Direct conversion in the heart: a simple twist of fate

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In a recent issue of Nature, Qian *et al* (2012) show that by injecting adult mouse hearts with a few transcription factors on a retroviral vector, they can switch cardiac fibroblasts—the workhorse supporting cells of the heart—into cardiomyocytes, the beating muscle cells driving the contractile forces that pump blood. When injected into the hearts of mice with induced myocardial infarctions, the treatment reduced the size of the infarct and improved cardiac function to a modest but significant degree. Until now, cell replacement therapies have dominated the research landscape of cardiac regenerative medicine. This study hints that a gene therapy approach for *in-situ* reprogramming may provide an alternative for generating new cardiomyocytes within failing hearts.

The science of regeneration is moving in two parallel and complementary tracks. In one, stem-cell biologists are determining how to direct differentiation of primitive pluripotent stem cells (ES and iPS cells) into functional cells and tissues for research and cell transplantation (Figure 1A, top). Moving in parallel is transdifferentiation (or ‘direct conversion’) whereby highly differentiated, specialized cells are directly channelled to another cell type by introducing panels of transcription factors important for embryonic development of the cell type of interest (Figure 1A, bottom). These transcription factors effectively jump-start that developmental programme in the starting cells. Importantly, for producing cells for transplantation therapies, transdifferentiating cells do not appear to pass through an intermediate teratoma-forming pluripotent stage, which reduces the potential for inadvertently introducing into patients such cells that might contaminate manufactured cell preparations.

Ex-vivo examples of transcription factor-directed transdifferentiation are becoming abundant, following the first studies decades ago by Weintraub and colleagues, who showed that muscle differentiation could be induced in other differentiated cell types by the addition of just one transcription factor—myoD (Davis *et al*, 1987). Later, conversion of cells from myeloid to erythroid fate was similarly induced by a single transcription factor (Kulesa *et al*, 1995). *Ex-vivo* studies reporting heart stromal cell to cardiomyocyte (Ieda *et al*, 2010), fibroblast to functional neurons (Pang *et al*, 2011), and a host of others have followed (Vierbuchen and Wernig, 2011).

In 2008, Melton and colleagues provided the first demonstration that this type of conversion can be done *in situ*, in the pancreas of a living adult mouse. They found that by adenoviral delivery of a combination of three transcription factors, important for pancreatic development; Ngn3, Pdx1, and Mafa, up to 20% of the treated pancreatic exocrine cells were converted into insulin-secreting β -islet cells (Zhou *et al*, 2008).

Just as generating islet cells has been a focus for the diabetes field, generating functional cardiomyocytes to replace fibrotic tissue resulting from myocardial infarction has been hotly pursued in the cardiac field. When a myocardial infarction occurs, oxygen deprivation (ischaemia) causes necrosis and scar tissue formation in a localized region of the heart. Because the heart does not effectively regenerate new cardiomyocytes, the accumulation of fibrotic tissue eventually impairs heart contraction, leading to disability and death, with heart failure being a leading cause of mortality worldwide. Current experimental approaches for stem-cell therapy include transplanting cultured ES-derived cardiomyocytes, with or without a scaffold ‘patch’, injecting bone marrow cells, or transplanting a variety of cardiac-derived adult stem-cell types. A phase I clinical trial with intercoronary delivery of cardiac stem cells cultured from patient biopsies was recently reported (Makkar *et al*, 2012), and whereas the approach appears to be feasible from a safety standpoint, further studies are needed to fully assess safety and efficacy.

In 2010, Srivastava and colleagues reported that by delivering three transcription factors regulating heart development to cultured dermal or cardiac fibroblasts, they could rapidly produce cardiomyocyte-like cells (Ieda *et al*, 2010; Figure 1A, bottom). They pinpointed the 3 transcription factors out of a pool of 14 cardiac regulators by screening them in various combinations for their ability to drive cardiomyocyte differentiation from fibroblasts, similarly to the approach taken by Yamanaka to identify the four transcription factors that reprogram cells to a pluripotent state (Takahashi *et al*, 2007). The induced cardiomyocyte (iCM)-like cells were mostly in a primitive, partially functional state in terms of their contractile and electrophysiological properties, suggesting that factors in the native environment of the heart might be required to obtain fully functional adult cardiomyocytes.

Figure 1 Cardiac regeneration by two approaches: cell transplantation and *in-situ* reprogramming. **(A)** Cell transplantation. Top, pluripotent cells are generated either from reprogramming fibroblasts with four factors delivered by retroviral vector (iPSCs), or by extracting inner mass cells from blastocysts (ESCs); and cardiomyocytes are generated by differentiation. Alternatively, cardiac fibroblasts can be directly converted to cardiomyocyte-like cells by retroviral delivery of three genes for cardiac regulators. These cultured cardiomyocytes can be transplanted in or near heart infarcts. **(B)** *In-situ* reprogramming. Carried on a retroviral vector, cardiac regulator genes are delivered *in vivo*, directly to cardiac tissue surrounding infarct, where resident cardiac fibroblasts are converted to cardiomyocytes, reducing the size of infarct from original size (white dotted line).

In the current paper, Qian *et al* tested whether more complete reprogramming would occur in the native environment of the heart. They injected the three transcription factors, carried on a retroviral vector, directly into the heart of healthy control mice (Figure 1B). They also injected the hearts of mice with myocardial infarctions induced by coronary ligation. By lineage-tracing and following markers of various cardiac and circulating cell types, they found that resident cardiac fibroblasts converted to cardiomyocytes at an efficiency of 10–15%. This efficiency is similar to the *ex-vivo* experiments; however, the iCMs appeared to be more fully reprogrammed than iCMs produced in tissue culture based on features such as sarcomeric structure, cell–cell connectivity, action potentials, and the ability to beat. In the infarcted hearts, the treatment decreased infarct size and improved parameters of cardiac function. The effects were modest, but statistically significant. Addition of the peptide thymosin- β , which promotes angiogenesis, among other activities, further reduced the infarction and improved cardiac function.

For clinical application of *in-vivo* transdifferentiation, it is possible that the somewhat low efficiency observed in the *ex-vivo* and *in-situ* studies may be a barrier. However, since cardiac fibroblasts comprise >50% of all cardiac cells, the target cell population is large. In general, vectors used for *in-situ* reprogramming should be optimized for localized and controlled expression of the transgenes, which will minimize non-specific expression and potentially toxic effects. Improved tissue targeting, reversible transcription factor gene integration, and improvements in transforming efficiency are likely in the future. Improving the efficiency of expression and delivery are active areas of pursuit for the gene therapy field, which currently has over 1700 clinical trials in various stages (<http://www.abedia.com/wiley/phases.php>, data accessed on 20 January 2012).

It is possible that tissue regeneration using directed transformation of one cell type to another in the body will emerge as a therapeutic alternative, and small molecule discovery might play a role. Some transcription factors involved in

reprogramming can be replaced by small molecules (Shi *et al.*, 2008), thus high throughput approaches to identify small molecules that direct transdifferentiation might lead to drugs that could stimulate tissue regeneration in patients and elicit specific cell transformations. This may provide attractive, small molecule, or gene therapy-based alternatives to the expensive methods required for manufacturing cells for transplantation.

The common thread in regenerative science is the question of how to control cell fate, and how to do so in a therapeutically powerful way. Cell transplantation has been discussed extensively for the past decade and the barriers remaining are the production of mature functional cells, and their delivery in a way where the cells integrate to effectively overcome disease or injury. However, what is less clear is what might

be achieved in regenerative therapies using cells that are transdifferentiated—what hurdles this strategy introduces and whether it can be done *in vivo*, using alternatives to cell replacement. The history of transdifferentiation spans decades, however, the concept of controlling such cell versatility *in vivo* for therapeutic purposes is only starting to emerge.

It is worth noting that discovery of the valuable toolkit of genes to control cell fate is only possible because of classic developmental biology that defined factors required for embryogenesis—an object lesson in the useful and often wholly unanticipated consequences of basic research.

Conflict of interest

The authors declare that they have no conflict of interest.

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