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Broader Implications of Defining Standards for the Pluripotency of iPSCs

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We read with great interest the excellent review by Maherali and Hochedlinger (2008) that recommends standards for characterization of pluripotent stem cell lines, especially the many new lines being generated using factor-based reprogramming techniques (induced pluripotent stem cells, or iPSCs). Of note was the suggestion that iPSCs should be assessed for “functional differentiation through the highest-stringency test acceptable.” For murine iPSCs, this means germline transmission following blastocyst chimerism, and for human iPSCs this means assessment of teratoma pathology. Given the fast pace of discovery in the field, the value and relevance of time-consuming characterization of cell lines are bound to be debated. We'd like to highlight what's at risk when the pressure for rapid publication erodes the imperative for applying rigorous and uniform standards before assigning the label “iPSC” to novel cell lines.

The term “pluripotency” can be assigned according to lax or stringent criteria. A diverse array of stem cell types have been labeled pluripotent: multipotential adult progenitor cells (MAPCs); amniotic fluid-derived stem cells (AFS); marrow-isolated adult multilineage inducible cells (MIAMI); testes-derived stem cells; and a variety of embryonic stem cells derived by parthenogenesis, blastomere culture, and somatic cell nuclear transfer. In the loosest sense, a pluripotent cell includes in its progeny elements of all three embryonic germ layers (ectoderm, endoderm, and mesoderm), regardless of experimental context. In the strictest sense, pluripotency pertains to cells

whose progeny can reconstitute an entire organism, and is measured most stringently in the mouse using tetraploid embryo complementation (a standard achieved by only a limited subset of ESCs). To date, murine iPSCs have not yielded live pups in the tetraploid complementation assay, and thus the standard routinely applied is transmission of cells through the germline of chimeric animals to yield live pups (and not just gametes). Given practical and ethical limitations on testing of human embryonic stem cells, the gold standard for assessing pluripotency is the capacity to generate well-differentiated teratomas following injection into immunodeficient mice (Brivanlou et al., 2003). Although nonquantitative and subjective, teratoma histology in the hands of a skilled pathologist can distinguish tumors composed predominantly of poorly differentiated neuroectodermal elements from cystic masses composed of well-differentiated tissue from all three embryonic germ layers. The former behave as malignancies and are akin to teratocarcinomas, while the latter behave as encapsulated, benign masses and are true teratomas that arise from pluripotent stem cells (Lensch et al., 2007).

Importantly, in the mouse there are several types of embryo-derived stem cells that all share the most basic capacity for differentiation into all three germ layers: classical ESCs, epiblast-derived stem cells (EpiSCs), and Fibroblast Growth Factor/Activin/Bio cultivated stem cells (FABSCs), among them. These different types of embryo-derived stem cells behave differently when subjected to specific *in vivo* assays, in some instances forming

teratomas but failing the criterion of blastocyst chimerism and germline transmission. Choosing to ignore the need to clearly establish the behavior of these and other novel classes of stem cells has both scientific (and we might point out, political) consequences. MAPCs and AFS cells have been touted as viable alternatives to human ESCs. While these cells may be valuable for generating differentiated cell types *in vitro*, they meet very different criteria for pluripotency. For nearly a decade after human ESCs were first isolated, stem cell scientists operated under the presumption that human ESCs were equivalent to mouse ESCs. Recent evidence gleaned from a careful comparison of growth factor requirements and behavior in various assays of differentiation now indicates that human ESCs are most similar to EpiSCs. How much do we know about iPSCs given that factor-based reprogramming is barely more than two years old? The initial iPSCs isolated by Takahashi and Yamanaka (2006) behaved quite differently from the subsequently cultured lines; the initial iPSC lines chimerized embryos but didn't yield live pups or chimerize the germline. Partially reprogrammed colonies can be identified and pushed toward full pluripotency with subsequent chemical treatment (Meissner et al., 2008), and human colonies that might be mistaken for faithfully reprogrammed iPSCs indeed fail a number of criteria for pluripotency, including marker expression and formation of highly differentiated teratomas. In fact, the mere detection of cell-type-specific markers on cells grown in culture is a less stringent criterion for functional differentiation than the presence of

well-differentiated cells in teratomas, as assessed by histological criteria.

Practitioners of reprogramming appreciate that the process produces a range of colony morphologies, and some that appear morphologically similar to ESCs do not share essential molecular features and behave quite differently in culture. Variability in epigenetic remodeling, the extent of methylation, and the persistence of expression of integrated proviruses all might alter the differentiation potential of iPSC lines. Our concern is not whether a cell line fails certain pluripotency criteria; the essential scientific imperative is that we know as much about the nature of the cell line as possible before we label it. When one lab produces a pluripotent cell using a particular protocol, and another lab produces pluripotent cells with a different protocol, we need to know whether the two different labs are producing comparable cell lines. Otherwise, our collective ability to make cross-

lab comparisons will be significantly compromised. Applications in regenerative medicine may favor one type of pluripotent cell type over another; indeed, because of safety concerns, lines that don't form teratomas may be preferable as sources of cell products for clinical applications. However, if we accept lax rather than strict criteria before assigning the "iPSC" label, we deprive the label of its integrity and risk muddying the literature with data from a disparate array of diverse cell lines.

The field of factor-based reprogramming is in its infancy. For the foreseeable future, as we are learning more about what signaling pathways and epigenetic modifications distinguish one particular pluripotent stem cell state from another, and until such time as reliable molecular surrogates of the reprogrammed state can be validated, we believe it is best to encourage everyone to characterize their cells according to the most stringent test

of pluripotency available. We need to understand the behavior of iPSCs in a standard set of assays to enable cross-lab comparisons and to move toward a deeper understanding of the molecular basis of pluripotency and lineage restriction.

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