RESEARCH HIGHLIGHT

Towards capturing of totipotency

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In June issue of *Cell Research*, Xu et al. demonstrate that totipotent-like stem cells (TPS cells) can be derived directly from a 2-cell mouse embryo and extended pluripotent stem (EPS) cells by a chemical cocktail (CPEC condition). In addition, the study shows the advanced potential of TPS cells in lineage differentiation and blastocyst-like structure generation, and provides insight into mechanistic regulation of totipotency.

Totipotency, the ability of a single cell to generate all the differentiated cells in an organism, is present in the mouse zygote as well as in the 2-cell blastomeres. How to capture the totipotent state in a dish has been a challenge for many decades. Recently, studies reported the successful conversion of pluripotent stem cells (PSCs) into totipotent-like cells by spliceosomal repression[1] or modulation of chromatin-remodeling enzymes.[2] However, whether the totipotency can be better captured and maintained in culture directly from 2-cell embryos remains less well characterized.

Totipotent embryonic cells have significant advantages over other pluripotent stem cell types in modeling embryo development due to their unlimited differentiation potential into both embryonic and extraembryonic tissues. Despite extensive advances in characterizing 2-cell-like cells, it is still challenging to stably capture and maintain this developmentally transient state as a long-term cell culture, and preserve their developmental potential as in totipotent embryo. Of particular interest is the direct derivation of totipotent-like stem cells (TPS cells) from early totipotent embryos.

In June issue of *Cell Research*, Xu et al.[3] performed a chemical screen in extended pluripotent stem (EPS) cells and identified chemicals that can significantly increase 2-cell marker (MERVL-tdTomato/Zscan4-GFP) expression and positive subpopulation. A condition containing CD1530 (a RARγ agonist), VPA (an HDAC inhibitor), EPZ004777 (a DOT1L inhibitor) and CHIR99021 (a WNT agonist) (collectively, the CPEC condition) turns out to largely and stably induce and maintain cells preserving the totipotent molecular features with intense expression of totipotency markers. Interestingly, RA signaling was recently identified as a critical regulator of totipotency,[4] and DOT1L inhibition was also used in another study as a component for sustaining totipotency in vitro.[3] The finding that the CPEC condition as a whole supports totipotency is interesting and novel. Besides several molecular totipotent features, CPEC-converted cells are also capable of differentiating into extraembryonic cells in vitro. Importantly, the cocktail can be successfully employed to derive totipotent-like cells directly from 2-cell mouse embryos without any adaption step.

Transcriptomic analysis provides a systematic approach to characterize cell states and examine molecule features. To this end, the authors performed bulk RNA-seq and single-cell RNA-seq to examine the transcriptome shift from EPS to TPS during conversion. These analyses revealed more close resemblance of TPS to 2-cell embryos in comparison to other reported totipotent and pluripotent stem cells. Intriguingly, the single-cell RNA-seq also revealed varied expression levels of totipotency marker genes among the TPS cell populations, which is also observed in other totipotent cell cultures, suggesting that a certain level of heterogeneity exists in the totipotent culture. In addition, at the epigenetic level, TPS cells share many features with 2-cell embryos as revealed by the chromatin accessibility and global DNA methylation analysis.

To further substantiate their claims in vivo, the authors examined whether TPS cells have an unbiased ability to contribute to both embryonic and extraembryonic lineage development. For this, the authors designed and performed chimera formation assays with multiple cells or more stringently, just as a single cell injected into 8-cell embryos. The resulting chimeric embryos showed germline contribution of TPS cells and the presence of TPS-derived trophoblast and visceral endoderm cells. Marker expression and single-cell RNA-seq analysis at early (E10.5) and late (E17.5) stages revealed a high contribution of TPS cells in the placenta. The author substantiated this finding by ruling out the possibility of having artificially fused cells of TPS cells and host cells in the placenta using differentially labeled host and injected TPS cells. To further investigate the potential of TPS cells, the authors attempted the formation of blastocyst-like structures (blastoids) from TPS cells and successfully generated blastoids with both epiblast and trophectoderm markers expression. Single-cell RNA-seq data showed that the TPS-blastoid cells cluster well with the respective cell lineages from blastocysts. Moreover, by comparing with blastoids generated by other methods, the author found that trophectoderm cells in TPS-blastoids resemble preimplantation trophectoderm in blastocyst instead of postimplantation extraembryonic ectoderm. It is worthy of mentioning, compared to EPS-blastoids,[5] EPS-converted TPS-blastoids contain much fewer intermediate cells, which further confirms the superior potency of TPS cells on lineage differentiation. TPS-blastoids were able to implant and induce decidualization after transferring into a surrogate. Taken together, these observations suggest that TPS cells have the potential to differentiate into both embryonic and extraembryonic lineages both in vitro and in vivo.

Beyond the mouse, in vitro capture of human 2- or 8-cell-like cell type has attracted a lot of interest for years. Recently, two studies reported the existence of a small subpopulation of 8-cell-

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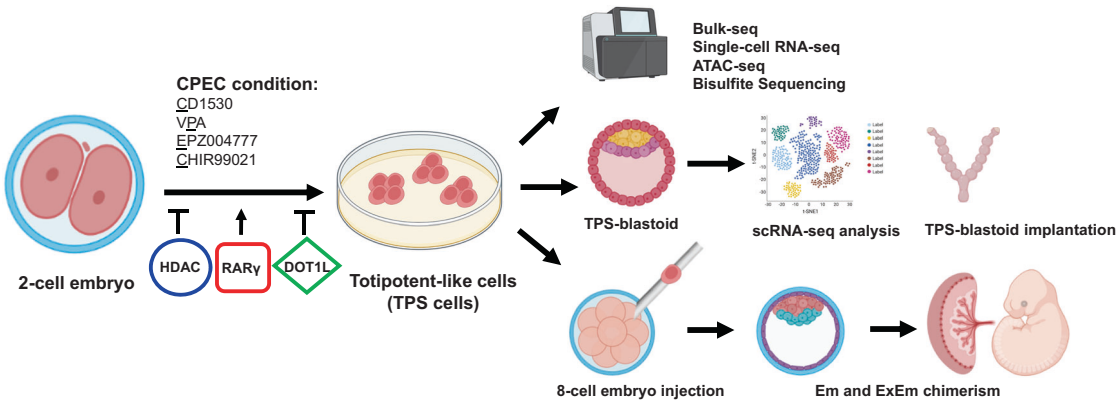

Fig. 1 Summary of derivation of totipotent-like stem cells (TPS cells) and the characterization and evaluation of totipotency in vitro and in vivo. TPS cells can be directly derived from 2-cell embryos under CPEC condition, and stably cultured in vitro for many passages. The molecular features of TPS cells were examined by multi-omics sequencing (upper). To functionally evaluate the differentiation potential of TPS cells, TPS-blastoid formation was performed in vitro and the transcriptome was analyzed by single-cell RNA-seq (middle). Further, in vivo contribution of TPS cells to embryonic and extraembryonic lineages was analyzed by chimerism (lower). Em, embryonic; ExEm, extraembryonic.

like cells in cultured naïve human PSCs, which can be enhanced by epigenetic modulation.[6,7] We envision that the study by Xu et al. presents a valuable resource that can potentially be applied in human or other mammalian species for totipotency capture and has a broad application in regenerative medicine. Although the current TPS cells are not capable of generating fully functional blastoids, TPS-blastoids seem to outperform previous attempts and generate cell lineages very close to those in the blastocyst. With further improvements, such as the reduction of heterogeneity in TPS cells as well as optimizing the induction protocol to guide the precise self-differentiation and organization capability of TPS cells during the formation of blastoids, TPS cells might be able to reconstitute a fully functional synthetic embryo in the future.

Altogether, the study by Xu et al. establishes a novel culture condition for totipotency capture and maintenance directly from 2-cell embryos and EPS cells (Fig. 1). The study demonstrates the developmental potential of TPS both in vitro and in vivo and unravels some of the molecular mechanisms that underlie the pathways for totipotency regulation. This study provides a useful approach and exciting opportunity for both future basic and clinical research on studying totipotency regulation and embryogenesis, as well as for other areas like species evolution and preservation of endangered animals.

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ADDITIONAL INFORMATION

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