Point of VIEW
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Induction of Pluripotency in Fibroblasts through the Expression of Only Four Nuclear Proteins

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ABSTRACT Pluripotent cell lines have the potential to provide an unlimited supply of cells to therapeutically replace those damaged by various diseases. Understanding the nature of the pluripotency of these cells could result in more controlled methods for their propagation and differentiation and could help work around the politically based policy restrictions currently dogging the field. A recent paper describes how it is possible to generate pluripotent cell lines from differentiated adult murine fibroblast cells. Establishing a similar method for human cells would offer tissues for transplantation that elicit no rejection response and would provide an embryo-independent and patient-specific source of therapeutic cells that would quell ethical and political issues.

Cells that have the power to develop into any differentiated precursor or into a fully differentiated cell type are said to be "pluripotent". It has been speculated that these cells could be incorporated as part of therapies to reverse cellular degeneration in diseases such as Alzheimer's and Parkinson's or used in tissue engineering and *ex vivo* organ growth applications. These cells could also provide illumination into human development by opening the door to *in vitro* experimentation. The most established method to derive human pluripotent cells is to isolate the inner cell mass from embryonic blastocysts to create human embryonic stem cell (hESC) lines (*1*). Many challenges must be overcome before these lines can be used as the basis of therapeutic technologies (*2*). Currently, most of the conditions to propagate the cells in a pluripotent state are complex, expensive, and ill-defined. Methods used to differentiate hESCs also tend to be low yielding and nonspecific and require multiple steps. Recently, combinatorial techniques have been explored with the intent to rapidly solve these problems (*3*). An additional challenge is perhaps less easily overcome. All of the early hESC lines were derived in a similar manner and were propagated on a feeder layer of mouse embryonic fibroblasts (MEFs). They all have been shown to express murine-specific antigens (*4*). Because of the current political climate, all federally funded labs in the U.S. are restricted to only these cell lines. Although hESC lines grown in humanized conditions have been established (*5*), they are not available to federally funded researchers in the U.S. In order to work around these restrictions, scientists have sought to more fully understand pluripotency. Researchers have already determined that cells can be de-differentiated by transferring their nuclei to unfertilized eggs (*6*) and that pluripotency can be transferred by fusing a stem cell with a differentiated cell (*7*). These results suggest that pluripotent cell lines could be established by expressing certain unknown key factors in differentiated cells of a non-embryonic origin. An additional benefit would be that transplantation rejection could be prevented when pluripotent cells are derived from the patient to be treated.

A recent paper by Takahashi and Yamanaka (*8*) published in *Cell* suggests that only a small number of factors expressed in concert are necessary to induce pluripotency in differentiated cells. The article describes a screen in which the authors select, from a pool of 24 embryonic nuclear proteins, factors pivotal in the maintenance of ESC-like properties. The screen links G418-resistance in MEFs to induction of pluripotency and thereby establishes lines of induced pluripotent stem (iPS) cells. The results of the study demonstrate that iPS cells expressing all 24 factors have mor-

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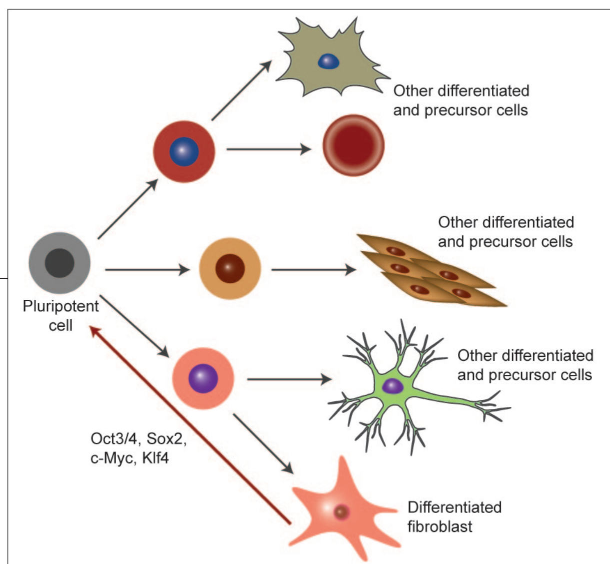

Figure 1. Differentiated mouse fibroblasts can be induced into a pluripotent state through the expression of only four nuclear factors.

phologies and growth rates and express many key markers similar to those of pluripotent stem cells. Interestingly, methylation patterns of key genes associated with pluripotency were similar but not identical to those of ESCs. By winnowing down the factors, the authors demonstrate that it is possible to maintain the pluripotency properties even if only 4 of the 24 are expressed (Figure 1). The established iPS cell lines could form embryoid bodies similar to those of ESCs. Additionally, the cells were injected under the skin of nude mice to induce teratoma tumor formation. These teratomas differentiated into all three developmental germ layers, demonstrating the pluripotency of the iPS cells. These four factors also could induce pluripotent properties in fully differentiated adult fibroblasts. Interestingly, these cells were injected into mouse blastocysts, but no chimeric mice were produced; this suggests that these cells cannot contribute to embryo development.

The four factors that were determined to induce pluripotency were Oct3/4, Sox2, c-Myc, and Klf4. It is not surprising that Oct3/4 and Sox1 are involved because they have been shown to be key for maintaining pluripotency (*9*). It is surprising, however, that the factor Nanog, which has also been shown to be important, is non-essential for the establishment of iPS cells in this study. The factors c-Myc and Klf4 are oncoproteins. The authors suggest that together their expression might balance the levels of p53 or p21^{CIP1} and thereby regulate apoptosis or cell proliferation; however, c-Myc in particular is rather nonspecifically global in its expression of downstream proteins.

The results described in this paper are intriguing. This study implies that the total deprogramming of differentiated cells might be much easier than was previously thought. If true, then a stripped-down, rational method could be envisioned to supply cells for therapies in human patients. This method would circumvent government restrictions and make available tissues that would be safe from immune rejection upon transplantation. However, as promising as these results are, a great deal more research is required to determine whether similar approaches can be used for human therapies. Pluripotency in murine models seems to be regulated differently than in humans. For example, proliferating mouse ESCs is possible with the addition of a single growth factor. For hESCs, no magic factor has been discovered, and a complex cocktail of compounds must be added to maintain pluripotency and promote proliferation. Therefore, reprogramming human somatic cells would probably not be as simple as for their murine counterparts. In addition, the iPS cells, unlike ESCs, seem to be at an intermediate stage of pluripotency. The DNA methylation of key pluripotency genes resembles a half-way position between that of ESCs and that of the fibroblasts from which the iPS cells were derived (*10*). Furthermore, the failure of chimeric mouse development suggests that the cells may have the appearance of pluripotent cells in some ways but may not be "pluripotent enough". Interestingly, this suggests that different degrees of pluripotency exist and that it may prove wise for the field to revisit its criteria for the determination of this important cellular property. To understand how these results can contribute to stem cell science and developmental biology requires much more experimentation. It is unclear whether de-differentiating human somatic cells can really be done so simply or even at all.

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