This article is the introduction of a new thematic series on Wnts in Cardiovascular Development and Disease, which includes the following articles:

An Updated Overview on Wnt Signaling Pathways: A Prelude for More [Circ Res. 2010;106:1798–1806]
The Multiple Phases and Faces of Wnt Signaling During Cardiac Differentiation and Development [Circ Res. 2010;107:186–199]
The Role of Wnt Signaling in Physiological and Pathological Angiogenesis

Wnt Signaling in Cardiac Hypertrophy and Remodeling: Lessons Learned From Cardiac Development

Abstract: On pathological stress, the heart reactivates several signaling pathways that traditionally were thought to be operational only in the developing heart. One of these pathways is the WNT signaling pathway. WNT controls heart development but is also modulated during adult heart remodeling. This review summarizes the currently available data regarding WNT signaling during left ventricular (LV) remodeling. Upstream, soluble frizzled-related proteins (sFRPs) block WNT-dependent activation of the canonical WNT pathway. By inhibition of WNT activation, these factors also reduce β-catenin–dependent transcription by altering the ratio of cytoplasmic/nuclear β-catenin. In experimental settings, sFRPs injected into the heart attenuated LV remodeling. sFRPs are secreted from autologous bone marrow–derived mononuclear cells. Disheveled is a signaling intermediate of both the canonical and noncanonical WNT pathway. Similarly to the effect of sFRP, depletion of a disheveled isoform attenuated LV remodeling. In contrast, disheveled activation led to progressive dilated cardiomyopathy. Inhibition of nuclear β-catenin signaling downstream of the canonical WNT pathway significantly reduced postinfarct mortality and functional decline of LV function following chronic left anterior descending coronary artery ligation. WNT signaling also affects mobilization and homing of bone marrow–derived vasculogenic progenitor cells. Finally, heart-specific WNT/β-catenin interaction partners have been identified that will possibly allow targeting this pathway in a tissue-specific manner. In summary, the WNT pathway plays a pivotal role in adult cardiac remodeling and may be suitable for therapeutic interventions.

Key Words: heart failure ■ progenitor cells ■ angiogenesis ■ WNT ■ β-catenin

On pathological stress, the heart reactivates several signaling pathways that traditionally were thought to be operational only in the developing heart.1 Recently published data prove the adult heart to be capable of limited regeneration from cardiac-endogenous precursors.2 Such adult cardiac progenitor cells (CPCs) appear similar to prespecified cardiac stem cells in the embryo.3 Canonical WNT signaling controls the proliferation and differentiation of embryonic cardiac precursors toward mature cardiomyocytes.4,5 Both positive and negative WNT signaling is observed during cardiac...
remodeling of the adult heart on stress or injury: WNT factors, as well as frizzled-related proteins inhibiting WNTs, are detected.6–8 This review summarizes the available data regarding WNT signaling in the adult heart. Because the role of WNT in cardiac development may allow understanding of the mechanisms also relevant in adult cardiac remodeling, the review begins with a brief summary of the existing knowledge regarding WNT signaling in cardiac precursor cells.

WNT/β-Catenin Signaling Axis in Embryonic and Adult Cardiac Precursor Cells

The heart develops from a common precursor. One of the earliest cardiac-specific transcription factors expressed both in cultured embryonic stem cells, as well as in situ, is the basic helix–loop–helix factor MesP1 (Figure 1).9 It is appearing at approximately embryonic day (E)5.5 in the murine cardiac development and vanishes by E8.5.10 In vitro, MesP1 is required and sufficient to induce cardiovasculogenesis from embryonic stem cells. The key factor downstream of MesP1 is the inhibitor of the WNT/β-catenin canonical signaling cascade, dickkopf-1. Whether dickkopf-1 is the sole transcriptional target of MesP1 responsible for cardiac cell fate determination remains a matter of debate: following MesP1 activation, the whole panel of cardiac transcription machinery is found to be upregulated.9–11 More precisely, forced expression of MesP1 in ES cells leads to the appearance of cardiomyocytes with intact gap junctions, direct expression of Nkx2.5, MEF2c, and GATA4, as well as sprouting of vasculogenic cells positive for von Willebrand factor (vWF) and CD31.11 In summary, inhibition of the canonical WNT- pathway via dickkopf appears to mediate early differentiation of several independent cardiac precursor cell pools from a common cardiac stem cell marked by MesP1 expression (Figure 1). These cells have the capacity to restore functional myocardium in the adult heart following

Figure 1. Schematic summary of WNT/β-catenin signaling regarding developmental and adult CPC proliferation and differentiation. CPCs expressing markers of first and second heart field progenitors were identified in both the murine embryo and adult heart. To date, no MesP1+ multipotent CPCs have been described in the adult heart. WNT/β-catenin activation is required for amplification of CPCs in both the embryo and the adult heart. WNT/β-catenin inhibition, together with activation of other signaling pathways (ie, BMP), not depicted in the scheme controls cell fate determination (ie, first vs second heart field or vasculogenic) and differentiation toward contractile LV cardiomyocytes.
damage: a population of CPCs from rhesus monkey pluripotent embryonic stem cells was able to integrate into an infarct scar without forming teratomas. This maneuver replaced \( \approx 20\% \) of the scar tissue in rhesus monkey heart with contractile myocardial tissue.12

Similarly to the role of dickkopf downstream of MesP1, the insulin-like growth factor binding protein (IGFBP)-4 was found to induce cardiomyocyte differentiation from P19CL6 cells. Independent of IGF, IGFBP-4 leads to the expression of cardiac troponin T and other markers of cardiomyocyte differentiation in P19CL6 cells. IGFBP-4 attenuated activation of a \( \beta \)-catenin–dependent reporter gene on stimulation with Frizzled 8. Several independent experiments then proved IGFBP-4 to inhibit WNT signaling directly at the cell membrane receptor complex by binding to Frz8 and LRPR.6

Other pathways known to be involved in cardiomyocyte differentiation, namely the bone morphogenetic protein (BMP) signaling cascade, were not affected. IGFBP-4 was not only sufficient but also required for cardiomyocyte differentiation in this system: knockdown of IGFBP-4 abrogated cardiomyocyte differentiation.13 Interestingly, we identified IGFBP-5 to be a target for \( \beta \)-catenin–dependent transcription: gene array experiments with adult left ventricular (LV) tissue from mice with heart-specific, conditional \( \beta \)-catenin stabilization (\( \beta \)-catenin\(^{\alpha\text{MHC}-\Delta\text{exon3}} \)) revealed IGFBP-5 to be a transcriptional target of \( \beta \)-catenin.14 The role of this observation is currently unclear because IGFBP-1, -2, -4, and -6 but not IGFBP-5 and -3 were found to inhibit WNT-dependent signaling. Possibly the IGF pathway itself is involved; forced, cardiac-specific expression of miIGF was found to attenuate LV remodeling following myocardial infarction. This was not mediated by the AKT pathway but via the alternate pathway of phosphoinositide-dependent protein kinase (PDK1) and serum glucocorticoid kinase (SGK1). The authors suggested endogenous regeneration to be involved in this process.15

In cardiac development, WNT/\( \beta \)-catenin has a stage-specific, at least biphasic, role.4,5 This state of affairs is covered in more detail in another review of this series.16 Experiments with embryonic stem cell cultures revealed WNT/\( \beta \)-catenin inhibition plus activation of the BMP pathway to enhance differentiation toward a cardiomyocyte phenotype but block hematopoietic and vascular lineage development in the early stage (Figure 1).17 Similar observations were made in vivo, where at approximately E5.5, upregulation of WNT/\( \beta \)-catenin is required to amplify the cardiomyogenic precursor cell pool of both the first and second heart fields, giving rise to the left and right ventricle, respectively (Figure 1).18 The segregation of these 2 distinct cardiogenic lineages appears early and results in very distinct populations of CPCs possibly also relevant to adult heart CPCs (Figure 2).19 The second heart field is marked by the expression of Islet (Isl)1.20 Using conditional mice, where Cre expression is driven by the Isl1 promoter, Notch1 was found to negatively regulate \( \beta \)-catenin–dependent transcription.21 Inhibition of \( \beta \)-catenin resulted in upregulation of cardiac transcription factors Myocd and Smyd1. In turn, these 2 factors were required to drive differentiation of Isl1 CPCs to a cardiomyocyte phenotype (Figure 3). In summary, \( \beta \)-catenin upregulation causes expansion of different CPC lines including Isl1\(^{\text{pos}} \) second heart field progenitors. Negative regulation of \( \beta \)-catenin appears to drive CPC cell fate and differentiation.22

Similarly, the nuclear protein Chibby was found to facilitate cardiomyocyte differentiation from ES cells. Chibby directly binds to \( \beta \)-catenin and antagonizes its transcriptional activity (Figure 3). Chibby expression was associated with upregulation of Nkx2.5, \( \beta \)-myosin heavy chain (\( \beta \text{MHC} \)), and Mef2c. Chibby expression is also found at high levels in adult cardiomyocytes. Apparently, the cardiac-specific transcription factor Nkx2.5 controls Chibby expression. The authors suggested the clinical perspective that antagonizing the WNT/\( \beta \)-catenin pathway by this cardiac signaling molecule will allow to promote CPC differentiation toward a cardiomyocyte phenotype similar to cardiac development.23

Several other independent lines of evidence find positive regulation of WNT/\( \beta \)-catenin to be required for CPC amplification, whereas negative regulation of WNT/\( \beta \)-catenin is required for CPC differentiation toward adult cardiomyocytes (Figure 1). In the murine embryo development, upregulation of WNT/\( \beta \)-catenin is required at the time of MesP1 activation (approximately E5.5) to start amplification of the different CPC pools. Most prominently, this was observed for first and second heart field CPCs marked by expression of Tbx5 and Isl1, respectively.24 At later stages (from approximately E7.5) negative regulation of the WNT/\( \beta \)-catenin pathway is required to drive differentiation of the 2 CPC populations to adult cardiomyocytes.18,25 Other proteins regulated at this time point of cardiac development include Nkx2.5, the first peak of \( \alpha \text{MHC} \), and GATA4 and eHand (first heart field) as well as eHand (second heart field).26,27 It is often overlooked that \( \alpha \text{MHC} \) is long known to have 2 expression peaks: the first peak is observed around E7.5 in the developing left ventricle.28,29 Subsequently, \( \alpha \text{MHC} \) is downregulated and is replaced by \( \beta \text{MHC} \). After birth, \( \alpha \text{MHC} \) is upregulated again and is replacing \( \beta \text{MHC} \).30

We have developed a technique that allows for flow cytometric analysis of adult heart CPCs in the noncardiomyocyte cell fraction of either murine heart tissue or human left atrial appendage.31 In brief, heart tissue is mechanically minced and then subjected to 2 steps of size filtration where the smallest mesh will filter for cells larger than 30 \( \mu \text{m} \). This technique is almost completely filtering adult cardiomyocytes. This allows analyzing the noncardiomyocyte cell fraction of the adult heart without alteration of protein expression associated with the use of collagenase.16 Analysis for several markers known from the developing heart to be characteristic for CPCs revealed the results depicted in Figure 2. In the adult heart, to date, no MesP1\(^{\text{pos}} \) CPCs have been described. Similarly, only a few CPCs of the second heart field marked by islet-1 expression have been detected. However, we readily detected CPCs positive for several markers of the first heart field (Tbx5, \( \alpha \text{MHC} \), eHand) in adult murine left ventricle, as well as human left atrial appendage (Figure 2). Modulation of proliferation and differentiation of first heart field CPCs in the adult heart may allow boosting endogenous regeneration. The precise knowledge of these developmental pathways has implications for adult cardiac remodeling as discussed below.
Soluble Frizzled-Related Proteins Attenuate Adult Cardiac Remodeling Following Experimental Infarct

WNT proteins are secreted glycoproteins that act locally in a paracrine fashion. WNT proteins bind to frizzled receptors. These are 7-pass transmembrane proteins characterized by an extracellular N-terminal domain. sFRPs lack the transmembrane domain and compete locally for WNT binding (Figure 3). Interestingly, sFRP-3 and sFRP-4 levels were found to be elevated in hearts of patients with both dilated cardiomyopathy and coronary heart disease. FrzA, related to mouse sFRP1, has been detected during cardiovascular maturation and in the adult heart. More specifically, sFRP1 is transiently upregulated following experimental infarct predominantly in the infarct border zone together with frizzled receptors 1, 2, 5, and 10, as well as WNT 10b. These data suggest sFRPs to be involved in infarct healing and tissue homeostasis following injury.

FrzA/sFRP1 was proven to regulate vascular cell proliferation and to induce an angiogenic response. Moreover, a genome-wide screen revealed sFRP2 to be the key stem cell paracrine factor that mediates myocardial survival and repair after experimental myocardial infarct treated with intracardiac injection of AKT-modified mesenchymal stem cells. This paracrine secretion of sFRP2 lead to a dramatic reduction of infarct size and restoration of cardiac function. These effects were observed as early as 72 hours after AKT–mesenchymal
Global knockout of sFRP2 lead to reduced cardiac fibrosis and improved function after experimental myocardial infarction.8 In conclusion, several lines of evidence suggest sFRP2 to be required and sufficient to mediate myocardial repair following ischemic injury. In line with the general function of sFRPs known to sequester WNTs away from the active receptor complex, sFRP2 was shown to significantly inhibit β-catenin transcriptional activity as assessed by reporter gene activation and target gene expression.36

Global knockout of sFRP2 lead to reduced cardiac fibrosis and improved function after experimental myocardial infarction. The proposed mechanism was increased activation of metalloproteinases that are inhibited in the presence of sFRP2. It remains unclear in this study whether knockout of sFRP2 affected WNT pathway activation. In addition, a global knock-out of sFRP2 might affect WNT signaling in other tissue compartments like the bone marrow important for mobilization of endothelial progenitor cells that indirectly could influence LV remodeling following experimental infarct.37

Transgenic mice with cardiac-specific overexpression of FrzA (similar to mouse sFRP1) resembled the phenotype described above: αMHC-dependant FrzA expression increased survival by preventing myocardial rupture following experimental infarct. Mechanistically, this phenotype was associated with reduced infarct size, less apoptosis, increased collagen deposition, and reduced activity of metalloproteinase-9 activity. This latter point is in line with the finding in global sFRP2 knockout mice, although the functional phenotype is opposite.37 Interestingly, the percentage of muscleized vessels was significantly higher in mice with αMHC-dependent FrzA/sFRP1 overexpression, together with an increase in open vessel area.7 In addition, FrzA/sFRP1 is sufficient and required for the effect of preconditioning on infarct area: in the absence of FrzA/sFRP1, no effect of preconditioning is observed, which can be restored by the conditional, αMHC-dependent FrzA expression.38 Similarly, direct injection of sFRP2-secreting mesenchymal stem cells in to the border zone of an experimental infarct decreased infarct area, increased ejection fraction and reduced dilation of the left ventricle in association with increased vascular density. No increase in angiogenic-positive (PECAM-1) or cardiomyocyte marker–positive (anti-α-actinin) cells was observed, implicating a paracrine mechanism rather than any form of direct transdifferentiation of mesenchymal stem cells.8 Direct treatment of isolated adult rat ventricular cardiomyocytes with sFRP2 reduced caspase activity and exerted a cytoprotective effect via upregulation of Birc1b.35

In summary, the majority of data finds expression of sFRPs in the heart following experimental infarct to be beneficial for

![Schematic summary of signaling molecules described to inhibit WNT/β-catenin signaling in the cardiac compartment.](http://circres.ahajournals.org/)

**Figure 3.** Schematic summary of signaling molecules described to inhibit WNT/β-catenin signaling in the cardiac compartment. Inhibition of WNT and several downstream signaling components including β-catenin–dependent transcription was found to attenuate LV remodeling and improve adult cardiac function following ischemia or pressure overload. This scheme therefore summarizes both positive and negative signaling components of the WNT pathway in the cardiovascular system. These signaling intermediates may be suitable for therapeutic interventions. For example, sFRPs were found to be released from bone marrow–derived mononuclear cells homing to the heart after injury. Experimentally injected or genetically upregulated sFRPs generate similar responses. sFRPs scavenge paracrine-secreted WNTs, preventing them from binding to membrane-bound Frz receptors. sFRPs therefore inhibit both the canonical, as well as the noncanonical, WNT pathway. Disheveled binds to FrzR and is required for activation of the noncanonical and probably also the canonical pathway (see text). Dickkopf blocks canonical WNT signaling upstream of GSK3β. KLF15, Notch1, and Chibby are nuclear interaction partners of β-catenin blocking β-catenin/Tcf3-activated gene transcription. (Illustration Credit: Cosmocyte/Ben Smith).
cardiac remodeling (Figure 4). The mechanism is not entirely clear, and the published literature suggests a cardiomyocyte cytoprotective effect, as well as increased vascular density in the infarct area (Figure 4). However, the general role of WNTs in other tissue compartments always remains unclear, and the published literature suggests a cardiomyocyte cytoprotective effect, as well as increased vascular density in the infarct area. The mechanism whereby WNT inhibition by secreted frizzled related proteins improves cardiac remodeling therefore remains unclear. A detailed analysis of signaling molecules downstream of WNT/frizzled interaction may allow identifying this missing link.

WNT activates the noncanonical pathway including the axis of phospholipase C (PLC). PLC in turn leads to calcium release within the cell. Calcium release induces Ca/calmodulin kinase II and calcineurin activation (Figure 3). Calcium/calmodulin kinase II and calcineurin including downstream signaling to frizzled/disheveled stimulation. Calcium/calmodulin kinase II is activated linking Ca-dependent signaling to frizzled/disheveled stimulation. Calcium/calmodulin kinase II is activated linking Ca-dependent signaling to frizzled/disheveled stimulation. The mechanism whereby WNT inhibition by secreted frizzled related proteins improves cardiac remodeling therefore remains unclear. A detailed analysis of signaling molecules downstream of WNT/frizzled interaction may allow identifying this missing link.

Disheveled is an intracellular protein that is an essential positive signal intermediate of both the WNT-initiated canonical and also noncanonical signal transduction cascade. These branches are interconnected as described in detail in another part of this review series. Some data support the notion that the canonical/noncanonical branches separate at the level of disheveled where activation of disheveled is specific for the noncanonical pathway. Mutants in drosophila however indicate disheveled to be part of the membrane-bound protein complex of frizzled receptor regarding all downstream signaling pathways. Disheveled also links the intracellular domain of the Frizzled receptor to activation of phospholipase C (PLC). Downstream of PLC and the subsequent intracellular calcium release, Calcium/calmodulin kinase II is activated linking Ca-dependent signaling to frizzled/disheveled stimulation. Calcium/calmodulin kinase II is activated linking Ca-dependent signaling to frizzled/disheveled stimulation. Disheveled expression was shown to be upregulated on transaortic banding (rat) and atrial-fibrillation induced heart failure (porcine). Cardiac-specific overexpression of disheveled under the control of an α-MHC promoter results in severe cardiomyopathy at 3 months of age with significantly enhanced mortality attributable to pulmonary congestion. The phenotype resembles a dilated cardiomyopathy with grossly enlarged hearts (end-diastolic diameter of 5.5 ± 0.2 mm [TG] versus 4.4 ± 0.1 mm [CT]) and reduced ejection fraction. A compensatory increase in myocyte cross-sectional area and myocardial fibrosis was observed. Downstream of disheveled, the 2 noncanonical pathways JNK and CaMKII, as well as c-Myc, were constitutively activated. Mechanistically, cell culture experiments with small interfering RNA-mediated knockdown of disheveled suggest the protein to be required and sufficient for β-adrenergic (isoproterenol)-induced cardiomyocyte hypertrophy. Activation of the canonical WNT pathway with β-catenin as the downstream target may also contribute to this phenotype. Interestingly, myofibroblasts known to contribute to myocardial wound healing are also regulated by WNT signaling. Migration of cardiac fibroblasts immortalized by stable transfection of telomerase was attenuated by active WNT signaling. The study indicates that myofibroblast migration and differentiation, but not proliferation, can be modulated by interventions in Wnt/Frzd signaling. In summary, attenuation of cardiac fibrosis after infarct may also contribute to the positive effects on LV remodeling seen by WNT inhibition (Figure 4).
Similarly to this heart-specific gain-of-function mutation regarding disheveled, a disheveled isoform knockout strain was characterized regarding an adult cardiac phenotype. No baseline phenotype was observed. Because β-catenin knock-out mutations are embryonic lethal this either indicates that disheveled is predominantly involved in the noncanonical WNT pathway or other isoforms compensate for the loss of disheveled 1. Interestingly, cardiac hypertrophy as assessed by heart weight, LV wall thickness in echocardiography and gene expression (atrial natriuretic factor, brain natriuretic peptide) on pressure overload was attenuated in disheveled 1 knockout animals.50 The cellular mechanism of this observation remains unclear; an association to increased GSK3β and AKT phosphorylation was described. The phenotype though is also similar to knockout models of the noncanonical pathway, i.e., modulations of CaMKII.51

The phenotype in disheveled-1 knockout mice might also be linked to the role of WNT signaling in cardiac development and adult cardiac remodeling already summarized above. Specifically, disheveled is both necessary and sufficient for the fusion of early heart precursors. Diversins links the Frz/disheveled membrane complex to the downstream noncanonical pathway with activation of Rho and Rac. Diversin depletion resulted in 2 independently beating hearts in the zebrafish. This phenotype of cardiac bifida was rescued by Rhoa injection.52 The data prove the noncanonical WNT pathway to be important for cardiac development. Given the role of disheveled/diversin in WNT signaling, these signaling molecules may be interesting therapeutic targets because the downstream effectors Rhoa, JNK, and CaMKII are known to be important mediators of maladaptive cardiac remodeling.

Frazzled receptors couple to heterotrimeric G proteins including Goq and GoqG.53 This state of affairs links WNT signaling to the activation of MAPK JNK. The role of Goq and GoqG, both classic 7-transmembrane receptors, in cardiac hypertrophy is well established.54 WNT activation also leads to recruitment of factors like β-arrestins, which mediate signal transduction from Goq to the MAPK signaling cascade.55 In summary, several lines of evidence support both the noncanonical and the canonical WNT pathway as involved in WNT/Frz-mediated maladaptive cardiac hypertrophy.

Evidence for β-Catenin Inhibition to Attenuate LV Remodeling

More than 90% of the cellular β-catenin protein content is bound in the membrane. Loss of β-catenin, however, does not lead to a “membrane” phenotype because plakoglobin (γ-catenin) functionally substitutes for β-catenin in the membrane in various tissue compartments including the heart.55 Modulations of β-catenin levels in the heart by WNT signaling or genetic modifications therefore predominantly affect β-catenin–dependent transcription.

Both gain- and loss-of-function mutations of β-catenin are embryonic lethal between E5.5 and E10.5.32 To study the role of β-catenin in the adult heart, several conditional mutants have been generated. Such mice exhibited no phenotype under baseline conditions.14,56 On pressure overload, chronic angiotensin (Ang) II stimulation or experimental infarct αMHC-dependent β-catenin loss-of-function mutations led to attenuated LV remodeling and improved ventricular function.14,31,55 Conversely, increased mortality and a phenotype of dilated cardiomyopathy were observed in β-catenin gain-of-function mutations.14,51 Conditional deletion of β-catenin was achieved by either using the αMHC-MerCreMer or αMHC-CrePR1 strains. When studied in a TAC model of pressure overload, mice with αMHC-MerCreMer mediated β-catenin deletion subjected to the same gradient as control mice had significantly reduced cardiac hypertrophy (heart weight CT: 138±11 g versus TG: 111±4 g) with increased fractional shortening (CT: 36.2±2.7%; TG: 39.1±2.4%) and less LV dilation (echo LV end diastolic dimension: CT, 3.56±0.14 mm; TG, 3.5±0.09 mm).56

We studied both gain-of-function (αMHC-CrePR1×ΔN-catenin) and loss-of-function (αMHC-CrePR1×β-catΔex3-6) mutants on 2 weeks of Ang II stimulation. Mice with stabilized β-catenin in αMHC-positive cells had severely impaired fractional shortening at the end of the treatment course (TG: 22.±4.5% versus CT: 36.2±1.6%), whereas mice with β-catenin depletion had no significant phenotype compared to their control littermates.14 Following chronic ligation of the left anterior descending coronary artery, β-catenin loss-of-function mutants had significantly decreased mortality, reduced infarct size, and improved fractional shortening four weeks after the infarct.51 No significant effect on ejection fraction was observed at 2 weeks post infarct, suggesting that modulation of LV remodeling rather than an acute effect on the infarct size is responsible for this functional effect. This phenotype is very similar to the observations following expression of soluble frizzled receptors in the border zone of the infarct.7,8,35

Several mechanisms whereby β-catenin depletion improves LV function following pressure overload or ischemia have been described (Figure 4). Among other targets, we consistently found β-catenin to regulate Tbx5, a key transcription factor in differentiation of first heart field progenitors toward a cardiomyocyte phenotype.31 In association with the maladaptive LV remodeling observed in gain-of-function αMHC-restricted β-catenin mutants subjected to chronic Ang II stimulation, heart Tbx5 gene expression was reduced. In contrast, Tbx5, as well as GATA4, was upregulated in heart tissue of transgenic mice with β-catenin loss-of-function mutations restricted to cells with activation of the αMHC-Cre construct. This phenotype was associated with increased differentiation of cardiac-endogenous progenitor cells toward a cardiomyocyte cell type in cell culture experiments with the noncardiomyocyte fraction of heart cells from such transgenic mice. The cells affected expressed αMHC and Tbx5 protein but not troponin T or islet-1.34 This suggests first heart field progenitors to be a target for negative WNT signaling in the adult heart. Such cells could be identified both in the murine left ventricle and in specimens from human left atrial appendage (Figure 2). The amount of cells present in the adult heart as quantified by FACS of the noncardiomyocyte cell fraction from murine left ventricle is sufficient to link differentiation of these cells to the phenotype of attenuated LV remodeling in αMHC/β-catenin–depleted mice. The identification of specific markers for first heart field CPCs in the adult heart is required to further analyze this hypothesis;
Tbx5, αMHC, and other currently known markers are not suitable because they are also expressed in adult cardiomyocytes.

**WNT Inhibition Enhances Mobilization of Endothelial Lineage Progenitor Cells From Bone Marrow**

As described above, the embryonic heart develops from several different CPCs. Aside from first heart field CPCs marked by αMHC and Tbx5, cells from the endothelial lineage are also affected by WNT signaling.57 WNTs play a key role in embryonic vasculogenesis by modulating expansion of primitive VEGF receptor2pos and postnatal angiogenesis.40 Mobilization and homing of vascular progenitor cells have shown potential to enhance myocardial tissue repair via enhanced neovascularization in the adult.59 Modulation of the WNT/β-catenin signaling axis in the adult might allow for enhanced mobilization and homing of such cells from bone marrow to the heart.59

The intracellular WNT signaling antagonist dickkopf (Dkk)-1 was studied in the context of vasculogenic progenitor cells resident in the bone marrow. Recombinant Dkk1 injected intraperitoneally induced mobilization of Flk1pos/sca-1pos progenitor cells. Secretion of the osteoclast differentiation factor RANKL was identified as the molecular mechanism. RANKL in turn induced release of cathepsin K responsible for selective mobilization of vasculogenic progenitor cells. The inhibitory effect of Dkk-1 on WNT signaling was measured by expression analysis of axin-2. This mobilization was sufficient to enhance in vivo vascularization of Matrigel plugs implanted subcutaneously. Different from other growth factors stimulating release of bone marrow–derived vascular CPCs like G-CSF, Dkk1 did not lead to an induction of inflammatory cells from the bone marrow. Even more intriguing was that the mechanism of vasculogenic cell mobilization is quite different between the 2 stimuli: whereas G-CSF reduces the amount of osteoclasts, Dkk1 indirectly cleaves osteoclast–derived SDF1 via cathepsin K. SDF1 is required for homing of such progenitor cells to ischemic areas including the heart (Figure 4).59

Interestingly, Flk1pos cells are multipotent cardiovascular progenitors that also form the endocardium and directly respond to both BMP and WNT signaling regarding differentiation once homed to the myocardium.60 In vitro, WNT activation of such cells suppressed myocyte specification, whereas early Noggin+Dkk1 exposure significantly enhanced cardiomyocyte specification. These data suggest that the WNT pathway not only controls mobilization, proliferation, and differentiation of CPCs but also determines cell fate decisions from early progenitors.60 Key markers of cell fate decision monitored in this study were similar to other studies: Tbx5 and GATA4 for first heart field CPCs,Isl1 for second heart field CPCs and Flk1, and CD31 for hematopoietic/vascular progenitor cells.

The canonical WNT pathway is activated by ischemic events. In addition to affect endothelial cells it was found to regulate smooth muscle cell proliferation and apoptosis.61 Several studies have analyzed the role of WNTs in peripheral ischemia, which probably can be transferred to the ischemic situation in the heart regarding angiogenesis but not myogenesis. These studies support the notion that paracrine effects of implanted cells are sufficient and required to enhance wound healing and neangiogenesis. One such study evaluated the healing potential of human aorta-derived CD133pos progenitor cells and their conditioned medium in an experimental model of ischemic diabetic ulcer.39 The CD133pos cells were found to initially secrete high levels of WNT molecules, which were downregulated on differentiation into CD133pos cells. The latter instead secreted soluble frizzled-related proteins sFRP1, -3, and -4 known to downregulate WNT-dependent signaling. Consistently, conditioned medium from CD133pos cells accelerated wound healing and reparative angiogenesis in comparison to vehicle.39 As depicted above, sFRP2 was identified to be the key paracrine factor secreted by mesenchymal stem cells injected into the heart to mediate myocardial survival and repair after an ischemic event.

CD133pos cells have been studied in human trials on chronic ischemic cardiomyopathy. One of the studies analyzed the effect parallel to surgical revascularization: patients were subjected to autologous CD133pos cell transplantation into the ischemic border zone during the operating procedure after autologous bone marrow harvest the day before. The therapy was safe and resulted in increased ejection fraction and other parameters of LV function compared to a well-matched control group.62

In summary, the WNT pathway is pivotal for vascular and angiogenic development. Yet different conclusions depending on the experimental settings have been drawn whether stimulation of WNTs ie, by paracrine activation or inhibition of this pathway by dickkopf or sFRP is sufficient to improve angiogenesis. One possible explanation is that biphasic control of WNT signaling is required for a beneficial effect where WNT activation promotes proliferation of the vasculogenic precursors, whereas inhibition is required for mobilization, homing, and differentiation. This interpretation would be consistent with the observation that constitutive WNT stimulation leads to stem cell exhaustion and multi-lineage blockade.63

**Nuclear Negative Interaction Partners of β-Catenin Attenuate LV Remodeling in Association With Cell Fate Determination**

Krüppel-like factors (KLFs) are a large family of zinc finger–containing transcription factors involved in regulating cell differentiation, cardiac remodeling, hematopoiesis, angiogenesis, and stem cell fate determination by interacting with coactivators and corepressors.64 Recent studies revealed the important role of KLFs as regulators of cardiac biology.65 The family of KLFs was found to control β-catenin–dependent transcription in several tissue compartments like the gut and the heart. Possibly, KLF proteins allow for tissue specific control of β-catenin transcription because KLF4 regulates transcription in the small intestine whereas we found another member of the same family, namely KLF15, to control β-catenin–dependent transcription in the heart.

Specifically, we searched for cardiac-specific β-catenin interaction partners able to modify the Wnt/β-catenin transcriptional activity specifically in the heart using a yeast 2-hybrid system with a cardiac-specific library. Our own unpublished data
describe a novel interaction between the KLF15 and β-catenin resulting in an inhibition of β-catenin/TCF3 transcriptional activity via the N-terminal domain of KLF15 in cardiomyocytes. Moreover, Nemo-like kinase (NLK), a Wnt/β-catenin signaling inhibitor, was found to interact with KLF15. NLK is activated downstream of the noncanonical WNT pathway via CaMKII linking the 2 pathways.

KLF15 is expressed at high levels only in the heart. KLF15 was previously reported to modulate cardiomyocyte hypertrophy and fibrosis under stress.66,67 We found that mice with global KLF15 deletion develop normally with no apparent phenotype at baseline. However, on aging and after pathological stress the mice exhibit progressive cardiac deterioration whereas no apparent defects were observed in other organs. The cardiac phenotype was associated with a reduction of cardiogenic precursor cells (Sca1pos/cKitpos and Tbx5pos/cTnTnegpos) and upregulation of the vascular (CD31) and endocardial Flk1 progenitor pool: the total CD31pos/Ki67pos, as well as the Flk1pos/Ki67pos, CPC pool was increased, as revealed by FACS analysis of the cardiomyocyte fraction of the left ventricle from KLF15 knockout mice as compared to control mice. We conclude that KLF15 determines CPC cell fate via β-catenin in the adult heart. Moreover, these data support the concept that maintenance of CPC-driven cardiomyocyte regeneration is required for cell homeostasis during cardiac aging. In addition, cell fate regulation is important for adaptive remodeling on cardiac injury by ischemic events or increased afterload as observed in arterial hypertension.

As a note of caution to this concept, one must recognize that until the identification of an unequivocally accepted marker of adult LV cardiac progenitor cells, these data only provide associations of heart physiology with biochemical and cellular events in the CPC pool but no causal relationship. New concepts are warranted to decipher the role of CPCs in the adult heart. Interestingly, second heart field progenitors giving rise to the right ventricle are marked by islet-1 and have been characterized in detail.20–22,68 Proliferation and differentiation of islet-1pos cells isolated from the embryo are also controlled by WNT/β-catenin, however only few islet-1pos cells could be isolated from the adult heart suggesting a limited role of this precursor cell pool after birth. In contrast, we found ~10% of the noncardiomyocyte cell fraction of the left ventricle to express a reporter gene controlled by the αMHC-promoter with 2 different conditional mouse strains, namely the αMHC-CrePR1 and the αMHC-MerCreMer.31 This cell fraction overlaps in part with previously described adult heart CPCs, namely the c-kitpos cells, as well as the sca-1pos cell population.31 However, both c-kit and sca-1 are global markers of stem and progenitor cell pools rather than being heart-specific. Future research needs to identify specifically the cardiac progenitor cell pool; currently available data suggest several different cell pools including locally resident cardiomyogenic CPCs, as well as angiogenic cells possibly homing from the bone marrow, to be involved in adult cardiac remodeling.

On the basis of the currently available data from our group and others, we believe that negative WNT/β-catenin signaling enhances first heart field CPC differentiation toward functional cardiomyocytes in the adult heart. This is sufficient for functionally relevant adult heart regeneration in the context of aging as well as following injury.

**Clinical Implications of Available Data Regarding WNT Signaling in Adult Cardiac Remodeling**

The data summarized above prove the WNT pathway to be required and sufficient for adult LV remodeling. Namely, inhibition of the WNT pathway on several levels (soluble frizzled receptors, dickkopf, inhibition of disheveled, β-catenin depletion) has proven beneficial for cardiac remodeling (Figures 3 and 4). The noncanonical WNT pathway links WNT signaling to well known players in adult cardiac remodeling, namely RhoA and CaMKII. Given the new findings of an interplay between the noncanonical and canonical pathways, the latter comes into focus.6 The role of β-catenin in cardiac development is now well established. The canonical WNT/β-catenin pathway has a conserved role in vertebrate heart development, regulating and restricting cardiac precursor cell formation and subsequent heart muscle differentiation.

Several lines of evidence, both in vitro and in vivo, suggest cardiac CPCs and their responsiveness to WNT signaling to be a possible therapeutic target. Some authors suggest the ex vivo amplification by WNT stimulation and reinsertion of amplified cells to be a favorable model.3 Given the high prevalence of cells resembling first heart field cardiac progenitors in the adult (Figure 1), boosting endogenous repair mechanisms also appears feasible. One such approach building on the above summarized data would aim to inhibit WNT signaling temporarily in the heart. This can be achieved by local injection of bone marrow–derived mononuclear cells or subpopulations thereof like CD133pos cells, which were found to be capable of secreting sFRP.39 These molecules scavenge WNT proteins and prohibit binding of WNTs to membrane-bound frizzled receptors.

In summary, the WNT pathway plays a pivotal role in adult cardiac remodeling and may be suitable for therapeutic interventions. Among several currently discussed molecular and cellular mechanisms whereby WNT inhibition attenuates LV remodeling (Figure 4), reactivation of the developmental program building the left ventricle from first heart field progenitors in the embryo appears feasible. This maneuver may restore functional LV myocardium from resident precursor cells without the need of exogenous cell therapy. To prove this hypothesis, consensus must be found regarding which markers are specific for adult heart LV CPCs. It is now clear that several different adult heart CPCs can be identified similar to the situation in the developing heart. Tissue-unspecific markers like c-kit or sca-1 are indirect markers that not suitable for cell tracking. Islet-1pos cells will only be relevant for the second heart field progenitors restricted to the right ventricle and the left ventricle outflow tract in the developing heart. The population of αMHCpos/cTnTnegpos/Tbx5pos/eHandpos cells similar to first heart field progenitors in the developing heart (Figure 2) is a possible candidate for adult LV cardiac precursor cells. These cells are responsive to negative WNT signaling and appear to be able to regenerate functional LV myocardium in vivo.31 Whatever the mecha-
nism, inhibition of WNT in the adult heart may allow for additional therapeutic interventions on top of currently available therapy.

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