The Wnt/Frizzled pathway as a therapeutic target for cardiac hypertrophy: where do we stand

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REVIEW

The Wnt/Frizzled pathway as a therapeutic target for cardiac hypertrophy: where do we stand?

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Abstract

Cardiac hypertrophy is an enlargement of the heart muscle in response to wall stress. This hypertrophic response often leads to heart failure. In recent years, several studies have shown the involvement of Wnt signalling in hypertrophic growth. In this review, the role of Wnt signalling and the possibilities for therapeutic interventions are discussed. In healthy adult heart tissue, Wnt signalling is very low. However, under pathological condition such as hypertension, Wnt signalling is activated. In recent years, it has become clear that both β-catenin-dependent signalling and β-catenin-independent signalling are involved in hypertrophic growth. Several studies, both *in vitro* and *in vivo*, have shown that genetic interventions in Wnt signalling at different levels resulted in an attenuated or diminished hypertrophic response. Therefore, inhibition of Wnt signalling could provide a new therapeutic strategy for cardiac hypertrophy, but further research on the Wnts and Frizzleds involved in the different forms of cardiac hypertrophy will be needed to achieve this goal.

Keywords frizzled, heart, hypertrophy, therapy, Wnt.

Cardiac hypertrophy is an enlargement of the heart in response to an increase in wall stress. It is an adaptive response of the heart muscle to compensate for the decrease of cardiac output. It is characterized by an increase in cardiomyocyte size, increased protein synthesis, increased fibrosis and the upregulation of foetal genes that are normally activated in the developing heart, such as α-actin, β-myosin heavy chain (β-MHC), atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) (Oka *et al.* 2007, Rohini *et al.* 2010).

Several subtypes of hypertrophy can be distinguished. In the concentric form, the thickness of the cardiomyocytes is increased by the addition of parallel sarcomeres. Concentric hypertrophy is caused by pressure overload, which is seen in patients with hypertension or aortic stenosis. This form of cardiac hypertrophy results in an increase in wall thickness and a reduced left ventricular lumen. Eccentric hypertrophy is characterized by an elongation of the cardiomyocytes, resulting in ventricle dilation and thinning of the ventricle wall. Sarcomeres are added in series. This is caused by volume overload, for instance, due to valve defects or myocardial infarction (Frey *et al.* 2004, Ellery *et al.* 2006, Barry & Townsend 2010). Although hypertrophy is at first a beneficial process, in the longer term, it progresses into decompensation, contractile dysfunction and heart failure. One to two percent of the population is affected by heart failure and the 5-year mortality is almost 50% (Barry & Townsend 2010).

Intensive exercise and pregnancy also lead to hypertrophy, but this enhances rather than deteriorates cardiac function. This form is called physiological hypertrophy, and does not lead to heart failure. Re-expression of foetal genes and fibrosis does not occur (Dorn 2007, McMullen & Jennings 2007). The different forms of cardiac hypertrophy are shown in Figure 1. In this review, our focus will be on the pathological forms of cardiac hypertrophy. We will provide an overview of the literature and discuss the findings in a context of therapeutic potential of the pathway.

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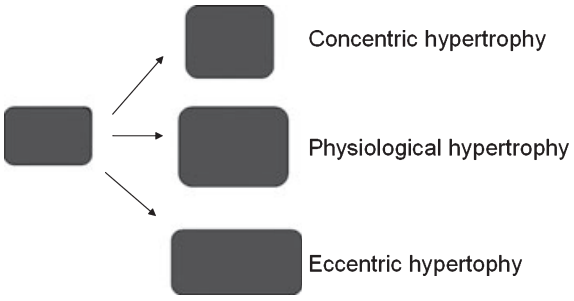

Figure 1 The different shapes of cardiomyocytes in concentric, eccentric and physiological hypertrophy.

Signalling pathways

Pathological hypertrophy is the result of the activation of several signalling pathways. Hypertrophic stimuli like angiotensin II (AngII), endothelin 1 (ET-1) and Phenylephrine (PE) can activate phospholipase (PLC), which hydrolyses PIP_2 to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 then releases intracellular Ca^{2+}, which in turn activates calcineurin. Transcription factor binding sites for nuclear factor of activated T-cells (NFAT), Mef2, Gata, Nkx, Foxo, C/Ebp and Irf (Hannenhalli *et al.* 2006) have been found in human heart failure patients. NFAT is dephosphorylated by calcineurin and translocates to the nucleus, where it initiates the transcription of hypertrophic genes (Rohini *et al.* 2010). This leads to the expression of c-Jun, c-Fos and c-Myc and foetal genes ANF, BNP and β-MHC (Usui *et al.* 2006). Interestingly, NFAT is phosphorylated by GSK3β, a key component of β-catenin-mediated Wnt signalling, which will be discussed in more detail below.

Physiological hypertrophy, on the other hand, is mainly activated by growth factors such as insulin-like growth factor (IGF) and growth hormone (GH). Phosphoinositide 3′ kinase (PI3K) is activated *via* tyrosine kinase receptors. PIP_2 is then phosphorylated to PIP_3, which activates the kinase Akt by phosphorylation and the activated Akt causes the transcription of hypertrophic genes (Barry & Townsend 2010), as shown in Figure 2.

Wnt signalling

Several studies have shown the involvement of Wnt signalling in cardiac hypertrophy. Wnts are highly conserved secreted glycoproteins with 350–400 amino acids. Wnts contain large, cysteine rich domains, and are extensively palmitoylated (Kikuchi *et al.* 2007). Because of this palmitoylation, the Wnt proteins are hydrophobic, making it difficult to purify Wnts in an active form. Until now, 19 Wnt ligands and ten Frizzled receptors have been identified. The Wnt ligands can be divided into two subclasses: the Wnt1 class, consisting of Wnt 1, 3, 3a, 7a, signals *via* the canonical or β-catenin pathway and the Wnt5a class, consisting of Wnt 2, 4, 5a, 5b, 6, 7b and 11, can activate the calcium

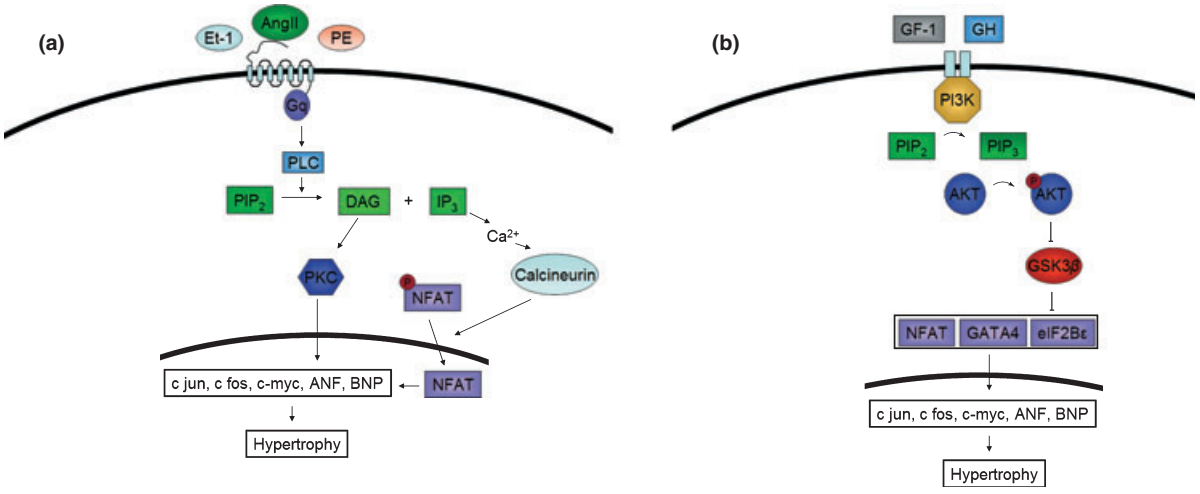

Figure 2 Signalling pathways leading to pathological (a) and physiological hypertrophy (b). In pathological hypertrophy, the stimulation by endothelin-1 (ET-1), Angiotenin II (AngII) or Phenylephrine (PE) lead to activation of Phospholipase-C (PLC) which hydrolyses PIP_2 to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 then releases intracellular Ca^{2+}, which activates calcineurin. NFAT is dephosphorylated by calcineurin and activates transcription of hypertrophic genes. Dag activates protein kinase C (PKC), which leads to hypertrophic gene transcription. Physiological hypertrophic growth is activated by growth factors as insulin-like growth factor (IGF) and growth hormone (GH). These growth factors activate Phosphoinositide 3′ kinase (PI3K) *via* tyrosine kinase receptors. PIP_2 is then phosphorylated to PIP_3, which activates Protein kinase B (Akt). The activated Akt phosphorylates glycogen synthase kinase-3β (GSK3β) at Ser^9, which inactivates its kinase activity. In resting cells, activated GSK3β phosphorylates and inhibits a number of transcription factors that are involved in hypertrophy.

pathway. However, over the last years, it has become clear that such a rigid classification is not correct because several Wnts have been shown to modulate both canonical and non-canonical Wnt signalling. Which pathway is activated depends on the cell type, the receptor, and co-receptor that is involved.

The receptors that bind the Wnt ligands are the seven transmembrane Frizzled receptors. The Frizzled family consists of 10 members. The size of these receptors varies between 500 and 700 amino acids, and the N-terminal part contains a cysteine-rich domain. This domain is the binding site for the Wnt ligand. Several co-receptors for Frizzled have been identified (Hendricks *et al.* 2008). The lipoprotein receptor-related proteins (LRP) 5 and 6 can act as co-receptor for Frizzled, and provide a binding site for Axin (Cadigan & Liu 2006).

Three Wnt signalling cascades have been described, namely the β-catenin, the RhoA/Jnk and the calcium pathway (Fig. 3). The best studied signalling cascade is the β-catenin-dependent or canonical Wnt signalling pathway. A key molecule in β-catenin-dependent signalling is glycogen synthase kinase-3β (GSK3β). In the absence of Wnt signalling, GSK3β forms a complex including Axin and adonomatous polypolis coli (APC). This complex is known as the β-catenin destruction complex. It binds β-catenin, which is then phosphorylated by GSK3β and casein kinase-1. The phosphorylated β-catenin is then ubiquitinated and degraded by the proteasome (Kimelman & Xu 2006), which results in low levels of β-catenin in the cytoplasm under baseline conditions.

When Wnt signalling is activated by the binding of Wnt to a Frizzled receptor and LRP co-receptor, Dishevelled (Dvl) binds to this Wnt-Frizzled-LRP complex. The Axin-Apc-GSK3β complex is then able to bind to the intracellular domain of LRP, preventing GSK3β to phosphorylate β-catenin (Cliffe *et al.* 2003). The β-catenin then accumulates and is transferred to the nucleus, where it can interact with the transcription factors Tcf/Lef1, and activate the transcription of the target genes, including Cyclin D1 and c-Myc (Cadigan & Liu 2006).

Wnt can also signal *via* Jun N-terminal kinase (Jnk) and *via* calcium. Wnt/Jnk signalling is similar to the planar cell polarity pathway in *Drosophila,* which regulates the orientation with neighbouring cells of the planar epithelial cells in the wing (Simons & Mlodzik 2008). Activation by Wnt leads to activation of the GTPases Rac, Cdc42 and Rho, which then activates Jnk and Rho kinase (Rock). Wnt is also able to increase the intracellular Ca^{2+} concentration and activate calcium-dependent signalling *via* G-proteins. These proteins activate phospholipase-C (PLC). The released Ca^{2+} activates Ca^{2+}-dependent enzymes, like the calcium/calmodulin-dependent kinase II (CamKII), PKC and the phosphatase calcineurin. Activation of CamKII inhibits the translocation of NFAT to the nucleus in cardiomyocytes by phosphorylation (MacDonnell *et al.* 2009). The activated calcineurin can dephosphorylate NFAT, leading to an accumulation of NFAT in the nucleus (Dejmek *et al.* 2006), whereas the phosphorylated NFAT is degraded in the cytoplasm.

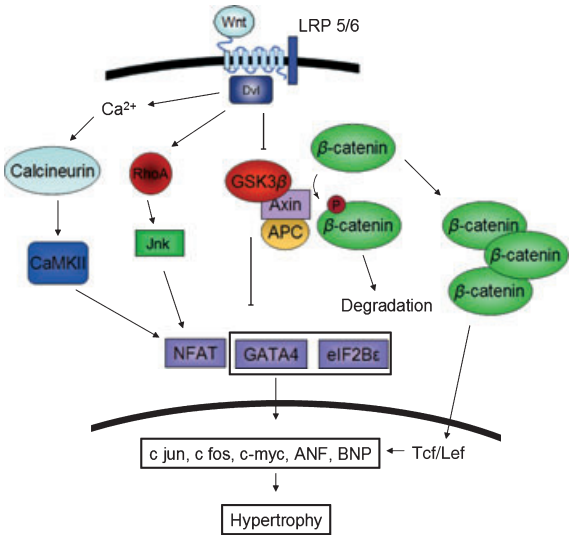

Figure 3 The three different Wnt signalling pathways. In the canonical pathway, activation of Wnt signalling leads to β-catenin accumulation by the disruption of a complex consisting of glycogen synthase kinase-3β (GSK3β), Axin and adenomatous polypolis coli (APC). In absence of Wnt signalling, this complex phosphorylates β-catenin, which is then degraded. When Wnt signalling is activated, β-catenin degradation is prevented and β-catenin can be transferred to the nucleus, where it interacts with the transcription factors Tcf/Lef and initiate transcription. In the Wnt/Jnk pathway, Wnt signalling leads to activation of Rho, which in turn activates jun N-terminal kinase (Jnk) and Rho kinase (Rock). The third Wnt pathway is the Wnt/Ca^{2+} pathway. Wnt signalling increases the intracellular Ca^{2+} concentration. The released Ca^{2+} activates Ca^{2+}-dependent enzymes, like the calcium/calmodulin-dependent kinase II (CamKII), protein kinase C (PKC) and calcineurin. Activation of CamKII inhibits the translocation of NFAT to the nucleus in cardiomyocytes by phosphorylation.

Antagonists of Wnt signalling

Several endogenous antagonists of Wnt signalling have been described. Dickkopf (Dkk), which by itself is a target of Wnt signalling, inhibits the formation of a complex of LRP, Frizzled and Wnt (Mao *et al.* 2002), thereby inhibiting Wnt signalling. Besides this complex formation, interaction of Dkk1 with Kremen is able to internalize the LRP co-receptor, which leads to its degradation (Krishnan *et al.* 2006). Another class of

Wnt antagonists are the secreted Frizzled related proteins (sFRPs). These proteins are similar to Frizzled, but lack the transmembrane domain and can scavenge Wnts by competing for binding with the Frizzleds. Also Wnt inhibitory factor (WIF) and Cerberus proteins can bind Wnts and affect the binding to the Frizzled receptors (Krishnan *et al.* 2006, Bovolenta *et al.* 2008).

Wnt signalling and cardiac hypertrophy

During the development of cardiac hypertrophy, several genes are expressed that are also expressed in the developing foetal heart (McMullen & Jennings 2007, Oka *et al.* 2007). Wnt signalling is involved in heart development (Bergmann 2010) and therefore this could suggest that Wnt signalling is also activated in cardiac hypertrophy. Several studies showed the involvement of Wnt signalling in cardiac hypertrophy. A study performed in our lab showed the upregulation of Frizzled-2 during the development of cardiac hypertrophy in rat heart tissue after aortic constriction (Blankesteijn *et al.* 1996). A more extensive micro-array study with three different hypertensive rat models showed a positive correlation between the left ventricular mass and Frizzled-2 mRNA levels and a negative correlation with Dickkopf-3 (Cerutti *et al.* 2006).

Downstream of Frizzled, expression of Dishevelled (Dvl) was shown to be upregulated after aortic constriction in rats and mice (van de Schans *et al.* 2007, Malekar *et al.* 2010). Intervention in Wnt signalling at the level of Dvl can modulate the hypertrophic growth; in our laboratory, we found that in Dvl-1 knockout mice, the hypertrophic response induced by 14 days of thoracic aortic constriction (TAC) was attenuated compared with wild-type mice. Also an increased activity of GSK3β, increased AKT phosphorylation and decreased β-catenin levels were observed (van de Schans *et al.* 2007, Blankesteijn *et al.* 2008). In a recent study by Malekar *et al.*, overexpression of Dvl-1 led to hypertrophy, with reduced ejection fraction and increased heart weight. In these mice, the β-catenin, as well as the Jnk and CamKII, was activated. Depletion of Dvl-1 with siRNAs in cultured cardiomyocytes led to an abrogated response to β-adrenergic stimulation, suggesting that dishevelled is required and sufficient for β-adrenergic induced hypertrophy (Malekar *et al.* 2010). These observations indicate that interventions at the level of Dvl could be a fruitful approach to modulate the hypertrophic response of the heart.

Surprisingly, little is known about the Wnts that are involved in cardiac hypertrophy. We have performed an analysis of the mRNA levels of most of the Wnts in hypertrophic hearts by quantitative PCR, but no upregulation of any of the Wnts was observed (P. ter Horst and W.M. Blankesteijn, unpublished observations). Therefore, it is still unclear which Wnt(s) is/are responsible for the activation of the downstream signalling that takes place in cardiac hypertrophy. However, it has to be noted that these observations should be confirmed by analyses of Wnt protein levels. Unfortunately, this research is hampered by the lack of specific antibodies for most of the Wnt family members.

GSK3β

GSK3β is a Ser/Thr kinase, which plays an important role in canonical/β-catenin-dependent Wnt signalling. It was originally identified as a factor regulating glucose metabolism. GSK3β phosphorylates glycogen synthase, which then inhibits its activity. The kinase is catalytically active in resting cells. The activity of the kinase can be inhibited by the phosphorylation of Ser^{9} residue (Cohen & Goedert 2004). The phosphorylation of the Ser^{9} residue occurs by a variety of kinases, including AKT, p60/p85–s6 kinases (S6Ks) and RSKs (Sugden *et al.* 2008). To phosphorylate a protein, GSK3β requires a priming phosphate group on its target. The phosphorylation of the Ser^{9} prevents the interaction with this priming phosphate, because GSK3β interacts intramolecularly with the Ser^{9} phosphate (Dajani *et al.* 2003). To be active, GSK3β also requires phosphorylation of the Tyr^{216} residue. This residue is probably phosphorylated by autophosphorylation by GSK3β itself (Cole *et al.* 2004).

Several studies have shown the involvement of GSK3β in the development of cardiac hypertrophy. Active GSK3β prevents hypertrophic growth by inhibition of transcriptional regulators, such as NFAT, Gata4 and eIF2Bϵ. When GSK3β is overexpressed, the development of hypertrophy is attenuated (Michael *et al.* 2004, Kerkelä *et al.* 2007). Several hypertrophic stimuli lead to a decreased GSK3β activity. Stimulation by either ET-1 (Haq *et al.* 2000), β-adrenoreceptor activation (Morisco *et al.* 2000) or aortic constriction (Haq *et al.* 2003) leads to Ser^{9} phosphorylation and inhibition of GSK3β activity. When the Ser^{9} residue was replaced by an alanine, the hypertrophic response diminished in response to calcineurin activation, adrenergic stimulation and aortic constriction (Antos *et al.* 2002). And when GSK3β was inhibited by LiCl, rats developed a higher heart weight, and had increased levels of ANF after abdominal aortic constriction (Tateishi *et al.* 2010). In human hearts, inhibition of GSK3β was seen in the end-stage of heart failure, but it was not observed in chronic hypertrophy (Haq *et al.* 2001). Inhibition of GSK3β induced compensated physiological hypertrophy in GSK3β-dominant negative mice (Hirotani *et al.* 2007). These mice had an increased cardiac function, less apoptosis and fibrosis after TAC.

β-catenin and hypertrophy

GSK3β is also able to phosphorylate β-catenin, as part of the β-catenin degradation complex. β-catenin has two functions in the cell: it is part of adherens junctions in the catenin/cadherin complex, necessary for cell structure and adhesion, and it serves as a transcription factor. The localization of β-catenin depends on the phosphorylation of its Tyr^{654} residue, which leads to complex formation with cadherin and phosphorylation of Tyr^{142}, leading to a decreased affinity for the adhesion complex and migration to the nucleus (Harris & Peifer 2005).

In cardiomyocytes, β-catenin levels are increased by the hypertrophic stimuli ET-1 and PE. Overexpression of β-catenin induced hypertrophic growth of cardiomyocytes (Haq *et al.* 2003, Hahn *et al.* 2006). In mutant mice carrying a cardiomyocyte-specific deletion of β-catenin, the hypertrophic response after TAC was attenuated. In another study also, an attenuated hypertrophic response upon TAC was found in mice haploinsufficient for β-catenin, but in these mice, the expression of foetal genes was upregulated (Chen *et al.* 2006, Qu *et al.* 2007). When β-catenin is knocked down in cultured cardiomyocytes, they showed attenuated protein synthesis, cell area and inhibition of the upregulation of ANF after treatment with PE. A Lef1 binding site is present in the ANF promoter (Zhang *et al.* 2009). Treatment with PE as well as inhibition of GSK3β with LiCl led to the binding of β-catenin and Lef1 to the ANF promoter. The overexpression of β-catenin led to an increase in protein synthesis, but was not sufficient to induce the expression of ANF (Zhang *et al.* 2009); therefore, also other factors play a role.

In contrast to these results, in a study performed by Baurand *et al.*, the cardiomyocyte-specific deletion of β-catenin increased the hypertrophic response after 2 weeks of AngII infusion. The stabilization of β-catenin reduced the cardiomyocyte area and ventricular wall thickness at baseline and an abrogated hypertrophic response after AngII infusion (Baurand *et al.* 2007, Zelarayan *et al.* 2008). Overall, these observations lead to the conclusion that β-catenin has the potential to regulate the hypertrophic response upon various stimuli, but there is no clear relationship between β-catenin levels and the amount of cardiac hypertrophy.

Non-canonical Wnt signalling

Besides the β-catenin-dependent Wnt signalling, also other Wnt pathways are involved in cardiac hypertrophy. The activation of Jnk *in vitro* resulted in a hypertrophic phenotype, *in vivo* overexpression led to a lethal restrictive cardiomyopathy and induction of foetal gene expression. Inactivation of Jnk stopped the hypertrophic response after pressure overload (Wang 2007).

The Wnt/Ca^{2+} pathway is also involved in the hypertrophic response. In human heart failure patients, both calcineurin and NFAT are activated. Their activation was found in pathological but not in physiological hypertrophy (Wilkins *et al.* 2004). Activation of calcineurin or NFAT induced hypertrophy in mice and the inhibition of calcineurin inhibited the hypertrophic response (Bueno & Molkentin 2002). In another study, inhibition of both CamKII and calcineurin attenuated the cardiac remodelling (Diedrichs *et al.* 2004, van Empel & De Windt 2004). Interestingly, GSK3β is able to counteract the calcineurin activity by phosphorylating NFAT.

At present, it remains unclear what the contribution of non-canonical Wnt signalling to the development of hypertrophy is. Recently, Malekar *et al.* found that in mice overexpressing Dvl-1, Jnk signalling was enhanced and also CamKII was activated. Additional research is needed to determine the precise role of non-canonical signalling (Malekar *et al.* 2010).

Wnt signalling and heart failure

A major problem of cardiac hypertrophy is that it frequently leads to heart failure. Little is known about the role of Wnt/Frizzled signalling in heart failure. In human heart failure patients, decreased Wnt signalling was observed compared with non-failing hearts. The expression of the pro-apoptotic sFRPs-3 and -4, but not -1 and -2, were upregulated, and β-catenin levels were decreased (Schumann *et al.* 2000). This upregulation of sFRPs -3 and -4 might lead to increased apoptosis and contribute to the transition to heart failure. More studies regarding the expression and function of sFRPs in animal models of heart failure should be performed to get more insight into the role of these sFRPs.

Next to cardiac hypertrophy, myocardial infarction (MI) is one of the main causes of heart failure. It is the result of an occlusion of a coronary artery, leading to cardiac ischaemia and cardiomyocyte death. MI leads to a number of changes in the heart: the workload of the remaining cardiomyocytes increases leading to hypertrophy, dilatation and collagen formation, a process referred to as adverse cardiac remodelling (Sutton & Sharpe 2000). The infarction leads to inflammation, and the dead cardiomyocytes are replaced by granulation tissue. This granulation tissue eventually matures into a scar (Frangogiannis 2006). When this scar is not properly formed, the ventricles can dilate, which eventually leads to heart failure (van den Borne *et al.* 2010). In the infarct area, myofibroblasts are present, which are part of granulation tissue. These cells migrate into the infarct, and are thought to counteract the dilatation

by maintaining the extracellular matrix (van den Borne *et al.* 2009) (Blankesteijn *et al.* 2001). A number of studies have shown a role for Wnt signalling in myofibrosblasts. Frizzled-1 and -2 and Dvl-1 are expressed in the area, where the myofibroblasts are migrating after MI (Blankesteijn *et al.* 1997, Chen *et al.* 2004). Another study showed the upregulation of Wnt10b, and Frizzled 1,2,5,10 and the downregulation Wnt7b (Barandon *et al.* 2003). Myofybroblast migration is attenuated by Wnt signalling in a myofibroblast cell line immortalized with telomerase. Migration and differentiation of myofibroblasts are affected by Wnt signalling (Laeremans *et al.* 2010). Overexpression of FrzA, a sFRP-1 homologue, reduced the infarct size by 50% after 15 days and the cardiac function was improved. Also, reduced cardiomyocyte apoptosis and increased amount of collagen was observed. The same results could also be obtained in rats by overexpression of β-catenin in the border zone (Hahn *et al.* 2006). sFRP2 was upregulated in rat hearts after MI. Injection of sFRP2 into the rat infarct reduced collagen disposition and ventricular fibrosis after 2 weeks, and after 4 weeks, wall-thinning was reduced and the cardiac function was improved (He *et al.* 2010). In conclusion, Wnt signalling plays a role during the separate phases of infarct healing and Wnt inhibition might have a positive effect on ventricular remodelling after MI.

Wnt signalling as therapeutic target

Wnt signalling is involved in a variety of processes that are known to modulate the hypertrophic response of the heart. Both β-catenin-dependent and non-β-catenin-dependent pathways are likely to be involved. Wnt signalling can inhibit the GSK3β activity, which inhibits the hypertrophic process. Also, calcineurin is activated by Wnt signalling, which in turn activates NFAT and leads to hypertrophic growth. Modulation of Dvl-1 regulates the hypertrophic growth, both *in vitro* and *in vivo*. The Wnt pathway might counteract the ET-1, AngII and PE-induced signalling pathways because GSK3β, Jnk and CamKII act upstream of NFAT and other hypertrophy-related transcription factors. Therefore, inhibition of Wnt signalling could be a promising target for anti-hypertrophic therapy, although our knowledge on the interactions of the different pathways is currently limited. Unfortunately, most research has focused so far on the downstream signalling pathways. Only little data are available on signalling at the level of Frizzled and the role of the different Wnt ligands in cardiac hypertrophy.

Most research has been performed on concentric hypertrophy after aortic constriction. Currently, data on the role of Wnt signalling in eccentric or physiological hypertrophy are scarce. Moreover, at present, there are no cardiac-specific ways to block the Wnt pathway. Therefore, research in this area should focus on the Wnts and Frizzleds involved in the hypertrophic response, and study the effect of interventions at the ligand-receptor level on the development of cardiac hypertrophy. This should be accompanied by a search for frizzled antagonists that block the interaction between Wnts and Frizzleds.

Conflict of interest

The authors declare no conflicts of interest.

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