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Review article

From stem cells to germ cells and from germ cells to stem cells

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Abstract

Germline and somatic stem cells are distinct types of stem cells that are dedicated to reproduction and somatic tissue regeneration, respectively. Germline stem cells (GSCs), which can self-renew and generate gametes, are unique stem cells in that they are solely dedicated to transmit genetic information from generation to generation. We developed a strategy for the establishment of germline stem cell lines from embryonic stem cells (ES). These cells are able to undergo meiosis, generate haploid male gametes in vitro and are functional, as shown by fertilization after intra-cytoplasmic injection into mouse oocytes. In other approach, we show that bone marrow stem (BMS) cells are able to trans-differentiate into male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells. The ability to derive male germ cells from ES and BMS cells reveals novel aspects of germ cell development and opens the possibilities for use of these cells in reproductive medicine. Conversely, we showed that adult male germline stem cells, spermatogonial stem cells (SSCs), can be converted into embryonic stem cell like cells which can differentiate into the somatic stem cells of three germ layers. Understanding how SSC can give rise to pluripotent stem cells and how somatic stem cells differentiate into germ cells could give significant insights into the regulation of developmental totipotency as well as having important implications for male fertility and regenerative medicine.

***Key words**: Stem cells, Germ cells, Bone Marrow stem cells, Spermatognial stem cells.*

Germline stem cells

Spermatogenesis depends on stem cells, which have the ability to self-renew and generate a large number of differentiated germ cells in most species. In mammals, millions of spermatozoa are produced every day from spermatogonial stem cells (SSCs), the germ-line stem cells in the testis. Although SSCs are infrequent in the testis, presumably ~1 in 3,000 – 4,000 cells in adult mouse testis, SSCs can be identified unequivocally by a functional transplantation assay. To maintain spermatogenesis, the processes of self-renewal and differentiation of SSCs must be precisely regulated by intrinsic gene

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expression in the stem cells and extrinsic signals, including soluble factors or adhesion molecules from the surrounding microenvironment, the stem cell niche. We identified and characterized several genes playing essential role during germ cell development (1-5).

Spermatogonial stem cells are descendants of the primordial germ cells (PGCs), which migrate from extra-embryonic sites to colonize the gonadal ridge early during embryonic life. In the females, the primordial germ cells proliferate extensively and enter meiotic prophase at around the time of birth. In males, cessation of germ cell proliferation and blockade of meiotic entry are important steps in the morphogenetic cascade initiated by the expression of the SRY gene. The male germ cells associate with somatic cells of the presumptive gonad and form testicular cords, which mark the differentiation of PGCs into gonocytes.

We established different in vitro culture systems for study the process of germ cell differentiation and development (6-9). On molecular level, we are studying molecular mechanism involving germline stem cell proliferation and differentiation with focusing on Piwil2 gene which plays very important role in this process and interestingly in tumorigenesis (10-15)

From stem cells to germ cells

Today, there is much evidence suggesting that organ-specific stem cells need not rely completely on their own sources for maintenance and regeneration of an organism. In certain circumstances, mostly related to tissue damage, stem cell populations residing past the affected organ can contribute to its recovery--that means from different cell lines and also in tissues from another germ layer. The key factor in formation of self-renewing cellular clones is the presence of stem cells either from the tissue of origin or stem cells migrating from other areas and their successful settlement in an empty niche of the damaged tissue. Stem cell plasticity is the ability of adult tissue-specific stem cells to switch to new identities. The term plasticity also means stem cell phenotypic potential, which is broader than phenotypes of differentiated cells in their original tissues.

One study published in recent month has dramatically changed the outlook on infertility and germ cell development, as the message it delivered is that male and female gametes can be produced in vitro from embryonic or teratocarcinoma stem cells (16). Recently, we showed that ES cells can differentiate to spermatogonial stem cells using a promoter based selection strategy (Figure 1). These cells are able to undergo meiosis, generate haploid male gametes in vitro during 72 h and are functional, as shown by fertilization after intra-cytoplasmic injection into mouse oocytes. Resulting two cell embryos were transferred into oviduct and live mice were born. Seven of eight animals developed to normal adult mice. This is a clear indication that male gametes derived from embryonic stem cells by this strategy are able to induce normal fertilization and development. Although we succeeded in obtaining progeny from ES-cell derived male gametes, technical improvements are necessary. Improvement of oocyte microinjection is important to obtain more 2 cell embryos. The fertilization rate might have been affected by damage during oocyte injection or by heterogeneity of microinjected male gametes. This research is particularly important in helping us to understand more about spermatogenesis, the biological process in which sperm is produced. We must know this if we are to get to the root of infertility (9).

In another approach, we demonstrated the potential of bone marrow stem cell to differentiate to male germ cells. Bone marrow-derived stem cells can be trans-differentiated into multilineage cells, such as muscle of mesoderm, lung and liver of endoderm, and brain and skin of ectoderm. We showed that bone marrow stem cells are able to Trans-differentiate into male germ cells. For derivation of male germ cells from adult bone marrow stem (BMS) cells we used a genetic selection strategy using a transgenic mouse line expressing EGFP specifically in male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells like fragilis, stella, Rnf17, Mvh and Oct4, as well as molecular markers of spermatogonial stem cells and spermatogonia like Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90a, beta1- and alpha6-integrins. However, the BMS-derived germ cells were not able to enter meiosis. Our finding addresses one of the critically important questions in the field of stem cell plasticity. For the first time, we demonstrated that a somatic adult stem cell can differentiate to germ cells.

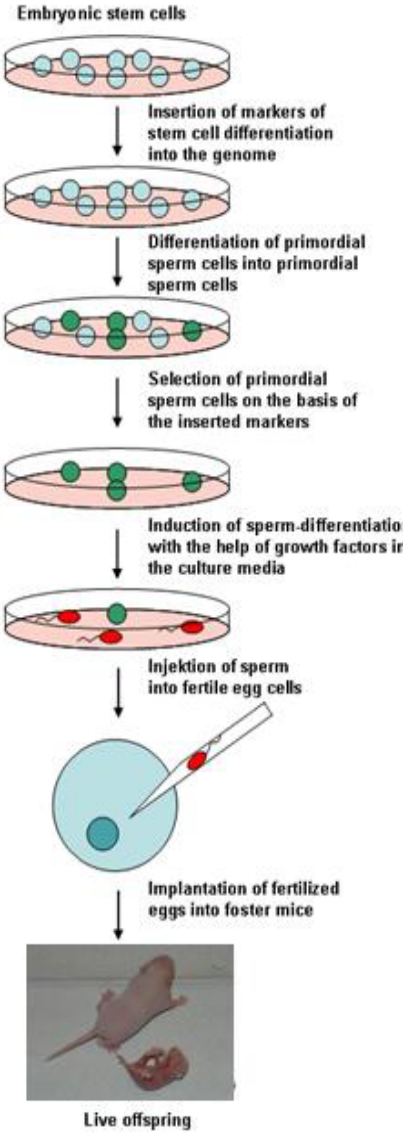

Figure 1- Schematic presentation of promoter based strategy used for generation of ES cell derived male gametes.

From germ cells to stem cells

Recent advances in cellular therapies have led to the emergence of a multidisciplinary scientific approach to developing therapeutics for a wide variety of diseases and genetic disorders. Although most cell-based therapies currently consist of heterogeneous cell populations, it is anticipated that the standard of care needs well-characterized stem cell lines that can be modified to meet the individual needs of the patient. Extensive research in the area of regenerative medicine is focused on the development of cells, tissues and organs for the purpose of restoring function through transplantation. The general belief is that replacement, repair and restoration of function is best accomplished by cells, tissues or organs that can provide the appropriate physiologic/metabolic functions more efficiently than any mechanical devices, recombinant proteins or chemical compounds. Several cell-based strategies are currently investigated including cell preparations from autologous parenchyma or established cell lines, as well as cell therapies derived from a variety of stem cell sources such as bone marrow or cord blood stem cells, embryonic stem cells, as well as cells, tissues and organs from genetically modified animals. Several lines of evidence have suggested extensive proliferation activity and pluripotency of germline stem cells, including spermatogonial stem cells. These characteristics provide new and unprecedented opportunities for the therapeutic use of spermatogonial stem cells for regenerative medicine.

We succeeded in developing a procedure for isolation and purification of spermatogonial stem cells from adult mouse testis. We were able to isolate and culture these cells in culture medium containing the precise combination of cellular growth factors needed for the cells to reproduce themselves in vitro. These cells were characterized concerning their molecular profiling and these were compared to molecular profiling of ES cells using a stem cell array which contains relevant genes related to stem cell metabolism. The results indicate that spermatogonial stem cells share many molecular characteristics with embryonic stem cells. On cellular level, spermatogonial stem cells resemble embryonic stem cells; they form embryoid body structure after two weeks of culture. Stem cell potential of isolated spermatogonial stem cells was examined using the transplantation technique. This method allowed spermatogonial stem cells to recolonize the seminiferous tubuli of germ cell depleted mice and regeneration of spermatogenesis (Figure 2). These cells are able to differentiate into various cell types of three germ layers in vitro. In contrast to ESCs, use of SSCs for cell transplantation will allow establishment of individual cell-based therapy, because the donor and recipient are identical. In addition, the ethical problem is avoided (17).

Our approach provides an accessible in vitro model system for studies of mammalian gametogenesis, as well as for developing new strategies for reproductive engineering and infertility treatment (Figure 3).

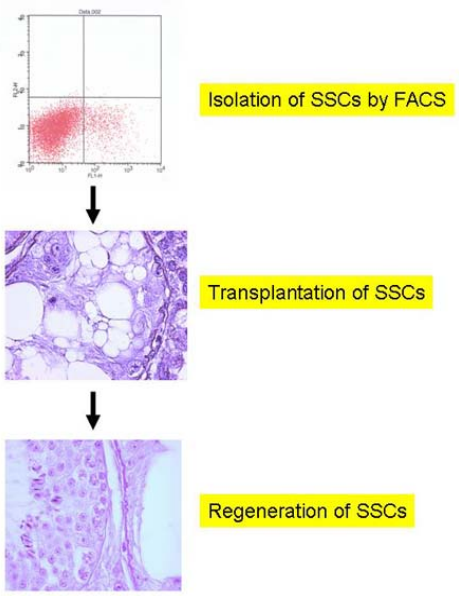

Figure 2- Regeneration of spermatogenesis after transplantation of isolated spermatogonial stem cells (SSCs).

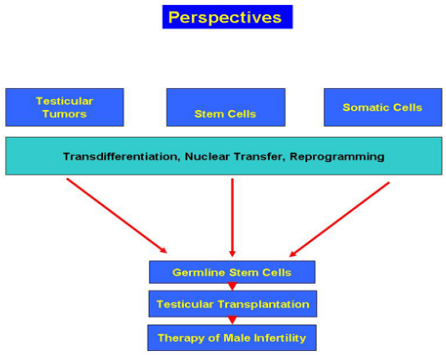

Figure 3- Perspectives of different approaches of in vitro gametogenesis.

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