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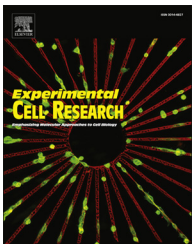

Research Article

Cardiac regeneration in non-mammalian vertebrates

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ARTICLE INFORMATION

Article Chronology:
Received 27 June 2013
Received in revised form
26 July 2013
Accepted 1 August 2013

Keywords:
Heart
microRNA
Regeneration
Teleost
Urodele

ABSTRACT

The heart is a robust organ, capable of pumping nutrients and transferring oxygen throughout the body via a network of capillaries, veins and arteries, for the entirety of a human's life. However, the fragility of mammalian hearts is also evident when it becomes damaged and parts of the organ fail to function. This is due to the fact that rather than replenishing the damaged areas with functional cellular mass, fibrotic scar tissue is the preferred replacement, resulting in an organ with functional deficiencies. Due to the mammalian hearts incapability to regenerate following damage and the ever-increasing number of people worldwide suffering from heart disease, tireless efforts are being made to discover ways of inducing a regenerative response in this most important organ. One such avenue of investigation involves studying our distantly related non-mammalian vertebrate cousins, which over the last decade has proved to us that cardiac regeneration is possible. This review will highlight these organisms and provide insights into some of the seminal discoveries made in the heart regeneration field using these amazing chordates.

Review

Mammalian adults lack the ability to regenerate damaged hearts

The multitude of steps involved with cardiac development proceeds in a similar fashion throughout the mammalian animal kingdom [1]. One such important step is the wave of embryonic precursor proliferation prior to differentiation into the cellular lineages that make up the functioning heart—cardiomyocytes, endothelial cells, smooth muscle cells, epicardium cells, fibroblasts, pacemaker cells, purkinje fibres and telocytes [2,3]. Non-cardiomyocyte cell populations predominate in the adult heart, with more than 50% of the cells identified as cardiac fibroblasts. The hyperplastic response is halted soon after birth leaving a fully functioning, beating heart with enough cardiac cells to sustain the organism for the rest of its life [4]. So for all intense purposes, the heart is a terminally differentiated organ. This lack of proliferative potential within the adult human heart leaves this major organ vulnerable, when the cardiomyocytes, cells that are essential for the rhythmically coordinated pumping action of the heart, become damaged and need replacing. Unlike the mesoderm related skeletal muscle that has an inherent dedicated stem cell to replenish damaged myofibres [5], the heart relies upon replacing the damaged or dying cardiomyocytes with collagenous scar tissue that retains the structural integrity of the organ [6]. However, the laying down of scar tissue comes at a price, as the heart no longer functions as it once did before the damage occurred, which can result in heart failure and sometimes death. Some elegant work using two novel approaches to label proliferating cardiomyocytes with carbon isotopes in humans and mice has altered our perception of the adult heart being a terminally differentiated organ [7,8]. Both studies revealed that the cardiomyocytes do have a

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0014-4827/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.yexcr.2013.08.001

capability to turnover during the animal's lifetime. However, the rate of cardiomyocyte hyperplasia is inadequate to provoke a regenerative response in damaged hearts. A population of cells resident within the adult myocardium, positive for the stem cell markers c-kit, MDR1 and Sca1, have been shown to mount a proliferative response following myocardial injury, leading to the production of new cardiomyocytes [9–11]. However, much like the cardiomyocyte turnover, the hyperplastic index of these cell-types is insufficient to mount a true cardiac regenerative response. In addition, further work is needed to distinguish whether these cell types are true cardiac stem cells or migrating cell populations that function in a cardiac independent manner within a myocardial niche. Research performed since these initial discoveries have provided promising breakthroughs in the identification and isolation of cardiac stem cells, resulting in the initiation of pre-clinical and clinical studies aimed at repairing injured myocardium [12]. Irrespective of the presence of cardiac stem cells or negligible proliferation of cardiomyocytes, mammalian hearts have a regeneration deficiency, which reflects why heart disease is a global public health issue that needs to be addressed with new therapeutic interventions [13].

Functional cardiac regeneration models in adult mammals

Previously it has been shown that foetal mammalian hearts possess the ability to regenerate [14]. Using a heart-specific conditional knockout approach to inactivate the X-linked gene encoding holocytochrome c synthase, it was demonstrated that 50% of the cardiomyocytes degenerated in heterozygous female mice at a mid-gestational time-point. Remarkably, prior to birth, there was a progressive reduction in damage, with only 10% of the cardiomyocytes showing signs of degeneration. These results demonstrated that the embryonic mammalian heart is conducive to regeneration. The adult mammalian heart, on the other hand, cannot regenerate! This provides an obvious obstacle, as there is no gold-standard adult mammalian model organism to study that allows us to comprehensively delineate the mechanisms that drive cardiac regeneration. For a short period of time an answer to this problem presented itself in the shape of the MRL mouse. Late in the 20th century this mouse strain was presented as a new model for mammalian regeneration, due to its ability to regenerate large ear-hole punches used to identify the animals [15]. Several years following this initial discovery, the same group presented work detailing how the MRL mouse could regenerate a cryogenically induced myocardial infarction without scarring [16]. However, the excitement of this discovery was short-lived as several groups disproved this finding by reporting that MRL mice induce a wound healing, scarring response to numerous methods of cardiac damage [17–19]. However, some intriguing work published in 2011 demonstrated that one-day old mouse pups were able to fully regenerate their hearts following a ventricular resection injury [20]. The hearts regenerated morphologically and functionally, with minimal signs of scarring or fibrosis. However, this regenerative response was transient, with mouse pups only several days older resorting to the deficient cardiac regenerative phenotype seen in mammalian adults. The findings presented in this work have been corroborated in an alternate mouse model of cardiac damage, using left anterior descending artery (LAD) ligation to induce a ventricular infarction [21]. The results from both publications indicate that very early post-natal mammalians still possess the ability to activate developmental pathways to cause cardiomyocytes to proliferate and replenish damaged myocardium. Exciting as these results are, the time-scale for this regenerative capacity is short lived after birth, which means we still lack a comparative adult mammalian model organism to study cardiac regeneration.

A rather fishy solution to adult cardiac regeneration

In order to study adult cardiac regeneration, comparable non-mammalian organisms were sort after to help solve the mysteries as to why mammalians hearts are so resistant to repair. The search delivered a rather unlikely candidate in the guise of the zebrafish (*Danio rerio*), a chordate organism displaced from humans by several hundred million years of evolution on the phylogenetic tree of life [22]. Up until the beginning of the 20th century, zebrafishes were being used primarily to define pathways associated with development and organogenesis [23]. However, an article published in 2002 reporting that adult zebrafish could regenerate their two-chambered hearts, elevated this bony fish's profile to even higher heights [24]. For the first time, zebrafish hearts were shown to regenerate without scarring within 30–60 days, following resection of 10–20% of the ventricle. The regeneration was due to elevated cardiomyocyte hyperplasia in close proximity to the newly regenerated myocardium but the source of the newly formed proliferating cardiomyocytes remained a mystery. That was until 2006, when the same group reported that the regenerated myocardium in zebrafish was derived from cardiac progenitor cells [25]. These results were based on measuring timed cardiomyocyte expression patterns for fluorophores under the control of a cardiac myosin light chain promoter. The identification of cardiac progenitors residing in a myocardial niche that can replenish damaged cardiomyocytes, in an adult vertebrate model organism, provided hope that similar progenitors could be found in humans. However, the authors stated themselves that a more reliable lineage trace was needed to corroborate their findings, which was justified several years later when two independent studies revealed cardiomyocytes as the source of the newly formed myocardium following resection injury [26,27]. Both papers utilised inducible Cre–Lox transgenics to lineage trace cardiomyocytes and indicated that cardiomyocytes go through dedifferentiation steps in order to achieve their proliferative potential.

Since the discovery that zebrafish hearts can regenerate, other modes of cardiac damage have been applied to determine their true regenerative capacity. One study using a genetic ablation of cardiomyocytes determined that only a minority of cardiomyocytes are necessary to induce a fully functioning regenerative response in the heart [28]. Again, using cardiac inducible Cre–Lox transgenics, over 60% of the cardiomyocytes were specifically ablated via the expression of diphtheria toxin A (DTA). This elegant technique overcame the obvious limitation associated with mechanical damage to the zebrafish heart and came to the conclusion that spared cardiomyocytes were able to dedifferentiate, proliferate and repopulate the damaged myocardium. Intriguingly, using a similar genetic ablation model to deplete ventricular cardiomyocytes, it has also been shown that progenitor-like cells could be partly responsible for the myocardial replenishment that is observed during zebrafish ventricular regeneration [29]. The methodology of this ablation study relied on creating transgenic zebrafish that has a ventricular myosin heavy chain

Please cite this article as: C. Garcia-Gonzalez, J.I. Morrison, Cardiac regeneration in non-mammalian vertebrates, Exp Cell Res (2013), http://dx.doi.org/10.1016/j.yexcr.2013.08.001