Title:

Reprogramming for cardiac regeneration – Strategies for innovation

Running Title: iPSCs and cardiac regeneration

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Abstract

It is well-known that the human myocardium has a low capacity for self-regeneration. This fact is especially important after acute myocardial infarction with subsequent heart failure and adverse tissue remodeling. New potential strategies have recently emerged for treating heart diseases, such as the possibility of generating large quantities of cardiomyocytes through genetic iPSC reprogramming, transdifferentiation for *in vitro* disease modeling, *in vivo* therapies or telomerase gene reactivation. Approaches based on these techniques may represent the new horizon in cardiology with an appropriate 180-degree turn perspective.

Keywords: acute myocardial infarction; heart failure; remodeling; iPSCs; miRNA; telomerase; regenerative medicine.

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Acute myocardial infarction (AMI) impairs the regenerative capacity of a tissue, the myocardium, with a low capacity for self-regeneration (Boengler et al., 2009). No major treatment advances have been made in this area and mortality rates within 1 year post-AMI remain high, prompting a need to explore novel cardiac regeneration strategies that might also benefit other heart disorders.

Several *in vivo* animal models, mostly with mice, have been developed to unveil the biological mechanisms underlying cardiac diseases and to assess regenerative strategies, notably gene therapy (Mauritz et al., 2011). A recent study indicated that telomerase gene reactivation delivered to adult mice hearts after AMI elongated myocardial telomeres, attenuated cardiac remodeling and increased survival rates (+17%) compared with non-treated controls (Bar et al., 2014). The authors suggested that telomerase activation could be used to regenerate an infarcted heart. However, mouse models do not always mimic human heart phenotypes (Ma et al., 2013). One ideal tool for disease-modeling could be the use of single cardiomyocytes isolated from patients, although they are rarely available and cannot be kept in culture for a long time nor can they be expanded *in vitro*.

The potential of regenerative medicine has grown considerably since the Nobel Prize laureate Yamanaka reported that genetic reprogramming by ectopic expression of transcription factor genes (Oct4, Sox2, Klf4, c-Myc) induces the pluripotent state in somatic cells, which then become ‘induced pluripotent stem cells’ (iPSCs) (Takahashi et al., 2007). iPSCs have major advantages including self-renewal and their capacity to differentiate into patient-specific cell lineages (Galera et al., 2016; Zurita et al., 2016). Nonetheless, iPSCs-derived cardiomyocytes feature immature, fetal-like cardiomyocytes rather than adult cells. They have a round shape, sarcomeric structures with different degrees of organization (**Figure 1A**), limited calcium handling ability, or

spontaneous contractions. In contrast, mature cardiomyocytes are larger, rod-shaped, they are often multinucleated and have more organized sarcomeres as well as a more negative resting potential. Thus, iPSCs-based disease modeling is overall more suitable for early-onset cardiac diseases. There is thus an urgent need for new and robust experimental systems allowing the modeling of heart diseases irrespective of the age of onset. Several approaches have been used to convert iPSCs-derived cardiomyocytes into cells with more mature phenotypes such as long-term culture, electric stimulation, increased substrate stiffness, the use of special scaffolds and the Matrigel mattress method (Feaster et al., 2015; Mummery et al., 2003), although more in depth research is still needed. Lee and coworkers recently showed that co-culturing ESC-derived cardiomyocytes with endothelial cells improved the maturation stage of cardiomyocytes (Lee et al., 2015). By comparing microRNAs (miRNA) expression profiles in cardiomyocytes derived from ESC co-cultured with endothelial cells *versus* ESC cultured alone, these authors identified a combination of 4 miRNAs (miR-125b, miR-199a, mir-221, miR-222) that were differentially up-regulated only in the former. Further, transfection of this miRNA combination mimicked the aforementioned ‘maturation’ effect in the cardiomyocytes that were derived from ESC cultured alone. Interestingly, a previous study had demonstrated that miR-222 modulates cardiomyocyte growth while conferring protection against adverse myocardial remodeling (Liu et al., 2015).

Current medical options for protecting the heart against pathological remodeling, notably after AMI, are sparse. Although there is not yet an ‘ideal’ perfect cellular model allowing to fully imitating the adult cardiomyocyte phenotype, pioneering results might set a starting point towards the development of a robust experimental methodology allowing generation of mature human- or iPSCs-derived cardiomyocytes for assessing

cardiac regeneration therapies (see **Figure 1B** for a summary). Finally, several emerging therapy options such as transplantation of iPSCs-derived cardiomyocytes, *in vivo* reprogramming of cardiac fibroblasts in the scar region to induce cardiomyocytes, generation of engineered heart muscle, or even reactivating the telomerase gene, represent the new horizon in cardiology with an appropriate 180-degree turn perspective.

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Conflict of interests

None of the authors have any conflict of interest.

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Figure Legend

Figure 1.

A) Human cardiomyocytes generated from induced pluripotent stem cells (iPSCs) in our laboratory: these cells show varying degrees of organization within the same preparation, *i.e.*, a more immature phenotype (left photograph) *versus* a more differentiated phenotype with more organized, adult-like sarcomeres (right photograph). Structural characterization of cardiomyocytes obtained from iPSCs by modulating Wnt/ β -catenin signalling (Lian et al., 2013). iPSCs were first generated from NHDF control fibroblasts, acquired in Promocell, using a Sendai virus-based reprogramming methodology (Cytotune 2.0, Life Technologies). Immunostaining for α -actinin (in pink) and cardiac troponin T (cTnT) (in green) shows sarcomere organization. Nuclei were stained with DAPI (in blue).

B) Generation of patient-specific cardiomyocytes for cardiovascular disease therapy. Cardiomyocytes can be generated *in vitro* using (i) iPSC reprogramming and subsequent differentiation or (ii) direct reprogramming ('trans-differentiation') into induced cardiomyocytes (Zhu et al., 2015). Disease-specific mutations within iPSCs can be previously corrected by genomic edition approaches such as the CRISPR/Cas9 system. In both cases, cardiomyocytes must undergo additional maturation before they can serve as models of adult cardiomyocytes. Direct *in vivo* reprogramming therapy to regenerate damaged cardiac tissue is also indicated as a future therapy.

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A) Human cardiomyocytes generated from induced pluripotent stem cells (iPSCs) in our laboratory: these cells show varying degrees of organization within the same preparation, i.e., a more immature phenotype (left photograph) versus a more differentiated phenotype with more organized, adult-like sarcomeres (right photograph). Structural characterization of cardiomyocytes obtained from iPSCs by modulating Wnt/ β -catenin signalling (Lian et al., 2013). iPSCs were first generated from NHDF control fibroblasts, acquired in Promocell, using a Sendai virus-based reprogramming methodology (Cytotune 2.0, Life Technologies).

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