Multi-author Review
Cardiovascular development: towards biomedical applicability

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From developmental biology to heart repair

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Abstract. Advances in our understanding of cardiac development have fuelled research into cellular approaches to myocardial repair of the damaged heart. In this collection of reviews we present recent advances into the basic mechanisms of heart development and the resident and non-resident progenitor cell populations that are currently being investigated as potential mediators of cardiac repair. Together these reviews illustrate that despite our current knowledge about how the heart is constructed, caution and much more research in this exciting field is essential. The current momentum to evaluate the potential for cardiac repair will in turn accelerate research into fundamental aspects of myocardial biology.

Keywords. Heart development, cardiac repair, stem cells, T-box genes, animal models.

Extensive recent developments in our understanding of the molecular and cellular events underlying heart formation have raised the possibility that this new knowledge could be used to manipulate stem cells to repair the damaged adult heart [1]. Heart disease is of primary biomedical significance, and the possibility of regenerating or repairing damaged myocardium, traditionally considered a non-regenerating tissue, is currently being explored and holds major promise for 21st-century medicine. A thorough understanding of myocardial biology and development is a prerequisite for clinical application. Recent developments stem from a combination of the application of molecular analysis to heart morphogenesis, the power of experimental animal models, in particular the mouse, to elucidate the genetic control of developmental processes, and advances in human genetics and the identification of genes in which mutations lead to syndromic and non-syndromic heart defects [2]. As a result of these approaches, we now have major insights into the transcriptional networks, signalling pathways and cellular events that regulate heart development from the earliest specification of mesodermal progenitor cells, through the identification of the distinct progenitor cell populations which contribute to the tubular heart, to the complex remodelling events associated with formation of a four-chambered heart and the development of the cardiac conduction system [3, 4]. Despite these advances, the keys to their successful application to biomedicine lie in ongoing research into cardiomyocyte biology and cardiovascular development. We need to know what are the best cellular sources for cardiac repair? What are the cell intrinsic and paracrine factors required for cardiomyocyte specification? How do the diverse cell types which constitute a functional heart become specified and organised? How is electrical coupling initiated and maintained?

In this multi-author review we explore current findings in developmental biology which address these questions and their application to the issue of cardiac repair. The reviews highlight contemporary issues in fundamental cardiac biology and present ongoing and future steps towards biomedical application.

Transcriptional regulation of heart development is not controlled by a single family of master genes, as is the case for other tissues such as skeletal muscle, but

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rather by the combinatorial activity of multiple different factors [5]. Among these, T-box-containing transcription factors regulate multiple aspects of heart development. Brachyury, the founding member of this gene family, was the first developmental mutation to be characterised [6]. Mutations in T-box genes contribute to cardiac disease, including great artery and atrial septal anomalies, and research into T-box function has provided an entry point into understanding congenital and electrical malformations of the heart. Hoogaars and colleagues review how this gene family regulates cardiac design, including cardiomyocyte specification, venous and arterial pole morphogenesis, chamber ballooning and development of the cardiac conduction system.

Current approaches to cardiac repair include either the manipulation of resident progenitor cells or the addition of non-resident progenitor cells [1]. The idea that the heart contains endogenous resident stem cell populations is a relatively recent one. Indeed, the most promising approaches may rely on activation of endogenous cardiac cells that have the potential to contribute new cells to the damaged heart and development of techniques to exploit these cells by triggering greater regenerative responses. Resident stem cells include, among others, c-kit positive cells, which can be stimulated to adopt myocardial fates and limit myocardial damage, as discussed by Torella et al. Another class of resident progenitor cells is positive for the LIM-homeodomain transcription factor Islet-1, a marker of embryonic progenitor cells known as the second heart field which contribute to the poles of the elongating heart tube in the early embryo and which may persist in the postnatal heart [4]. Moretti et al. discuss how enriching for Isl1 positive cells can potentially provide a framework for isolating and studying the pathways for differentiation of multipotential cardiac stem cells. Essential challenges to be overcome before any clinical application include defining the extracellular triggers that activate resident progenitor cells towards myocardial and non-myocardial fates and understanding the lineage relationships, if any, between different resident stem cell populations.

While research into resident stem cell biology holds great potential for future therapies, the use of non-resident stem cells for cardiac repair has been the subject of much research and a number of clinical trials. Franco and colleagues provide an overview of current thinking with respect to non-resident cell therapy approaches. Non-resident cells, in particular bone marrow-derived stem cells, were initially thought to adopt a myocardial cell fate [1]. Extensive recent work has now shown that non-resident cell therapy acts primarily by providing a paracrine contribution to heart repair rather than contributing to new myocardium. Such approaches are now considered to facilitate cardiac repair. Cells derived from the epicardium contribute extensively to non-myocardial components of the heart during normal development, in particular to the cardiac vasculature [2]. Promotion of vascularisation is crucial for repairing damaged myocardium, and Winter and Gittenburger de Groot propose that epicardium-derived cells have potential as a source of stem cells which can contribute to cardiac repair through vascularisation and paracrine effects on damaged myocardium.

One source of non-resident progenitor cells, however, embryonic stem cells, holds significant promise as a potential source of new myocardium as our knowledge of the series of events required to drive these totipotent cells to a myocardial fate is unravelled. Filipczyk and colleagues discuss our current understanding of the signalling events and potential of the embryonic stem cell approach. This work relies on a detailed understanding of the series of signals required to drive early mesoderm development and subsequently to specify and diversify myocardial fates *in vivo* in the embryo. In addition, given the pluripotent and tumorogenic potential of stem cells, it is important to understand the brakes required to block their unwanted proliferation and differentiation into other cell types.

Our current knowledge of heart development relies on animal models, including not only mouse but also chick, *Xenopus*, zebrafish and *Drosophila*. Analysis of the fruitfly heart led to the isolation of Tinman, a homeodomain transcription factor-encoding gene in 1993 [7]. Nkx2.5, the mammalian homologue of Tinman, was subsequently shown to be essential for multiple steps of mouse heart development and heterozygous mutations in this gene contribute to congenital heart defects in man [8]. Research into the fly heart is now providing insights into the establishment and maintenance of cardiac rhythm, ageing and myocardial diversification [9, 10]. Xavier-Neto and colleagues explore the evolutionary relationship between fly and mammalian hearts and discuss how chambered hearts originated during animal evolution. They conclude that homology between fly and human hearts is not at the level of sophisticated pumping organs but at that of a primitive peristaltic vessel represented by a concentric layer of myoepithelial cells (or myocytes associated with a primitive vessel). This explains why transcriptional networks are so highly conserved across evolution despite pronounced morphological differences between species and suggests that similarities of design between these organs originated from parallel, independent adaptations to common haemodynamic constraints.

This collection of reviews thus provides an overview of some of the ongoing research into basic questions about cardiovascular development which have the potential of being applied in a biomedical setting. Central issues for cardiac repair are the exploration of resident stem cell populations, determining how these cells can be activated though paracrine effects which could eventually be used to replace cell therapy, and the signals controlling positive and negative regulation of stem cell behaviour in vivo. Clinical applicability of non-resident cells will require refinement of issues such as what the best source of non-resident cells is and how can they be optimally delivered to the hearts and home to the damaged region. Addressing these issues will require developing a thorough understanding of the sequence of events that drive mesodermal progenitor cells to a myocardial fate in the early embryo and their subsequent differentiation into mature and functionally coupled cardiomyocytes.

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