Niche, 2014; 3: 24-7 • DOI : 10.5152/niche.2015.217

A Brief Review: From Spermatogonial Stem Cell to Spermatids in Mammals

Niche

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Review

Abstract

Spermatogonial stem cells exist in the seminiferous tubules of the testes and function as a source of spermatids. They can be produced in in vitro cultures; animal models of allo- and auto-transplantation of these stem cells have been developed to find a solution for the patients suffering from infertility owing to chemotherapy, testicular cancer, or azoospermia. In this brief review, we summarize the developments in this striking research field during the last 20 years.

Keywords: Spermatogonial stem cell, infertility, , xenograft

Background

Spermatogonial stem cells (SSC) exist in the basement membranes of the seminiferous tubules of the testis (1, 2). They have the function of self-renewal and differentiation into haploid spermatids in adults (3). Because of their high proliferative capacity, they are prone to damage by chemo- and radiotherapy (4). To help the patients who survived cancer to bear their biological child, SSC culturing as well as freezing and thawing methods have been researched at many centers.

SSC produce sperms in vivo, but they are proven to be pluripotent in vitro; in many studies, embryonic stem cells (ESC) are produced from them in cell cultures (5-7). Cells of three embryonic germ layers were produced in many researches by generating ESC from SSC of both mice and humans (8-10). Functional neurons and glial cells and mature cardiac and endothelial cells are some of the striking end-products obtained in research field (11, 12). Direct transdifferentiation of SSC from the neonatal mice into cells of all three germ layers, such as uterine, prostate, and skin epithelia, was also reported (13). Glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor 2 (FGF 2), leukemia inhibitory factor (LIF), and epidermal growth factor (EGF) enhanced long-term spermatogenesis in in vitro cultures of mouse SCC (14). Addition of recombinant bone morphogenetic protein 4 (BMP 4) increases the number of human primordial germ cells (PGC) in the cultures and promotes the differentiation of ESC as well as induces the development of pluripotent stem cells (iPSC) into PGC (15). Retinoic acid is another important regulator improving the in vitro differentiation of prepubertal mouse SSC into spermatids (16), and it possibly performs its role via the phosphatidyl inositol 3-kinase/AKT (PI3K/AKT) pathway and KIT protein (17). Pelota (Pelo) is a recently elucidated gene, and its protein is important in the maintenance of spermatogenesis of germ cells in male mice. Pelo protein is suggested to control the PI3K/AKT pathway (18).

Male germ cells have been generated from ESC and iPSC in many studies, but it is observed that offsprings obtained in researches starting from PGC are normal when compared with the ones obtained in studies starting from ESC or iPSC (19). The interest of this review is the new developments in producing spermatids from SSCs. Hereafter, we will discuss about the researches investigating this subject in mammals.

Developments in non-human mammals

In 1994, Brinster et al. (20, 21) demonstrated that the transplanted SSC from the donor mice could engraft the seminiferous tubules of the recipient mice, which was treated with chemotherapy before transplantation to terminate host spermatogenesis. Spermatogenesis was successfully restored, and viable progeny could be produced through normal breeding. In this year, another group also managed to produce haploid spermatids from mice in vitro but the fertilization capacity of these spermatids remained unknown (22).

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Submitted: 08.06.2015
Accepted: 12.10.2015
Available Online Date: 05.01.2016

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Staub et al. (23) demonstrated the differentiation of spermatocytes and spermatids from the rat testicular cell mixture in vitro. The results were confined by morphological and biochemical analyses. Testicular tissues from newborn mice, pigs, and goats completed spermatogenesis when they were grafted under the skin of nude mice (24). The sperm product of this ectopic tissue was also demonstrated to be capable of producing offspring (25). Subsequently, the same experiment was tested on the prepubertal rhesus macaques; fertilization competent sperm could be obtained from the ectopic testicular tissue that was transplanted into nude mice (26). The grafts from the prepubertal animal tissues were more successful with regard to survival and establishment of spermatogenesis than the grafts from the adult tissues (27).

Subsequently, donor-derived progeny had been obtained in other mammals such as mice, rats, sheep, and goats (27, 28). The researches in rodents continued to result in the successful production of SSC in tissue cultures, and the products were competent in spermatogenesis and restored fertility upon transplantation (29- 31). Sperm could be recently produced from SSC in serum-free cultures of fresh and cryopreserved neonatal mouse testes (32, 33). Hermann et al. (34) demonstrated that chemotherapy-induced infertility can be accomplished by autologous or allogeneic SSC transplantation, resulting in the restoration of spermatogenesis in non-human mammals.

The genomic stability of SSC is very important because these cells transmit genetic information to next generations. The stability of androgenetic imprinting and normal karyotype was shown in mouse SSC after 24 months of culturing. The offspring of recipient mice proved to be fertile and had normal imprinting pattern (35). But the genetic alteration of isolated and cultured human SSC still remains to be determined.

Developments in human SSC studies

In 1999, the pioneering study of Radford et al. (36) made the researchers more convenient about the desire of the patients in protecting their fertility and supported the development of new experiments. In this study, before the exposure of chemotherapeutic agents, testicular tissues obtained from 12 male non-Hodgkin's lymphoma patients were cryopreserved. These cellular suspensions were returned to seven of those patients. However, the follow-up regarding patients' fertility is lacking.

Sousa et al. (37) obtained late-stage spermatids from the coculture of mixture of cells isolated from the testicular biopsies of non-obstructive azoospermic patients with the Vero cell-conditioned medium, FSH-containing medium, or both FSH and testosterone-containing medium. Significant number of meiosis could be obtained, and the fertilization potential of the in vitro matured spermatids were controlled by microinjecting them into human oocytes. Normal elongating and elongated spermatids elicited 30.5% of fertilization and 42.9% of blastocyst rates. Most of the embryos could not reach the morula stage and expressed major abnormalities in the sex chromosome. In another study, haploid spermatids could be obtained in vitro from the SSC of non-obstructive azoospermia and Sertoli cells-only syndrome patients. They possessed normal chromosomal structure and could also activate human oocytes (38).

When the SSC of immature human testes frozen with dimethyl sulfoxide were xenotransplanted to mice, they showed migration ability toward the basement membrane of seminiferous tubules without differentiation into mature germ cells (39). Nagano et al. (40) isolated human SSC from six infertile men and showed that these cells could make colonies and were able to survive for 6 months in recipient mice testes after a freezing–thawing procedure. The number of SSC decreased significantly after 2 months from transplantation, and meiotic differentiation of these cells could not be observed.

During the last 10 years, human SSC were produced in many centers (27, 41, 42), and 3 of whichfrom prepubertal human testes (39, 43, 44). Testicular biopsies from nine boys with cancer aged 2–10 years resulted in the isolation of SSC showing stem cell activity after xenotransplantation into mouse testes. Only 3% of the cell population in the biopsy specimen were SSC (43). The efficiency of SSC transplantation mainly depends on the number of transplanted SCC. Therefore, in vitro increase in the amount of SSC is necessary before transplantation (45). This was achieved in both adult (46) and prepubertal (39) human testes. A feeder layer from the patients' own testicular somatic cells was required to support SCC in these systems (45). Human SSC were viable up to 15 weeks with a doubling time of 3–7 days in an in vitro environment.

The lack of standardization of culturing techniques and the inability of transplantation of cultured cells into human testes to evaluate their spermatogenic potential are the points that need to be further studied and improved.

Although the testicular tissue transplants in non-human mammals resulted in functional sperms and healthy offspring (25, 26), the human testicular tissue xenografts to nude mice have not completed spermatogenesis nor haploid gametes could be obtained from them (47-49). Spermatocytes were the most advanced cell stage in them. The observation of primary spermatocytes in the xenograft of testicular tissues of a 3-month-old boy by Sato et al. (49), even after 1 year from grafting, gives us hope about the viability of the grafts.

Conclusion

SSC researches provide hope to patients suffering from infertility owing to gonadotoxic treatments, cancer, or azoospermia. Freezing and thawing methods as well as the experiments of allo- and xenotransplantation of testicular tissues are improved to obtain viable and fertile sperms from SSC. Although offsprings could be obtained in some non-human vertebrates, there is a long way for human SSC to be used as the source of functional sperms in these patient groups.

The risk of malignant contamination of the testicular tissue, limited availability of human samples, and non-standardization of cryopreservation and culturing methods of the testicular tissue samples are the challenging subjects of this area. The use of xenobiotics in xenotransplantion is another concern.

Because of uncertainty methods that will be used in clinics in the future, it is appropriate to preserve testicular tissue in a manner in which viability and functionality can be maximized.

Peer-review: Externally peer-reviewed.

Author contributions: Consept - B.G., S.H.K.; Design - B.G., S.H.K.; Supervision - S.H.K., T.D.; Funding B.G., S.H.K.; Materials - B.G., S.H.K., T.D.; Data Collection and/or Processing - B.G.; Analysis and/or Interpretation - B.G., S.H.K., T.D.; Literature Review - B.G., S.H.K., T.D.; Writer - B.G., S.H.K.; Critical Review - S.H.K., T.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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