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INVITED RESEARCH HIGHLIGHT

Sperm Biology

How to make a human germ cell

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How the primordial germ cell (PGC) lineage, which eventually gives rise to spermatozoa in males and oocytes in females, is established in the developing mammalian embryo has been a critical topic in both developmental and reproductive biology for many years. There have been significant breakthroughs over the past two decades in establishing both the source of PGCs and the factors that regulate the specification of this lineage in mice,[1] but our understanding of the factors that control PGC development in the human is rudimentary. The SRY-related HMG-box (SOX) family of transcription factors consists of 20 genes in humans and mice that are involved in the maintenance of pluripotency, male sexual development, and other processes. A recent paper in *Cell* has identified one member of this family, SOX17, as an essential factor for inducing the PGC lineage in humans.[2] Surprisingly, this protein does not appear to have a role in PGC specification in mice. This work not only introduces a new and important player to the field of germ cell specification, but also emphasizes the uniqueness of human PGC development compared to more extensively studied mouse models.

In the early mouse embryo, PGCs develop from epiblast cells in response to bone morphogenetic protein 4 (BMP4) by the end of the first week of embryonic development. The BMP4 in turn induces expression of BLIMP1 and other downstream proteins,[1,3] and these factors induce the differentiation of the PGCs. Due to ethical considerations, it is not possible to directly study PGC development in early human embryos, and there are key differences in the early development of the mouse and human embryo that suggest that the factors regulating PGC development in mouse and human embryos may differ.[4] The spontaneous differentiation of primate as well as human embryonic stem cells (hESCs) into human PGC-like cells (hPGCLCs) *in vitro* has been observed,[5,6] but these studies have not provided a mechanistic understanding of the factors that regulate key developmental processes in these cells.

Recent work by investigators from the Weizmann Institute of Science in Israel and Cambridge University in England[2] uses a new culture methodology developed by Gafni *et al.*[7] to induce hESCs or human induced pluripotent stem cells (hiPSCs) that have a knockin PGC-specific reporter gene to differentiate as PGCs. This system allows effective visualization and analysis of the early stages of PGCLC development. In this culture system, extensive development of PGCLCs from hESCs can be obtained, and this system was utilized by Irie *et al.*[2] to identify factors critical for human PGC differentiation and the pathways that regulate their subsequent development.

Initial studies indicated that SOX17 was expressed early in the differentiation of hESCs into PGCLCs.[2] Previous work with mouse systems has indicated that BLIMP1, induced by BMP4 exposure in these systems, was critical in the developmental progression of ESCs into PGCLCs.[8,9] The expression of SOX17 in early hPGCLCs appeared to occur before BLIMP1, suggesting that BLIMP1 might be downstream of SOX17. Subsequent work identified the SOX17 protein both as the key inducer of BLIMP1 as well as a major regulator of overall hPGCLC differentiation from hESCs. Notably, SOX17 appeared to be both necessary and sufficient for PGCLC differentiation from the hESCs. When hESCs that lacked SOX17 were cultured under the same conditions that produced strong differentiation of hPGCLCs from normal hESCs, cells with the molecular signature of hPGCLCs were essentially absent, as were key downstream regulators of this lineage (e.g, BLIMP1). Conversely, when an inducible SOX17 gene was inserted into and activated in these knockout cells, critical germ cell genes such as BLIMP1 were expressed, and immunohistochemical markers of germ cell differentiation were again seen.

The SOX17 protein is a most unlikely candidate to emerge as an essential regulator of BLIMP1 expression and overall human PGCLC development, as it does not appear to have a role in the differentiation of mouse PGCs.[10,11] Thus, although the critical role of BLIMP1 derived from studies of mouse PGC development has been confirmed by Irie *et al.*[2] using human cells, the unique critical role of SOX17 in the development of human, but not mouse, PGCs identifies critical species differences in molecular events that establish the PGC lineage.

The groundbreaking study of Irie *et al.*[2] was directed toward furthering understanding of PGC development in the human, but these results have broader implications in reproductive medicine. Infertility is a common problem in human medicine. The ability to produce mature or even immature sperm *in vitro* from iPSCs would potentially permit reproduction by infertile men. The previous few years have seen rapid and in some cases unexpected advances from mouse, primate and human studies that suggest that this could become a reality. In 2011, Hayashi *et al.*[12] demonstrated that they could use either ES cells or iPSCs from mice and induce these cells to form PGCLCs *in vitro*. Notably, when these cells were transplanted into testes of host mice lacking endogenous spermatogenesis, the ES- or iPSC-derived cells formed sperm that could fertilize eggs and produce viable offspring.

Viable human sperm has not yet been derived from hESCs, but recent work in both nonhuman primate and human systems suggests that this is feasible. In 2012, Easley

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et al.[13] reported that they could generate advanced haploid stages of spermatogenesis, in some cases up to round spermatids, using hESCs and hiPSCs. The successful transplantation of spermatogonial stem cells into the testes of a primate (rhesus monkey) and the subsequent establishment of spermatogenesis from these cells that resulted in the production of functioning sperm have also been reported.[13] These types of *in vivo* techniques may be useful in humans for producing mature sperm in cases where *in vitro* techniques can be used to produce early germ cells from hiPSC, and then these cells could be subsequently expanded and allowed to develop to maturity by transplantation *in vivo*.

Although serious scientific hurdles remain before this knowledge could be translated into a clinical setting, the present results of Irie *et al.*[2] have provided unexpected and valuable information that advances our understanding of PGC lineage specification in the human. This brings the day closer when advances in many aspects of understanding and manipulating male or female germ cell development may culminate in the ability to generate viable gametes, and thus allow reproduction, from men and women who are presently infertile.

COMPETING INTERESTS

The authors declare no competing interests.

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