How are germ cells produced and what factors control their production?

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With every heartbeat, a man produces ~1,000 sperm. At this rate, 72,000 sperm are generated in a minute, over 100 million in a day, and 3 billion in a month. Furthermore, male gametogenesis (spermatogenesis) continues for almost the entire life. Thus unlike women, men can father genetic children naturally at more advanced age beyond 60 yrs. How does the male reproductive system achieve this remarkable functionality?

The key to understand the biological mechanism of spermatogenesis is to understand the nature and function of stem cells. Indeed, other self-renewing tissues in our body that are able to replenish and maintain the structure and function of different organs rely on proper functioning of stem cells. Examples include our hematopoietic system, skin and intestinal epithelium. We have over 10 trillion erythrocytes in our blood circulation at any given time of our life despite a limited life-span of 120 days for each cell. Similarly, cells of the skin and intestinal epithelia die and slough off constantly. Yet these epithelia remain functional for a lifetime. As such, in these systems (so-called self-renewing tissues), cells are continuously lost but replenished to maintain the structure and function of an organ throughout life. This is made possible by stem cells, and spermatogenesis is one such self-renewing system supported by stem cells.

Stem cells of spermatogenesis are called spermatogonial stem cells (SSCs). They are part of the spermatogonia (diploid male germ cells). During steady-state spermatogenesis, one SSC divides into two cells: one remains as a stem cell (self-renewal) and the other differentiates to eventually become spermatozoa (differentiation) (FIG. 1). The one that has self-renewed is re-incorporated in the stem cell pool while the one that has taken a differentiation path is considered “lost”, as it leaves the testis for the reproductive tract after becoming spermatozoa. How an SSC chooses which path to take, a phenomenon termed “fate decision”, is currently the focus of intensive research.

SSCs are a sub-population of spermatogonia; thus, they are found within the seminiferous tubules, residing on the basal membrane and surrounded by Sertoli cells. In general, a microenvironment that contains stem cells is called a stem cell “niche”; it supports and regulates stem cells. The SSC niche is composed of Sertoli cells, the basal membrane, and daughter germ cells that SSCs have produced. Does the niche affect SSC activity? Although there is no strong evidence that hormones, e.g., FSH, LH, testosterone,

directly control SSCs, it is known that Sertoli cells secrete a soluble protein factor, GDNF (glial-cell-line-derived neurotrophic factor), that is important for SSCs to self-renew. If a functioning GDNF gene is found only in one allele in a mouse, SSC numbers become significantly reduced. In the preparation of an SCC culture, GDNF is an indispensable factor to maintain self-renewal of SSCs. Additional stimulators of SSC self-renewal include FGF2 from Sertoli cells and CSF1 (colony-stimulating factor 1) from Leydig cells and select peri-tubular myoid cells. Most investigators believe that there exist additional factors that can control and regulate SSC activity. More players of SSC fate regulation are expected to be identified in the future.

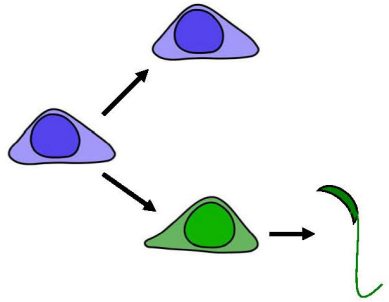

FIG. 1. **Self-renewal and differentiation of SSCs.** SSCs self-renew to maintain a stem cell pool. They can also commit to differentiation to produce sperm. Blue = SSCs. Green = committed male germ cells.

To maintain spermatogenesis at a steady state for daily sperm production, the input (stem cell differentiation) and output (the loss of spermatozoa from the testis) need to be balanced. It is known, however, that SSCs are very few (0.01 – 0.02 % in an adult mouse testis) and divide very infrequently. To compensate for these short-comings of SCCs, it is assumed that SCCs produce a subpopulation of spermatogonia termed “amplifying compartment” that divide more frequently to expand the size of the population.

SSCs are defined functionally by their ability to continuously self-renew and differentiate. In other words, they cannot be identified convincingly by morphology or biochemical / molecular properties at present. As SCCs cannot be visualized microscopically, the detection of SSCs requires a bio-assay that measures the stem cell function of self-renewal and differentiation. Spermatogonial transplantation is an example of such a bio-assay and has been developed using the mouse (FIG. 2). In this technique, cells derived from a donor testis are injected into the seminiferous tubules of sterile

recipients (no germ cells in their testes). Two months later, donor SSCs regenerate spermatogenesis in segments along the recipient seminiferous tubules. The regeneration of donor-derived spermatogenesis implies that there were cells in the donor testis cell preparation that were able to self-renew and differentiate for a long time. Since these are the activities unique to stem cells, successful regeneration and maintenance of spermatogenesis indicate the presence of SSCs in donor cells. This assay is analogous to the detection of gravity; gravity is invisible, but we can detect its presence through experimental intervention by dropping an object in the air. A problem of this SSC assay is that it detects SSCs only retrospectively and does not allow us to determine which cells are SSCs before transplantation. Therefore, researchers are now actively searching for marker molecules expressed specifically by SSCs to identify these cells prospectively; indeed, some markers have been discovered.

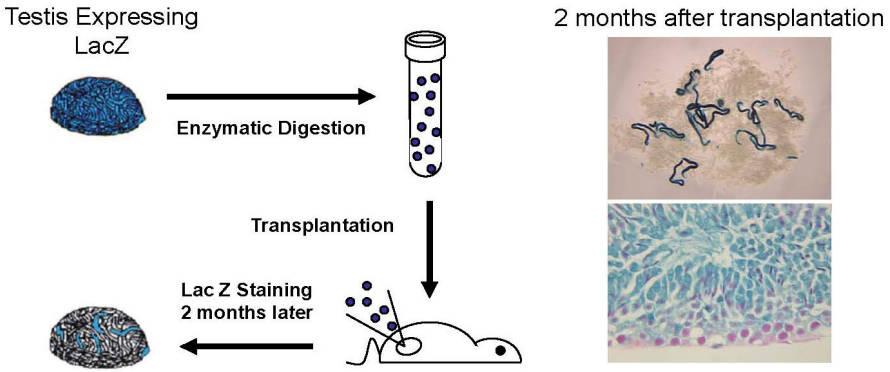

FIG. 2. **Spermatogonial transplantation.** The left side of the figure shows the transplantation procedure. The donor testis is derived from a transgenic mouse carrying the bacterial LacZ gene. The donor testis is digested into single cells using enzymes. These cells are injected into the seminiferous tubules of a recipient mouse that is infertile. Two months later, recipient testes are harvested and reacted with LacZ substrate, which makes donor-derived cells blue. As show in the right panels, SSCs derived from the donor testis regenerate spermatogenesis in the form of blue segments along the recipient seminiferous tubules, which is visualized by the LacZ reaction (blue). Each segment is made of fully regenerated spermatogenesis that the donor SSCs produced.

SSCs are expected to be a powerful resource to restore male fertility in the future. For instance, cancer treatment with chemotherapy is toxic to male

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germ cells including SSCs. Thus, even if the cancer is cured, the patient may lose his fertility. This treatment complication is of great concern for prepubertal boys who have not started spermatogenesis and therefore cannot benefit from sperm banking. Since SSCs exist from the time of birth, SSCs can be harvested from the testes of a patient at any age to be cryopreserved before chemotoxic therapy. When the patient is ready to have his own child, cryopreserved SSCs may be thawed and transplanted back into his testes. The transplanted SSCs, which were harvested before exposure to chemotherapeutic drugs, will regenerate spermatogenesis and produce sperm, making it possible to achieve conceptions through natural intercourse or with the use of assisted reproductive technologies. Further studies on SCC biology may allow this fertility preservation strategy to become a reality in the near future.

Suggested reading

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