging.

In 1994, Brinster and Zimmerman first demonstrated the presence of stem cells in the undifferentiated spermatogonia populations (12). They mechanically and enzymatically dissociated seminiferous tubules from neonatal and prepubertal mouse carrying LacZ transgene and microinjected the cell suspensions into adult wild type recipient testes devoid of germ cells. The donor cells colonized the recipient seminiferous tubules and restored spermatogenesis (12). The donor cells gave rise to mature sperm which were able to fertilize oocytes and produce offspring (12). Although these experiments demonstrated the presence of stem cells in the undifferentiated spermatogonia population, the identification of the true spermatogonial stem cells remains elusive as a result of lack of specific molecular markers. It has been demonstrated that spermatogonia expressing the membrane receptor GFRalpha1, such as A single and some A paired spermatogonia, are more likely to colonize seminiferous tubules and restore spermatogenesis (38). In addition, transgenic mice over-expressing glial cell line-derived neurotrophic factor (GDNF), the ligand for GFRalp1, show an increase in the self-renewal of spermatogonial stem cells in their testes (39).

It was previously thought that the putative SSCs are unipotent and can only differentiate into sperm. In 2003, the group of T. Shinohara demonstrated that if SSC's from neonatal mice are cultured in a defined medium specific for hematopoietic stem cells and supplemented with specific growth factors including GDNF, SSCs can self-renew for months in culture while retaining they potential to differentiate into sperm (40). Moreover, under this culture condition, some SSCs formed colonies that appear similar to ES cells colonies, and could be subsequently maintained in standard ES cell culture conditions containing LIF (14). These cells were subsequently shown to produce derivatives of the three embryonic germ layers upon differentiation in specific conditions (14). Results obtained by Guan et al and Seandel et al also showed that ES-like cells can also derive from the testes of juvenile and adult mice (15, 32). When these ES-like cells are derived from adult testes, they express markers of pluripotency such as Oct4, Nanog, SSEA-1 and alkaline phosphatase in vitro, and form teratomas after being transplanted into immunodeficient mice. SSCs from adult testes are able to form embryoid bodies upon LIF withdrawal and can also differentiate into derivatives of the three germ layers (41). SSCs from adult testes can also be induced to differentiate into functional neurons and glia in the presence of noggin, which blocks endogenous BMP signaling and inhibits mesodermal differentiation, eventually leading to neural induction (42). More recently, it was demonstrated that human SSCs also transdifferentiate into cells belonging to the three embryonic germ layers (17, 18). These results demonstrate that the SSC's possess pluripotency in contrast to what was originally believed.

3.3. Germinal stem cells in adult ovaries

In contrast to testes that are capable of producing sperm throughout the postpubertal life of the male, mammalian ovaries only produce a finite number of eggs. Once settled in the gonadal primordium, female germ cells enter meiosis and arrest at prophase of meiosis I, which will not resume until the time of ovulation when the female reaches sexual maturity. The number of female germ cells or oocytes in the ovary reaches its peak in fetal life and declines in late pregnancy and after birth. Whether the numbers of female germ cells are fixed in fetal life or female germ cells retain self renewal ability in adult life just like spermatogonial stem cells has always been in the center of debate. In the 1920s, it was believed that the ovary is endowed with a fixed number of oocytes. This view was challenged by Allen in 1923 who supported the theory that the formation of oocytes continued throughout reproductive life (43). Allen proposed that cyclic proliferation of the germinal epithelium gives rise to oocytes. This became a widely held view until the 1950s when Zuckerman, by performing intensive counting of follicles, demonstrated that oocytes were not produced throughout the adult postpubertal lifespan in most mammals (44), suggesting that germline stem cells are not present in adult ovaries. Mitotically active oocytes are found in some species of prosimian primates; however, whether these proliferating female germ cells contribute to the oocyte pool and folliculogenesis remains to be determined (45-48). As a result, the idea that the female reproductive life span is determined by a non-renewing pool of oocytes produced during fetal development has become the central dogma of female reproduction (49).

In 2004, Johnson et al. reported that the ovarian surface epithelium of adult mice is a source of proliferating germinal stem cells, which are able to sustain postnatal follicular renewal (50). In a subsequent paper, Johnson et al. further demonstrated that bone marrow or peripheral blood serves as reservoirs of putative stem cells for germ cell regeneration in the adult mouse ovary (51). In addition, Lee et al. demonstrated that bone marrow transplantation could generate immature oocytes and rescue long-term fertility in chemotherapy-treated female mice, suggesting the existence of the cells with germine potential in the bone marrow of adult mammals (52). Specifically, Bukovsky et al. proposed that the pool of primary follicles in adult human ovaries represents a dynamic population of differentiating and regressing structures in which primitive granulosa and germ cells differentiate from the bipotential mesenchymal cell precursors of tunica albuginea in adult human ovaries (53). Virant-Klun et al. (2008) demonstrated that small round cells expressed early ES cell markers in the ovarian surface epithelium of adult postmenopausal women and in women with premature ovarian failure (54).
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These results suggest the possibility of extra-ovarian and intra-ovarian sources of stem cells that contribute to female germ line formation and renewal in adulthood.

However, like other paradigm-shifting discoveries, the findings of germ line stem cells in the adult ovary have been extensively scrutinized (57-61). Most of the criticisms derive from concerns on histological quantitative measurements, fixation artifact, and others. The extra-ovarian source of female germ line stem cells was further examined by Eggan et al. (48) using parabiotic mouse pairs, which were created by joining wild-type mice with transgenic animals that ubiquitously expressed GFP (green fluorescent protein). Although a common circulatory system was established between the wild type and GFP animals, none of the oocytes collected from wild type partners expressed GFP, indicating a lack of contribution of blood cells or bone marrow stem cells from the GFP parabiotics. In addition, combined chemotherapy and radiotherapy fully depleted oocyte reserves, and transplantation of bone marrow cells did not restore fertility in these mice (60). In a separate study, Liu et al. evaluated the possibility of neo-oogenesis in adult human ovaries and found no evidence of the presence of proliferating germ cells (61). These results are in favor of the theory that neo-oogenesis via self-renewal of extra- or intra-ovarian germline stem cells are unlikely in the adult ovary.

4. STEM CELL POTENTIAL OF SOMATIC CELLS IN GONADS

3.1. Somatic stem cells in testes

In contrast to spermatogonia, which undergo proliferation and differentiation in adulthood, somatic cells in the testis are relatively quiescent. The three major somatic cell types, Sertoli cells, Leydig cells, and peritubular myoid cells, are established in fetal life (Figure 1B) and once the expansion of their populations is completed in the neonatal period, proliferation of these cells is seldom observed in sexually mature individuals. Sertoli cells, the only cells that express the Y-linked Sry gene in the testis, are considered as terminally differentiated postmitotic cells (62). Trans-differentiation of Sertoli cells into other cell types has never been found when Sertoli cells were transplanted into other tissues. For example, when Sertoli cells are transplanted into brain, they maintain their morphological characteristics (63). Peritubular myoid cells that exhibit smooth muscle cell properties are also terminally differentiated cells. To date, the presence of stem cells for Sertoli cells and peritubular myoid cells has yet to be proven. Stem cells for Leydig cells, on the other hand, have been identified in adult rodent testis. Leydig cells, which reside in the interstitium of testes, produce androgens that are essential for the establishment of male characteristics and spermatogenesis. In rodent models where adult Leydig cells were abolished by ethane-dimethanesulfonate (EDS) treatment, a new population of Leydig cell replenished the testis interstitium and restored androgen production (64). Spindle-shaped interstitial cells, which are negative for luteinizing hormone receptor (LHR) but positive for platelet-derived growth factor receptor alpha (PDGFRα), appear to be the source of stem cells for adult Leydig cells (65). When these spindle-shaped LHR-negative- PDGFRα-positive cells were injected into Leydig cell-depleted testes, they populated the testis interstitium and expressed steroidogenic markers for adult Leydig cells. In addition to the spindle-shaped interstitial cells, perivascular and peritubular smooth muscle cells and interstitial cells with low nuclear Hoechst dye uptake were proposed as putative stem cells for adult Leydig cells (66-68). It was reported that nestin-positive perivascular smooth muscle cells underwent proliferation, protruded from the vessel wall, and then transformed into adult Leydig cells in EDS treated rats (67). On the other hand, Leo et al. identified an interstitial cell population, which has low Hoechst nuclear uptake similar to the side population (SP) of hematopoietic stem cells (see Storms et al., Blood, 2000) (69). When these cells with low Hoechst nuclear uptake were transplanted into LHR knockout testis where Leydig cells were depleted, testosterone production was restored (68). It remains to be determined whether the spindle-shaped interstitial cells, perivascular smooth muscle cells, and low Hoechst interstitial cells belong to the same stem cell population or they represent different stages of differentiation of a common stem cell. The possibility that multiple sources of stem cells contribute to the regeneration of adult Leydig cells cannot be excluded.

3.2. Stem cell potential of somatic cell in adult ovary

Adult ovaries undergo dramatic cyclic or seasonal (in some animals) changes. In female rodents, for example, ovaries go through a cyclic morphological and cellular transformation every 4-5 days. Granulosa and theca cells, the somatic cells in the ovary, proliferate, differentiate, and regress in response to cyclic changes in hormone levels. To accommodate this dramatic turnover of cells, one would assume that the somatic cells in the ovary must maintain the potential to self-renew while providing differentiated cells to sustain follicle formation. However, this idea of “neogenesis” of ovary is not supported by the structure of the ovary. As mentioned previously in this review, the adult ovary only contains a finite number of oocytes, which are enclosed in the follicle surrounded by granulosa and theca cells at the time of birth. In each reproductive cycle, only a set number of the follicles will be selected to differentiate and eventually being ovulated. The cyclic changes of the ovary could be merely a consequence of growth of the selected follicles instead of a generation of new follicles.

The stem cells for granulosa cells, the somatic cell type supporting follicle development, have yet to be identified. However, granulosa cells are not terminally differentiated and have the plasticity to develop into at least two different other somatic cell types: the luteal cells and Sertoli cells. After ovulation, estrogen-producing granulosa cells are transformed into luteal cells which produce progesterone to maintain pregnancy (70). When pregnancy does not occur or when its function is replaced by the developing placenta, luteal cells then degenerate. Granulosa cells in the preovulatory follicles also undergo spontaneous luteinization when oocytes were removed prematurely, indicating that the oocyte could prevent the transformation of granulosa cells into luteal cells (71).
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Figure 1. Establishment of cell lineages in gonads and their stem cell potential. (A) Stem cell potential of germ cells. Primordial germ cells or PGCs, the progenitor cells of male and female germ cells, can be coaxed to differentiate into embryonic germ (EG) cells in culture in the presence of LIF, bFGF, and Steel factor. EG cells, which exhibit characteristics similar to embryonic stem cells, are immortal and pluripotent. Days after PGCs migrate to the gonadal primordium, the fates of female and male germ cells become separated when female germ cells enter meiosis whereas male germ cell arrest at mitosis. Male germ cells become mitotically active until puberty and spermatogonial stem cells (SSCs) are maintained for the rest of the reproductive life. SSCs not only differentiate into sperm, but also show pluripotent ability similar to embryonic stem cells in specific culture environments. On the other hand in the ovary, female germ cells are enclosed in the follicle and surrounded by granulosa cells, and the numbers of follicles are set at the time of birth. Research is still needed to conclusively demonstrate the presence of ovarian germ-line stem cells and their differentiation ability. (B) Stem cell potential of somatic cells. Somatic cells in the testis and ovary derive from common gonadal precursor cells. In the presence of the Y chromosome or the Sry gene, the differentiation of Sertoli cells will be activated, which is followed by the appearance of other somatic cell types such as Leydig cells and peritubular myoid cells (PMCs). The stem cell populations for Sertoli cells and PMCs have yet to be identified. Adult Leydig cells, which have cellular characteristics different from their fetal counterparts, maintain a stem cell population. In the ovary, somatic cell precursors differentiate into granulosa and theca cells in the absence of the Y chromosome or Sry gene. Whether granulosa and theca cells derive from the same fetal progenitor’s population remains to be determined. In the adult ovary, putative theca cell stem cells have been identified. Although the presence of granulosa stem cells is not known, granulosa cells have the ability to transdifferentiate into Sertoli cells particularly in the absence of estrogen signaling. Granulosa and theca cells both differentiate into luteal cells after ovulation.

Granulosa cells can also transdifferentiate into Sertoli cells under certain circumstances. The most intriguing examples are the estrogen receptors alpha and beta double knockout (ER-alpha and –beta double KO) and aromatase knockout mice (72-77). When these knockout mice reach adulthood, seminiferous tubules and Sertoli cells start to appear in the ovary. Apparently the lack of estrogen and its signaling pathway allows the emergence of Sertoli cells, probably through transdifferentiation of granulosa cells. It is known that in nonmammalian vertebrates such as reptile and birds, estrogen, the product of aromatase activity, is responsible for the formation of the ovary and appearance of ovarian somatic cells such as granulosa cells (78). When estrogen synthesis or its action in chick or reptile embryos is inhibited, gonadal primordium takes on testicular fate and Sertoli cells and testis structure emerge. We speculate that during evolution this estrogen-sensitive mode of ovary determination in nonmammalian species has been overridden in eutherian mammals where pregnancy occurs. In the ERαβKO and
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Aromatase knockout mice, the ovary forms properly during embryogenesis and the transdifferentiation of granulosa cells to Sertoli cells did not occur until adulthood. These observations indicate that only adult granulosa cells but not their fetal counterparts have the potency to transdifferentiate in the absence of estrogen.

Theca cells, another somatic cell type in the ovarian follicle, have androgen-producing ability and cellular and molecular characteristics similar to adult Leydig cells in the testis. Theca cells are located in the ovarian interstitium and are believed to derive from fibroblast-like precursors in the mesenchyme. Putative stem cells for theca cells have been found in the neonatal mouse ovary (79). Theca stem cells, which have fibroblast morphology, can differentiate into androgen-producing cells in response to proper hormone and growth factor stimulation. Most importantly, when injected into ovaries, putative theca stem cells colonized the ovarian interstitium and became part of the theca layer of the follicles. Similar to granulosa cells, theca cells differentiated into luteal cells after ovulation at least in ruminant species. However, the molecular mechanism for this transition has yet to be thoroughly understood.

5. PERSPECTIVE

The identification of mesenchymal stem cells in many adult tissues such as the cardiovascular system, adipose tissue, skin, and bone marrow raises the possibility that such tissue-specific stem cells are present in most organs. In contrast to ES and EG cells, which are pluripotent, mesenchymal stem cells are multipotent and have the potential to develop only into certain cell types. In the gonads, germline-derived stem cells such as PGCs and SSCs are pluripotent. Somatic cell-derived stem cells such as Leydig and theca stem cells are able to give rise to androgen-producing cell types but their potential to differentiate into other cell types remains to be determined.

The differentiation potential of stem cells has raised the hope of using these cells for regenerative therapy or treatment for genetic disorders. The ethical concerns of embryo-derived ES cells have reduced the enthusiasm, but at the same time triggered new incentives to search for stem cells in adult organs. The identification of stem cells in gonads will certainly broaden the options for treatment of reproductive disorders such as infertility, and at the same time will provide potential alternatives to ES cells.

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Abbreviations: Blimp1: B-lymphocytes-induced maturation protein1; PGCs: primordial germ cells; EC: embryonal carcinoma cells; EG: embryonic germ cells; ERalpha-beta KO: estrogen receptors alpha and beta double knockout; BMP4: Bone morphogenetic protein 4; bFGF: basic fibroblast growth factor; LIF: leukemia inhibitory factor; SP: side population; GDNF: glial cell line-derived neurotrophic factor; EDS: ethane-dimethanesulfonate; LHR: luteinizing hormone receptor; PDGFRalpha: platelet-derived growth factor receptor alpha; SSCs: spermatogonial stem cells; TGFbeta: Transforming growth factor beta.

Key Words: Stem cell, Gonad, Germ Cells, Spermatogonia, Oocyte, Sertoli Cells, Leydig Cells, Granulosa Cells, Theca Cells, Review

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