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NEWS AND VIEWS

In vitro reconstitution of germ cell development

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Mammalian germ line cells undergo unique cellular and genetic changes under the regulation of specific regulators, in different stages as they develop and differentiate into functional gametes. It is a fundamental challenge to reconstitute gametes development *in vitro*, because of the complexity of the regulation process. In mice, embryonic stem cells (ESCs) develop into Epiblast stem cells at around embryonic day 6.0 (E6.0) induced by the bone morphogenetic protein 4 (Bmp4) (Lawson et al., 1999). At around E7.25, epiblast stem cells develop into primordial germ cells (PGCs) in the extraembryonic mesoderm regulated by the critical transcriptional regulator Blimp1(Prdm1) and Prdm14 (Yamaji et al., 2008). PGCs are the origins for the oocytes and the spermatozoa (a motile sperm cell).

A great part of the framework for the signaling and transcription in sperm specification of mice has been established in the last decade of intensive research. The *ex vivo* development from epiblast cells to PGC-like cells is viable; PGC-like cells formed functional sperm when transplanted into neonatal testes lacking endogenous germ cells (Ohinata et al., 2009). However, many basic questions still remain to be addressed. It was a challenge for the *in vitro* generation of pregastrulating epiblast-like cells from embryonic stem cells or induced pluripotent stem cells (iPSCs). Attempts both in mice and humans to isolate cells expressing a germ cell marker(s) in embryoid bodies differentiated spontaneously under undefined conditions were either inefficient or failed. In a paper published in a recent volume of *Cell*, Saitou's group in Department of Anatomy and Cell Biology from Kyoto University in Japan explored a condition under which ESCs and induced PSCs with naïve pluripotency are induced into pregastrulating epiblast-like cells. A defined culture system reconstituting the PGC specification pathway in mice was established in the paper (Hayashi et al., 2011).

They firstly induced epiblast-like cells with high competence for the PGC fate under a distinct strategy, similar to those used to induce EpiSC-like cells: ESCs cultured under a serum- and feeder-free condition with necessary cell signaling inhibitors, form PGCs within a few days when introduced into the blastocysts; and convert into pregastrulating epiblast-like cells with high competence for the PGC fate in the continuous presence of necessary factors. They quantified the expression of key genes in the induced EpiLCs, and confirmed that EpiLCs show properties that are consistent with pregastrulating epiblasts.

They next examined whether PGC-like cells could be induced from the EpiLCs induced in the previous step, under conditions that was used to induce epiblast cells to PGC. Multiple factors were examined, including gene expression dynamics, and they confirmed that at a later stage, the induced cells could be considered established PGCLCs. The culture system they used allows the generation of PGCLCs in a relatively large number and thus is very promising for elucidating unexplored areas of germ line cell biology. They also identified the surface markers for PGCLCs (SSEA-1 and Integrin-beta3), which had enabled the induction and purification of PGCLCs with a capacity for proper spermatogenesis from iPSCs, and the purification of PGCLCs from ESCs of other mammalian species, including humans, since human ESCs exhibit similar characteristics to mouse EpiSCs.

In order to determine the global transcription dynamics for PGCLC induction, they did microarray analysis. They isolated total RNAs from ESCs, EpiLCs, EpiSCs, epiblasts, and PGCLCs of comparatively the same developmental stage. Two sets of microarray analysis were performed: one with nonamplified RNAs and the other with amplified RNAs from cells listed above. Unsupervised hierarchical clustering (UHC) of nonamplified samples suggested the reproducibility of the PGCLC induction. Principle component analysis (PCA) suggested that EpiLC induction from ESCs is a directional and progressive progress. UHC of amplified samples demonstrated close similarities between EpiLCs and epiblasts and between PGCLCs and PGCs and a relative large difference between EpiSCs and epiblasts, given that all the compared cells were at comparable developmental stages. All these findings strongly confirmed that PCGLC formation from ESCs through EpiLCs recapitulates PGC formation from epiblasts.

They then evaluated the epigenetic profiles of PGCLCs. They showed that compared to non-PGCLCs, PGCLCs at

day 6 appeared to have reduced H3K9me2 and cytosine methylation (5mC) and elevated H3K27me3 levels by IF analysis. They also quantified the dynamics of H3K9me2, H2K27me3 and 5mC levels during the PGCLC induction by western/dot blot analysis. The dynamics of histone modification and 5mC changes during PGCLC formation have the similar pattern as the changes in PCG formation (Seki et al., 2005). They also explored the dynamics of PGCLC induction and proliferation, which provides further evidence that PGCLC formation is a recapitalization of PGC formation.

Finally they identified the surface markers for PGCLC isolation, and reconstituted the germ cell specification pathway from iPSCs. Three out of 18 tests with the cells from one of the iPSCs cell lines showed proper spermatogenesis, and the resultant sperm contributed to fertile offspring. The success of spermatogenesis indicates that although iPSCs exhibit different induction properties depending on different lines, they can form PGCLCs with proper function.

As the author said at the end of the paper, "Continued investigations aimed at *in vitro* reconstitution of germ cell development, including the induction of female PGCLCs and their descendants, will be crucial for a more comprehensive understanding of germ cell biology in general, as well as for the advancement of reproductive technology and medicine." (Hayashi et al., 2011)

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