

Recreating Pluripotency?

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Two Matters Arising articles in this issue challenge the conclusions of a previous *Cell Stem Cell* paper that found extensive transcriptional differences between hESCs and hiPSCs. The original authors provide a response and set in motion a discussion in the field about appropriate methods for microarray data analysis.

The decisive discovery that differentiated cells can be reprogrammed into induced pluripotent stem cells (iPSCs) has opened new lines for intellectual inquiry and promising avenues for medical therapies. However, many fundamental unresolved issues remain, such as what is the extent of the pluripotency of iPSCs. The pluripotency of many mouse iPSC lines has been brought into question, because they have attained some but not all of the diagnostic hallmarks of pluripotency; for example, many lines uniformly express pluripotency markers and can activate an *Oct4-GFP* reporter, but most lines are incapable of tetraploid complementation, which is the defining capability of a bona fide pluripotent stem cell line (Stadtfeld et al., 2010; Zhao et al., 2009). Thus, current methods of transcription factor-mediated reprogramming fail to fully recreate authentic embryonic pluripotency in the majority of differentiated mouse cells (Figure 1). A Matters Arising discussion in this issue of *Cell Stem Cell* addresses the extent of pluripotency of human iPSCs, a topic that has substantial implications for the use of human iPSCs in the laboratory and the clinic.

Are human iPSCs an exact reproduction of human embryonic stem cells (ESCs), or have they inherited the incomplete pluripotency of their mouse iPSC counterparts? It is currently not possible to use embryo complementation-based measures to assess the pluripotency of human iPSCs, so instead Chin and colleagues conducted a transcriptional comparison of five human iPSC lines and three human ESC lines to determine how closely human iPSCs resemble ESCs (Chin et al., 2009). They found that 318 genes and 16 microRNAs were

consistently differentially expressed between iPSC and ESC lines, and, based on these results, concluded that the induced pluripotency of human iPSC lines is transcriptionally distinct from the embryonic pluripotency of human ESC lines.

In this issue of *Cell Stem Cell*, Guenther and colleagues have revisited this topic, employing statistical algorithms and cell lines different from those used by Chin and colleagues (Guenther et al., 2010). After rigorous transcriptional comparison of six human iPSC lines and six human ESC lines, Guenther et al. concluded that only four genes are consistently differentially expressed between human iPSCs and ESCs. They also did not find significant differences between the genome-wide distributions of the activating H3K4me3 and repressive H3K27me3 histone modifications in human iPSCs and ESCs, consistent with results obtained by Chin et al. (2009). Based on their analyses, Guenther et al. (2010) assert that human iPSCs have accurately reinstalled the transcriptional and epigenetic controls of ESCs and that there are minimal overt molecular differences between human iPSCs and ESCs.

In a separate study, Newman and Cooper conducted a microarray comparison of 17 human ESC lines and 67 human iPSC lines that were produced in 7 independent laboratories (Newman and Cooper, 2010). After unsupervised transcriptome clustering, they do not find that human iPSCs segregate into one distinct cluster and that human ESCs segregate into their own cluster, as would be expected if there were consistent transcriptional differences between human iPSCs and ESCs. Instead, they find that

human iPSC and ESC lines cultured in the same laboratory reproducibly cluster together. This suggests that each unique laboratory's culture condition imposes a distinct transcriptional footprint in cell lines cultured within it, and that these laboratory-specific transcriptional signatures overshadow any possible transcriptional differences between human iPSCs and ESCs.

What could be the cause for the considerable discrepancies between the results reported by these three groups? In their response, Chin et al. (2010) discuss the statistical practices that they used in analyzing their microarray data and deliberate on the differences between their algorithms and those employed by Guenther et al. (2010) and Newman and Cooper (2010). Chin et al. (2010) also point out that they designed part of their analysis to be intralaboratory comparisons to control for the interlaboratory differences highlighted by Newman and Cooper (2010). At a broader level, Chin et al. (2010) challenge members of the field to unite and to develop standardized best practices for microarray data analysis. We agree that adoption of standard practices would be an advance for the field overall and would help ensure that different groups do not come to different conclusions when analyzing the same data set. Finally, Chin et al. (2010) remind us that the lineage and genetic background of the starting cell type as well as the type of reprogramming vector used (integrating or non-integrating) will significantly impact the properties of the resulting iPSCs (Stadtfeld et al., 2010).

This discussion elicited by Chin et al. (2010) also brings up broader questions

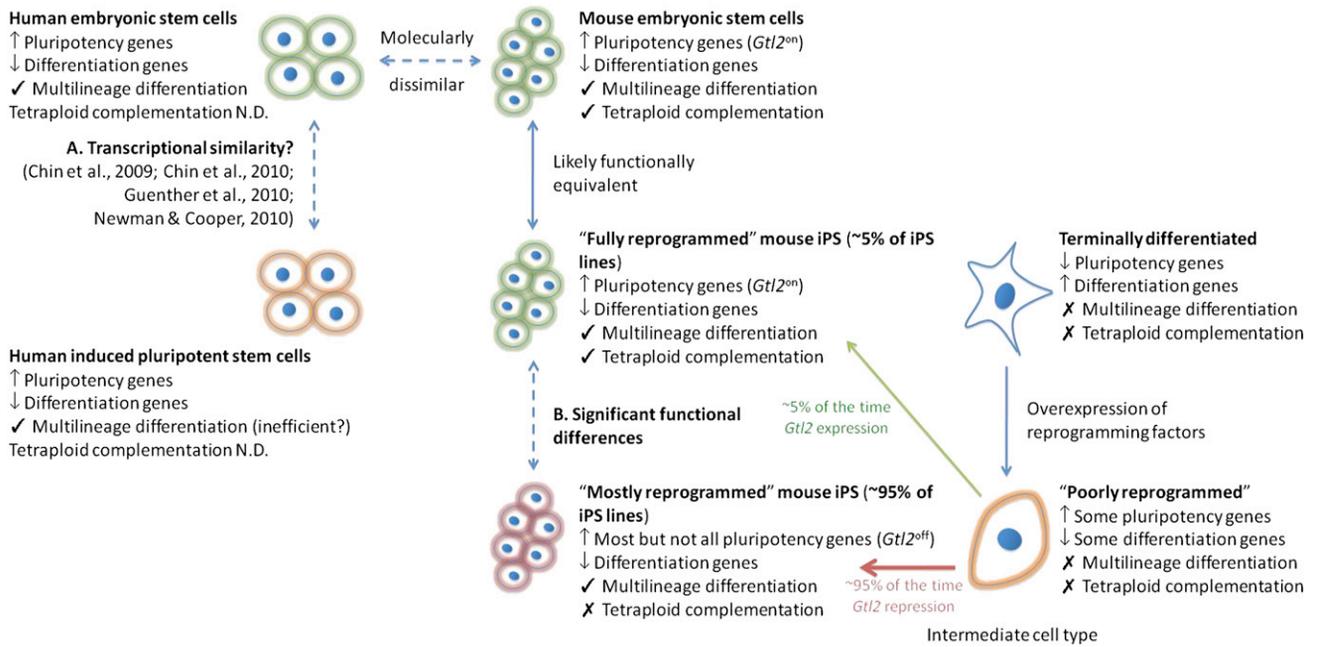


Figure 1. Recreating Pluripotency in Differentiated Human and Mouse Cells

Transcription factor-induced reprogramming in mouse cells frequently fails to recreate authentic embryonic pluripotency. This diagram defines an operational continuum of stable cell lines that arise after overexpression of the reprogramming factors—“fully reprogrammed” mouse iPSC lines (rare), “mostly reprogrammed” mouse iPSC lines, and “poorly reprogrammed” nullipotent cell lines. A critical question for human cells is whether human iPSC lines correspond to the “mostly reprogrammed” or “fully reprogrammed” iPSC lines present in the mouse system. The Matters Arising articles in this issue, and the original paper to which they refer, help address this question. N.D., not determined.

about using transcriptional analyses to determine whether or not a cell type is pluripotent. Is transcriptional similarity to embryonic pluripotency even pertinent when we are attempting to ascertain the pluripotency of iPSCs? In the mouse, there are several transcriptional programs that can produce a pluripotent cell, as shown by the fact that mouse ESCs, mouse embryonic germ cells, and mouse epiblast stem cells—which are all pluripotent stem cells—are all transcriptionally different from one another (Sharova et al., 2007; Tesar et al., 2007). The idea that there are many (transcriptional) roads to pluripotency could suggest that minor transcriptional or epigenetic differences between ESCs and iPSCs might be functionally inconsequential to the pluripotency of iPSCs.

However, an important study argues otherwise, by showing that differential expression of even a few genes between mouse iPSCs and ESCs can compromise the pluripotency of mouse iPSC lines (Stadtfield et al., 2010). They found that most established mouse iPSC lines (~95%) have downregulated several genes from the *Gtl2* locus relative to ESCs. These cell lines uniformly express

pluripotency markers and activate an *Oct4-GFP* reporter, but are consistently incapable of tetraploid complementation resulting from their repression of the *Gtl2* locus (Stadtfield et al., 2010). We refer to these cell lines—the majority of mouse iPSC lines—as “mostly reprogrammed,” despite their acquisition of many pluripotent hallmarks (Figure 1). They are distinct from previously described dedifferentiated “poorly reprogrammed” intermediates that express few pluripotency markers and fail to activate an *Oct4-GFP* reporter, but which can be coerced into a fully reprogrammed state (Figure 1; Mikelsen et al., 2008).

These striking observations raise the question of whether it is even possible to recreate authentic embryonic pluripotency in differentiated cells by the introduction of defined factors. We remain confident that it is possible. Rare mouse iPSC lines (~5%) have correctly upregulated expression of genes in the *Gtl2* locus and we call them “fully reprogrammed,” because they are capable of tetraploid complementation, and thus, they have recreated authentic embryonic pluripotency (Stadtfield et al., 2010). Given that gene expression is a function

of the transcription factors and epigenetic regulators in the nucleus (Yamanaka and Blau, 2010), it seems logical that it should be possible to recreate genuine pluripotency in iPSCs by introducing all the critical nuclear regulators that are present in ESCs into differentiated cells. Thus, our current inability to efficiently generate mouse iPSC lines capable of tetraploid complementation must reflect that our current reprogramming factors are an incomplete representation of the necessary critical regulators. To this end, we note that the transcription factor *Tbx3* can increase the germline transmission potential of mouse iPSCs when it is overexpressed during the reprogramming process (Han et al., 2010). It is likely that additional factors with similar effects will be found in the near future, thus allowing for the efficient recreation of authentic embryonic pluripotency in differentiated cells.

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Note Added in Proof

Recent publications by Kim et al. (Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., et al. (2010). *Nature*, in press. Published online July

19, 2010. 10.1038/nature09342) and Polo et al. (Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T. et al. (2010). *Nat. Biotechnol.*, in press. Published online July 19, 2010. 10.1038/nbt.1667) extend on the publications previewed here. In brief, Kim et al. and Polo et al. show that mouse iPSCs derived from distinct differentiated cell types show defining transcriptional and epigenetic similarities with their starting cell of origin. This retention of the starting cell's transcriptional program in iPSCs could explain why certain genes are differentially expressed between human iPSCs and ESCs, as discussed here. Furthermore, Polo et al. show that after extended passaging, mouse iPSCs largely censor these cell-of-origin-specific transcriptional programs, thus affirming Chin et al. (2009)'s findings that extended passaging of human iPSC lines increases their transcriptional similarity to human ESC lines.

Getting to the Heart of the Matter: Direct Reprogramming to Cardiomyocytes

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Since MyoD was used to convert fibroblasts directly to skeletal muscle, biologists have tried to apply this strategy to generate other cell lineages. In their recent *Cell* paper, Ieda et al. (2010) use selected cardiac transcription factors to directly reprogram fibroblasts to cardiomyocytes without passing through an intervening pluripotent state.

A landmark paper from Takahashi and Yamanaka (2006) showed that adult mammalian cells could be reverted to a pluripotent state with just four transcription factors. Before that time, pluripotent cell reprogramming was thought to require either somatic cell nuclear transfer into an unfertilized egg cell or fusion of somatic cells with pluripotent embryonic stem cells (ESCs) (Hochedlinger and Jaenisch, 2006). Direct reprogramming of terminally differentiated cells, also called lineage reprogramming, had been limited to skeletal muscle via MyoD. This transcription factor became recognized as a “master regulator gene,” because it was able to convert fibroblasts, chondrocytes, and retinal epithelium into contracting muscle in culture (Choi et al.,

1990). Subsequent examples included the conversion of B lymphocytes into macrophages by CEP/B (Xie et al., 2004) and inner ear support cells into sensory hair cells by Math1 (Izumikawa et al., 2005). Yet, despite years of research, master regulators for other lineages have remained elusive. Now, with an approach similar to Yamanaka's, Srivastava and colleagues demonstrate that fully functional cardiomyocytes can be derived from cardiac and skin fibroblasts (Ieda et al., 2010).

Master Regulators: A Team Approach

Yamanaka demonstrated that a selected group of transcription factors was sufficient to direct somatic cells to adopt

an immature pluripotent state and that this conversion involved the loss of the original imprint that determined the cells' functional characteristics. This finding helped shift the field's approach to lineage reprogramming. Instead of performing modest searches for single master regulator genes, hundreds of critical developmental factors were screened in multiple combinations with lineage reporter cells as readouts. Yamanaka's protocol reduced several hundred pluripotency candidate genes to just four. Why not try the same direct reprogramming paradigm to generate specific differentiated cell lineages?

Melton and colleagues applied this technique successfully to identify a small set of genes capable of converting