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Letter

Alternative iPSC Characterization Criteria for In Vitro Applications

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"Guidelines and Techniques for the Generation of Induced Pluripotent Stem Cells" (Maherali and Hochedlinger, 2008) is a timely and valuable review of this rapidly progressing and multiple-disease-relevant area of stem cell research. We agree that criteria and standards are important to allow for cross-lab data comparisons to ensure that new consensus-based interpretations are well grounded. The criteria are especially relevant for fundamental studies on the mechanisms of reprogramming and on interrogation into the pluripotent state. It is clear from the recent published literature that the breadth of investigators participating in technology development in the iPSC area should increase dramatically. New translational energy and activity should emerge by introducing iPSC technology to as many different laboratories as possible. Criteria that could be routinely established in an average lab setting could be beneficial to the field by promoting speedy in vitro applications for iPSCs. However, as one specific example, the proposed criteria may not be fully required for applications in which reprogrammed human cell lines are used to model disease processes in vitro, and to screen for novel drugs or drug toxicity. It is expected that many such cell lines from many different human genetic backgrounds and disease states will be generated in the near future. To stimulate a broad discussion of the fundamental minimal criteria that should define these iPSC lines, we offer the following comments.

Teratoma Formation Is Not Required for In Vitro Applications, and Germline Transmission Is Not a Feature of All Pluripotent Cells

Maherali and Hochedlinger agree that complete characterization of every iPSC line is not feasible and developed the following minimal set of criteria for iPSC characterization: (1) pluripotent stem cell morphology and unlimited self-renewal, (2) expression of pluripotency markers and downregulation of differentiation markers, (3) reprogramming factor independence, and (4) "proof of functional differentiation through the highest-stringency test acceptable." The most stringent functional criteria are that mouse iPSCs must be germline competent after chimera formation, while human iPSCs must form teratomas with tissues of all three germ layers when transplanted to immune-deficient mice. Human iPSC lines appear to be less efficient at forming teratomas and require more cells in comparison to hESCs (Park et al., 2008), suggesting that the teratoma assay employs many recipient mice to define the competence of perhaps a subset of contributing iPSCs. Furthermore, the teratoma assay, at least in its commonly used form, is qualitative in nature. In the context of in vitro applications and analysis of patient-specific iPSCs, is teratoma formation really the most appropriate assay?

We agree with the minimal criteria proposed by Maherali and Hochedlinger, except that we propose that cell lines that fail at the most stringent functional level of generating teratomas or transmission through the germline may be equally useful for in vitro studies. There are currently robust protocols available to drive hESCs toward early neuroectoderm, mesoderm, and endoderm in vitro that allow full quantitative assessment of differentiation capacity (Murry and Keller, 2008). Application of these methods could rapidly determine whether the lines retain the fundamental criterion of differentiation into the three primary germ layers or had restricted differentiation capacity. Importantly, as iPSC technology improves with the development of therapeutically compatible reprogramming methods, the most desirable iPSCs to use for transplantation might be those that do *not* form teratomas in vivo while retaining the capacity to efficiently generate curative cell types in vitro. This possibility can only be determined if non-teratoma-forming iPSC lines are fully studied in vitro. Finally, when mouse iPSCs are generated as a proof of principle for future human iPSC applications, the ability to generate germline chimeras may not always be the most relevant property. The equivalent experiments cannot be conducted in the human setting and contribution to the specialized germ cell lineage is noninformative for in vitro studies. Furthermore, future generation of mouse iPSCs with properties similar to pluripotent epiblast stem cells could have increased relevance to human ESCs and iPSCs (Rossant, 2008). Epiblast stem cells do not contribute to chimeras and yet show in vitro pluripotency, suggesting that pluripotency evaluation by directed differentiation may be a useful functional criterion for iPSCs that are developed for in vitro applications.

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The Utility of Reporter Constructs

We also wish to point out that Maherali and Hochedlinger propose that human iPSCs should be derived using morphological or live cell staining procedures that require "a considerable degree of ESC expertise" and that "selection methods were unnecessary and actually counterproductive." For iPSC technology to have its most profound impact, it is important to simplify the entrance requirements to recruit more laboratories to contribute new disease-specific lines and in vitro applications to the field. While we agree that iPSC lines can readily be isolated by morphological criteria and live cell staining, we contend that well-designed reporters containing fluorescent or selectable markers can facilitate isolation and expansion of reprogrammed colonies and would be critical for new investigators in the field. They also have utility for optimization of novel reprogramming methods and can monitor the presence of undifferentiated cells during directed differentiation protocols. Furthermore, looking into future therapeutic applications of iPSCs, reporter constructs could be engineered to encode a suicide gene to destroy residual undifferentiated iPSCs ex vivo prior to transplant, or to track and ablate any misbehaving cells that appear in vivo in the patient.

In summary, we welcome a discussion to establish a globally acceptable set of fundamental criteria that define iPSCs for in vitro applications. These criteria should be compatible with rapid characterization of the isolated iPSCs. In addition, development of reagents that facilitate entry into the field and that genetically modify iPSCs for safe future transplantations should be encouraged.

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