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Hallmarks of cardiac regeneration

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Abstract

Despite substantial recent advances, *bona fide* regeneration of the damaged human heart is still an unmet ambition. By extracting our current knowledge from developmental biology, animal models of heart regeneration, and clinical observations, we propose five hallmarks of cardiac regeneration and suggest a holistic approach to reconstituting human heart function.

Introduction

Regeneration of the cardiac muscle in patients with heart disease has been a holy grail, but despite decades of efforts and several successes in pre-clinical models, such outcomes have not yet positively translated into human clinical trials. It is thus clearly time to go back to the drawing board and rethink our approach. This Focus Issue of *Nature Reviews Cardiology* aims to catalyze this process by providing a broad overview of the most important recent advances in cardiac development and regeneration.

Over the past decade, the rise of revolutionary technologies such as single-cell genomics, CRISPR–Cas9 gene editing, and pluripotent stem cells has dramatically shifted our understanding of cardiac biology. Our knowledge of cardiogenesis and the underlying gene regulatory networks has been highly refined, and the heterogeneity and plasticity of the resulting cell states is becoming increasingly clear (reviewed in this Focus Issue by Meilhac and Buckingham, and by van Eif, Devalla, Boink, and Christoffels). We have not only identified and characterized the spatiotemporal function of crucial pathways involved in cardiomyocyte specification, proliferation, and survival (reviewed in this Focus Issue by Wang, Liu, Heallen, and Martin, and by MacGrogan, Münch, and de la Pompa), but we have moved well beyond the cardiomyocyte to increasingly appreciate the physiological importance and therapeutic potential of cell populations such as the epicardium, the stroma, the vasculature, and the immune system (reviewed in this Focus Issue by Cao and Poss, and by Forte, Furtado, and Rosenthal). Thus, today we face the unprecedented opportunity to integrate all this new knowledge with the lessons learned by the existing pre-clinical and

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clinical experience in order to devise the next generation of cardiac regeneration therapies (reviewed in this Focus Issue by Menasché, and by Hashimoto, Olson, and Bassel-Duby).

In 2000, the classic paper from Hanahan and Weinberg propelled a dramatic shift in cancer biology from a reductionist view focused on the neoplastic cell to one regarding the tumour as a complex, heterotypic tissue¹. Further, a number of ‘hallmarks of cancer’ were proposed to explain the staggering variation of cancer phenotypes down to a core of acquired properties. By analogy, we propose a minimal set of five ‘hallmarks of cardiac regeneration’ that are consistently observed in organisms with an innate capacity for cardiac regeneration, such as zebrafish and salamanders, or in the neonatal mouse heart² (Figure 1). We suggest that all these properties ought to be implemented in a new generation of holistic approaches to achieve *bona fide* reconstitution of the heart muscle.

Remuscularization

Regeneration of the cardiac wall must by necessity involve its primary functional component, the cardiac myocyte. A number of approaches have been devised and can be broadly classified in three groups: stimulation of endogenous cardiomyocyte proliferation through modulation of developmental signalling pathways or direct regulation of cell cycle regulators, among other strategies³; reprogramming of resident stromal cells into cardiomyocytes by forced expression of cardiogenic factors⁴; supplementation with exogenous stem or progenitor cells with putative, but still controversial, cardiogenic potential, or with *bona fide* cardiomyocytes derived from human pluripotent stem cells (hPSCs)^{5,6}. Overall, all these strategies have shown moderate to substantial promise, but care must be used when assessing efficacy of true remuscularization. For instance, it is now widely believed that current clinical strategies based on stem or progenitor cells mainly lead to paracrine effects that, while possibly beneficial, do not directly realize this central hallmark⁷. By contrast, transplanting cardiomyocytes derived from hPSCs has so far created the highest volume of new myocardium^{5,6}, but has not yet been attempted in humans. Looking forward, we anticipate that our improved understanding of cardiac development will inform improved approaches both to drive cellular proliferation and reprogramming, and to obtain hPSC-derived cardiomyocyte subtypes most suited for remuscularization.

Electromechanical stability

New cardiomyocytes will not only be mechanically feeble, but could even lead to adverse effects should they not be properly connected within the innate electrical topology of the heart. While these concerns were once purely theoretical, they are now corroborated by pre-clinical data showing that transplantation of human or monkey PSC-derived cardiomyocytes into non-human primates can indeed induce sustained ventricular arrhythmias^{5,6}. It is foreseeable that other approaches might have similar drawbacks once they are used for large-scale remuscularization. To overcome these limitations, regenerating cardiomyocytes will have to rapidly achieve adult-like functionality of several crucial components of the excitation–contraction coupling machinery (including ion channels, gap junctions, T-tubules, and myofibrils)⁸. Putting the brakes on the automaticity of new cardiomyocytes will be particularly important, and to achieve this we can leverage our emerging understanding of

the gene regulatory networks underpinning development of the cardiac pacemaking and conduction system. Finally, efficient and specific delivery methods for cells, gene vectors, and molecular therapies will be needed to ensure spatially precise and homogeneous remuscularization of the damaged region, so as not to interfere with healthy muscle.

Angiogenesis and arteriogenesis

An extensive and hierarchical vascular network is required to robustly supply the regenerating muscle with oxygen and nutrient exchange. While pre-existing vessels and vascular progenitor cells can innately contribute to angiogenesis⁵, this process can be markedly boosted by a number of paracrine factors that can be supplemented exogenously (such as growth factors, microRNAs, and exosomes). Endogenous pro-angiogenic signals are also secreted by the stroma, the immune system, and the epicardium. Regeneration could be further boosted by providing new vasculogenic cells either as progenitors or as differentiated progenies. Importantly, angiogenesis creates a fairly disordered plexus that is not robustly perfused. Complete myocardial regeneration will also require arteriogenesis. This can either result from biological remodeling, for example through enlargement of capillary-sized vessels into muscularized conduction vessels, or be designed through emerging bioengineering solutions for the generation of engineered heart tissues⁹.

Resolution of fibrosis

While production of extracellular matrix (ECM) in the heart was once considered primarily a pathological mechanism, it is now well appreciated that the ECM is not only pivotal to prevent early rupture of the damaged myocardium, but that it also facilitates cardiac regeneration in the neonatal heart and in regenerative organisms. Overall, cells of the stroma, primarily fibroblasts and myofibroblasts, represent a double-edged sword that must be carefully balanced to sculpt an environment appropriate to efficient remuscularization and vascularization. A number of approaches have been explored to modulate the plasticity of these populations and control the composition and stability of the ECM, including administration of antifibrotic paracrine factors either directly or *via* stem or progenitor cells. Excessive fibrosis represents a particular challenge when surgically applying epicardial cardiac ‘patches’, because the resulting scar strongly limits electromechanical coupling and angiogenesis of the graft, limiting its functional benefit and long-term survival⁹.

Immunological balance

The immune system has multiple crucial roles both in the early and chronic stages following cardiac injury. In particular, a balanced activation of the innate immune system is necessary to clear necrotic cells, initiate angiogenesis, and promote fibroblast ingrowth, while rapid resolution of inflammation is required to allow regeneration¹⁰. In this context, the interplay between the stroma and immune cells has a crucial role, offering promising therapeutic avenues. The adaptive immune system is particularly important in the context of cell transplantation^{5,6}, as rejection must be prevented by either inducing immunotolerance through administration of immunosuppressive drugs or biologics, or by using cells that are autologous or otherwise engineered to avoid immune rejection.

Closing thoughts

The first generation of regenerative therapies has been focused on individual agents or approaches, because of both practical and regulatory limitations. Looking ahead, we envision holistic approaches encompassing bioactive molecules, stem cells, bioengineering, and gene editing. By embracing complexity we can target these five cardiac regeneration hallmarks and, we think, achieve clinically meaningful heart repair in the next decade.

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Figure 1. Properties of the regenerating heart.

a | We propose that five aspirational hallmarks ought to be realized to truly reconstitute the human heart muscle. **b** | Example of optimal regenerating myocardium, in which new cardiomyocytes electromechanically couple with the surviving host myocardium, while stromal cells, new blood vessels, cells of the immune system create the necessary favourable microenvironment.