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Advances in understanding the mechanism of zebrafish heart regeneration

Kazu Kikuchi*

Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia
St. Vincent's Clinical School, University of New South Wales, Kensington, NSW 2052, Australia

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Abstract The adult mammalian heart was once believed to be a post-mitotic organ without any capacity for regeneration, but recent findings have challenged this dogma. A modified view assigns the mammalian heart a measurable capacity for regeneration throughout its lifetime, with the implication that endogenous regenerative capacity can be therapeutically stimulated in the injury setting. Although extremely limited in adult mammals, the natural capacity for organ regeneration is a conserved trait in certain vertebrates. Urodele amphibians and teleosts are well-known examples of such animals that can efficiently regenerate various organs including the heart as adults. By understanding how these animals regenerate a damaged heart, one might obtain valuable insights into how regeneration can be augmented in injured human hearts. Among the regenerative vertebrate models, the teleost zebrafish, *Danio rerio*, is arguably the best characterized with respect to cardiac regenerative responses. Knowledge is still limited, but a decade of research in this model has led to results that may help to understand how cardiac regeneration is naturally stimulated and maintained. This review surveys recent advances in the field and discusses current understanding of the endogenous mechanisms of cardiac regeneration in zebrafish.

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* Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Lowy Packer Building Level 7, 405 Liverpool Street, Darlinghurst, NSW 2010, Australia.
E-mail address: k.kikuchi@victorchang.edu.au.

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Introduction

Acute myocardial infarction (MI), typically caused by coronary artery occlusion and ischemia, is a leading cause of death worldwide. For those fortunate enough to survive MI, necrotic muscle induces a massive inflammatory response that activates reparative mechanisms including recruitment and activation of local fibroblasts, leading to the replacement of lost myocardium with collagen-rich scar tissue. The scar provides a rapid solution to cardiac injury by stabilizing the wound area; however it is not contractile and weakens cardiac output, increases susceptibility to aneurysm, induces compensatory pathology and eventually leads to heart failure. Therapies that facilitate survival or replacement of myocardium after ischemic injury in the human heart are urgently needed and would have enormous social and economic impact.

Patients with acute MI represent a significant test-bed for regenerative medicine. In principle, cardiomyocytes could be generated from a variety of cellular sources and transplanted into damaged tissues to restore functional myocardium. To date, multiple endogenous stem and progenitor cell populations have been isolated from the postnatal mammalian heart and these cells to varying degrees can differentiate into cardiomyocytes when transplanted into infarcted hearts (Bearzi et al., 2007; Beltrami et al., 2003; Bu et al., 2009; Chong et al., 2011; Ellison et al., 2013; Goumans et al., 2007; Hierlihy et al., 2002; Laugwitz et al., 2005; Matsuura et al., 2004; Messina et al., 2004; Oh et al., 2003; Pfister et al., 2010; Smith et al., 2007; Uchida et al., 2013; van Berlo et al., 2014; Ye et al., 2012). Other promising sources for generating cardiomyocytes in vitro are embryonic stem cells (He et al., 2003; Kattman et al., 2006; Kehat et al., 2001; Mummery et al., 2003; Yang et al., 2008) and induced pluripotent stem cells (Mauritz et al., 2008; Narazaki et al., 2008; Zhang et al., 2009), which have been used for treating damaged hearts in animal models (Chong et al., 2014; Laflamme et al., 2007). Cardiac stem and progenitor cells are extremely rare populations which diminish in quality with age, which makes it reasonable to explore other cellular targets for regenerative therapies. The epicardium, a mesothelial layer covering the heart, has been shown to be capable of contributing to the myocardial lineage at low frequency in infarcted mouse hearts pretreated with the natural secreted signaling peptide Thymosin β4 (Smart et al., 2011). More recently, cardiac fibroblasts have been induced to transdifferentiate into cardiomyocytes in vitro and in vivo when defined cardiac transcription factors (Ieda et al., 2010; Nam et al., 2013; Qian et al., 2012; Song et al., 2012) or miRNAs (Jayawardena et al., 2012) are overexpressed.

An alternative approach would be to identify successful examples of organ regeneration in nature, dissect their mechanisms, and then attempt to apply gained insights to humans via the provision of the appropriate regenerative stimuli. Urodele amphibians and teleosts are well-known examples of animals that possess remarkable regenerative capacity in a variety of structures and organs as adults (Brockes and Kumar, 2008; Poss, 2010). Among these, the zebrafish (*Danio rerio*) is a relatively new experimental model in regeneration biology, and has been quickly established as the standard for investigating mechanisms of natural organ regeneration, primarily due to its amenability to genetic approaches. Zebrafish are highly regenerative as adults and regrow injured or amputated tissues such as fins (Johnson and Weston, 1995), maxillary barbel (LeClair and Topczewski, 2010), retinae (Vihtelic and Hyde, 2000), optic nerves (Bernhardt et al., 1996), spinal cord (Becker et al., 1997), heart muscle (Poss et al., 2002), brain (Kroehne et al., 2011), hair cells (Ma et al., 2008), pancreas (Moss et al., 2009), liver (Sadler et al., 2007), and kidney (Diep et al., 2011).

Although cardiac regeneration and repair have been investigated in other teleost models (Grivas et al., 2014; Ito et al., 2014; Lafontant et al., 2012), zebrafish arguably display the most robust and best characterized cardiac regenerative responses known to date among non-mammalian vertebrate models (Chablais et al., 2011; González-Rosa et al., 2011; Parente et al., 2013; Poss et al., 2002; Schnabel et al., 2011; Wang et al., 2011). This review will summarize recent advances in the field of regenerative medicine and discuss cellular and molecular mechanisms underlying the cardiac regenerative response in zebrafish.

Origins of regenerated myocardium

Fate-mapping studies

Identifying cellular origins of regenerating tissues is a fundamental question in regeneration biology. Several studies have performed fate-mapping analyses to investigate the cellular origin(s) of cardiac muscle during heart regeneration in zebrafish. By using inducible genetic fate-mapping techniques (Buckingham and Meilhac, 2011), two studies directly examined the contribution of cardiomyocytes to the regenerating zebrafish heart (Jopling et al., 2010; Kikuchi et al., 2010). In both cases, two transgenic lines were used: one line carries a 4-hydroxytamoxifen (4-HT) – inducible Cre recombinase (CreER) gene from which expression is driven in cardiomyocytes by the promoter of the *cardiac myosin light chain 2* (*cmlc2*) gene, also known as *myosin light chain 7* (*myl7*) gene; and the other is an indicator line in which enhanced green fluorescent protein (EGFP) reporter expression can be induced in CreER-