REGENERATIVE MEDICINE

Short cut to cell replacement

Robert Blelloch

To make one differentiated cell type from another, a 'stopover' at an undifferentiated state is often required. An alternative method offering an efficient direct route could have implications for disease treatment.

Regenerative medicine aims to repair diseased or damaged tissues by replacing the affected cells with healthy, functional cells of the same type. The prospects of this discipline have been boosted by the promise of embryonic stem (ES) cells, which are pluripotent — that is, they can differentiate into any cell type — and which can be maintained in culture to 'self-renew' indefinitely. Indeed, recent breakthroughs both in the production of patient-specific ES-like cells and in inducing the differentiation of ES cells into functional adult tissues have provided further hope[1]. Like all promising therapies, however, the use of ES cells has its challenges, among them the difficulties associated with efficiently transplanting and integrating the generated tissue into the physiological framework of the body. On page 627 of this issue, Zhou *et al.*[2] describe an approach whereby differentiated adult cells of one type can be directly and efficiently converted into functional cells of another type within an organism and without the need to first reprogram them into an ES-cell-like state.

As an organism develops, its cells become increasingly specialized, losing their developmental potential (Fig. 1a). This differentiation process involves silencing of gene networks that are no longer needed and activation of other specific networks. These networks are regulated at various levels. First, the RNA and protein composition of a cell drives a specific gene-expression program that is often self-reinforcing. Second, the packaging of DNA — the epigenetic program — affects the access of gene transcription factors to specific genomic regions. Finally, in exceptional cases, the DNA sequence itself can be irreversibly altered. Each of these mechanisms further locks down the developmental potential of the differentiating cell.

For many years, it was presumed that once a cell differentiates, it burns all bridges behind it. But the discovery, first in amphibians and then mammals, that a fully differentiated cell can be manipulated to 'dedifferentiate' (Fig. 1b), and revert to a state resembling that of an early embryonic cell, proved this presumption incorrect. Dedifferentiation can be triggered by either placing the nucleus of a differentiated cell in the cytoplasmic milieu of an egg cell[3] or — as was shown relatively recently[4] — by introducing just four specific transcription factors into the differentiated cell. The latter finding has given a huge boost to the field of regenerative medicine as it indicates that the production of pluripotent cells from many sources, including patients with specific diseases[5,6], can be relatively straightforward. A crucial goal now is to ensure the safety of such induced pluripotent stem cells and to differentiate them into cells that can be used to repair damaged tissue.

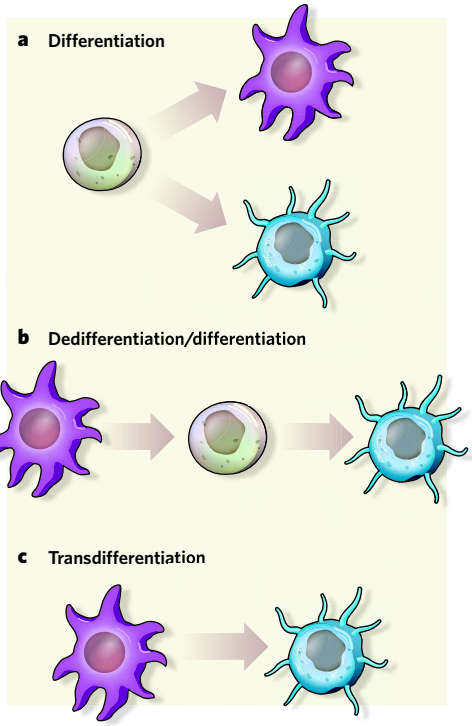

Figure 1 | The regenerative-medicine toolbox. a, During development, non-specialized cells with a broad developmental potential differentiate into various highly specialized cells that have limited developmental potential. **b**, Nonetheless, in the lab, these highly specialized cells can be induced to dedifferentiate — that is, revert back to an earlier stem-cell fate with a broad developmental potential. The cells generated in this way can then be triggered to differentiate into another cell type. **c**, Alternatively, in some circumstances, as Zhou *et al.*[2] show, a highly specialized cell can be induced to transdifferentiate into another specialized cell, bypassing the intermediate step of dedifferentiation.

Transdifferentiation — the direct conversion of one differentiated cell type to another (Fig. 1c) — provides an alternative strategy for repairing damaged tissue. In the early 2000s, this approach received a lot of publicity when several reports suggested that transdifferentiation occurs spontaneously across a wide range of tissues[7]. It was soon realized, however, that what was perceived as spontaneous transdifferentiation was, in fact, the result of cell fusion[7]. Although this realization dampened the general excitement, it did not deter many researchers, who continued to pursue the possibility of inducing transdifferentiation through the introduction of specific 'master regulators' to cells.

In the field of diabetes, for example, efforts were concentrated on generating pancreatic insulin-producing β-islet cells by inducing transdifferentiation of liver cells[8]. In 2003, two groups reported[9,10] that when the gene for either of the transcription factors Pdx1 or NeuroD1 — the latter was used together with the growth factor betacellulin — is directly introduced into the liver of adult mice (using adenoviruses as gene vectors), liver cells transform into long-lived insulin-producing cells. Moreover, these transdifferentiated cells could correct high blood-glucose levels following chemically induced injury of β-islet cells. Nonetheless, the efficiency of this approach was low, and it was unclear which liver-cell type underwent transdifferentiation.

Zhou *et al.*[2] now elegantly marry previous approaches used for inducing transdifferentiation with those that led to the discovery of the four main transcription factors required for dedifferentiation[4]. The outcome is efficient production of β-islet-like cells from a distinct, highly specialized cell type in the pancreas known as exocrine cells.

Using previous data from many labs[11], the authors identified nine genes that are essential to the embryonic development of β-cells. They used adenoviruses to introduce different combinations of these nine genes into the pancreas of adult mice and found that the introduction of a set of three transcription factors (Ngn3, Pdx1 and Mafa) induces transdifferentiation of an impressive 20% of the manipulated exocrine cells into β-islet-like cells. The authors used a combination of cellular markers and lineage-tracing experiments to prove that the cells that underwent transdifferentiation were, indeed, exocrine cells.

Zhou and colleagues' transdifferentiated β-islet cells resemble these cells' natural counterparts in several important aspects, including the proteins they express; their morphology; and their ability to secrete active insulin, which could diminish high blood-glucose levels following chemically induced pancreatic injury. However, unlike their natural counterparts, these cells do not form, or become incorporated into, islets — islands consisting of β-islet cells, other endocrine cells and blood vessels. Nonetheless, they do form intimate contacts with blood vessels, presumably allowing them to sense blood-glucose levels and release insulin into the circulation accordingly.

These exciting results lead to many intriguing questions. For instance, does transdifferentiation involve epigenetic reprogramming or is the nuclear content of exocrine cells already permissive for the activation of the transcriptional program required for β-islet-cell

formation? And how developmentally distant can the cell of origin and the target cell be for this approach to work? Exocrine cells and β-islet cells share a common precursor. When Zhou *et al.* used the same factors to induce transdifferentiation of more distantly related fibroblast cells or muscle cells into the β-islet-like cells, the approach failed. Could it be that the inclusion of other factors allows transdifferentiation of more distantly related cells?

Considering the many problems associated with the use of viral vectors in gene therapy[12], finding alternative ways to induce the expression of the three transcription factors in the exocrine cells of an organism would be especially useful. Can the virus-mediated introduction of genes for Ngn3, Pdx1 and Mafa be substituted with transient introduction of non-DNA elements such as RNAs, recombinant proteins or chemical mimics of these factors, which may be safer? Indeed, the transdifferentiation process that Zhou *et al.* describe occurred rapidly and did not require ongoing expression of the virally introduced genes, suggesting that transient non-DNA-based approaches might succeed. No matter what the answers to these questions are, the authors' findings[2] remind us that the field of regenerative medicine must pursue several strategies to uncover the best therapeutic solutions to degenerative diseases. ■

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1. Murry, C. E. & Keller, G. *Cell* **132,** 661–680 (2008).
2. Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. & Melton, D. A. *Nature* **455,** 627–632 (2008).
3. Hochedlinger, K. & Jaenisch, R. *Nature* **441,** 1061–1067 (2006).
4. Takahashi, K. & Yamanaka, S. *Cell* **126,** 663–676 (2006).
5. Park, I.-H. *et al. Cell* **134,** 877–886 (2008).
6. Dimos, J. T. *et al. Science* **321,** 1218–1221 (2008).
7. Rodić, N., Rutenberg, M. S. & Terada, N. *Trends Mol. Med.* **10,** 93–96 (2004).
8. Meivar-Levy, I. & Ferber, S. *Trends Endocrinol. Metab.* **14,** 460–466 (2003).
9. Ber, I. *et al. J. Biol. Chem.* **278,** 31950–31957 (2003).
10. Kojima, H. *et al. Nature Med.* **9,** 596–603 (2003).
11. Oliver-Krasinski, J. M. & Stoffers, D. A. *Genes Dev.* **22,** 1998–2021 (2008).
12. Hasbrouck, N. C. & High, K. A. *Gene Therapy* **15,** 870–875 (2008).

AIDS

Prehistory of HIV-1

Paul M. Sharp and Beatrice H. Hahn

The origin of the current AIDS pandemic has been a subject of great interest and speculation. Viral archaeology sheds light on the geography and timescale of the early diversification of HIV-1 in humans.

Human immunodeficiency virus type 1 (HIV-1) must have been spreading through the human population long before AIDS was first described in 1981, but very few strains from this 'prehistoric' period (pre-1980s) have been characterized. Viral sequences from earlier times can provide insight into the early spread of HIV-1, because the rapid rate of evolution of this virus — up to a million times faster than that of animal DNA — means that substantial amounts of sequence change occur in a matter of decades[1]. On page 661 of this issue, Worobey *et al.*[2] describe the sequences of partial genome fragments of HIV-1 from a lymph-node biopsy collected in 1960 in Léopoldville (now Kinshasa, Democratic Republic of the Congo). They compare these sequences with those of other HIV-1 strains, shedding light on the early evolution and diversification of this virus in Africa.

HIV-1 strains are divided into three groups, each of which was independently derived from a simian immunodeficiency virus (SIV) that naturally infects chimpanzees in west-central Africa[3]. Whereas two of these groups are rare, the third, group M, has spread throughout the world and is the cause of more than 95% of HIV infections globally. Group M can be further divided into many subtypes (A–K), which seem to have arisen through founder events. For example, subtype B, which encompasses all the strains originally described in North America and Europe, is very rare in Africa, and reflects such a founder event. Last year, Worobey and colleagues showed[4] that this subtype probably arose from a single strain that was carried from Africa to Haiti before spreading to the United States and onwards. The newly described[2] 1960 virus (DRC60) falls within, but close to the ancestor of, subtype A.

DRC60 is not the first 'ancient' HIV-1 sample to be characterized: viral sequences from a blood-plasma sample originally obtained in 1959 — also from Léopoldville — were published 10 years ago[5]. The importance of DRC60 is that it is highly divergent from the 1959 sample (ZR59), which was most closely related to the ancestor of subtype D, thus directly demonstrating that, by 50 years ago, group M HIV-1 strains had already undergone substantial diversification.

The ZR59 and DRC60 sequences differ by about 12%, a value similar to distances now seen between the most divergent strains within subtypes. As the positions of ZR59 and DRC60 within the group M phylogeny indicate that the various subtypes already existed 50 years ago, simple extrapolation suggests that these two viral sequences had a common ancestor at least 50 years before that. For a more robust estimate of the date of the common ancestor of HIV-1 group M strains, Worobey and colleagues used state-of-the-art statistical analyses, allowing a variety of models for the growth of the HIV-1 pandemic and variable rates of evolution. The different analyses gave broadly similar

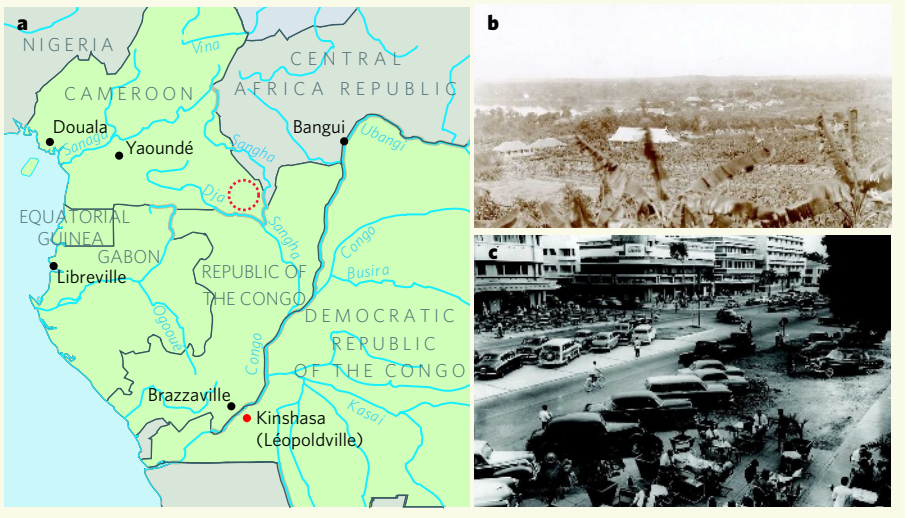

Figure 1 | Origin of pandemic HIV-1. a, Map of west-central Africa showing major rivers, and cities with explosive population growth in the twentieth century. Chimpanzees carrying the SIV strains most closely related to the viruses of HIV-1 group M, such as that described by Worobey *et al.*[2], have been found in southeast Cameroon (red ring). **b**, Léopoldville in 1896 (view from Mount Léopold) and **c**, around 1955 (the commercial centre).(Photos F. L. Michel (**b**) and C. Lamote (**c**), collection of the Royal Museum for Central Africa, Tervuren, Belgium.)