Rats, Cats, and Elephants, but Still No Unicorn: Induced Pluripotent Stem Cells from New Species

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Two independent studies in this issue of *Cell Stem Cell* (Liao et al., 2009; Li et al., 2009) derive rat induced pluripotent stem cells (iPSCs). In one report, the method used results in rat and human iPSCs that exhibit phenotypic traits similar to mouse embryonic stem cells.

Robust pluripotent embryonic stem cells (ESCs) have been derived from mouse, human, and several monkey species. However, attempts to develop pluripotent cell lines from other rodents, domestic animal species, and endangered animals has been largely unsuccessful to date (Keefer et al., 2007; Ueda et al., 2008). Two independent reports in this issue (Liao et al., 2009; Li et al., 2009) report the production of induced pluripotent stem cells (iPSCs) in the rat. These findings represent a significant and interesting development that carries important potential implications for medical science, cell biology, and animal conservation.

The studies reported by Liao et al. (2009) show that rat primary skin fibroblasts and bone marrow cells may be reprogrammed using lentiviral vectors that expressed the conventional reprogramming Oct4, Sox2,c-Myc, and Klf4 transcription factor genes. Interestingly, the authors were unable to achieve reprogramming using retroviral vectors or lentiviral constructs that expressed Nanog and Lin28 instead of c-Myc and Klf4. The rat iPSCs (riPSCs) could be stably maintained on mouse embryonic fibroblasts (MEF) with human ESC medium for at least 36 to 38 passages in vitro, were capable of differentiation into all three germ lineages (ectoderm, mesoderm, and endoderm) in vitro, and formed teratomas when injected into immune-compromised mice. In this regard the riPSCs behave much like human ESCs and iPSCs. An additional similarity between induced pluripotent cells from the two species is that neither have been shown to contribute to embryonic chimerism, although this failure may be due to the lack of suitable assays. It is interesting that reprogramming of rat cells by the conventional transcription factor cocktail gives rise to colonies that share at least some traits with human cells rather than with mouse iPSCs.

The apparent differences between mouse and human ESCs and iPSCs and how these growth factor distinctions might relate to the formation of riPSCs and human iPSCs (hiPSCs) was the principal focus of the studies reported by Li et al. (2009). In this case, the authors made use of the divergent pathways that control mouse and human pluripotent cell renewal and differentiation to design conditions for creating iPSCs. The conditions that succeeded, as well as those that did not, reveal particularly interesting outcomes. Their hypotheses were based on the observation that murine ESCs derived from preimplantation blastocysts give rise to distinct cell colonies that are different from the ESCs generated from the epiblast of implanted embryos. The latter resemble human ESCs in morphology, and in the response to growth factors and pathways for renewal and differentiation (Brons et al., 2007; Tesar et al., 2007). Specifically, mouse ESCs require BMP4 to inhibit ERK activation, whereas ERK is active in human ESCs and those made from mouse epiblasts. Therefore, the authors used a MEK inhibitor in combination with GSK3 inhibition to elevate the Wnt signaling cascade. They further stabilized the cultures with an inhibitor of the type 1 TGFβ receptor and reprogrammed rat liver cells to riPSCs using retroviral vectors that contained Oct4, Sox2, and Klf4. These riPSCs could be stably maintained as clonal lines with a phenotype resembling mouse ESCs. Like mouse ESCs, long-term maintenance of the riPSCs required the presence of LIF in renewal cultures. Consequently, these reprogrammed rat cells are very similar to mouse ESCs and, like these cells, can differentiate into all three germ lineages in vitro, produce teratomas in vivo, and result in chimerism when injected into wild-type rat blastocysts. It remains to be seen whether the difference in the phenotypes of the riPSCs derived by the two groups stems from the difference in starting cell population, reprogramming factors used, or the selection conditions applied during derivation of the lines.

Furthermore, Li and colleagues used a retroviral derivation method involving Oct4, Sox2, Nanog, and Lin28 genes and the growth factor/inhibitor cocktail that succeeded in maintaining riPSCs to produce hiPSCs that also resemble mouse ESCs in morphology and growth factor responses during renewal and differentiation. These cells were maintained for more than 20 passages and exhibited similar differentiation potential as has been observed for ESCs in vitro and in vivo. The authors propose that the hiPSCs derived in the presence of the inhibitor cocktail are at least phenotypically equivalent to mouse ESCs and also distinct from conventionally derived human ESCs. The opportunity to equate populations of human and mouse pluripotent cells may assist in using mouse ESC data to inform studies of human pluripotent stem cell function both in culture and after transplantation. However, while potentially significant, the conclusion that the hiPSCs described by Ding and colleagues are equivalent to mESCs remains to be tested in additional comparative experiments.

No matter how similar mouse, rat, and human iPSCs might be, it remains to be seen whether the usefulness of human iPSCs can be established, given the

presence of viral sequences and of multiple copies of the transcription factors in potentially unregulated sites in the human genome. Regulatory agencies are likely to consider these lines to be genetically engineered and will consequently hold them to a very high bar in order to establish safety for use in human therapy. On the other hand, the potential availability of riPSCs for modeling human diseases will be very attractive to researchers and companies involved in drug discovery, as the mouse has often proven inadequate as a model for candidate therapeutics. In order to serve as a useful research tool, gene targeting of the riPSCs must be accomplished with relative ease, and the production of chimeric offspring will need to become routine. With respect to the latter, Li et al. (2009) have shown that chimerism is possible with a small number of blastocyst injections.

The field of stem cell research will certainly embrace these new developments in iPSC research. In addition, one can predict that similar approaches will be undertaken to determine whether other species will prove amenable to reprogramming, such as such as horses and household pets. Indeed, iPSCs have recently been generated from nonhuman primates (Liu et al., 2008). The usefulness of iPSCs in pigs, cattle, sheep, etc., may also be demonstrated in time. However, the practice of cloning sheep, cattle, and pigs has not been sustained due to the developmental abnormalities that have been observed in cloned offspring. Thus, the potential of iPSC-derived chimeric males for use in breeding is of arguable benefit and unlikely to be widely adopted. Nonetheless, there will be interest from animal conservationists to explore the opportunity for recovery of endangered species by forming chimeras with closely related species, if available (Tecirlioglu et al., 2006; Beyhan et al., 2007), and perhaps even to rescue extinct species if appropriate cells or tissues have been cryobanked.

Clearly, the presence of viral elements and potentially unregulated genes is a danger for the sustained health of any chimeric species, and any endogenous genes that are not properly reprogrammed in the iPSCs are potentially hazardous, as has been observed in animal cloning. Much work is needed to remove the dangers that remain within genetically altered iPSCs if they are ever to be used for transplantation or human cell therapeutics. To this end, the rapid pace of the iPSC field continues, and progress toward the goal of eliminating oncogenes and viral genomic insertions has been reported recently (reviewed in Maherali and Hochedlinger, 2008). Overall, the availability of rat iPSCs offers a new and potentially powerful model for discoveries in human medicine.

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