Cardiac Hypertrophy and Target Organ Damage

Interruption of Wnt Signaling Attenuates the Onset of Pressure Overload-Induced Cardiac Hypertrophy

Veerle A.M. van de Schans, Susanne W.M. van den Borne, Agnieszka E. Strzelecka, Ben J.A. Janssen, Jos L.J. van der Velden, Ramon C.J. Langen, Antony Wynshaw-Boris, Jos F.M. Smits, W. Matthijs Blanksteijn

Abstract—The hypertrophic response of the heart has been recognized recently as the net result of activation of prohypertrophic and antihypertrophic pathways. Here we report the involvement of the Wnt/Frizzled pathway in the onset of cardiac hypertrophy development. Stimulation of the Wnt/Frizzled pathway activates the disheveled (Dvl) protein. Disheveled subsequently can inhibit glycogen synthase kinase-3β, a protein with potent antihypertrophic actions through diverse molecular mechanisms. In the Wnt/Frizzled pathway, inhibition of glycogen synthase kinase-3β leads to an increased amount of β-catenin, which can act as a transcription factor for several hypertrophy-associated target genes. In this study we subjected mice lacking the Dvl-1 gene and their wild-type littermates to thoracic aortic constriction for 7, 14, and 35 days. In mice lacking the Dvl-1 gene, 7 days of pressure overload-induced increases in left ventricular posterior wall thickness and expression of atrial natriuretic factor and brain natriuretic protein were attenuated compared with their wild-type littermates. β-Catenin protein amount was reduced in the group lacking the Dvl-1 gene, and an increased glycogen synthase kinase-3β activity was observed. Moreover, the increase in the amount of Ser473-phosphorylated Akt, a stimulator of cardiac hypertrophy, was lower in the group lacking the Dvl-1 gene. In conclusion, we have demonstrated that interruption of Wnt signaling in the mice lacking the Dvl-1 gene attenuates the onset of pressure overload-induced cardiac hypertrophy through mechanisms involving glycogen synthase kinase-3β and Akt. Therefore, the Wnt/Frizzled pathway may provide novel therapeutic targets for antihypertrophic therapy.

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Key Words: hypertrophy ■ Wnt ■ cell signaling ■ glycogen synthase kinase-3β ■ Akt

Cardiac hypertrophy is an adaptive response of the heart to an increased workload, caused by a variety of pathological stimuli, including hypertension, myocardial infarction, and valvular disease. Because cardiomyocytes are terminally differentiated, these cells can only respond by hypertrophic growth.1 This growth is initially beneficial but a sustained hypertrophic response often leads to heart failure.2 In this hypertrophic response, extracellular stimulation is translated into a cellular response, leading to changes in the contractile apparatus and to an activation of many signaling pathways.3 Several of these signaling pathways transduce prohypertrophic signals, but it has been shown that a number of endogenous molecules can regulate the hypertrophic response negatively.4

One of the most powerful negative regulators that can antagonize the hypertrophic response is glycogen synthase kinase-3β (GSK-3β), a ubiquitous serine/threonine protein kinase.5-7 GSK-3β is a downstream regulatory switch of multiple signaling pathways that regulates a wide range of cellular functions.6 Dysregulation of GSK-3β plays a role in many human diseases, including diabetes, Alzheimer’s disease, bipolar disorder, cancer,7 and heart failure.8 In cardiomyocytes, inhibition of GSK-3β augmented the hypertrophic response,9,10 whereas overexpression of GSK-3β had an inhibitory effect on the development of cardiac hypertrophy.11

The activity of GSK-3β can be modulated by several signaling pathways, including the insulin-like growth factor pathway,12 β-adrenergic13,14 and Wnt signaling.15 In this study, we focused on the role of the Wnt/Frizzled (Fz) pathway in the development of cardiac hypertrophy. In this pathway, a Wnt ligand binds to a membrane-bound complex consisting of a member of the Fz receptor family and a LDL receptor–related protein.16 This results in the activation of a member of the disheveled (Dvl) protein family, which, in turn, inhibits GSK-3β.17 The mechanism by which Wnt actually regulates GSK-3β is poorly understood.18 The inhibition of GSK-3β results in a decreased phosphorylation and subsequent accumulation of β-catenin, a second messenger of Wnt signaling, in the cytoplasm.

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To study the effect of interruption of Wnt signaling on the onset of cardiac hypertrophy development, mice lacking the Dvl-1 gene (Dvl-1<sup>-/-</sup>) were used. The development of pressure overload-induced cardiac hypertrophy was studied in these mice and their wild-type littermates (Dvl-1<sup>+/+</sup>) by subjecting the mice to thoracic aortic constriction (TAC). The effects of pressure overload were monitored by echocardiography and histological techniques. Moreover, the amounts of Akt, GSK-3β, and β-catenin were determined; GSK-3β activity was measured; and the expression of the hypertrophy-associated genes atrial natriuretic factor (ANF), brain natriuretic protein (BNP), and proto-oncogenes, was analyzed.

Methods
An expanded Methods section detailing the techniques and procedures mentioned here is available in an online supplement at http://hyper.ahajournals.org.

Animals

**Dvl-1** knockout (Dvl-1<sup>-/-</sup>) mice, generated as described previously, were inbred (>10 generations) into a 129/S6 background. Dvl-1<sup>-/-</sup> mice of both sexes and wild-type (Dvl-1<sup>+/+</sup>) littermates were used. All of the experimental procedures were approved by the Committee for Animal Research of Maastricht University and were in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.

**TAC Model**

Dvl-1<sup>-/-</sup> and littermate Dvl-1<sup>+/+</sup> mice were randomly distributed over the TAC group and sham-operated control group. Animals were anesthetized by intramuscular injection with ketamine (100 mg/kg body weight) and subcutaneous injection with xylazine (10 mg/kg body weight), and TAC was induced as described previously. 2D Guided M-Mode Echocardiography

Animals were anesthetized, and 2D guided M-mode echocardiography on the mouse was performed using a 20-MHz probe connected to an AU4 Idea device (Esaote Biomedica).

Histological Analysis

Hearts from Dvl-1<sup>-/-</sup> and Dvl-1<sup>+/+</sup> mice were arrested in diastole; one half was embedded in paraffin and cut in 4-μm sections and stained with hematoxylin/eosin. Myocyte cross-sectional areas (CSAs) were measured using a computerized morphometric system (version Leica Qwin 3.1, Leica). All of the sections were measured in 5 different areas of the left ventricle of the heart. Myocyte CSA was measured per nucleus; the outline of 100 to 200 myocytes was traced in each section. An examiner blinded to the genotype of the animals performed all of the measurements.

Real-time PCR

Total RNA was isolated from left ventricle (LV) tissue, purified by DNase digestion, and transcribed into cDNA using random hexamer primers. The number of transcripts of ANF, BNP, β-catenin, Dvl-1, Dvl-2, Dvl-3, Fzd2, c-fos, c-myc, c-jun, and cyclin D1 was determined on an ABI Prism 7700 Sequence Detection System (Applied Biosystems) using the SYBRGreen PCR Master mix kit (Eurogentec). Cardiac gene expression was normalized to the housekeeping gene β-actin. All of the samples were run in duplicate. Data analyses were performed using the Sequence Detection System software (Applied Biosystems).

Western Immunoblot Analyses

Total protein extracts (20 μg) were separated on a 10% SDS-PAGE gel and transferred to a Hybond C nitrocellulose membrane (Amersham Biosciences). The membranes were incubated overnight at 4°C with the following primary antibodies: β-catenin and GSK-3β (BD Transduction Laboratories); pSer<sup>9</sup> GSK-3β, pTyr<sup>216</sup>-GSK-3β, Akt, and pSer<sup>173</sup> Akt (Cell Signaling Technology); and α-tubulin (Santa Cruz Biotechnology). Anti-rabbit IgG and anti-mouse IgG horseradish peroxidase–conjugated antibodies (DAKO) were used as secondary antibodies as appropriate.

GSK-3β Kinase Activity Assay

Total GSK-3β was immunoprecipitated from 200-μg protein extracts of the heart in lysis buffer. The samples were precleared with protein G-Sepharose beads (Amersham Biosciences) and subjected to immunoprecipitation with an anti-mouse GSK-3β antibody (BD Transduction Laboratories) overnight at 4°C with gentle agitation, then incubated with protein G-Sepharose for 1 hour at 4°C with gentle agitation. Kinase activity was assayed in 40 μL of total reaction buffer containing GSK-3 peptide substrate (Biomol P-151) and 10 μCi [γ-<sup>32</sup>P] ATP (Amersham Biosciences). After 30 minutes of incubation at 30°C, 25-μL aliquots were spotted onto 1.5 cm × 1.5 cm pieces of Whatman P81 phosphocellulose paper (Whatman Nederland BV). Quantification of the kinase assay was achieved by counting the amount of <sup>32</sup>P incorporated into the substrate in the scintillation counter.

Statistical Analysis

All of the data are presented as mean±SE. Either unpaired Student’s t test or 2-way ANOVA was used as appropriate. *P<0.05 was considered to indicate statistical significance.

Postmortem and Echocardiographic Analysis of Aortic Banded Dvl-1<sup>-/-</sup> and Dvl-1<sup>+/+</sup> Mice

<table>
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<tr>
<th>Parameters</th>
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<th>14 Days</th>
<th>TAC</th>
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<td>HW/LW, mg/mm</td>
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<td>6.7±0.2</td>
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<td>7.1±0.4‡</td>
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<tr>
<td>Pwts, mm</td>
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<td>1.029±0.009</td>
<td>1.258±0.064*</td>
<td>1.106±0.049†</td>
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<td>1.014±0.056*</td>
<td>0.887±0.034†</td>
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</table>

BW indicates body weight; HW, heart weight; TL, tibia length; Lw, left ventricular weight; LuW, lung weight; Pwts, posterior wall thickness in systole; Pwtd, posterior wall thickness in diastole. Values are mean±SE.

*P<0.05 vs sham Dvl-1<sup>-/-</sup>; †P<0.05 vs sham Dvl-1<sup>-/-</sup>; ‡P<0.05 vs Dvl-1<sup>-/-</sup> 7 days after TAC; ††P<0.05 vs Dvl-1<sup>-/-</sup> 14 days after TAC.
Attenuated Development of Cardiac Hypertrophy in Dvl-1<sup>−/−</sup> Mice in Response to Pressure Overload

In the Table, the postmortem and echocardiographic data from Dvl-1<sup>−/−</sup> mice and Dvl-1<sup>+/+</sup> littermates, euthanized at 7, 14, and 35 days after TAC or after sham surgery, are shown. No significant differences were observed in the heart weight/tibia length ratio between sham-operated Dvl-1<sup>−/−</sup> and Dvl-1<sup>+/+</sup> mice. Two-way ANOVA showed a significant effect of both time after TAC (P<0.001) and genotype (P<0.05) on heart weight/tibia length ratio (Figure I). In Dvl-1<sup>−/−</sup> mice, the heart weight/tibia length ratio was significantly increased at all 3 time points after TAC (21%, 31%, and 72% increase compared with sham, respectively), whereas this ratio was only significantly increased at 35 days after TAC in the Dvl-1<sup>+/+</sup> mice. Similar differences in hypertrophic response were observed when the LV weights were compared. The lung weight/tibia length ratio showed a tendency toward increase at 35 days after TAC in both groups, but no statistical significance was reached.

Interstitial fibrosis, assessed by Sirius Red staining, was increased in both groups after TAC, with no significant differences between the genotypes (Figure II). To determine posterior wall thickness, 2D-directed M-mode echocardiography was performed (Table). Seven days after TAC, a statistically significant increase was observed in both end-systolic and end-diastolic posterior wall thickness in the Dvl-1<sup>−/−</sup> group (21% and 22%, respectively), which was sustained at 14 days after TAC. In contrast, end-systolic and end-diastolic posterior wall thickness did not increase significantly in Dvl-1<sup>−/−</sup> mice at 7 days after TAC (7% and 9% increase, respectively) and were mildly increased at 14 days after TAC (16% and 12%, respectively). Conclusively, these findings indicate that the lack of the Dvl-1 gene attenuated the onset of pressure overload-induced hypertrophy.

Attenuated Increase in Cardiomyocyte CSA, ANF, and BNP Expression in Dvl-1<sup>−/−</sup> LV on Pressure Overload

Histological analysis showed no significant difference in cardiomyocyte CSA between the 2 sham groups. At 7 days after TAC, the CSA was increased by 35% in Dvl-1<sup>+/+</sup> and 18% in Dvl-1<sup>−/−</sup> hearts (Figure 1A). The expression of ANF and BNP is generally used as a molecular marker for the development of cardiac hypertrophy. Real-time PCR analysis showed statistically significant increases in ANF mRNA levels in both groups at 7 days after TAC when compared with sham (Figure 1B). However, the increase in ANF expression was significantly attenuated in the Dvl-1<sup>−/−</sup> compared with the Dvl-1<sup>+/+</sup> LVs. The upregulated expression of BNP was only significant in the Dvl-1<sup>−/−</sup> mice at 7 days after TAC; although a trend toward an increased BNP expression was also observed in the Dvl-1<sup>−/−</sup> group, no statistical significance was reached (Figure 1C).

Increased Fz2 and Unaltered Dvl-1, Dvl-2, and Dvl-3 Expression in Dvl-1<sup>−/−</sup> LV After TAC

Previously, we have shown that the Fz2 expression was upregulated after induction of cardiac hypertrophy in rats. Therefore, we investigated whether the absence of the Dvl-1 gene affected the expression of different components of the Wnt/Fz pathway on TAC. Both in Dvl-1<sup>−/−</sup> and Dvl-1<sup>+/+</sup> LVs, a significant increase in Fz2 transcripts was observed (Figure 2A), which did not differ between the 2 groups. Next, we determined the number of transcripts of the 3 Dvl isoforms in the Dvl-1<sup>−/−</sup> and Dvl-1<sup>+/+</sup> LVs by real-time PCR and studied the effect of 7 days of TAC on their expression. As expected,
Dvl-1+/− LVs were completely devoid of Dvl-1 transcripts (Figure 2B), whereas the gene was readily expressed in LVs of the Dvl-1+/− group. No significant differences were observed in Dvl-2 and Dvl-3 expression between the Dvl-1+/− and Dvl-1+/+ groups (Figure 2C and 2D). In addition, TAC did not affect the expression of any of the 3 Dvl isoforms.

**Effect of TAC on Ser9-, Tyr216-Phosphorylated GSK-3β and the Activity of GSK-3β**

Because GSK-3β is considered to control β-catenin protein degradation, we analyzed the amounts of total GSK-3β, as well as its inactive Ser9-phosphorylated (pSer9-GSK-3β) and active Tyr216-phosphorylated (pTyr216-GSK-3β) form by Western blotting (Figure 3A). As shown in Figure 3B, the pSer9-GSK-3β/total GSK-3β ratio was not significantly different in sham-operated Dvl-1+/− and Dvl-1+/+ LVs. At day 7 after TAC, the pSer9-GSK-3β/total GSK-3β ratio tended to increase in both groups, although no statistical significance was reached. In contrast, TAC induced a significant increase in the pTyr216-GSK-3β/total GSK-3β ratio in Dvl-1+/− LVs but had no effect in the Dvl-1+/+ LVs (Figure 3C). As shown in Figure 3D, GSK-3β activity was significantly upregulated in Dvl-1+/− LVs after TAC, whereas in Dvl-1+/+ LVs, no significant effect of TAC on GSK-3β activity was observed.

Moreover, in the sham-operated Dvl-1+/− mice, the baseline activity of GSK-3β was significantly lower than in the sham-operated Dvl-1+/+ mice.

**Reduced Amounts of β-Catenin Protein in Dvl-1+/− LV After Pressure Overload**

Next, we examined the expression of β-catenin by real-time PCR (Figure 4A) and Western blotting (Figure 4B and 4C). The amount of β-catenin transcripts did not differ significantly between the LVs of sham-operated Dvl-1+/− and Dvl-1+/+ mice. Seven days after TAC, a significant decrease in the amount of β-catenin transcripts was observed in the Dvl-1+/− group but not in the Dvl-1+/+ group (Figure 4A). In Figure 4B, representative examples of β-catenin Western blots are shown. A quantitative analysis of the amounts of β-catenin protein, normalized to α-tubulin, is shown in Figure 4C. In sham-operated animals, we observed reduced levels of β-catenin protein in Dvl-1−/− LVs compared with the LVs of Dvl-1+/+ littermates. After 7 days of TAC, the amount of β-catenin protein increased significantly in the Dvl-1+/− group but not in the Dvl-1+/+ group. We extended our study to TAC-induced changes in the expression of β-catenin target genes. After TAC, the c-myc and c-fos
showed a tendency to increase; in contrast, c-jun and c-fos expression was downregulated after TAC (Figure III).

**Dvl-1 Inactivation Reduced the Amount of Ser473-Phosphorylated Akt**

Akt is one of the kinases that act as an upstream regulator of GSK-3β. However, Akt can stimulate the hypertrophic response through other mechanisms too. Phosphorylation of Akt at Ser473 results in its activation. The amount of active p-Ser473-Akt was studied by Western blotting with a phosphospecific antibody (Figure 5A). No significant difference was observed in the pSer473-Akt/total Akt ratio between the LVs of sham-operated and Dvl-1/−/− mice. After induction of hypertrophy, however, the ratio of pSer473-Akt/total Akt was significantly more increased in Dvl-1/−/− LVs compared with Dvl-1+/+ LVs (Figure 5B). This suggests that Dvl-1 plays a role in the activation of Akt by pressure overload.

**Discussion**

In the present study, we investigated the effect of the deletion of the Dvl-1 gene on the development of pressure overload-induced cardiac hypertrophy in mice. We observed an attenuated onset of cardiac hypertrophy in the Dvl-1−/− mice, which was sustained for ≥35 days.

Proteins from the Dvl family play a key role in the Wnt/Fz signaling pathway. In the present study, expression of all 3 isoforms of the Dvl protein was observed in the mouse LV. Pressure overload did not induce a significant change in the amount of transcripts of any of the 3 Dvl isoforms. Moreover, the inactivation of the Dvl-1 gene did not result in a compensatory upregulation of the expression of Dvl-2 or Dvl-3 in the Dvl-1−/− LVs. The upregulation of the Fz2 expression in the TAC mice confirms a previous observation from our laboratory, in which the upregulation of the Fz2 expression was observed after thoracic aortic banding in the rat.

GSK-3β is a downstream component of the Wnt/Fz pathway but has also been recognized as a negative regulator of cardiac hypertrophy. For the inhibition of GSK-3β activity, 2 mechanisms have been described. The first mechanism is the phosphorylation of GSK-3β at the Ser9 residue by protein kinases such as Akt, as in the signaling pathway of insulin.

In the present study, we have determined the effect of TAC on the pSer9-GSK-3β/total GSK-3β ratio and observed a similar trend toward increase in both groups, despite significantly higher levels of pSer473-Akt, the active form of Akt.
in the Dvl-1\(^{+/−}\) group. This suggests that the Akt-mediated Ser\(^{β}\) phosphorylation of GSK-3β is of minor importance for the hypertrophic response after 7 days of TAC. This observation is in agreement with a study by Haq et al., which Akt-induced Ser\(^{β}\)-phosphorylation of GSK-3β was shown to peak at 3 days after TAC but returned to baseline levels at 7 days after TAC in the rat.

The second mechanism of GSK-3β inhibition consists of a destabilization of the so-called β-catenin destruction complex, which consists of many proteins including axin, the adenomatous polyposis coli protein, and GSK-3β. On Wnt signaling, the activated Dvl protein can bind to this complex, which results in a decreased GSK-3β activity.\(^{17}\) The molecular details of this type of GSK-3β inhibition are poorly understood, but this mechanism is generally considered to form the link between Wnt/Fz activation and the decreased β-catenin degradation in the canonical Wnt pathway.\(^{18,25}\) In the present study, we observed an increased GSK-3β kinase activity in Dvl-1\(^{+/−}\) mice after TAC. This was paralleled by an elevated amount of pTyr\(^{216}\)-GSK-3β, the active form of GSK-3β, in this group. The elevated GSK-3β activity leads to an increased degradation of β-catenin, which explains the lower β-catenin protein levels in the Dvl-1\(^{+/−}\) TAC group compared with wild-type littermates. Our results underscore the concept that β-catenin levels are regulated at the protein level rather than through control of its transcription,\(^{26}\) because no correlation between β-catenin transcript numbers and protein levels was observed.

Stabilization of β-catenin has been shown to be necessary and sufficient for the hypertrophic response of cardiomyocytes, both in vitro and in vivo.\(^{24}\) The correlation between hypertrophic response and β-catenin protein levels could be confirmed in the present study. However, in this study, hypertrophic stimuli that inactivated GSK-3β through Ser\(^{β}\) phosphorylation mediated by Akt (protein kinase B) were used.\(^{23}\) From the present study, we conclude that the Wnt/fz pathway also plays a role in the control of GSK-3β kinase activity and in the stabilization of β-catenin in the hypertrophic response. Taken together, the 2 studies underscore the importance of GSK-3β activity in the regulation of cardiac hypertrophy, despite different underlying regulatory mechanisms.

As a next step, the transcription of the proto-oncogenes c-myc, c-fos, and c-jun, as well as cyclin D1, known to be controlled by β-catenin,\(^{27–30}\) was analyzed. No consistent pattern of regulation was observed for these genes. An explanation for this may be that the time point (7 days after TAC) is quite late to detect the expression of proto-oncogenes, which are typically upregulated within several hours after the onset of a hypertrophic stimulus.\(^{31,32}\) However, an alternative explanation may be that the increased GSK-3β activity in the Dvl-1\(^{+/−}\) LV exerts its antihypertrophic effect through other mediators than β-catenin. As shown in Figure 6, several mediators involved in the regulation of the hypertrophic response have been reported to be phosphorylated by GSK-3β, including GATA4,\(^{14}\) nuclear factor of activated T-cells,\(^{13}\) and eukaryotic translation initiation factor 2Be.\(^{34}\) Although the contribution of these mediators to the inhibition of the hypertrophic response observed in the Dvl-1\(^{+/−}\) mice is beyond the scope of this study, it further underscores the
importance of GSK-3β as a negative regulator of the hypertrophic response.

In the present study, the fraction of activated, phosphorylated Akt was increased in the LVs of the Dvl-1+/− mice and not the Dvl-1−/− mice, after 7 days of TAC. Apart from its role in the phosphorylation of GSK-3β at the Ser9 residue, Akt itself has been shown to be a modulator of the hypertrophic response as part of the insulin-like growth factor I signaling pathway.23

Figure 5. Western blot analysis of Akt and its active form, phospho-Ser473-Akt, in sham-operated animals and after 7 days of TAC. A, Representative Western blots of total Akt and pSer473-Akt protein expression. B, Densitometric analysis of pSer473-Akt and Akt blots. The pSer473-Akt /total Akt ratio is shown. S indicates sham; C, TAC. *P<0.05 vs sham Dvl-1+/−; †P<0.05 vs Dvl-1−/−; 7 days after TAC; all n=5 to 6.

Figure 6. Schematic representation of the effect of activation of the Wnt/Fz pathway on the expression of hypertrophy-associated genes. The effects of inactive (left) and activated (right) Wnt/Fz signaling are depicted. In the absence of Wnt stimulation, the level of free cytosolic β-catenin is kept low by GSK-3β phosphorylating β-catenin on serine and threonine residues at the amino-terminal region, thereby targeting it for ubiquitination and degradation in proteasomes. The phosphorylation of β-catenin by GSK-3β occurs in a complex with axin, adenomatous polyposis coli (APC) protein, and several other proteins (not shown), where axin is a scaffold protein that increases the efficiency and the specificity of the β-catenin phosphorylation. Apart from β-catenin, other hypertrophy-associated targets, like nuclear factor of activated T-cells (NFAT), GATA4, and eukaryotic translation initiation factor 2B (eIF2B), can be phosphorylated and inactivated by GSK-3β. In the right part of the scheme, the effect of activation of a Fz 7 transmembrane receptor by a Wnt ligand is shown. In a complex with LDL receptor–related protein 5 or 6, this leads to the activation of Dvl. The activated Dvl protein inhibits the GSK-3β activity by 2 distinct mechanisms: first it changes the conformation and promotes the dissociation of the GSK-3β/APC/axin complex and, second, it may increase Akt activity, resulting in phosphorylation of the Ser473 residue of GSK-3β. The inhibition of GSK-3β leads to stabilization and accumulation of β-catenin in the cytoplasm. β-Catenin can shuttle to the nucleus were it interacts with a transcription factor complex of the T-cell factor/lymphoid enhancer factor family. This can induce the transcription of genes involved in the hypertrophic response. Moreover, activated Akt by itself can also induce hypertrophic gene transcription, and inactive GSK-3β no longer inhibits the hypertrophy-associated proteins NFAT, GATA4, and eIF2B, providing activation of diverse pro-hypertrophic pathways.
Interestingly, overexpression of Dvl-1 has been shown to increase the amount of activated Akt in vitro.\(^{35}\) This suggests a direct link between the Wnt/Fz pathway and Akt activation, which may explain the attenuated activation of Akt in the hearts of the Dvl-1\(^{-/-}\) mice (see Figure 6).

**Perspectives**

This study shows that interruption of Wnt signaling through disruption of the Dvl-1 gene can attenuate the onset of pressure overload-induced cardiac hypertrophy by a mechanism involving both GSK-3 activity of endogenous negative regulators of hypertrophy pathways, as is today’s clinical practice, augmenting the hypertrophic process: rather than inhibiting prohypertrophic open a new therapeutic perspective for intervention in the hearts of the which may explain the attenuated activation of Akt in the.

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**Disclosures**

None.

**References**


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