Planar Cell Polarity Signaling Pathway in Congenital Heart Diseases

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1. Introduction

Congenital heart disease (CHD), the most common disorder of congenital disease in humans, occurs in approximately 1% of live births [1, 2]. Among many types of CHDs, septation and alignment defects make up the largest group of CHDs, including ventricular and atrial septal defects, tetralogy of Fallot, and double-outlet right ventricle defects [3]. In particular, congenital defects that involve the outflow tract are especially prevalent, including defects of the transposition of the great arteries (TGA), double outlet right ventricle (DORV), and persistent truncus arteriosus (PTA), where a single outflow tract vessel is observed in place of the normal aorta and pulmonary artery [4].

The prognosis, morbidity, and mortality are dependent on the type, size, location, number of defects, and the associated anomalies [5]. CHD represents the cause of one-tenth of all infant deaths worldwide and is the leading noninfectious cause of death in the first year of life [6]. Of great concern to pediatricians and cardiac surgeons are outflow tract defects, because babies that suffer from these problems typically require urgent and complex surgeries shortly after birth.

In recent years, a correlation has been made between dysregulation of the planar cell polarity signaling pathway and CHD.

2. Cardiac Development

2.1. Early Heart Development. In vertebrates, the heart is the first organ to form and has a vital role in the distribution of nutrients and oxygen in the embryo [7]. Formation of the vertebrate heart can be subdivided into distinct but partially overlapping phases, such as specification of cardiac progenitors and the formation of the linear heart tube by cell migration and morphogenetic movements, followed by cardiac looping, chamber formation, septation, and maturation [8].

Myocardial cells are derived from the mesoderm, which emerge from the primitive streak during gastrulation. Later, these cells migrate from the streak in an anterior-lateral direction to positions under the headfolds forming two groups of cells on either side of the midline [9]. The cells then extend across the midline to develop a crescent-shaped epithelium called the cardiac crescent, which fuses at the midline to form the early heart tube [7] called the primary heart field or the first heart field (FHF). These cells will form the left ventricle.

During the formation of a mature heart, the linear heart tube subsequently expands. This is achieved by two mechanisms: cell proliferation and recruitment of additional cells. The latter cells originate in the second heart field (SHF) a cardiac precursor cell population distinct from the first heart
field [10]. SHF will mainly develop into the outflow tract (OFT) and the right ventricle, but also into both atria [11]. Also, as demonstrated in the chicken system, cells of the SHF populate the right ventricle [12]. Frog hearts contain a single ventricle; therefore, cells of the SHF exclusively end up in the OFT [13].

2.2. Outflow Tract Formation. OFT formation involves interactions between diverse cell types in the region of the pharyngeal splanchnic mesenchyme and in SHF that gives rise to the myocardium of the OFT and its endothelial lining [14, 15]. Cardiac neural crest (CNC) cell-derived mesenchyme also plays an important role [16]. These cells form the greater part of the outflow tract cushions, and if they are removed physically or genetically, then outflow tract septation fails [17, 18].

OFT is normally divided by the fusion of a series of ridges or cushions within itself. There are two mechanisms. In the early stages, the dominant mechanism is myocardialization [19]. First during myocardialization, the cells within the thin layer of OFT myocardium fail to adhere to one another tightly. Then, the cells stop behaving like an epithelium and, instead, show protrusive activity and move into the adjacent outflow tract cushions. Thus, the cushions become directly populated by cardiomyocytes [20]. It is thought that myocardialization shares characteristics with the convergent and extension (CE) process, at least with respect to the polarized migration of cells [20]. Direct invasion, the second mechanism, may be complemented by the recruitment of cushion mesenchymal cells into the muscle lineage [21].

3. The Planar Cell Polarity Signaling Pathway

Signaling by ligands of the Wnt family, which controls cell proliferation and patterning, is important for a variety of crucial cell changes and morphogenetic events in development. Two branches of the Wnt pathway exist: a β-catenin-dependent canonical pathway and β-catenin-independent noncanonical pathways [22, 23]. Noncanonical Wnt signaling has been shown to be inhibitory for canonical Wnt signaling through multiple mechanisms [24]. In zebrafish, Wnt genes that activate the noncanonical Wnt pathway are pipetail/Wnt5 [25] and silverblick/Wnt11 [26].

Noncanonical Wnt pathways, also called the planar cell polarity (PCP) signaling pathway, work on planar cell polarity in Drosophila and gastrulation movements and cardiovascular development of the outflow tract for the following reasons: Vangl2 plays an important role in the myocardialization of the OFT and its endothelial lining [14, 15]. If the correct expression of proteins in PCP signaling is disrupted, then defects may be seen in the heart.

4. Planar Cell Polarity Signaling Pathway in CHD

Normal cardiac development is dependent on PCP signaling [3, 20, 36, 37], which contributes to correct cardiac specification in the mesoderm germ layer [8]. If the correct expression of proteins in PCP signaling is disrupted, then defects may be seen in the heart.

4.1. Vang-Like 2. Vertebrates have two Vang-like (Vangl) genes, Vangl1 and Vangl2, which are homologs of the Drosophila gene Van Gogh/Strabismus (Vang/Stbm). Vangl mutations disrupt the organization of various epithelial structures, causing characteristic swirled patterns of hairs on wing cells and disorientation of eye ommatidia [38]. Vangl1 and Vangl2 proteins share ∼70% sequence similarity, which underlies their conserved functions; Vangl1 and Vangl2 proteins bind to three mammalian Dvl proteins, and Lp mutations engineered in Vangl1 or Vangl2 abrogate interaction with Dvl [39]. The Vangl2 gene encodes a membrane protein comprising four transmembrane domains and a large intracellular domain with a PDZ-domain-binding motif at its carboxy terminus [40]. Mutations in Vangl2 can cause neural tube defects and cardiac abnormalities [41]. Loop-tail (Lp), a naturally occurring mouse mutant, develops severe cardiovascular defects in association with abnormal midline development [40, 42], and is often used when studying of Vangl2.

4.2. Outflow Tract. Vangl2 plays an important role in the development of the outflow tract for the following reasons: Vangl2 is strongly expressed in the outflow tract myocardium, including the cells that migrate into the outflow tract cushions [36]. Conspicuous abnormalities are found in the outflow trace of Lp mutants [3], and similarities exist between myocardialization of the OFT and the CE movement during gastrulation, in which Vangl2 is generally thought to be correlated [20].

Complex cardiovascular defects can be found in Lp homozygotes, including double-outlet right ventricle defects, with obligatory peri-membrane ventricular septal defects, and double-sided aortic arch defects, with associated abnormalities in the aortic arch arteries [3]. During the myocardialization of the Lp mice, both the extension of polarized membrane protrusions and the reorganization of the actin cytoskeleton are inhibited in myocardial cells at the muscle-cushion tissue, strongly suggesting that this is a defect in...
cell polarity and/or cell movement, rather than some other aspect of cell behavior [20]. RhoA and ROCK1 [43], the downstream mediators of the PCP signaling pathway, are required as well for Vangl2 function [36]. However, the Tetralogy of Fallot (ToF) abnormality, resulting from disturbances of morphogenetic processes in OFT development [44], does not show any specific mutations in Vangl2 gene that are responsible for the ToF phenotype [45]. Further research could include the possible role of other PCP components expressed in the development of the outflow tract [45]. Furthermore, a typical PCP phenotype in a mouse mutant for the Sec24b gene has been reported, including abnormally small ventricles and abnormally arranged OFT vessels [46]. Sec24b is a component of the coat protein complex II (COPII) that is essential for intracellular endoplasmic reticulum- (ER-) to-Golgi protein transport [47]. In Sec24b mutation mice, both abnormal Vangl2 expression or localization were detected; however, this abnormal expression or localization of Vangl2 may only be partially responsible for the Sec24b mutation caused cardiac defects [46].

As for Vangl1, its role in OFT is much smaller than Vangl2. No cardiac outflow abnormalities were detected in Vangl1ff/+ or Vangl2ff/+ double heterozygotes, or in Vanglff/gt homozygotes, only an aberrant right subclavian artery was found in the former, suggesting that the two genes genetically interact to regulate the proper development of the extracardiac structures [41].

4.3. Coronary Circulation. The coronary arteries that channel oxygen rich blood throughout the ventricular myocardium are formed from cells that originally derive from a region of the splanchnic mesoderm known as the proepicardium [48, 49]. Vangl2-PCP signaling can play a noncell autonomous role in coronary artery formation [50]. For example, in the animals with Lp/Lp hearts, the coronary vessels fail to develop a normal smooth muscle cell layer and instead develop enlarged ectopic vessels in the subepicardium. Reduced fibronectin deposition, because of loss of functional vangl2 in the subepicardial space, is associated with limited migration of epicardially derived cells (EPDCs) into the ventricular myocardium and likely contributes to those defects [50]. Fibronectin deposition has also been shown to be deficient at tissue boundaries in Xenopus embryos in which Vangl2 is disrupted and PCP signaling is abnormal [51, 52]. These defects were associated with defects in the polarized cell movements during the process of CE, which resembles the Lp/Lp heart, where fibronectin deposition is reduced at the epicardial-myocardial boundary and cell migration is impaired [50].

Similarly, mice deficient in connexin 43 also have defects in epicardial cell polarization, migration, and early remodeling of the coronary vascular plexus [53, 54]. However, aberrant expression of planar cell polarity pathway components was not detected in the connexin 43 knockout hearts, so it is currently unclear whether and how connexin 43 interacts with the planar cell polarity pathway [55].

4.4. Diversin/Inversin. The vertebrate ankyrin repeat protein, Diversin, is related to the Drosophila protein Diego, which controls PCP during fly development [56]. Diversin also acts in the canonical Wnt signaling pathway, where its centrosomal localization is crucial for its function in Wnt signaling [57]. Diversin is a modular protein containing N-terminal ankyrin repeats, a central casein kinase-binding domain, and a C-terminal domain that binds axin/conductin [58].

Early zebrafish embryos injected with Diversin mRNA that encodes a protein lacking the ankyrin repeat domain, Div-ΔANK, were found to develop cardiac bifida [59]; however, those cardiac bifida can be rescued by coinjection of an activated form of RhoA (RhoA-V14), suggesting that Diversin controls heart formation through RhoA [59]. Cardiac bifida in fish is generated when the bilateral heart anlagen fail to fuse because of defective migration of myocardial precursors to the dorsal midline [60, 61], which is regulated by PCP signaling [28]. These results suggest that Diversin can play a role in heart through PCP in the downstream of RhoA.

Meanwhile, Diversin and Dishevelled are accepted as mutually dependent players within the PCP signaling pathway. The Diversin orthologue of Drosophila, Diego, genetically interacts with and physically binds to Dishevelled [62]. However, during cardiogenesis, coinjection of both dominant-negative molecules, Div-ΔANK and Dvl-ΔDEP, did not synergize, suggesting that both Diversin and Dishevelled control heart formation and PCP signaling in zebrafish embryogenesis by similar mechanisms [59].

In the inv/inv mouse, carrying an insertional mutation in the inversin gene, some cardiopulmonary malformations were found, which are not rare in the mutant mice. The inv/inv mice have a propensity for defects in the development of the right ventricular OFT and the interventricular septum.

4.5. Dishevelled. Dishevelled (dsh in Drosophila or Dvl in mice) proteins, of which three have been identified in humans and mice, are highly conserved components of both the canonical Wnt pathway [63], and the PCP pathway [64]. They function as essential scaffolding proteins that interact with diverse proteins, including kinases, phosphatases, and adaptors proteins [65, 66].

In zebrafish, it is reported that injection of the dominant-negative Dishevelled lacking the DEP domain (Dvl-ΔDEP) into zebrafish embryos induced cardiac bifida phenotypes and CE defects. Dishevelled regulates heart formation via the activation of RhoA. Meanwhile, during cardiogenesis, Div-ΔANK and Dvl-ΔDEP, the frequency of cardiac bifida was not increased [28].

In mice, mutations in the Dvl2 gene, one of three vertebrate homologues of Drosophila Dishevelled, developed OFT defects similar to those seen in Lp mice, including double-outlet right ventricle and ventricular septal defects [37]. Dvl1/Dvl2 double mutants develop the neural tube defect craniorachischisis [37], which is associated with disruption of PCP signaling in mice [40]. Since both Vangl2 and Dvl2 are expressed in the OFT myocardium [36], Dvl2 is suggested to act in PCP pathway in OFT. The PCP signaling pathway can regulate cell migration processes during gastrulation and neural crest cell migration in vertebrates [22, 67]. Dvl2 is a core PCP member in canonical Wnt signaling. But Dvl2 is
also suggested to influence OFT formation through neural crest cell migration in PCP signaling since a defect is found in cardiac neural crest development during OFT formation in Dvl2 null mutants [37].

Dvl3/− mice died perinatally with cardiac OFT abnormalities, and the mutants displayed a misoriented stereocilia in the organ of Corti, suggesting that Dvl3 is required for cardiac OFT development in the PCP pathway [64]. However, OFT in Dvl3/− mice were not due to an absence of CNC or SHF Cells. Moreover, Dvl2+/−; Dvl3/− mice can survive to adulthood and are fertile. In an inbred background, conotruncal abnormalities were seen, while Dvl2+/−; Dvl3/− hearts had similar morphologies to Dvl3/− hearts, suggesting Dvls are functionally redundant [64].

4.6. Wnt5a and Wnt11. In vertebrates, Wnt5a and Wnt11 can activate the Wnt/JNK pathway, which resembles the PCP pathway in Drosophila [68]. Wnt5a [69, 70] and Wnt11 [4, 29, 69–72] represent the PCP pathway that have been implicated in cardiogenesis.

4.6.1. Wnt5a. Wnt5a primarily signals through the PCP signaling pathway, although it also has the potential to activate the canonical Wnt signaling pathway [73], by functioning as an antagonist of the canonical Wnts [74]. A mutation in Wnt5a in mice can lead to PTA. In the model, Wnt5a produced in the OFT, by cells originating from the pharyngeal mesoderm, signals adjacent CNC cells during the formation of the aortopulmonary septum through a PCP pathway via localized intracellular increases in Ca2+ [16].

Wnt5a is thought to be supportive but not required for cardiogenesis [8] because in discussing Notch signaling in cardiac progenitors, Wnt5a synergizes with BMP6 and Sfrp1 to promote formation of troponin-positive cells, likely through noncanonical Wnt signaling activities. However, adding individually SFRP1, Wnt5a, and BMP6 does not significantly increase cardiac development [75].

4.6.2. Wnt11. Wnt11 is a secreted protein that signals through the PCP pathway and is a potent modulator of cell behavior and movement. In human, mouse, and chicken, there is a single Wnt11 gene, and in Xenopus and zebrafish, there are two, Wnt-11 and Wnt-11R [71]. Wnt11 can activate PCP signaling and at the same time inhibit Wnt signaling [24]. Wnt-11 shows a spatiotemporal pattern of expression that correlates with cardiac specification [76, 77] and loss-of-function experiments also demonstrated that Wnt11 is required for normal heart development and cardiac marker gene expression [29, 78]. Wnt11 leads to cardiac specification in the PCP pathway [68] and is conserved [29].

The mouse Wnt11 gene is expressed within or in close proximity to the precardiac mesoderm, and later in the myocardium of the primitive heart tube [79]. Thus, the Wnt-11 expression domain overlaps with the first and secondary heart fields that contribute to the majority of the tissues establishing the heart [69–71, 79]. At later stages, Wnt11 is expressed in OFT, where both Wnt5a and Wnt11 signaling have morphogenetic roles [4, 16]. Interestingly, human Wnt11 has been proposed to be expressed in the adult heart [80].

In cell culture models, Wnt11 signaling determines the fate of the cardiomyocytes and promotes differentiation of the already committed cardiomyocytes, a conclusion based in part on its capacity to induce the expression of certain cardiac transcription factor genes [29, 81–85], suggesting that Wnt11 signaling may have a broader role in the control of mammalian heart development [86].

5. Heart Morphogenesis

In Xenopus, Wnt11-R is expressed in neural tissue, dorsal mesenchyme derived from the dermatome region of the somites, the brachial arches, and the muscle layer of the heart, similar to the expression patterns reported for mouse and chicken Wnt11 [72]. Inhibition of Wnt11-R function using morpholino oligomers causes defects in heart morphogenesis, in fact, 10% of the hearts exhibit a pronounced cardiac bifida phenotype, suggesting Wnt11-R functions in regulation of cardiac morphogenesis [72].

In cardiocytes of Xenopus, Wnt11 is required for heart formation and is sufficient to induce a contractile tissue in embryonic explants by PCP signaling which involves protein kinase C and Jun amino-terminal kinase [29].

When embryos injected with Wnt11-R MO on one side only were examined in section, it was clear that the myocardial layer on the injected side was thicker than on the control side. This is also visible in double-sided MO1-injected embryos. Quantitation of differentiated myocardium showed that the area of the Wnt11-R-depleted side was 26% larger than the control side [72].

However, in a mouse model in which Wnt-11 function has been inactivated, Wnt-11 signaling serves as a critical cell adhesion cue for the organization of the cardiomyocytes in the developing ventricular wall. In the absence of Wnt-11, the coordinated organization, intercellular contacts, colocalized expression of the cell adhesion components N-cadherin and β-catenin, and the cytoskeleton of the differentiating ventricular cardiomyocytes are all disturbed. Moreover, the ventricular wall lacking Wnt-11 signaling is thinner [86].

5.1. Outflow Tract. Wnt11 signaling can affect extracellular matrix composition, cytoskeletal rearrangements and polarized cell movement required for morphogenesis of the cardiac OFT [4]. In fact, Wnt11 plays this role in the integration and crosstalk between three major signaling pathways: Wnt pathway, PCP pathway, and TGFβ signaling. In Wnt11 mutants, penetrance of the outflow tract phenotype was 100% accompanied by ventricular septal defects (VSD) [4].

5.2. Ptk7. Protein tyrosine kinase 7 (Ptk7) is a transmembrane protein containing seven extracellular immunoglobulin domains and a kinase homology domain [87]. Ptk7 is regarded as a regulator of PCP signaling that could modulate the dsh localization as well as the interaction with pathway-specific effector proteins [87], while someone regarded it as an essential component of PCP pathway [32]. In Xenopus, Ptk7 is required for neural convergent extension [88] and
can regulate neural crest migration by recruiting dishevelled (dsh) to the plasma membrane [89].

In chick, disruption of off-track (the chick Ptk7 homologue) causes abnormal heart development [90]. In mouse, chuzhoi (chz) mutants, carrying a splice site mutation in Ptk7, exhibit several defects in cardiovascular development, including OFT defects with VSD, while they exhibit minor defects in neural crest cell distribution [89]. A genetic interaction between chuzhoi mutants and both Vangl2+/− and Celsr1Crh mutants was demonstrated, strengthening the hypothesis that chuzhoi is involved in regulating the PCP pathway [89].

5.3. Scribble. Scribble (also known as Scrbl) is orthologous to the Drosophila scribble gene, which regulates apical-basal polarity and functions as a tumor suppressor, regulating cell growth; scribble mutants exhibit disrupted cellular architecture [91–93]. Scribble is a putative cytoplasmic protein and is a member of the LAP protein family that is characterized by the presence of four PDZ domains. Scribble plays essential roles in cell–cell adhesion [94]. In human, hScrib protein displays highly polarized localization in mammalian epithelial cells and could play an important role in the suppression of human tumors [93]. Scribble has not been implicated in planar cell polarity [95], although some scholars identify it as PCP protein [30, 31].

In mutations in mouse Scribble (circle tail mutant, Crc), cardiac looping and chamber expansion are disrupted and abnormal development of the arterial wall and early abnormalities in myocardial organization are found. Finally, spectrum of congenital heart defects are developed, such as smaller and abnormally shaped ventricular chambers, cardiovascular defects, and atrioventricular septal defects [96], suggesting that Crc can develop heart malformations and cardiomyopathy attributable to abnormalities in cardiomyocyte organization within the early heart tube [96].

The mechanism of Scribble involved in heart development may refer to N-cadherin. The integrity of the heart tube is dependent on N-cadherin, which is tightly localized to the lateral membranes of cardiomyocytes from the earliest time of heart tube formation [97]. The N-cadherin zebrafish mutant can develop a disorganized myocardium with abnormally shaped and loosely-aggregated cardiomyocytes [98], which is very similar to Crc. Scribble is required for the correct localization of N-cadherin and β-catenin at the lateral cell membrane in the primitive myocardium [96].

Moreover, Scribble and Vangl2 can interact in heart development. Scribble is required for the correct localization of Vangl2 within the membrane compartments of cardiomyocytes and that Scribble is acting to direct the PCP pathway in the developing myocardium. Double heterozygosity for mutations in both Scribble and Vangl2 can cause cardiac defects similar to those found in homozygous mutants for each gene but without other major defects. Those are in accord with the fact [31] that proteins interact physically through discrete PDZ-binding domains, observed in yeast 2 hybrid and coimmunoprecipitation studies [99], in addition, heterozygotes (Lp+/−,Crc+/−) also exhibit craniorachischisis, which is equal in severity to either Crc/Crc or Lp/Lp mice [100], suggesting the overlapping expression of Scrb1 with Vangl2.

6. Conclusions

The planar cell polarity (PCP) pathway is a highly conserved signaling pathway that mediates changes in cell polarity and cell motility during cardiogenesis, through activation of cytoskeletal pathways, such as RhoA and Rho kinase (ROCK). Several components of the pathway are expressed within the developing heart, and the disrupted function of pathway members in chick, Xenopus, zebrafish, and mouse are associated with some heart defect, which leads to congenital heart disease (CHD). The interaction of proteins within the PCP pathway and the intercross of the PCP pathway with the other pathways such as the Wnt signaling pathway are also demonstrated. No genes within the PCP pathway that cause cardiovascular defects in humans have been described thus far.

Abbreviations

PCP: Planar cell polarity
CHD: Congenital heart disease
FHF: First heart field
SHF: Second heart field
CNC: Cardiac neural crest
OFT: Outflow tract
CE: Convergent and extension
EPDCs: Epicardially derived cells.

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