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Previews

Reprogramming a Broken Heart

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Fibrosis resulting from cardiac injury presents a major challenge to restoring heart function after myocardial infarction. Two recent papers in *Nature* report successful in vivo reprogramming of fibroblasts to cardiomyocytes in injured mouse hearts (Qian et al., 2012; Song et al., 2012), resulting in improved cardiac function and reduced scar formation.

In the beginning, there was MyoD. The discovery of this master muscle gene, which has the capacity to convert fibroblasts into muscle, over 25 years ago (Davis et al., 1987) transformed the field of developmental biology and formed a scientific foundation for modern day cell reprogramming. The many iterations of this technology now include reprogramming into iPSCs (Takahashi and Yamanaka, 2006), direct reprogramming of hematopoietic lineages and exocrine pancreatic cells to pancreatic beta islet cells, and direct conversion of fibroblasts to distinct differentiated cell types, including neurons (see Graf, 2011 for a recent review).

Despite intense efforts and the relative ease with which fibroblasts can be reprogrammed into skeletal myocytes, reprogramming fibroblasts to cardiomyocytes remained elusive for many years. To date, none of the cardiac transcription factors by themselves have been shown to dominantly activate a cardiac cell phenotype in nonmuscle cells, and early fibroblast-cardiomyocyte heterokaryon studies had suggested that such a single master cardiac gene may not exist (Evans et al., 1994). Recent progress in reprogramming into the cardiac lineage was reported by Ieda et al. (2010). In this study, cultures of mouse fibroblasts established from the heart or skin were transduced with retroviruses encoding Gata4, Mef2C, and Tbx5 (GMT). This resulted in a subset of the transduced fibroblasts acquiring features of cardiomyocyte-like cells, including activation of the cardiomyocyte-specific transgenic promoter α-MHC, expression of structural proteins specific for cardiomyocyte sarcomeres, and, with low efficiency, spontaneous beating. Now, a duo of studies (Qian et al., 2012; Song et al., 2012) extend these findings by reporting the conversion of fibroblasts into cardiomyocyte lineages with viral vectors for three or four cardiac transcription factors after in vivo cardiac injury, which has clear, important implications for cardiac developmental biology, physiology, and regenerative therapeutics (see Figure 1).

In these studies, the laboratories of Deepak Srivastava (Qian et al., 2012) and Eric Olson (Song et al., 2012) transduced cardiac cells with retroviruses encoding GMT (Qian et al., 2012) or GMT supplemented with Hand2 (GHMT; Song et al., 2012) in vivo following experimentally induced myocardial infarction with coronary artery ligation. Following the infarct, cardiac fibroblasts become activated, migrate to the site of injury, and proliferate, thus rendering these cells susceptible to retroviral infection and subsequent GMT/GHMT expression. The transduced hearts were examined 3 to 4 weeks later, and both groups report a conversion of transduced fibroblasts, identified by genetic lineage tracing of noncardiomyocyte cells in the heart, to an "induced cardiomyocyte-like (iCM)" (Qian et al., 2012) or "induced cardiac-like myocyte (iCLM)" (Song et al., 2012) state. Interestingly, both studies show that reprogramming is significantly more efficient in situ than under tissue culture conditions, with a reprogramming efficiency of up to 12% in the heart (Qian et al., 2012), and a substantial number of cardiomyocytes in the border region of the myocardial infarct, where the retrovirus was delivered, were iCMs/iCLMs (Figure 1). The vast majority of iCMs/iCLMs were derived from cardiac fibroblasts. Genetic labeling of cardiomyocytes prior to virus exposure showed that the labeled cells were diluted by newly reprogrammed cells, which ruled out the possibility that cell fusions between transduced cardiac fibroblasts and cardiomyocytes were erroneously regarded as reprogramming events. Importantly, the reprogramming procedure resulted in reduced scar size and an increase in the ejection fraction of the left ventricle, a functional readout of the contractile capacity of the heart, and is of immediate interest from a translational perspective.

The physiology of in vivo myocardial repair appears to be a critical element promoting the conversion of cardiac nonmyocytes to functional cardiomyocytes. In vitro, the same combination of transcription factors exhibit low efficiency of cardiac reprogramming when assessed by spontaneous beating of transduced fibroblasts in culture (Ieda et al., 2010; Song et al., 2012), and in certain cases the GMT combination does not induce a complete cardiac muscle cell phenotype (Chen et al., 2012; Protze et al., 2012). This suggests that the endogenous milieu, or more likely paracrine factors that accompany the mobilization and expansion of cardiac fibroblasts after cardiac injury, represent a critical checkpoint for the reprogramming event, and might markedly enhance the efficiency of conversion (Figure 1). Notably, Zhou et al. recently reported that epicardial cells are activated upon cardiac injury and secrete paracrine factors that may also contribute to the enhanced reprogramming in vivo (Zhou et al., 2011). The improvement in cardiac function may be due, in part, to a decrease in fibrosis or improved function of preexisting cardiomyocytes, in addition to an augmentation in functional cardiomyocyte mass after injury. On the developmental front, these studies define a combinatorial pathway for cardiogenesis

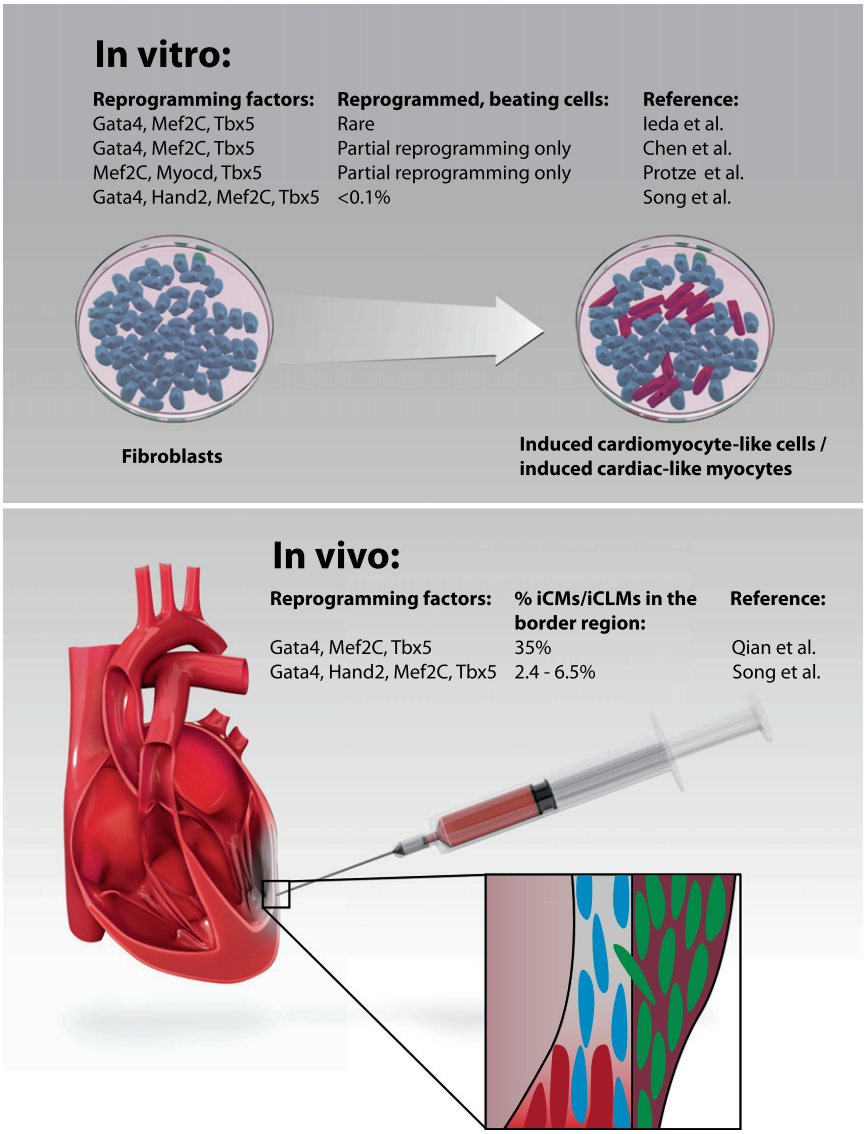

Figure 1. Schematic Figure Illustrating the Strategies and Efficiencies in Attempts to Reprogram Fibroblasts to Cardiomyocytes In Vitro and In Vivo

In vitro, although induced expression of a subset of cardiac genes is fairly efficient, very few transduced fibroblasts exhibit full reprogramming as determined by spontaneous contractile activity. In vivo, in a myocardial infarction, activated fibroblasts (blue cells) proliferate and migrate into the area of the infarct, and the epicardial cells (green) overlying the injured area also proliferate and modulate the injury through secretion of paracrine factors. Red cells indicate surviving cardiomyocytes. Upon transduction with GMT/GHMT, a substantial fraction of the total number of cardiomyocytes in the border region of the infarcted area are iCMs/iCLMs, and when examined in vitro, these iCMs/iCLMs appear to exhibit many characteristics of endogenous cardiomyocytes.

that has been actively pursued since the discovery of MyoD. The possibility of utilizing other combinations of transcription factors to drive the formation of other heart cell lineages, such as pacemaker cells, should become of interest.

From a clinical standpoint, the studies raise the possibility of finding small molecules or defined paracrine factors that might be utilized in lieu of transcription factors that traditionally have been difficult as drug targets. For example, the ability to find small molecules that enhance iPSC reprogramming suggests that such a strategy might prove fruitful. As these technologies move closer toward clinical translation, however, a number of issues need to be considered. The relative maturity and normal function of reprogrammed cardiomyocytes, as well as their durability, will become important parameters to assess in long-term studies. Even subtle differences may have important effects on cardiomyocyte function, as illustrated by the facts that haploinsufficiency of a number of muscle genes can lead to cardiomyopathy, and that failing heart muscle cells are also fully differentiated and express many of these same cardiac genes. Therefore, the potential physiological differences between iCMs/iCLMs and endogenous cardiomyocytes derived from multipotent heart progenitor cells will become a particularly important point as we go toward a therapeutic goal. Nevertheless, a new potential therapeutic paradigm has been established for heart failure after cardiac injury, joining cell-based and paracrine-factor-based approaches. For the 5 million heart failure patients in the USA alone, this long road from MyoD to novel regenerative therapeutics is welcome news indeed.

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