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Wnt/β-catenin signaling in heart regeneration

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Abstract

The ability to repair damaged or lost tissues varies significantly among vertebrates. The regenerative ability of the heart is clinically very relevant, because adult teleost fish and amphibians can regenerate heart tissue, but we mammals cannot. Interestingly, heart regeneration is possible in neonatal mice, but this ability is lost within 7 days after birth. In zebrafish and neonatal mice, lost cardiomyocytes are regenerated via proliferation of spared, differentiated cardiomyocytes. While some cardiomyocyte turnover occurs in adult mammals, the cardiomyocyte production rate is too low in response to injury to regenerate the heart. Instead, mammalian hearts respond to injury by remodeling of spared tissue, which includes cardiomyocyte hypertrophy. Wnt/β-catenin signaling plays important roles during vertebrate heart development, and it is re-activated in response to cardiac injury. In this review, we discuss the known functions of this signaling pathway in injured hearts, its involvement in cardiac fibrosis and hypertrophy, and potential therapeutic approaches that might promote cardiac repair after injury by modifying Wnt/β-catenin signaling. Regulation of cardiac remodeling by this signaling pathway appears to vary depending on the injury model and the exact stages that have been studied. Thus, conflicting data have been published regarding a potential role of Wnt/β-catenin pathway in promotion of fibrosis and cardiomyocyte hypertrophy. In addition, the Wnt inhibitory secreted Frizzled-related proteins (sFrps) appear to have Wnt-dependent and Wnt-independent roles in the injured heart. Thus, while the exact functions of Wnt/β-catenin pathway activity in response to injury still need to be elucidated in the non-regenerating mammalian heart, but also in regenerating lower vertebrates, manipulation of the pathway is essential for creation of therapeutically useful cardiomyocytes from stem cells in culture. Hopefully, a detailed understanding of the *in vivo* role of Wnt/β-catenin signaling in injured mammalian and non-mammalian hearts will also contribute to the success of current efforts towards developing regenerative therapies.

Keywords: Wnt, Beta-catenin, Cardiomyocyte, sFrp, Regeneration, Heart, Fibrosis, Hypertrophy, Zebrafish

Introduction

All organisms have evolved means of repairing tissue loss after injury or disease. In most species, healing of epidermal wounds and other epithelia is an efficient repair process, whereas the ability to recuperate the damage in other tissues varies widely. Mammals (including humans) can repair injury of skeletal muscle, regenerate large parts of the liver, and repair damage to the epithelia of the kidney and the lung but have limited regenerative capacity in other organs [1]. In contrast, other vertebrates, such as urodele amphibia (salamanders and newts) and certain teleost fish species, can completely regenerate lost limbs and tails and repair damage to the lens, the retina, and the central nervous system [2–5]. Importantly, zebrafish and newts can also replace lost heart tissue in adults. A thorough understanding of the cellular and molecular mechanisms of regeneration in urodele amphibia and fish is therefore very likely to be informative for the development of regenerative therapies in humans.

One of the pivotal signaling pathways regulating the regenerative process in many systems is the Wnt/β-catenin signaling pathway. In addition, Wnt/β-catenin signaling is activated in response to cardiac injury in adult mammals and plays important roles in hypertrophy and cardiac remodeling [6]. Here, we review the cellular mechanisms underlying heart regeneration in lower vertebrates and the known functions of Wnt/β-catenin signaling in the cardiac

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injury response both in mammals and in non-mammalian vertebrates.

Mammalian heart injury responses

The adult mammalian heart has a very limited capacity to repair loss of cardiomyocytes (CMs) after infarction or cardiac overload disorders [7]. In contrast, adult zebrafish can regenerate the heart in response to several injury paradigms, including surgical removal of myocardial tissue, cryoinjury, and genetic ablation of cardiomyocytes [3, 8–12]. Intriguingly, neonatal mice can regenerate the myocardium after partial surgical resection as well, but this ability is lost by 7 days after birth [13]. During both zebrafish and neonatal mouse heart regeneration, differentiated CMs re-enter the cell cycle to proliferate and genetic lineage tracing experiments indicate that the majority of the newly forming myocardium is derived from pre-existing CMs [13–15]. Thus, zebrafish and neonatal mice regenerate lost myocardium not by differentiation of CMs from progenitor cells but by activating the re-entry of differentiated CMs into the cell cycle. In contrast, adult mammalian CMs have long been thought to be terminally differentiated and thus post-mitotic [16–18] and much of the growth of the postnatal mammalian heart occurs via enlargement of pre-existing CMs, *i.e.*, hypertrophy [18, 19]. While some studies in adult mouse and human myocardial cells have suggested that certain CMs are not terminally differentiated and can reinitiate the cell cycle under physiological or pathological conditions [20–22], others have found little evidence for CM proliferation in the adult heart [23–25]. Indeed, using transgenic mice that facilitated unambiguous identification of CM nuclei, Soonpaa and Field found that only 1 out of 180.000 adult mouse ventricular CMs incorporate [^{3}H] thymidine, and this increased to only 3 in 36.000 nuclei (0.0083 %) in the injured heart [23–25]. The consensus conclusion from these and other studies is that DNA synthesis is very rare in differentiated adult rodent CMs even after injury, and CM hypertrophy after injury appears to largely occur without increase in DNA content in the adult heart [24, 26].

Nevertheless, there is good evidence that some new CMs do form during adult mammalian life, including humans. A pulse of atmospheric carbon-14 (^{14}C) was generated by nuclear bomb tests during the Cold War and rapidly declined after atmospheric tests were banned. Since ^{14}C makes its way through the food chain into human cells, the ^{14}C levels found in DNA of human CMs corresponds to the atmospheric levels at the time when these cells were born. Bergmann and colleagues have measured the ^{14}C concentrations in DNA of human myocardial cells [27]. They have found that in subjects born up to 22 years before the onset of bomb tests, ^{14}C concentrations were elevated compared to the levels before the tests, indicating that myocardial cells contained DNA synthesized years after birth. Thus, human CMs are capable of renewal during adulthood [27]. CM renewal, however, is very slow as estimated from this study; 1 % of the CMs are renewed per year at the age of 25 and only 0.45 % at the age of 75. Thus, approximately 45 % of all CMs are exchanged during a human lifetime while 55 % remain from neonatal stages [27].

While it is not possible to identify the cellular source of newly forming CMs in the adult human heart, several studies in mice have addressed whether CM renewal during homeostasis and the low-rate CM replacement after cardiac injury are due to proliferation of existing CMs or due to CM differentiation from progenitor cells [26, 28–33]. Unfortunately, the conclusions drawn by several studies based on genetic lineage tracing of CMs or cardiac progenitor cells differ considerably. While most reports using different means to track the fate of differentiated CMs conclude that renewal of CMs during homeostasis is due to proliferation of existing CMs [26], one study concluded that progenitor cells contribute to CM formation after cardiac injury, since the progeny of differentiated CMs get diluted with other cells in injured hearts [28]. In contrast, other studies using lineage tracing of differentiated CMs and multi-isotope imaging mass spectrometry concluded that the limited CM replacement after injury is due to CM proliferation [29, 32]. However, c-kit-positive cardiac progenitor cells have been reported to contribute significantly to CM production after cardiac injury as well [30]. In contrast, another study found that c-kit-positive cells form negligible numbers of CMs both during homeostasis and after injury [33]. The dissimilar results obtained by these studies are a reminder of the fact that albeit genetic lineage tracing tools are the most powerful way to address questions of cellular lineage, each tool needs to be very critically evaluated. In particular, it is possible that some Cre lines are not specifically expressed in a particular cell type; Cre expression could cause toxicity, and the fact that most tools fail to label all cells of a particular cell population could result in failure to appreciate that the labeled population is actually heterogeneous. Yet, while further studies are needed to clarify how CMs are formed in the adult mammalian heart, the consensus is that the rate of CM formation is too low to result in significant myocardial regeneration after heart injury [26].

A possible reason for the failure of adult mammalian CMs to sufficiently proliferate in response to cardiac insult might be their increased DNA content. Most adult mammalian CMs contain more than two sets of chromosomes. Starting at 4 days post birth, rodent CMs grow and become binucleated with each nucleus remaining diploid [18]. In contrast, most human CMs maintain a single nucleus, which however increases its DNA content to tetraploidy or even higher ploidy [34–36]. Binucleation, as seen in rodents, likely represents an impediment to