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REVIEW

Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation

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Abstract Cardiomyocyte proliferation and progenitor differentiation are endogenous mechanisms of myocardial development. Cardiomyocytes continue to proliferate in mammals for part of post-natal development. In adult mammals under homeostatic conditions, cardiomyocytes proliferate at an extremely low rate. Because the mechanisms of cardiomyocyte generation provide potential targets for stimulating myocardial regeneration, a deep understanding is required for developing such strategies. We will discuss approaches for examining cardiomyocyte regeneration, review the specific advantages, challenges, and controversies, and recommend approaches for interpretation of results. We will also draw parallels between developmental and regenerative principles of these mechanisms and how they could be targeted for treating heart failure.

Contents

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Introduction

Cardiomyopathies constitute a group of heart muscle diseases of varied etiology that are treatable with medical therapies, but cardiac function generally declines despite therapy. Most cardiomyopathies at the stage of heart failure exhibit cardiomyocyte loss. Adult mammals do not sufficiently regenerate cardiomyocytes to compensate for lost cardiomyocytes. Cardiac transplantation is the only proven method to replace lost cardiomyocytes. In light of insufficient numbers of donor organs, stimulating myocardial regeneration offers a potential strategy for treating heart failure.

In contrast to adult mammals, amphibians, reptiles, and zebrafish regenerate cardiomyocytes after myocardial injury (Jopling et al., 2010; Oberpriller and Oberpriller, 1974). The research community has directed attention to two principal strategies to regenerate myocardium: Use of stem and progenitor cells to repair damaged myocardium and enhancement of endogenous regenerative mechanisms (Garbern and Lee, 2013). This review focuses on endogenous regeneration mechanisms by cardiomyocyte proliferation.

Historical and current perspectives in cardiac regeneration

Anecdotal reports over the past 100 years have shown mitoses of cardiomyocyte nuclei; however, evidence for cardiomyocyte division remained elusive. The first systematic and quantitative examinations of the cellular mechanisms of human heart growth were published in the 1950s (Linzbach, 1950; Linzbach, 1960). One study examined the increase of cardiomyocyte cross-sectional area in the left ventricular papillary muscle in humans and concluded that cardiomyocyte enlargement could fully account for physiologic myocardial growth between birth and adulthood (Linzbach, 1950, 1960). Because examining orthogonally sectioned papillary muscles is associated with biases, the validity of extrapolating these results to the entire heart is limited. Still, historically, the mammalian heart has been viewed as a post-mitotic organ in which the primary parenchymal cells, cardiomyocytes, do not increase in number after birth (Zak, 1973; Linzbach, 1950).

Additional classical studies of cardiomyocyte proliferation used microscopy to visualize mitotic figures. However, these approaches probably did not have the precision necessary for definitive visualization of cardiomyocyte cytokinesis nor the throughput for quantifying rare events. Although not conclusive, these studies led to two paradigms: First, unlike skeletal muscle, the adult heart does not have progenitors supporting the generation of new cardiomyocytes. The second paradigm argued that there is a single cellular mechanism of post-natal developmental and pathological heart growth: cardiomyocyte enlargement (Rumyantsev and Carlson, 1991; Borisov and Claycomb, 1995). In the latter-20th century, technical advances, including use of confocal microscopy to visualize cell cycle events, automated analyses of large cell populations, and genetic and metabolic labeling for cellular fate mapping, have provided new data. This has advanced a new cellular paradigm, which includes adult cardiomyocyte renewal, while incorporating the significant role that cardiomyocyte enlargement plays in physiologic and pathologic heart growth.

The recognition of endogenous cardiomyocytes as a source for new cardiomyocytes raises the possibility of stimulating this process for myocardial repair. By understanding the mechanisms limiting the regenerative response of mammalian myocardium, therapeutic strategies could be developed. To realize this therapeutic potential, the scientific community needs to address questions involving the precise cellular source for new cardiomyocytes, the regulating signaling pathways, the underlying cellular mechanisms, and the genesis rates and dynamics.

Mechanisms of cardiomyocyte regeneration pose challenges for investigation

Cardiomyocyte generation in the adult mammalian heart is a slow process compared with the blood, skin, and the digestive system, which makes it difficult to characterize turnover dynamics. In situ time lapse imaging of the beating mammalian heart at cellular resolution is technically challenging (Hashimoto et al., 2014; Hesse et al., 2012). Myocardial biopsies from patients provide only a small amount of tissue for analysis. The cellular heterogeneity and close spatial packing of cells in the myocardium can obscure the identification of cardiomyocyte nuclei from neighboring non-cardiomyocytes (Soonpaa and Field, 1997; Soonpaa et al., 2012). Perhaps the most challenging aspect is that cardiomyocytes exhibit non-proliferative cell cycles that increase the DNA content without cell division. Despite these intrinsic biological features that complicate studying regeneration, several new methods have enabled the advance of our understanding of cardiomyocyte proliferation.

Classical and new methods have advanced cardiac regeneration research

Labeled thymidine and thymidine analogs are stably incorporated into the genome during DNA synthesis and can be used to