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Review

Wnt/β-Catenin Signaling during Cardiac Development and Repair

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Abstract: Active Wnt/β-catenin signaling is essential for proper cardiac specification, progenitor expansion and myocardial growth. During development, the mass of the embryonic heart increases multiple times to achieve the dimensions of adult ventricular chambers. Cell division in the embryonic heart is fairly present, whereas cell turnover in the adult myocardium is extremely low. Understanding of embryonic cardiomyocyte cell-replication, therefore, could improve strategies for cardiac regenerative therapeutics. Here, we review which role Wnt signaling plays in cardiac development and highlight a selection of attempts that have been made to modulate Wnt signaling after cardiac ischemic injury to improve cardiac function and reduce infarct size.

Keywords: Wnt/β-catenin; heart; development; cardiomyocyte proliferation; regenerative medicine

1. Introduction

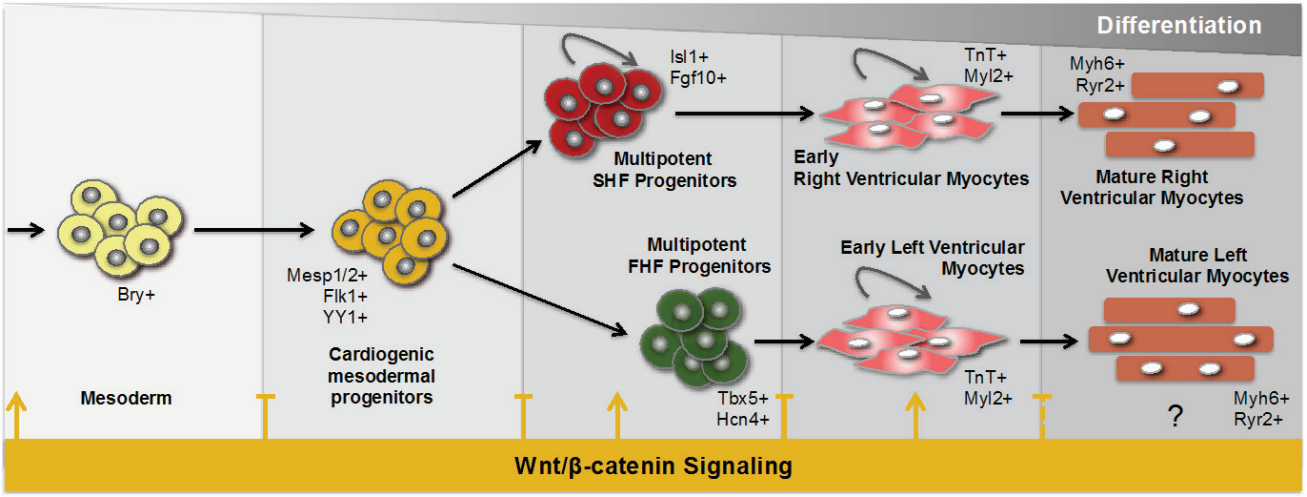
Cardiovascular diseases and especially heart failure are among the most frequent disease entities worldwide. A loss of functional cardiomyocytes overtime can perturb the balance between the body's oxygen demands and the blood supply generated by the heart. Current therapies aim to prevent adverse cardiac remodeling, but do not restore the number of myocytes lost after myocardial infarction. Regeneration of myocardial tissue after myocardial infarction through endogenous renewal of cardiomyocytes is minimal [1]. Therefore, it is pivotal to understand the molecular and genetic factors that control cardiomyocyte proliferation and differentiation during early cardiac development, since it can provide crucial insights for cardiac regeneration. The heart is the first organ to be formed in the mammalian embryo, where its role becomes essential to supply the exponential increasing demands in nutrients as is required for growth [2,3]. The Wnt/β-catenin signaling pathway plays an important role in embryonic cardiac specification, cardiovascular progenitor expansion and cardiomyocyte proliferation [4]. Wnt signaling is rarely reported to be active in the adult heart; however, recent evidence suggests that after ischemic damage the myocardium and epicardium exhibit active Wnt signaling [5,6]. Here, we focus on Wnt signaling and its role in cardiac development. We also display a selection of efforts that have been made to modulate Wnt signaling after cardiac ischemic injury to improve infarct healing and functional outcome.

2. Wnt Signaling during Cardiogenesis

Cardiogenesis is a highly complex process that is governed by a dynamic interplay between embryonic growth pathways and transcriptional regulators controlling cell fate and specification [3,7,8] Previous work from numerous laboratories has shown that the embryonic Wnt signaling pathway is essential during cardiogenesis and development [9]. Wnt signaling involves multiple complex signaling cascades, of which the β-catenin mediated canonical pathway and the non-canonical pathway are the most widely known [10]. The Wnt signaling system, consisting of 19 lipophilic proteins, controls wound repair and regeneration in simple organism such as planaria and hydra [11,12] to hair follicle, sweat gland and intestinal crypt regeneration in mammals [13–15]. Furthermore, Wnts are evolutionary conserved for their role in early development of the mammalian heart [16–18]. The heart in mammals is embryonically derived from the mesodermal layer, which rises from the primitive streak directly after gastrulation. Wnt signaling is required for the gastrulation process and in embryos from β-catenin knockout mouse the mesoderm failed to arise from the inner layer of endoderm cells [19]. During normal mouse cardiac development, the cells from the primitive streak migrate anteriorly to form the cardiac crescents of the splanchnic Mesp1/2+ mesoderm [20,21]. To achieve cardiac specification of these Mesp1/2+ cells Wnt/β-catenin is repressed in the cardiac mesoderm. The presence of a constitutive active β-catenin molecule in Mesp1+ cells abrogated cardiac tube formation in the mouse embryo, while antagonizing Wnt/β-catenin through Dkk-1 initiates cardiogenesis (Figure 1) [4,22,23]. Around E7.5, the cardiac crescents arise from cardiac mesoderm, of which the posterior located crescent is characterized as the First-Heart-Field (FHF), whereas the anterior crescent is identified as the Second-Heart-Field (SHF). Tbx5 and Hcn4 were identified as markers of the early FHF and fate

mapping experiments have shown that, in the posterior cardiac crescent, these Tbx5+ and Hcn4+ cells give rise to the left ventricle and parts of the atria [24–27]. Isl1 and FGF10 were found as specific markers of the SHF and lineage tracing revealed the contribution of Isl1+ cell populations to the right ventricle and outflow tract on the arterial pole of the heart, whereas FGF10 also contributed to the venous pole of the early heart structure [28–31]. In mouse, the FHF and SHF regions fuse by E8 into a linear heart tube, followed by a looping process to eventually form the four-chambered heart [2,32]. From E8.5 on, bipotent Nkx2.5+ progenitors, contributing to the myocardial and smooth muscle lineages fuel an early increase in cardiac mass [33]. Isl1, a LIM homeodomain transcription factor, moreover, marks a distinct population of multipotent Isl1+ cardiovascular progenitors that play an essential role in sourcing the right ventricular myocardium and outflow tract [28]. Undifferentiated Isl1+ progenitors were shown to have the ability to give rise to endothelial cells, smooth muscle cells and cardiac myocytes [34]. Furthermore, it was shown that Wnt/β-catenin signaling controls the clonal expansion of Isl1+ progenitors. Mice with constitutively activated β-catenin in the Isl1 lineage showed to have a massive accumulation of Isl1+ cells in the SHF-derived structures as the right ventricle and the outflow tract, while conditional β-catenin loss of function studies revealed an arrest in development through attenuated expansion of Isl1+ progenitors (Figure 1) [18,35,36]. After the specification of multipotent progenitors into committed ventricular progenitors or early cardiac myocytes, growth continues. And while the whole fetal heart is growing extensively, tight regulation per area is required. For many years, it is known that the outside layer, also called the compact myocardium, proliferates more rapidly when compared to the trabecular myocardium in luminal regions of the heart [37,38]. This region specific proliferation of fetal cardiomyocytes is necessary for proper morphogenesis of ventricular myocardium, trabeculae and volume of chamber cavities. Recent work showed that Wnt/β-catenin regulates this regional expansion of ventricular myocytes (Figure 1). β-catenin was predominantly observed in the compact zone of the myocardium and when β-catenin was knocked out in ventricular cardiomyocytes this resulted in a reduction of the compact zone of the myocardium and an arrest in development around E12.5. Conversely, ubiquitous activated β-catenin in ventricular myocytes caused an increase in trabecular proliferation at E12.5. This increased ventricular proliferation was sustained until birth [39,40]. To date, it remains unknown what exact role Wnt/β-catenin has in homeostasis of the adult myocardium. Moreover, it might play a role in endogenous cardiac repair and remodeling, since fetal gene programs, including Wnt signaling, are re-expressed upon myocardial infarction [5,41].

Figure 1. Distinct Phases of Wnt/β-catenin Signaling in Cardiac Development. Stage specific roles of Wnt/β-catenin signaling in proliferation and differentiation of cardiac progenitors and ventricular myocytes. Wnt/β-catenin is spatiotemporally activated or repressed to orchestrate normal cardiac formation; whereas activation of Wnt/β-catenin is required for mesodermal specification [42], repression of Wnt/β-catenin is mandatory for specification of cardiogenic mesodermal precursors and multipotent progenitors [43]. Subsequently, activated Wnt/β-catenin signaling has proliferative effects in multipotent progenitors [18] and early ventricular myocytes, while the repression of Wnt/β-catenin signaling in this stage promotes further differentiation and exiting of the cell cycle [39]. To date, it is unknown what exact effects Wnt/β-catenin exerts on adult cardiomyocytes. Arrows represent activated Wnt/β-catenin signaling; T's indicate repressed Wnt/β-catenin signaling; FHF, first-heart-field; SHF, second-heart-field.

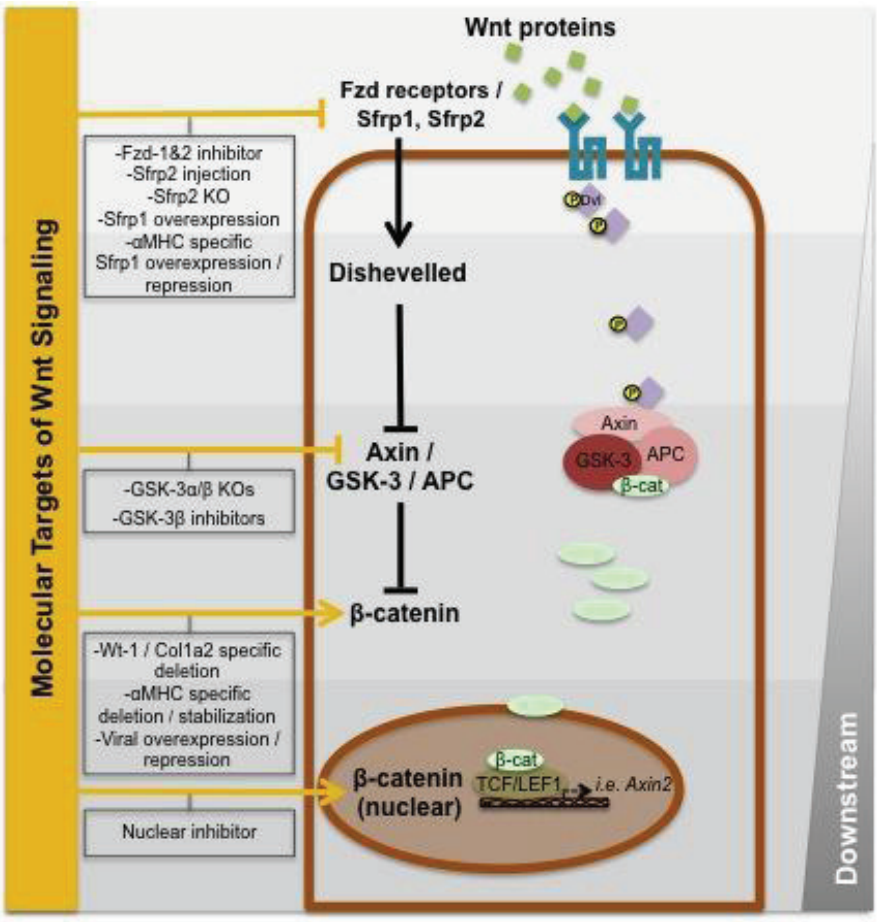


3. Wnt Signaling during Cardiac Repair

Unlike neonatal heart tissue, the mammalian postnatal myocardium exhibits a very low turnover of cardiomyocytes or replenishment by resident progenitors, and therefore almost completely lacks the potential to regenerate [1,44–46]. Multiple tissues in the body with regenerative capacities, such as skin and liver, however, rely on embryonic growth pathways to compensate for lost cells or to refresh damaged tissue [15,47]. After ischemic cardiac injury the former functional adult myocardium is largely replaced by fibrotic scar tissue. Current therapies successfully minimize the duration of ischemia and pharmacologically prevent (adverse) remodeling of the remaining myocardium, but do not lead to restoration of the number of lost ventricular muscle cells [48,49]. At the cellular level, fetal gene programs are reactivated in response to myocardial damage [41]. Work from several groups has shown that modulation of canonical Wnt signaling in murine or rat cardiac ischemia models improved post-infarct outcomes, with various results (Table 1). Wnt signaling is activated when Wnt protein ligands occupy the Frizzled protein receptors [50]. In cooperation with lipoprotein receptor-related proteins, the Wnt signal is transferred over the membrane [51] Subsequently, activated Dishevelled proteins disrupt the degradation-complex of glycogen synthase kinase 3 (GSK-3), APC and Axin, resulting in stabilization of cytoplasmic β-catenin and leading to nuclear accumulation [52]. Active nuclear β-catenin will activate the Wnt target genes with its cofactors LEF-1 and TCF [53,54].

Wnt signaling, therefore, can potentially be modulated at these described levels (Figure 2). In a permanent left anterior descending artery (LAD) ligation mouse model, Wnt signaling and its downstream effectors are activated upon infarction, manifested by increased levels of β-catenin, Dishevelled-1 and adenomatous polyposis coli (APC) protein in resident endothelial cells and newly formed vessels and an increase of Dishevelled-1 in the infarcted areas and border zones [55,56]. Using an Axin2 promotor-driven LacZ-expressing murine myocardial infarction (MI) model, we confirmed that post-MI several cell types react and express LacZ; 4 days after LAD ligation active Wnt/β-catenin signaling was observed in cardiomyocytes, smooth muscle cells, endothelial cells and progenitors [5]. Using a similar model with the TOPGAL (beta-galactosidase gene driven by a T cell factor (TCF) beta-catenin responsive promoter) mice, others subsequently showed that canonical Wnt signaling is present 4 days post-MI in both subpericardial and perivascular endothelial cells and an increase in endothelial-to-mesenchymal transition, most likely upon Wnt activation [57].

Figure 2. Selected Molecular Targets of Wnt Signaling Studied for Cardiac Regeneration. When Wnt proteins attach to the Frizzled-receptors, to activate Wnt/β-catenin signaling, Dishevelled is subsequently phosphorylated. Next, Dishevelled targets the Axin/GSK-3/APC destruction complex and thereby inhibits the degradation of β-catenin. As a result, β-catenin accumulates in the cytoplasm and will be translocalized to the nucleus where it binds to TCF and LEF-1 transcription factors to start transcription of direct downstream Wnt target genes as Axin2. Fzd, Frizzled; Sfrp, soluble frizzled related protein; GSK, Glycogen Synthase Kinase; KO, knockout; MHC, myosin heavy chain.



At the membrane level, overexpression of Secreted Frizzled Related Protein 1 (Sfrp1), which causes a decrease in Wnt signaling by occupying its target receptors as decoys, causes a reduction in infarct size and improved cardiac function [58]. The injection of recombinant Sfrp2 two days post-LAD ligation gave similar results [59]. Then again, genetic deletion of Sfrp2 showed reduced fibrosis and better cardiac function compared to littermate controls two weeks after LAD ligation [60]. Synthesized peptides, targeting the Frizzled-1 and Frizzled-2 receptor and thereby antagonizing Wnt signaling, also showed reduction of infarct area and increase in repair [61]. Furthermore, alpha myosin-heavy-chain-driven overexpression of Sfrp1 caused decreased cardiac function and increase of the infarct size after ischemia-reperfusion and annihilated the effect that preconditioning has on reducing infarct size [62]. Presumably, timing and technical differences explain these controversies.

At the level of cytoplasmic degradation of β-catenin, inhibition of GSK-3, APC and Axin results in activation of Wnt signaling. Knocking-down both the α- and β-isoform of GSK-3 showed no difference in cardiac function after ischemic injury compared to wild type controls [63]. Knocking down GSK-3α caused an increase in mortality following LAD ligation, potentially via increased apoptosis and fibrosis and a decrease in cardiac function [64]. Targeting the other isoform GSK-3β, using either a genetic knock-out model or small inhibitory molecules (Lithium and SB216763), was associated with preserved cardiac function, less apoptosis and increased capillary density in the infarcted area [65,66]. However, it remains unknown if there is an actual refreshment of cardiomyocytes underlying this improved cardiac outcome. Furthermore, β-catenin itself, as a downstream target of GSK-3, APC and Axin, was targeted in several studies using different approaches. Vector based expression of β-catenin in mice caused better functional outcomes and decreased apoptosis compared to wild type controls [67]. Down-regulation of β-catenin in cardiac fibroblasts caused increased left ventricular dilatation *in vivo* and a decrease in fibroblast proliferation *in vitro* following ischemia [6]. Alpha myosin-heavy-chain (αMHC) specific deletion of β-catenin was superior over αMHC specific β-catenin stabilization, with significantly improved functional outcomes and an upregulation of cardiac fetal gene expression in animals lacking β-catenin in the heart [68]. A recent study used a murine LAD ligation model and treated mice immediately after ligation with an one-time injection of a Wnt inhibitor ICG-001, thereby antagonizing nuclear β-catenin; 10 days post-LAD occluded animals had better cardiac function and showed an increase in endothelial-to-mesenchymal transition compared with controls [69].

These conflicting results may very well be due to the difference in timing, dosage and strategies. And while Wnt/β-catenin exerts highly stage specific effects during cardiac development, the same might be true for the process of ischemic cardiac damage and the endogenous repair mechanisms of the heart.

In conclusion, Wnt/β-catenin signaling plays a pivotal role in cardiovascular development. Moreover, Wnt signaling is evidently associated with the cellular mechanisms following cardiac ischemic injury. Thus far identified targets are Frizzled, GSK-3 and β-catenin. Current evidence, however, is lacking clear definite conclusions regarding cellular renewal of the myocardium, so further studies should aim to investigate the cell-specific stage-specific manipulation of Wnt/β-catenin signaling after cardiac ischemic injury and enhancement of endogenous repair.

Table 1. Selection of literature on Wnt modulation in cardiac ischemic injury. All studies used a left anterior descending artery (LAD) ligation model in mice or rats. An ischemia-reperfusion model was used in some studies ([62,66]). Fzd = Frizzled. Sfrp = soluble frizzled related protein. GSK = Glycogen Synthase Kinase. KO = knockout. MHC = myosin heavy chain. MI = myocardial infarction. LVEF = left ventricular ejection fraction. CM = cardiomyocytes. LV = left ventricle. EMT = endothelial-to-mesenchymal transition.

References	Target	Treatment/modulation	Wnt/β-cat	Timing	Outcome
[61]	Fzd-1/Fzd-2	Peptidergic Fzd-1/Fzd-2 antagonist UM206	↓	0 or 14d post-MI (similar results)	Reduction of infarct expansion and increased repair in infarct area
[59]	Sfrp2	Recombinant Sfrp2 injection	↓	Injection 2d post-MI	Reduced fibrosis and improved LVEF
[60]	Sfrp2	KO mice	↑	-	Reduced fibrosis and significantly higher LVEF compared to controls
[62]	Sfrp1	αMHC-specific Sfrp1 overexpression with doxycycline inducible repression	↓	Overexpression/repression (1wk prior to MI)	Ischemic preconditioning caused improved outcomes after MI due to GSK-3β inhibition, but this effect was diminished in Sfrp1-overexpressing CM
[58]	Sfrp1	Overexpression of Sfrp1	↓	-	Reduction of infarct size, fibrosis and improved cardiac function (7 d or 30 d post-MI)
[64]	GSK-3α	KO mice	↑	-	Increased mortality (10 d post-MI), more LV dilatation, dysfunction, hypertrophy, fibrosis and heart failure (8 weeks post-MI) and increased apoptosis in border zone (2 d post-MI) in KO mice
[65]	GSK-3β	KO mice (tamoxifen inducible)	↑	KO at 3d post-MI	Improved LVEF and LV dilatation with less hypertrophy post-MI in GSK-3β KO mice (8 weeks post-MI)
[63]	GSK-3	KO mice	↑	-	No functional difference between GSK-3 KO mice and controls
[66]	GSK-3β	Inhibitors (Lithium/SB216763)	↑	Directly after MI	GSK-3β inhibition mimicked ischemic precondition, resulting in less apoptotic cardiomyocytes and increased capillary density
[69]	β-catenin	Nuclear inhibitor (ICG-001)	↓	Directly after MI for 10d	Improved LVEF (10 d post-MI) and increased EMT in epicardial cells in treated mice
[6]	β-catenin	Downregulation (tamoxifen inducible) in cardiac fibroblasts	↓	10d prior to MI	Increased left ventricular dilatation (8 d post-MI) and decreased cardiac fibroblast proliferation *in vitro*
[68]	β-catenin	αMHC specific depletion or stabilization of β-catenin	↓/↑	-	Upregulation of fetal gene program (GATA4, Tbx5) and improved MI outcomes (LVEF and mortality) in β-catenin depleted animals compared to stabilization
[67]	β-catenin	Overexpression of β-catenin	↑	Directly after MI	Decreased left ventricular dilatation, increased fractional shortening and decreased apoptosis compared to controls (7 d post-MI)

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Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

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