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RESEARCH HIGHLIGHT

Derivation of male germ cells from induced pluripotent stem (iPS) cells: a novel and crucial source for generating male gametes

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One of the most significant findings in recent stem cell research is the establishment of the induced pluripotent stem (iPS) cells, because they could have critical implications in both regenerative and reproductive medicine. Male gametes play a crucial role in transmitting genetic information to subsequent generations, and notably there are more and more patients with azoospermia, due to genetic and environmental factors. Recent advancements on generation of male gametes from human iPS cells would bring great promise to produce patient own male gametes for treating male infertility and provide an excellent platform for unveiling molecular mechanisms of male germ cell development.

Exciting progress has recently been made in the derivation of primordial germ cells (PGCs), various kinds of male germ cells, and eventually male gametes from mouse and human induced pluripotent stem (iPS) cells.[1–5] These studies would provide an ideal platform for unveiling molecular mechanisms of male germ cell development and open new opportunities for treating male infertility in the future.

Male gametes play a critical role in transmitting genetic information to subsequent generations. Spermatogenesis is a complex process by which male germ-line stem cells (also known as spermatogonial stem cells) self-renew and differentiate into haploid spermatozoa, which involves three major stages: mitosis, meiosis and spermiogenesis. Any error at these stages could result in male infertility, which affects millions of people worldwide. It has been estimated that 10%–15% of couples are infertility, and male factor accounts for half of these cases. Currently sperm donation by genetically unrelated donors is the only available option for many patients with azoospermia. Embryonic stem (ES) cells possess the capability to differentiate into male gametes *in vivo* or *in vitro*.[6–8] However, human ES cells involve egg donation and embryo damage and human ES cell-derived male gametes are genetically unrelated to the patient in need of fertility treatment, which would discourage this route of treatment. Male gametes derived from patient-specific iPS cells would overcome these issues, and thus there is a great potential to obtain male gametes with genetic information from iPS cells of patients.

Hayashi *et al.*[1] combined the approach using the *in vitro* induction and transplantation assay to obtain male germ cells from mouse iPS cells. They first induced iPS cells to differentiate into epiblast-like cells that were committed to become the PGC-like cells with the stimulation of bone morphogenetic protein 4.[1] Mouse PGC-like cells derived from iPS cells were transplanted into the seminiferous tubules of mice and they exhibited proper spermatogenesis.[1] Significantly, functional assay further revealed that spermatozoa generated from iPS cell-derived PGC-like cells were able to fertilize the oocytes by intracytoplasm sperm injection and eventually gave rise to fertile offspring after embryo transfer.[1] It has been verified that PGC-like cells derived from mouse iPS cells can form functional sperm.[5] Mouse iPS cells derived from adult hepatocytes and fibroblasts were capable of differentiating into PGC-like cells,[3,4,9] which reflects the independence of the origin of somatic cells. More recently, mouse iPS cell-derived PGC-like cells were shown to reconstitute seminiferous tubules by ectopic grafting into the dorsal skin of mice or transplantation to seminiferous tubules of mouse testes.[3,4] Mouse PGC-like cells could differentiate into late stages of male germ cells, including various spermatocytes and haploid spermatids.[3,4] These studies illustrate that derivation of PGC-like cells in culture is an essential step for male germ cell specification pathway from iPS cells. Nevertheless, there is no report showing that functional male gametes can be obtained from mouse or human iPS cells *in vitro*.

Human PGCs could also be generated from human iPS cells when cocultured with human fetal gonadal cells,[10] although they did not initiate efficiently the process of imprint erasure. Strikingly, human iPS cells were induced to differentiate into post-meiotic male germ cells including haploid cells.[2] Even though functional sperm was not determined, this study may bring great promise to produce human personalized male gametes from iPS cells of patients with male infertility.

Several issues remain to be solved before iPS cell-derived male gametes could be used for clinical application. First of all, safety risk is a major concern of iPS cell-derived male gametes, because pluripotent transcription factors were used to reprogram somatic cells to become iPS cells[11] and some of these transcription factors (e.g., Myc) belong to oncogenes. Interestingly, recent study has shown that other combinations of factors excluding Myc could produce mouse and human iPS cells.[12] Notably, recombinant proteins or chemical compounds have been used to generate iPS cells.[13,14] These reprogramming approaches using recombinant proteins or chemical compounds could reduce significantly the risk of

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cancer formation of iPS cells. Secondly, the efficiency of generating male gametes from iPS cells is relatively low. This may be improved by optimizing the induction protocols. A number of growth factors and/or cytokines, including bone morphogenetic protein 4, stem cell factor, epidermal growth factor and Forskolin could induce iPS cells into male germ cells *in vitro*. Moreover, retinoic acid promotes mouse germ cells entering meiosis.[15,16] Thirdly, not all iPS cell lines have the potentials to produce male germ cells. In Hayashi's study,[1] only the iPS cell line 20D17 possessed the highest capacity for germ-line transmission among three iPS lines. Yang *et al.*[3] and Zhu *et al.*[4] used two iPS cell lines and only one of them was able to generate male germ cells. The iPS cells could produce male germ cells *in vitro*, despite their origin outside the germ line, but gene combinations for iPS cell induction may affect the result. Thus, reprogramming approaches of iPS cells have to be further defined for their germ-line competence. Finally, functional male gametes have not yet obtained from human iPS cells *in vitro* or *in vivo*. Although haploid male germ cells were generated from human iPS cells,[2] it is required to determine whether these cells can fertilize the oocytes and gave rise to healthy offspring.

It is worth noting that derivation of male gametes from human iPS cells have advantages over human ES cells in the following aspects: (i) there is no ethical issue using human iPS cells compared with human ES cells; (ii) the source of human iPS cell is abundant; and (iii) most importantly, patients with male infertility could get offspring with their own genetics. In summary, recent progress in generation of male germ cells from mouse and human iPS cells is bringing us closer to the production of patient-specific iPS cell-derived male gametes, which would offer a crucial source of generating male gametes for treating male infertility and provide an excellent paradigm for elucidating the underlying mechanisms of spermatogenesis.

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