STEM CELLS

The definition of naive

Scientists propose a more human-centric benchmark for assessing naive and primed pluripotency in human embryonic stem cells.

Among mammals, the early embryos of rodents are a bit peculiar. Stem cells derived from the inner cell mass of a young mouse embryo, before it implants in the uterus, are able to generate every tissue in the body. These cells are considered to represent a 'naive' developmental ground state, whereas the same cells cultured from other mammalian embryos are generally more restricted in their lineage options. The less pluripotent 'primed' cells, which include traditional human embryonic stem cells (ESCs) and induced pluripotent stem cells, resemble epiblast stem cells derived from a rodent embryo after its implantation.

A lot of work has gone into finding conditions that can lead primed human cells to revert to a naive state, resulting in a bounty of protocols. But a complication hangs over all of these efforts. Unlike with rodent cells, for which the contribution to tissues of a chimeric animal is a gold standard for pluripotency, there is no stringent way to assess the naiveté of human cells. Prior efforts to assess human cell pluripotency largely focused on transcriptional and epigenetic similarities to naive mouse ESCs. But a team led by Didier Trono of the École Polytechnique Fédérale de Lausanne in Switzerland, Joseph Ecker of the Salk Institute and Rudolf Jaenisch of the Massachusetts Institute of Technology argue that cultured cells should be compared to human embryos because early rodent and human development diverge. They advance a set of molecular comparisons to human embryos as a benchmark for pluripotency.

The researchers compared traditional (primed) human ESCs with naive cells converted using one of three protocols. As a first criterion, the researchers compared transposable element expression to that derived from single-cell RNA sequencing data from human embryos, finding an unexpected induction of transposon signatures corresponding to earlier stages (morula to inner cell mass of the early blastocyst) in naive ESCs than previously thought.

The higher density of integrated transposons compared with genes in the genome generated more sharply differentiated profiles between naive and primed cells than traditional gene expression profiles. In particular, a subgroup of the hominid-specific SINE-VNTR-Alu (SVA) family of transposons and two classes of HERVK-associated LTR retroelements exhibited clear expression differences in primed and naive cells, which were mirrored by differences in histone modification state.

The second criterion focused on epigenetic information. Bisulfite sequencing revealed a global drop in DNA methylation in naive cells compared with primed cells, similar to what occurs in cleavage-stage embryos. Imprinted regions, which are protected from demethylation in the embryonic context, were also demethylated in naive cells, highlighting a key difference from cells in the developing embryo.

Both copies of the X chromosome are active in female human preimplantation embryos despite the expression of the XIST repressor, which inactivates one copy of X later on. Using allele-specific expression of X-linked genes as a third criterion, the researchers found that only one protocol (using five kinase inhibitors, leukemia inhibitory factor and activin A) produced reactivation of both X copies when naive cells were generated from primed cells, again consistent with a morula or early-blastocyst-stage embryo.

The creation of interspecies chimeras has been attempted with human pluripotent stem cells and early mouse embryos as a functional test for pluripotency, with little success. Using a highly sensitive detection scheme for human mitochondrial DNA, the researchers attempted these experiments again with naive cells generated in five different ways, but they found levels of chimerism that were far too low for assessing lineage contributions. Thus studies of human ESCs will remain limited to molecular profiling until new ways are found to assess the cells' *in vivo* developmental potential. For now, comparing transposon expression, epigenetic signatures and X inactivation against a human embryonic reference may provide a more human definition of the developmental state of cultured stem cells.

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RESEARCH PAPERS

Theunissen, T.W. *et al.* Molecular criteria for defining the naive human pluripotent state. *Cell Stem Cell* http://dx.doi.org/10.1016/j.stem.2016.06.011 (2016).