Review

The role of Wnt signalling in cardiac development and tissue remodelling in the mature heart

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Received 3 February 2006; received in revised form 2 June 2006; accepted 22 June 2006

Available online 29 June 2006

Time for primary review 22 days

Abstract

The heart is one of the first organs to function in the developing embryo. Vertebrate heart development can be subdivided into different phases, e.g. specification of myo- and endocardial progenitor cells during the establishment of heart-forming fields within the anterior lateral plate mesoderm, formation of the linear heart tube by merging of the paired heart-forming fields in front of the foregut, looping of the heart tube and transformation of the tubular embryonic heart into the four-chambered heart. The molecular mechanisms underlying these processes are phylogenetically remarkably conserved and involve the activation of specific transcriptional programs by different extracellular growth factors. In this review, we will focus on the functions of the Wnt family of growth factors in normal cardiac development and in tissue remodelling in the mature heart under pathological conditions.

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Keywords: Wnt; Frizzled; Cardiac development; Cardiac specification; Cardiac morphogenesis; Cardiac remodelling

1. Introduction: a short synopsis of vertebrate heart development

Development of the vertebrate heart can be subdivided into different phases such as specification of myo- and endocardial precursor cells during the establishment of paired heart-forming fields within the anterior lateral plate mesoderm, formation of the linear heart tube by merging of the paired heart-forming fields in front of the developing foregut, cardiac looping, chamber formation, septation and formation of the cardiac valves. As this complex process of heart development has been recently reviewed in several excellent reviews [1–4], we will here provide just a short synopsis of heart development outlining the major steps of this process and indicating some of the most important signalling molecules and marker genes. The reader is referred to the above-mentioned reviews for a more in depth summary on heart development.

Specification of myo- and endocardial precursor cells occurs early during embryonic development within the mesodermal germ layer. In Xenopus laevis, myocardial progenitors are specified at the onset of gastrulation. They define paired heart-forming fields that are located on each side of the Spemann organizer, the equivalent of Hensen’s node. In chicken and mouse embryos, specification of myocardial precursor cells starts during their ingestion through the anterior region of the primitive streak and is completed within the so-called heart-forming fields of the anterior lateral plate mesoderm (Fig. 1A). Two sources of diffusible signalling molecules required during cardiac specification have been identified by explantation and transplantation studies. These are the embryonic organizer as well as the anterior endoderm [1–4]. Signalling molecules that are required for induction and/or maintenance of early myocardial marker genes are members of the FGF, BMP and Wnt families of growth factors. The particular influence of

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doi:10.1016/j.cardiores.2006.06.025
Wnt proteins during this phase of cardiac development is discussed below in more detail. Once committed to a myo- or endocardial fate, the cells of the heart-forming fields move anteriorly and medial and fuse to form the cardiogenic crescent (Fig. 1B). These cells express the homeodomain transcription factor Nkx2.5 as one of the earliest myocardial marker genes. Additional early marker genes of the heart-forming fields are zinc finger transcription factors of the GATA family (particularly GATA-4, -5 and -6) and the T-box transcription factor Tbx20. Classical fate-mapping studies on chicken embryos have suggested that all myo- and endocardial precursors derive from a single pair of heart-forming fields [5]. More recent data from chicken and mice, however, implicate the presence of an additional pair of heart-forming fields, called the anterior or secondary heart-forming field (Fig. 1B) ([6], for review see Refs. [4,7]). Cells from the latter field are characterized by the expression of the LIM homeodomain transcription factor Isl-1 (Isl-1), the growth factor FGF-10, the transcription factor Me2c and other marker genes [4]. The “classical” heart-forming field has been found to deliver the myocardium of the left ventricle and the atria. It also contributes some myocardial cells to the right ventricle. The secondary heart-forming field delivers the myocardium of the right ventricle and the outflow tract (OFT). It also contributes some myocardial cells to the atria as well as to the inflow of both ventricles [6].

During gastrulation and neurulation, the heart-forming fields move towards the midline of the embryo where they merge ventral to the developing foregut to establish the single linear heart tube (Fig. 1C). The process of merging of the paired heart-forming fields starts in the region of the embryonic right (avian embryos) or left (mouse embryos) ventricle from where it proceeds in cranial and caudal directions during the phase of cardiac looping [7,8]. Thus, at the linear heart tube stage, the embryonic heart is a relatively short tube-like organ that consists of the embryonic ventricles, only. The other building units of the embryonic heart (outflow tract, atrio-ventricular segment, atria, sinus venosus) become added to its arterial or venous pole by continued merging of the secondary and classical heart-forming fields during cardiac looping. The merging of the heart-forming fields is completed by the end of cardiac looping. At this time point, distinct regions can be identified that roughly correspond to the different chambers of the mature heart. The formation of the heart chambers is accompanied by differential gene expression within each part of the heart and different cardiac chambers thereon express a distinct set of transcription factors and contractile proteins [9].

The wall of the linear heart tube is composed of an outer layer of myocardial cells and an inner layer of endocardial cells. These two cell layers are connected with each other by a thick extracellular matrix layer, called cardiac jelly. Shortly after the linear heart tube is formed, its myocardium starts peristaltoid contractions. The pacemaking area has been mapped to the putative sinus venosus region (Fig. 1C). During the subsequent looping process (Fig. 1D), the initially linear heart tube elongates and undergoes dramatic positional and morphological changes that bring the building units of the embryonic heart approximately into their definitive topographical relationship to each other [10,11]. Furthermore, the looping heart tube becomes colonized by primarily extracardiac progenitor cell populations. Cardiac neural crest cells, for example, invade the OFT that connects the embryonic ventricles to the aortic sac, pharyngeal arch arteries and dorsal aortae. These cells constitute the aortico-pulmonary septum which divides the OFT into systemic and pulmonary flow paths. The importance of cardiac neural crest cells for the normal development of the heart is demonstrated by the chicken neural crest ablation model resulting, for example, in congenital malformations of the OFT such as Tetralogy of Fallot and persistent truncus arteriosus. Furthermore, defects in patterning of the aortic arches have been observed [12]. A second primarily extracardiac progenitor cell population is the proepicardium (PE). The PE forms near to the venous pole of the heart. It delivers the epicardial mesothelium, the majority of the cardiac interstitium and the entire coronary vasculature [13]. Furthermore, PE-derived tissues play important regulatory roles in the development of the ventricular myocardium (for review, see Ref. [7]).

Another major step in vertebrate heart development is the transformation of the tubular embryonic heart loop with a single undivided lumen into the four-chambered heart. This step encompasses several morphogenetic processes such as ballooning of the walls of the chambers (atria and ventricles) [10], remodelling of the wall of the inner heart curvature and OFT, the formation of interatrial and interventricular septa and the formation of valves at the atrio-ventricular and ventriculo-arterial junctions. Failure in any of these processes can lead to severe congenital heart defects. Therefore, a detailed analysis of the molecular mechanisms underlying these processes is an important task. Formation of the cardiac valves, for example, includes several steps, starting with the formation of endocardial cushions filled with cardiac jelly. The primarily cell-free extracellular matrix of these cushions becomes colonized by a subpopulation of endocardium-derived mesenchymal cells as indicated in Fig. 1D [14]. Some specified endothelial cells, covering the endocardial cushions of the atrioventricular canal (AVC) and the OFT, undergo an epithelial-mesenchymal transition (EMT). This is a very complex process (reviewed in [14]), involving specification, delamination from the endocardial epithelium, transdifferentiation from endothelial to mesenchymal cells as well as proliferation and migration (see Fig. 1D). Several signalling molecules have been implicated in one or more steps of this process, including Wnt/β-catenin signalling as discussed below. The so formed endocardial cushions undergo a not well-understood remodelling process to finally form the thin tapered leaflets of the mature heart valves (Fig. 1F). Finally, the terminal differentiation of the conduction system and the transition from the primitive
peristaltoid contraction pattern of the heart tube to the mature contraction pattern of the four-chambered heart contributes to the morphological and functional maturation of the developing heart.

2. Wnt signalling molecules—Wnt signalling pathways

Individual Wnt genes are defined by their sequence homology to the first described members, *wingless* in...
Drosophila and int-1 in the mouse. They code for secreted, lipid-modified glycoproteins that can activate different intracellular signalling pathways through interaction with transmembrane receptor complexes (see Fig. 2). The best-characterized Wnt-mediated signalling pathway is the canonical Wnt/β-catenin pathway [15]. In the absence of Wnt, cytoplasmic β-catenin is degraded through interaction with and phosphorylation by the destruction complex consisting of the scaffolding proteins APC and Axin as well as the serine/threonine kinase GSK-3β. Upon binding of Wnt proteins to seven transmembrane receptors of the Frizzled family as well as the co-receptor LRP-5/6, β-catenin is stabilized and the destruction complex is inhibited. This leads to the stabilization of TCF/LEF transcription factors and activation of the canonical Wnt/β-catenin pathway.

Fig. 2. Short synopsis of Wnt signalling pathways in vertebrates. The canonical Wnt/β-catenin pathway is shown on the left, whereas non-canonical, β-catenin-independent pathways are shown on the right. Some components are involved in the planar cell polarity pathway in Drosophila melanogaster, as indicated. In vertebrates, this pathway regulates morphogenetic movements. For details, see main text.

Fig. 1. Development of the embryonic mouse heart. (A) At E 6.5, specification of myocardial precursor cells starts during their ingression through the anterior primitive streak region and becomes completed within the heart-forming fields. Extracellular growth factors of different families are involved in this process as indicated. BMP: bone morphogenetic protein, FGF: fibroblast growth factor, dkk: dickkopf. (B) At E 7.5, the anterior portions of the heart-forming fields have merged to form the cardiogenic crescent. The classical (primary) and the secondary heart fields can be distinguished. (C) At E 8.0, the linear heart tube has formed by progressive merging of the paired primary (red) and secondary (blue) heart fields. Note that the outflow tract is not present at this stage of development and that the areas of the heart-forming fields representing the atria and sinus venosus have not started merging. AV: atrio-ventricular segment, LA: future left atrium, LSV: future left sinus horn, LV: embryonic left ventricle, RA: future right atrium, RSV: future right sinus horn, RV: embryonic right ventricle. (D) At E 9.5, cardiac looping is in progress and the formation of the endocardial cushions (indicated in yellow) is initiated which includes specification, delamination, epithelial-mesenchymal transition (EMT) and proliferation (see right panel). The outflow tract has formed by merging of the secondary heart-forming fields. The myocardium of the ventricular outer curvature starts formation of trabeculations. AoS: aortic sac, OFT: outflow tract. (E) A cross section of the heart at E 10.5 is shown. The four chambers are preformed but not separated yet. PAS: primary atrial septum, VS: muscular anlage of the ventricular septum. (F) A schematic drawing of the heart at E 14.5 is shown. The conduction system is given in blue. Ao: aorta, DA: ductus arteriosus, L SVC: left superior vena cava, RSVC: right superior vena cava, IVC: inferior vena cava, PT: pulmonary trunk, PV: pulmonary vein orifice, SA-node: sinuatrial node.
cytoplasmic β-catenin is stabilized, enters the nucleus and regulates gene expression through interaction with HMG-box transcription factors of the TCF/LEF family. Recent papers indicate that this Wnt/β-catenin pathway involves G50 and Gβq subunits of heterotrimeric G-proteins [16,17]. In contrast, non-canonical Wnt signalling pathways act independently of β-catenin and LRP-5/6 and involve a G-protein-mediated intracellular calcium influx, activation of calcium sensitive enzymes like protein kinase C (PKC), calcium/calmodulin-dependent kinase II (CaMKII) or calcineurin (CaCN) as well as GTPases of the rho family and jun N-terminal kinase (JNK); see Ref. [18] for review. The Wnt/JNK pathway resembles the planar cell polarity pathway in Drosophila, which includes additional proteins like strabismus (van gogh), flamingo and prickle. During planar cell polarization, these proteins are asymmetrically distributed within the cell and thereby provide positional information. In vertebrates, the Wnt/JNK/PCP signalling pathway is involved in regulating cell migration processes during gastrulation and neural crest cell migration [18,19]. A critical component for all Wnt signalling branches is the cytoplasmic protein Dishevelled (Dsh, dvl) [20]. In mice, three dishevelled homologues are known. Recent data indicate that non-canonical Wnt signalling might also involve the co-receptor Ror-2 in some cells [21]. It needs to be noted here that these pathways can regulate each other, e.g. Wnt proteins, such as Wnt-11, can activate non-canonical signalling and at the same time inhibit β-catenin signalling [22]. In the following sections, we summarize our current understanding of how Wnt proteins influence different aspects of cardiac development and remodelling processes in the adult heart.

3. Wnt signalling during myocardial specification and early cardiac morphogenesis

Vertebrate heart development starts with specification of myocardial precursor cells within the mesodermal germ layer of the early embryo. Data from embryology suggest the active participation of different growth factors in this context and several studies have analyzed the function of different Wnt factors during myocardial specification. Several Wnt ligands and Frizzled receptors have been shown to be expressed in a spatially and temporally regulated manner consistent with a function during early heart development. Wnt-11 homologues (there are two in Xenopus and zebrafish, named Wnt-11 and Wnt-11R) show a conserved expression in the heart-forming fields in quail, chicken, mouse and Xenopus (Wnt-11) [23–26]. Later on Wnt-11 can be found in the primary heart tube of mouse, chicken and Xenopus (Wnt-11R) embryos [24–27]. Interestingly, human Wnt-11 has been reported to be expressed in the adult heart [28]. At gastrula stages of mouse embryos, Wnt-2 expression overlaps with that of Wnt-11 before it becomes restricted to the pericardium [29]. Within or lateral to the primitive streak, a number of different Wnt ligands were detected in avian and mouse embryos: Wnt-3a, Wnt-5a, Wnt-5b and Wnt-8c [30–34]. At day 10.5 dpc, murine Wnt-8 is detected in the myocardium and four additional members of the Wnt family are expressed in the tubular heart of chicken embryos: Wnt-2b, Wnt-5a, Wnt-7a and Wnt-14 (also named Wnt-9) [26,34]. In addition to this enormous number of Wnt ligands, many different Wnt receptors of the Frizzled family have been detected in the mesoderm of the heart-forming fields, cardiac neural crest cells or the adult heart, including mouse Fz-2, -4, -9, Xenopus Fz-7, -8, -10a, -10b or human Fz-1, -2, -7, -8, -9 [35–42]. This huge number of different ligands and receptors makes it very likely that Wnt proteins influence different aspects of cardiogenesis but also indicate potential redundancies.

Functional analysis in chicken and Xenopus embryos indicate an inhibitory role of the canonical Wnt/β-catenin pathway during myocardial specification. In Xenopus, inhibition of canonical Wnt signalling by the extracellular Wnt inhibitor dickkopf (Dkk) or other Wnt inhibitors results in ectopic cardiomyocyte formation [43]. Similarly, inhibition of Wnt/β-catenin signalling is required for cardiogenesis in the early chicken embryo [44,45]. In the mouse embryo, tissue specific depletion of β-catenin results in the formation of multiple, additional hearts [46]. Consistent with these data is the observation that overexpression of Wnt-3a or Wnt-8 in Xenopus explants blocks cardiogenesis and, similarly, Wnt/β-catenin signalling blocks formation of cardiomyocytes in chicken explants [43–45]. However, recent publications suggest a positive role for Wnt/β-catenin signalling during differentiation of P19 teratocarcinoma stem cells [47,48]. It needs to be noted though that in this system an indirect effect for Wnt/β-catenin signalling on cardiomyocyte formation cannot be ruled out.

Two recent studies shed some light onto this discrepancy using murine embryonic stem cells. Kouskoff et al. [49] demonstrated that differentiating embryonic stem cells expressing the panmesodermal marker Brachyury (Bra+) and the hemangioblast marker Flk-1 (Flk+) will not form cardiomyocytes, whereas Bra+/Flk+ cells do, indicating that Flk+ cells will contribute to the hemangioblast lineage. However, Yamashita et al. demonstrated that Flk+ cells can give rise to cardiomyocytes under certain culture conditions, the OP9 co-culture system [50]. A particular fraction of cells (Flk+/CXCR4+/VE-cadherin) was shown to be the cardiac progenitor cell type in this setting. Moreover, treatment of Flk+ cells with Wnt inhibitors led to cardiomyocyte formation whereas Wnt-3a inhibited this process. As this experimental setting analyzed the influence of Wnt/β-catenin signalling on a purified cell population, it also suggested that the Wnt/β-catenin pathway favours the formation of Flk1+ endothelial and hematopoietic precursor cells but inhibits myocardogenesis.

Just recently, Dbf4 has been identified as an endogenous Wnt/β-catenin inhibitor that is clearly required for heart development in Xenopus [51]. Things become more complicated, however, with the observation that a direct inhibition of Wnt/β-catenin signalling at the level of TCF/
LEF transcription factors by dominant-negative TCF constructs is not sufficient to trigger formation of a contractile phenotype in *Xenopus* explants. This indicates that additional signalling branches of the Wnt signalling network are involved in cardiogenesis [52,53]. Dkk, for example, has been shown to activate JNK signalling in addition to inhibiting Wnt/β-catenin signalling [52,54]. In summary, inhibition of Wnt/β-catenin signalling seems to be an important step during specification of cardiomyocytes.

Several publications indicate that non-canonical Wnt signalling plays an important role during cardiac development as well. In quail embryos, Wnt-11 promotes ectopic formation of cardiomyocytes in posterior mesoderm [55] and, consistently, in the quail mesodermal stem cell line QCE-6 cardiac differentiation requires functional Wnt-11 [25]. In *Xenopus* and zebrafish, two Wnt-11 homologues are known [27,56]. In gain of function studies, Wnt-11 and Wnt-11R induce myocardial marker gene expression in *Xenopus* embryonic explants and this activity has been linked to activation of a non-canonical Wnt signalling pathway involving PKC and JNK [27,52]. Indeed, biochemical analyses revealed that PKC seems to be upstream of JNK in non-canonical Wnt signalling [52,57]. In a mesodermal context, Wnt-11 was sufficient to trigger a contractile phenotype, as does the Wnt/β-catenin inhibitor Dkk [52]. The myocardium promoting activity of Wnt-11 is also substantiated by the findings that Wnt-11 increases the number of Nkx2.5 positive cells in mouse embryonic stem cells [58], triggers cardiomyocyte formation in P19 cells [52], and activates myocardial marker gene expression in human circulating progenitor cells [59], in mouse mesenchymal stem cells [60] and in bone marrow stem cells [61]. In human circulating progenitor cells and mouse bone marrow stem cells this activity of Wnt-11 has also been linked to non-canonical Wnt signalling involving PKC [59,61]. In summary, these data suggest a myocardium promoting function of Wnt-11 through a non-canonical Wnt pathway.

Furthermore, loss-of-function studies indicate a requirement of non-canonical Wnt signalling for cardiac morphogenesis. In *Xenopus*, loss of Wnt-11 leads to defects in linear heart tube formation [52] and loss of Wnt-11R, the second Wnt-11 gene in *Xenopus* which is expressed in cardiac tissue after cardiac specification, leads to morphogenetic defects at later stages [27]. In zebrafish, loss of non-canonical Wnt signalling results in cardiac bifida, suggesting defective fusion of the heart-forming fields [56]. Interestingly, the activation of myocardial marker genes by Wnt-11 in human circulating progenitor cells and in bone marrow stem cells requires direct cell-cell contacts [59,61], which may be mediated by cadherins [62,63]. Cadherin-mediated cell adhesion itself is an important regulatory mechanism of cardiac differentiation. N-Cadherin deficient mice initially form cardiomyocytes, however, these cells loose their cell contacts and do not form a linear heart tube [64]. These observations raise the question whether both aspects, non-canonical Wnt signalling and cadherin-mediated cell adhesion, are linked to each other. Indeed, the ability of Wnt members that activate non-canonical Wnt signalling to regulate cadherin-mediated cell adhesion has long been recognized [65]. In particular, Wnt-11 has been shown to regulate N-cadherin expression in the quail mesodermal stem cell line QCE-6 and the function of E-cadherin during zebrafish gastrulation [25,66]. Furthermore, Wnt signalling has been shown to regulate the level of N-cadherin and the strength of N-cadherin-mediated cell adhesion in neonatal cardiac myocytes [67,68], a process that also involves PKC [69]. Cadherin-mediated cell adhesion is important for cell migration and morphogenesis during development and early work in amphibians indicated the requirement of cell adhesion for cellular differentiation, the so-called “community effect” [70]. Interestingly, a similar situation is found during eye development. Non-canonical Wnt/Frizzled signalling is simultaneously required for cell migration and differentiation of early retinal precursor cells in *Xenopus* and zebrafish [71–74], possibly by inhibiting the canonical Wnt/β-catenin pathway and regulating coherence of cells within the early retina field [72].

In summary, these data suggest several Wnt activities during early cardiac development: canonical Wnt/β-catenin signalling inhibits cardiogenesis and therefore needs to be inhibited itself for proper heart development. Simultaneously, a non-canonical Wnt pathway serves at least two functions: (1) It contributes to the inhibition of β-catenin signalling and promotes myocardial differentiation and (2) it is involved in cardiac morphogenesis by regulating cadherin-mediated cell adhesion and cell polarity. All these aspects of Wnt signalling contribute to proper differentiation and morphogenesis of the early vertebrate heart.

### 4. Wnt signalling during the transformation of the tubular embryonic heart into the four-chambered heart

During later stages of development, Wnt signalling has been implicated in development of the OFT, formation of cardiac valves and the development of the cardiac conduction system.

As outlined above, cardiac neural crest cells play important roles during cardiac development. In this cell population, Wnt signalling has been linked to cell type specific cell proliferation [75]. Here, Wnt triggers the expression of the transcriptional regulator Pitx2 through activation of β-catenin. Pitx2 then regulates expression of cyclin D2. Cell proliferation is thereby upregulated in a tissue specific manner in the cardiac OFT, the pituitary gland, muscles and other tissues. Reversely, in mice that lack β-catenin in cells expressing Wnt-1 (in Wnt-1 Cre/β-catenin<sup>−/−</sup> mice) but also in Pitx2<sup>−/−</sup> and Dvl2<sup>−/−</sup> mice [76–78] defects in cardiac neural crest cell proliferation were observed. In consequence, this led to a lack of separation of the great arterial trunks (persistent truncus arteriosus) and further malformations of the OFT, i.e. double outlet right ventricle, or transposition of the great arteries.
Non-canonical Wnt signalling has also been reported to be necessary for proper development of the proximal OFT. In *Drosophila*, *Xenopus* and zebrafish, the planar cell polarity pathway has been reported to regulate morphogenetic processes during different developmental events (reviewed in [18,79,80]). Van gogh, also named strabismus (Stbm, Vangl, Lpp1, Ltap), is a known component of this β-catenin-independent Wnt signalling pathway. The van gogh gene is disrupted in the *looptail* mouse. This natural occurring mouse mutant is characterized by severe cardiovascular defects, e.g. double outlet right ventricle as well as improper aortic arch development. Interestingly, during remodelling of the proximal OFT, Wnt-11 and Dvl2 are expressed in a similar pattern as Vangl2 in the myocardium of the OFT as well as in cardiomyocytes extending into the proximal outlet septum [81]. These migrating cardiomyocytes display characteristics of polarized cells, which are not seen in Lp−/− mice. Therefore, it has been suggested that non-canonical Wnt signalling regulates polarized cell movements through Vangl2 as one component of a multiprotein complex by which Dvl activates RhoA. Activation of RhoA subsequently activates Rho kinases (ROCK) whose activities can reorganize the cytoskeleton and affect cell movements. Indeed, ROCK1 is co-expressed with RhoA in the myocardial cushion interface. This co-expression is disrupted when the vangl2 gene is mutated. As mentioned above Dvl2−/− mice display severe OFT defects, which were related to cardiac neural crest defects [78]. Disruption of the homologue of the planar cell polarity gene flamingo, Celsr1, seems to induce OFT defects as well [81]. These data consistently suggest an important function of non-canonical Wnt signalling for proper development of the OFT and disruption of this signalling pathway leads to severe abnormalities.

As mentioned above, canonical Wnt signalling has been implicated to play a fundamental role in endocardial cushion development. Clevers and co-workers [82] have analyzed a zebrafish mutant, in which the APC gene is disrupted by a premature stop codon, resulting in a C-terminally truncated APC protein. Due to the mutated APC protein, β-catenin signalling is constitutively active in these fish that display signs of severe heart failure such as pericardial oedema and blood pooling as well as other organ abnormalities. Rescue experiments showed that the heart failure is specific to the constitutively active β-catenin. Histological examination revealed that the endocardial cushion located between atria and ventricle (see Fig. 1D,E), the future atroventricular canal (AVC), was massively expanded, comprising an excessive endocardial layer fused to the AVC. All endocardial cells in this region seemed to have undergone EMT. Injection of APC or Dkk-1 mRNA in wildtype embryos, both inhibitors of β-catenin signalling, induced a complete lack of endocardial cushion tissue. Therefore, the canonical Wnt signalling pathway seems to be essential for this process. Analysis of marker gene expression in APC mutant fish indicated that Wnt/β-catenin signalling is functional only in those cells that are located in the valve-forming region. This conclusion was further supported by the analyses of an Axin 1 mutant, which displayed comparable heart defects. Consistently, in hearts of wildtype embryos, β-catenin was detected only in the designated valve-forming region.

In line with these observation in fish, Wnt-9a, as well as the secreted canonical Wnt signalling antagonist Frlazzled-b (Frzb), have been described to play pivotal roles during chicken heart valve formation [83]. The spatial expression pattern of Wnt-9a comprises endocardial cells in the AVC region of the chicken heart. Frzb is detected in the same cell population but is also found in transformed mesenchymal cells. Specific overexpression of Wnt-9a in the AVC region using a retroviral approach in an *ex vivo* AVC explant assay led to a massive increase in mesenchymal cell number compared to mock infected explants. When Wnt signalling was inhibited by a truncated Wnt-9a, the formation of mesenchymal cells was strongly reduced. Activated Wnt signalling resulted in a significant increase in proliferation, while inhibiting Wnt signalling in the AVC explant had no effect. When assaying the apoptosis rate, the reverse result was obtained. These results were also confirmed *in vivo*. The AVC explant system was also used to study the role of Frzb as a known antagonist of Wnt/β-catenin signalling. Inhibition of Frzb translation resulted in a marked increase in cell number, while overexpression of sFRP-3, the mouse orthologue of Frzb, lead to a normal proliferation rate or, in high doses, to a decreased number of mesenchymal cells. Like the study in zebrafish, this report argues for a role of Wnt signalling in promoting proliferation in the AVC cushion region. The authors propose the following model for Wnt signalling in avian endocardial cushion formation. Wnt-9a is secreted by endocardial cells and acts as a proliferation promoting factor in the endocardium. Mesenchymal cells express Frzb inhibiting Wnt-induced cell proliferation and thereby directing cushion outgrowth. Downregulation of Wnt signalling may promote differentiation of mesenchymal cells which contributes to mature valve leaflet formation. Wnt-9a and Frzb, however, are absent from mouse endocardial cushions; thus, the described role for Wnt-9a in regulating cell proliferation is not transferable to the mammalian system.

Furthermore, a recent study implicates β-catenin signalling in proper heart valve formation in the mouse, although it is still unclear which Wnt protein activates β-catenin in this context [84]. By using a β-catenin reporter mouse, it could be demonstrated that EMT in the AVC is accompanied by an upregulation of β-catenin signalling activity. Furthermore, when the endothelial expression of β-catenin is impaired, heart valve development is disrupted. α-smooth muscle actin (αSMA) expression marks mesenchymal cells and the expression of this protein depends on TGFβ2 signalling. In β-catenin deficient cells, αSMA is detectable only at a low level, which in turn indicates that TGFβ2-induced EMT depends on β-catenin signalling. As other marker genes
(Notch1, delta-like4, snail and VE-cadherin) were not altered upon deprivation of β-catenin and the phosphorylation state of Smads was not changed in knockout cells it could be concluded that TGFβ2 signalling is not altered in the absence of β-catenin. Taken together, these data demonstrate a role for β-catenin signalling in heart valve development and indicate an interaction of both signalling pathways, TGFβ2 and Wnt/β-catenin. In summary, all three papers clearly argue that Wnt/β-catenin is essential for normal heart valve development during zebrafish, chicken and mouse embryogenesis.

The development of the cardiac conduction system starts as early as the tubular heart is formed. The first functional element of the cardiac conduction system is the anlage of the sinusatrial node, the so-called pacemaker, situated in the sinus venosus region of the linear heart tube (Fig. 1C). During cardiac looping and chamber formation, the cardiac conduction system is further developed. The cells of the cardiac conduction system and of the working myocardium both originate from a common, multipotent progenitor lineage. Treatment of embryonic cardiomyocytes with endothelin-1 can trigger the development of a Purkinje fibre phenotype. A recent publication demonstrates that looped hearts from chicken that had been treated with Endothelin-1 exhibit an upregulation of Wnt-7a and Wnt-11 [85]. Expression of Wnt-7a and Wnt-11 could be verified in peripheral and central portions of the conduction system, respectively. Although no functional data is available just yet, it is tempting to speculate that Wnt signalling is involved in the formation of central (His bundle) as well as peripheral (Purkinje fibres) portions of the cardiac conduction system.

Finally, there is evidence that Wnt proteins are also involved in signalling from the epicardium to the myocardium [86]. Merki et al. analyzed the function of retinoic acid signalling through the retinoid X receptor α (RXRα) during heart development through several tissue specific knock-outs of RXRs including the epicardium, cardiac neural crest and the endothelium. Interestingly, only removal of RXRα in the epicardium results in thinning of the myocardium, which is the identical phenotype to systemic removal of RXRα signalling. Detailed analyzes identified Wnt-9b and FGF-2 as being responsive to RXRα signalling in the epicardium. In this study, epicardial Wnt-9b was than sufficient to upregulate FGF-2 in the myocardium and to induce its proliferation. In summary, these data highlight the function of the epicardium for cardiac development and indicate that Wnt signalling has an important role for the interplay of epicardium and myocardium.

5. Wnt signalling during tissue remodelling in the mature heart

The adult heart can react upon different stimuli, including exercise (physiological) or pressure overload and hypertension (pathological), by a cardiac enlargement. As the mature cardiomyocyte is withdrawn from the cell cycle, this is rather due to an enlargement of cells (hypertrophy) and not due to proliferation (hyperplasia). Under pathological conditions, this hypertrophy may result in dilated cardiomyopathy and sudden death. It is also widely accepted that the inability of mature cardiomyocytes to divide is the main reason why a damaged heart after myocardial infarction will not return to its previous state in most cases. A recent publication however indicates that the proliferative capacity of cardiomyocytes can be reactivated under certain conditions [87]. In contrast to humans, mice and birds, amphibians and fish can regenerate even severe myocardial defects [88–93]. Several adult myocardial stem cell populations were described in higher vertebrates that differ in their expression of marker genes. These include Sca-1 [91,92], c-kit [93], abcg-2 [94] or Islet-1 [95]. It is not known so far, whether these cells are independent cell populations with a myocardiogenic potential or whether they reflect descendents of a common precursor, isolated at different levels of differentiation. Despite the presence of those adult stem cells, it needs to be pointed out that they are insufficient to repair the damaged myocardium after infarction under normal, physiological conditions and without any external intervention. There is hardly any information available about the effect of Wnts on adult myocardial stem cells; therefore, our focus in this section will be on the role of Wnt signalling during cardiac hypertrophy and cardiac wound healing after myocardial infarction. The latter issue has just recently been summarized by van Gijn et al. [96], and the reader is referred to this review for a more detailed description. Here, we will summarize some key publications suggesting the involvement of Wnt signalling in both of these aspects.

Several publications indicate a crucial role for GSK-3β and β-catenin in the development of a hypertrophic response. During Wnt signalling, GSK-3β is inhibited thereby allowing the accumulation of cytoplasmic β-catenin. It has been shown convincingly that an increased activity of GSK-3β inhibits cardiac hypertrophy [97]. This opens the possibility that Wnt ligands and Frizzled receptors may favour hypertrophy although this link has not been established so far. Interestingly, well-known hypertrophic stimuli, e.g. phenylephrine or endothelin-1, induce stabilization of β-catenin through activation of protein kinase B (PKB), which phosphorylates and inhibits GSK-3β [98]. Strikingly, the Wnt/β-catenin target gene cyclin D1 was not activated in this setting, which fits with the inability of mature cardiomyocytes to re-enter the cell cycle. The authors conclude that hypertrophic cardiomyocyte growth may involve activation of β-catenin signalling in a Wnt-independent manner. In the hypertrophic heart, the intercalated discs have been shown to be disorganized [99]. Within this study, the Wnt signalling mediator β-catenin, however, was found to be increased due to changes in GSK-3β activity and an increased expression of Wnts. Subcellular, β-catenin was found to be enriched in the intercalated discs. Interestingly, Wnt signalling has also been linked to skeletal muscle hypertrophy [100–102]. Cardiac overload can finally
lead to heart failure. This process is accompanied by an increased rate of apoptosis of cardiomyocytes. Schumann et al. investigated the gene expression profile of failing and non-failing left ventricular tissue and identified the Wnt antagonists sFRP-3 and -4 to be elevated in failing ventricles [103]. Interestingly, expression of these two genes could be linked to pro-apoptotic markers. These data suggest that modulating Wnt signalling plays a role during late cardiac remodelling processes. In summary, these findings with respect to the role of Wnt signalling during hypertrophic changes also point towards a new target for therapeutic interventions of pathological hypertrophy.

The contribution of Wnt signalling during wound healing after myocardial infarction has been most intensively studied by Blankesteijn and colleagues and data are available that suggest that Wnt signalling may be important in this process. In a rat infarct model, Frizzled-2 is expressed in myofibroblasts that migrate into the infarcted area [104]. In addition, Dvl1 was detected in these myofibroblasts [105,106]. Interestingly, it has been reported that Dvl1 knock out mice may experience problems in infarct healing. Levels of β-catenin in cardiomyocytes were found to be diminished in Dvl1−/− mice, in particular in the intercalated discs [107]. Most likely as a consequence of this change in β-catenin content, infarct rupture was observed more frequently in Dvl-1 knock out mice in comparison to wild type mice. However, as indicated before in this review, Wnts are also involved in regulating cell–cell adhesion between different cardiomyocytes, which nicely fits into this scenario. In summary, the data by the group of Blankesteijn implicates that Wnt signalling may play a crucial role during infarct healing. Gene expression profiling analyzes after myocardial infarct also indicated an elevated level of Wnt signalling [108]. However, recent data also suggested a protective function of the Wnt inhibitor sFRP-1 after myocardial infarction [109]. Mice overexpressing sFRP-1 showed a reduced infarct size. Taken together, these data indicate the involvement of Wnt and Frizzled signalling during infarct healing. However, more detailed analyzes have to reveal the cell type specificity and the time dependencies of the described effects in the future (Table 1).

6. Outlook

As discussed in this review, Wnt signalling is required for different aspects of cardiac development, including myocardial specification, cardiac morphogenesis, and cardiac valve formation. In the adult, changes in β-catenin signalling are linked to pathological hypertrophy, and after myocardial infarction modulation of Wnt signalling has been implicated in wound healing. By learning more about the function of Wnt signalling during normal development, we will gain more insight into the molecular mechanisms of pathological changes or during infarct healing in the future. Finally, it remains to be seen, whether this knowledge can be adopted to adult cardiac stem cells.

Acknowledgements

Work in the lab of MK is supported by the Deutsche Forschungsgemeinschaft (DFG, SFB 451, Tp B13 and Ku1166/3-1/2). Work in lab of JM is supported by the Deutsche Forschungsgemeinschaft (MA 2377/4-1). We would like to thank Petra Pandur for critical reading of the manuscript.

References


Table 1

<table>
<thead>
<tr>
<th>Analyzed process</th>
<th>Canonical Wnt signalling</th>
<th>Non canonical Wnt signalling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration and linear heart tube formation</td>
<td>Not implicated</td>
<td>Required for morphogenesis [27,52,56]</td>
</tr>
<tr>
<td>Cardiac looping and OFT development</td>
<td>Required for cardiac neural crest proliferation [75–78]</td>
<td>Required for proper outflow tract development [78,81]</td>
</tr>
<tr>
<td>Heart valve development</td>
<td>Required [82–84]</td>
<td>Not implicated</td>
</tr>
<tr>
<td>Conduction system development</td>
<td>Not implicated</td>
<td>Wnt-11 and Wnt-7a are expressed, but no functional data available [85]</td>
</tr>
<tr>
<td>Interaction of epicardium and myocardium</td>
<td>Epicardial Wnt-9a implicated to signal to myocardium [86]</td>
<td>Not implicated</td>
</tr>
<tr>
<td>Cardiac hypertrophy</td>
<td>Involved downstream of GSK-3β Wnt ligand not implicated [97–99]</td>
<td>Not implicated</td>
</tr>
<tr>
<td>Wound healing in myocardial infarct</td>
<td>Regulation of β-catenin might be involved [107–109]</td>
<td>Implicated in myofibroblast migration [104–106]</td>
</tr>
</tbody>
</table>
differentiation and contributes a majority of cells to the heart. Dev Cell 2003;5:877–89.


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