Genome Biology

RESEARCH HIGHLIGHT

Creating a blood line from human skin

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Abstract

A recent study has generated blood cell progenitors with therapeutic potential by direct lineage conversion of human fibroblasts, thus circumventing reprogramming to pluripotent stem cells.

The possibility of reprogramming somatic cells into pluripotent stem cells by defined sets of transcription factors, which has been delineated in landmark discoveries over the past few years [1], has removed a major roadblock to stem cell applications. The ability to generate patient-specific stem cells by this method has greatly extended the potential for autologous stem cell therapy and cell culture models of specific diseases. As with embryonic stem cells, however, these strategies still depend on complex differentiation protocols to derive mature cells of a defined specificity, a task that is often hindered by our limited understanding of cell fate deterination. For practical applications, any residual cells escaping a differentiation stimulus engender a high risk of tumor formation *in vivo*, as pluripotent stem cells are tumorigenic. In addition, differentiation protocols might yield cell types that are close, but not identical, to the required mature cells. For example, hematopoietic differentiation of pluripotent stem cells typically gives rise to red blood cells expressing embryonic globin genes, and it has proved more difficult to induce expression of the adult genes.

A recent series of papers has explored a short cut - the direct conversion of fibroblasts into differentiated cell types of other lineages, such as macrophage-like cells [2], neurons [3] or cardiomyocytes [4], without prior generation of pluripotent stem cells. This is typically achieved by the expression of transcription factors implicated in the developmental program of the desired lineage. These studies used mouse fibroblasts, but a paper published recently in *Nature* by Mick Bhatia and colleagues (Szabo *et al.* [5]) shows that direct lineage conversion is also possible in human cells. The authors obtained hematopoietic cells with multilineage potential by the ectopic expression of the transcription factor OCT4 in both neonatal and adult human fibroblasts. Interestingly, lineage conversion did not involve passage through pluripotent intermediates and, in contrast to hematopoietic cells obtained by embryonic stem cell differentiation, the fibroblast-derived cells primarily expressed adult (β) and some fetal (ε) globins rather than embryonic (ξ) globins.

The study by Szabo *et al.* [5] was triggered by the observation that after expression of a standard cocktail of reprogramming factors (OCT4, SOX2, Nanog and LIN28) in human fibroblasts, only a subset of cells acquired stable and full pluripotency, whereas most cells remained in an intermediate state of reprogramming. The authors noticed that some of these cells showed a hematopoietic phenotype, including the expression of the pan-hematopoietic marker CD45, and that such colonies expressed OCT4, but not the other reprogramming factors, at a high level. Following up on this observation, Szabo *et al.* noted that expression of OCT4 on its own (but not the other factors) could generate CD45-positive ($CD45^+$) colonies. The hematopoietic nature of these fibroblast-derived cells was then confirmed by a panel of classical *in vitro* and *in vivo* assays.

Cultivation of the $CD45^+$ colonies in a cocktail of hematopoietic cytokines led to their differentiation along multiple lineages. Colony assays, surface-marker analysis by flow cytometry, and cytological staining demonstrated the potential for myeloid lineage differentiation to macrophages and granulocytes as well as differentiation to erythroid and megakaryocytic cells in the presence of the erythroid cytokine erythropoietin. Notably, the conversion rate of the fibroblast-derived $CD45^+$ cells in these assays was comparable in efficiency to that of umbilical cord blood cells, a common source of human hematopoietic progenitors. Interestingly, a subpopulation of the cytokine-treated cells appeared to maintain blood cell progenitor potential, as indicated by expression of the progenitor marker CD34. This potential was confirmed by their ability to engraft and give rise to blood cells several weeks after transplantation into NOD/SCID Il-2Rγc-null mice (NSG), a common model for the

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xenotransplantation of human cells. Although the efficiency of transplantation was lower than that of umbilical cord blood cells and differentiation was significantly biased towards myeloid cells, this is still a remarkable demonstration of the hematopoietic progenitor potential of these fibroblast-derived cells.

Most notably, molecular analysis revealed that lineage conversion from fibroblasts to hematopoietic cells did not involve passage through a pluripotent state. OCT4-transduced cells activated the expression of endogenous OCT4, but in contrast to induced pluripotent stem cells (iPS cells), activation of any of the other reprogramming factors was not observed. In addition, the cells did not acquire typical markers or a global gene expression signature of pluripotency, and they did not give rise to teratomas *in vivo*, a standard test of pluripotency. By contrast, the cells showed increased expression of hematopoietic transcription factors and cytokine receptors as early as 4 hours after tansduction, consistent with their acquired responsiveness to hematopoietic cytokines. Significantly, this hematopoietic specification also did not involve the activation of genes usually associated with mesodermal transitions from the embryonic pluripotent state. Some other direct lineage conversions have also been found not to require dedifferentiation or a reversion to progenitor stages, such as from B cells to macrophages [6], from pancreatic exocrine cells to pancreatic β-cells [7] and from fibroblasts to cardiomyocytes [4]. Recent reviews cover other examples of direct lineage reprogramming and discuss similarities and differences between iPS cells and direct lineage reprogramming in relation to normal cell fate decisions [8,9]. It will now be important to determine the molecular mechanisms and genomic changes underling direct lineage conversions. A question of particular interest will be whether the epigenetic signature and phenotypic stability of directly converted cells is equivalent to that of cells derived through successive stem and progenitor cell stages during normal development or differentiation in culture.

Perhaps the most surprising observation of the study by Szabo *et al.* [5] is that lineage conversion was achieved by ectopic expression of OCT4, which has not been implicated in hematopoiesis before and has been considered a stringent cellular marker of pluripotency and the germline in normal development. Although it is conceivable that OCT4 could be mimicking the activity of the related OCT1 and OCT2 transcription factors, which play a role in hematopoiesis and can bind the same DNA octamer sequences as OCT4, the expression of OCT1 and OCT2 remained unchanged during lineage conversion and the ability of OCT1 and OCT2 to specify multilineage hematopoietic fate remains to be demonstrated. In particular, OCT1 and OCT2 have roles in lymphoid differentiation, rather than that of the other hematopoietic lineages, and the fibroblast-derived hematopoietic cells did not show lymphoid potential, although this may have been due to lack of appropriate culture conditions.

The lineage conversion achieved by Szabo *et al.* with OCT4 is in contrast to previous experiments, where lineage-converting transcription factors have been identified either by hypothesis-driven or experimental selection from a pool of transcription factors with known functions in the normal development of the desired lineage. In this way, for example, the transcription factors C/EBPα or C/EBPβ and PU.1 were found to induce a macrophage fate in lymphoid cells and fibroblasts [3,8]; Ascl1, Brn2/Pou3f2 and Myt1l to induce neuronal identity in fibroblasts [3]; Gata4, Mef2c and Tbx5 to induce fibroblast differentiation to cardiomyocytes [4]; and Pdx1, Ngn3 and MafA to induce conversion of exocrine panceatic cells into β-islet cells [7]. Together these recent direct reprogramming results raise the question of whether there is a particular transcription factor signature for each cell type that defines its identity and from which one can select the appropriate cocktail of factors to achieve lineage conversion. It will also be important to determine whether the nature of this cocktail depends not only on the identity of the target but also on the source cell type. The transcription factor cocktail of OCT4, SOX2, KLF4 and cMyc can reprogram cells of many different tissues into iPS cells [1], and a combination of PU.1 and C/EBPαβ appears to induce a macrophage fate in several cell types [2,8], but such generally valid reprogramming combinations may not always be available for lineage specification, in which successive transcription-factor interactions drive the differentiation process, and the responsiveness to a lineage-converting factor may depend on the presence or absence of antagonistic and cooperative factors in the source cells [8]. Alternatively, epigenetic changes in response to external stimuli, such as cytokines or growth factors, may become possible upon induction of a plastic state that is susceptible to lineage conversion. This latter mechanism could be compatible with the puzzling observation that a factor such as OCT4, which is implicated in pluripotency rather than lineage identity, can induce a hematopoietic fate in an unrelated cell type.

The study of Szabo *et al.* [5] has increased our armory of methods for deriving autologous cells with therapeutic potential from accessible tissues such as skin, and has provided proof of principle that direct lineage conversion is possible in human cells. Could this strategy be the way of the future - avoiding a detour via pluripotency and all its complexities? Although this might be possible, many questions remain to be addressed to identify the best strategy for each application. As well as a detailed, functional characterization of the directly converted cells

compared with their normal counterparts and the demonstration of their true potential in a therapeutic context, cells derived by direct lineage reprogramming lack one major advantage of pluripotent stem cells - the possibility of indefinite amplification in culture. This advantage should not be underestimated, as envisaged therapeutic and drug-screening applications require large numbers of cells. By generating progenitor cells with limited proliferative potential, Szabo *et al*. go beyond previous studies with one-to-one cell conversions, but proliferation was still restricted to a few divisions and cannot match the nearly unlimited self-renewal potential of pluripotent stem cells. This may not be an intractable problem, however, as it appears that extended self-renewal potential can also be activated in functionally differentiated cells without tumorigenic transformation [10]. Differentiated cells thus appear to be capable of more than could have been imagined only a few years ago, and the challenge of the coming years will be both to re-evaluate cherished conceptual limits and definitions and to determine how this newly uncovered potential can be harnessed in useful applications.

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Published: 20 December 2010

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doi:10.1186/gb-2010-11-12-143
Cite this article as: Sieweke MH: **Creating a blood line from human skin.** *Genome Biology* 2010, **11:**143.