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Perspective

Modulation of Pluripotency: Progressive Poising for Lineage Differentiation

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DESCRIPTION

The transformation of ESCs from their naive condition to various states of pluripotency has been documented in a number of studies. Early Primitive ectoderm-Like (EPL) cells are formed when ESCs are cultivated in a medium that has been conditioned by human HepG2 hepatocarcinoma cells.

In vitro differentiation of EPL cells into derivatives of the three germ layers suggests that they may still be pluripotent. EPL cells can no longer create chimaeras, nevertheless. Changes in gene expression patterns, such as the down-regulation of early epiblast markers Gbx2 and Rex1 and the up-regulation of late epiblast markers Fgf5, are associated with the establishment of EPL cells, indicating that they resemble the epiblast population of the post-implantation mouse embryo. To compare EPL cells through an epiblast from the implanting blastocyst stage to the gastrula stage, we still lack a comprehensive transcriptome characterization. Furthermore, it is currently unknown if EPL cells may be generated directly from the epiblast of a postimplantation embryo under the MEDII condition.

Epiblast Stem Cells (EpiSCs), which are produced directly from the epiblast of a postimplantation embryo, can be created from ESCs. This is performed by growing ESCs as tiny colonies in a chemically specified culture medium supplemented with Knockout Serum Replacement Factors (KOSR), FGF2 and Activin A. The converted cells, like the EPL cells, are still capable of differentiating into derivatives of the three germ layers *in vitro*, but they no longer possess chimeric competency, indicating that ESC-derived EpiSCs may be developmental equivalents to the epiblast population of the post-implantation mouse embryo.

The epiblast of mouse embryos that are E6 to E8 post-implantation can be used to generate epiSCs directly. These cells exhibit a global gene expression profile that is unique from ESCs yet comparable to the epiblast of the post-implantation embryo. The idea of a primed state of pluripotency, which is presumably closer to the commitment of lineage differentiation, has emerged as a result of the distinctive characteristics of EpiSCs.

The transition from naive to primed pluripotency is accompanied by transcriptomic reconfiguration, genome-wide hyper-methylation, increased DNA methylation activity, ATP-dependent chromatin modification and nucleosome remodelling, differential expression of miRNA clusters and selective enhancer activity, and a switch from oxidative phosphorylation to glycolysis for energy production.

With 2i cells positioned at the naive end of the order and EpiSCs at the primed end, distinct states of pluripotency may be represented by 2i/LIF ESCs, serum/LIF ESCs, EPL cells, and EpiSCs. Epiblast cells of a late blastocyst will settle in various locations along a falling gradient of pluripotency depending on the *in vitro* settings for derivation.

The similarity in gene expression profiles suggests that 2i/LIF settings were able to preserve the epiblast cells at the pluripotent stage of the E3.75–E4.5 blastocyst. Without examining the molecular characteristics of individual cells, it is difficult to determine whether there is an amalgamation of disparate cell types in the 2i/LIF colonies. One example is the discovery of a small number of stem cells that appear to be "totipotent" and have a predilection towards extraembryonic cell lineages in the LIF/Serum and the 2i/LIF ESC colonies.

CONCLUSION

It is unknown if these "totipotent" cells are created especially under circumstances that encourage a naive state of pluripotency. A different condition of naive pluripotency, possibly corresponding to the pluripotency of cells in the diapause (delayed) blastocyst, may be captured by LIF and serum. Compared to LIF/Serum ESCs, EPL cells are positioned further down the gradient. The cells are stopped at a higher level of pluripotency, similar to the epiblast in gastrula-stage embryos, by FGF2 and Activin.

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