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Dapper-1 is essential for Wnt5a induced cardiomyocyte hypertrophy by regulating the Wnt/PCP pathway

Marco Hagenmueller [a], Johannes H. Riffel [a], Elmar Bernhold [a], Jingjing Fan [a], Hugo A. Katus [a,b], Stefan E. Hardt [a,b,*]

[a] Department of Cardiology, University Hospital of Heidelberg, INF 410, 69120 Heidelberg, Germany
[b] DZHK (German Center for Cardiovascular Research), Partner Site Heidelberg/Mannheim, Heidelberg, Germany

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ABSTRACT

The Wnt signaling pathway was identified as crucial mediator of cardiomyocyte hypertrophy. In this study we found that activation of non-canonical Wnt signaling by Wnt5a stimulates protein synthesis and enlargement of cardiomyocyte surface area. These hypertrophic features were inhibited in Dapper-1 (Dpr1) depleted cells. On the molecular level, we observed inhibition of the non-canonical Wnt/planar-cell-polarity (PCP) pathway denoted by reduction of c-jun-n-terminal-kinase (JNK) phosphorylation. Upstream of JNK, increased protein levels of the Wnt/PCP trans-membrane receptor van-Gogh-like-2 (Vangl2) were observed along with an enrichment of Vangl2 in perinuclear located vesicles. The findings suggest that Dpr1 is essential for execution of the Wnt/PCP pathway and regulation of the Vangl2/JNK axis. Depletion of Dpr1 inhibits non-canonical Wnt signaling induced cardiomyocyte hypertrophy by blocking Wnt/PCP signaling.

Structured summary of protein interactions:
Dpr1 and **Vangl2** colocalize by fluorescence microscopy (View interaction)

1. Introduction

Cardiac hypertrophy is an adaptive response to balance the increased workload of the heart in state of pathological stress. It is mainly characterized by increased protein synthesis and an enlargement of single cardiomyocytes [1]. The contribution of Wnt signaling in the development of cardiac diseases is increasingly recognized in basic cardiovascular research [2–5]. Generally, the Wnt signaling pathway is differentiated between the canonical and the non-canonical branch [6,7]. Both pathways require Wnt ligand binding to membrane receptors and Dishevelled (Dvl) activity in the cytoplasm [8,9]. Canonical and non-canonical pathways recruit different downstream proteins to transduce Wnt signals. The non-canonical pathway is separated into three branches: the planar-cell-polarity (PCP) pathway, the Ca^{2+}/calmodulin-dependent-protein-kinase-2 pathway (CamKII), and the protein-kinase-C (PKC) pathway [10]. Among the Frizzled (Fzd) receptors, receptor-tyrosine-kinase-like-orphan-receptor (ROR2) in conjunction with van-Gogh-like-2 (Vangl2) mediates the transmission of extracellular signals by binding Wnt ligands [11]. Following ligand/receptor binding, Dvl proteins are crucial coordinators of signal transduction in all non-canonical branches. Dpr1 interacts with Dvl in cell and context dependent manner [12,13]. Previous studies allocated a functional role of both proteins in the Wnt/PCP pathway which is activated through the ROR2/Vangl2/c-jun-terminal-kinase-1(JNK) axis [11]. In a recently published work we have demonstrated that over-expression of the Wnt component Dapper-1 (Dpr1) in a transgenic mouse model activates canonical Wnt signaling in a Dishevelled-2 (Dvl2) dependent manner and induces cardiac hypertrophy [12]. Although in cardiomyocytes Dpr1 is an activator of canonical Wnt signaling its role in non-canonical Wnt signaling is not investigated to date. Here, we address the role of Dpr1 in non-canonical Wnt signaling transduction.

We provide evidence that Dpr1 is essential to activate the non-canonical Wnt/PCP pathway in cardiomyocytes. Moreover, we demonstrate that Wnt5a induced activation of the Wnt/PCP pathway induces cardiomyocyte hypertrophy and that Dpr1 depletion inhibits cells from hypertrophic growth by interruption of the Vangl2/JNK axis.

Abbreviations: PCP, planar cell polarity; Vangl2, Van Gogh like 2

* Corresponding author at: Department of Cardiology, Angiology and Pulmology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. Fax: +49 6221/56 8068.
E-mail address: stefan.hardt@med.uni-heidelberg.de (S.E. Hardt).

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2. Methods

2.1. Cardiomyocytes culture and determination of myocyte surface area

Isolation and culture of cardiac myocytes was performed as described previously [14]. For determination of surface area cardiomyocytes were cultured on coverglasses and transfected with siRNA as described [14] or incubated with JNK Inhibitor SP600125 [10 μM] or DMSO as a control. 48 h later (for siRNA treated cells) or 1 h later (for SP600125 treated cells) cells were stimulated with Wnt5a conditioned medium (1:50 dilution) for further 2 days. Myocytes were washed twice with ice cold phosphate-buffered saline and fixed in PBS containing 4% paraformaldehyde for 30 min. For permeabilization cell membrane cells were incubated for 10 min in phosphate-buffered saline containing 0.1% Triton X. Fixed cells were blocked with 2% BSA in phosphate-buffered saline for 1 h. Immunostaining was performed using Texas Red phalloidin (Molecular Probes) and DAPI staining for 30 min. After staining coverglasses were fixed on microscope slides and microscope images were captured by a digital camera. Myocyte surface area from at least 100 cells per group in each experiment, were analyzed by using the Image J program.

2.2. RNA analysis

Quantitative real-time PCR was performed as described previously [14]. Real time PCR was performed using the Universal Probe Library from Roche Diagnostics. Primers and Probes used are HPRT 5′-GTCAAGGGGGACATAAAAG-3′ and 5′-TGCATTGTTTTACCAGTGT CAA-3′, probe #22; Vangl2 5′-GCGAGCCGCTTCTATAATGT-3′ and 5′-TCTCCAGGATCCACACTGC-3′, probe #42.

2.3. siRNA transfection

Cardiomyocytes were cultured in serum and antibiotic free medium for at least 5 h. For transfection of 2×10^6 cardiomyocytes in a 6 cm dish 250 pmol siRNA was diluted in 650 μL serum- and antibiotic free OptiMEM (Invitrogen). In another tube 4.5 μL of Lipofectamin 2000 (Invitrogen) was diluted in 200 μL OptiMEM and incubated for 15 min. After complex formation the solution and OptiMEM were added to the cells. The cells were incubated for 72 h at 37 °C. siRNAs used were Dpr1 5′-GCAGAUAAGUGACCU GAGA-3′ and non-specific control siRNA 5′-AGGUAGUGUAAUC GCCUUGTT-3′. In all siRNA mediated knockdown experiments myocytes transfected with non-specific (scrambled) siRNA were used as controls. siRNAs were synthesized by MWG (Ebersberg, Germany). Knockdown of Dpr1 was confirmed by Western blotting.

2.4. Adenoviral infection of isolated cardiomyocytes

Cardiomyocytes were cultured with serum free medium. The next day cardiomyocytes were infected over night with adenovirus encoded Dpr1 (Applied Biological Materials Inc., Richmond, Canada) or an empty adenovirus vector as a control. After washing the cells 3 times with PBS the culture medium was changed. 48 h later protein lysates were prepared. Overexpression of Dpr1 was confirmed by Western blotting.

2.5. Protein analysis

Immunoblot analyses were performed as described previously [14]. Primary antibodies used were anti-Dapper1 (Santa Cruz, for rat), anti JNK1 (Santa Cruz), anti phospho-SAPK/JNK (Thr183/185) (Cell signaling), anti-CaMKII (BD Transduction Laboratories), anti-ActiveCaMKII (Thr286) (Promega), PKCα (Cell Signaling), phospho-PKCα/βII (Thr 638/641) (Cell Signaling), anti-Vangl2 (Santa Cruz). Anti-α-actin (Sigma–Aldrich) and anti-GAPDH (US-biological). Horseradish peroxidase-conjugated Anti-rabbit IgG, anti-mouse IgG and anti-goat IgG antibodies (Santa Cruz) were used as secondary antibodies. Bands were quantified by densitometry using the Image J program.

2.6. [^{3}H]-Glycine incorporation assay

Cardiomyocytes were cultured in 12 well plates. The next day cells were transfected with Dpr1 specific siRNA for 48 h or incubated with JNK inhibitor SP600125 [10 μM] for 1 h. Then, cells were stimulated with Wnt5a conditioned medium (1:50 dilution) and incubated with 1 μCi/ml [^{3}H]-Glycine overnight. Cells were washed twice with ice cold PBS. Proteins were precipitated for 1 h with 10% TCA in a freezer. Precipitate was solved in 1 ml 1 M NaOH. [^{3}H]-Glycine incorporation was determined by scintillation counting.

2.7. JNK activity assay

JNK activity assay was performed with the JNK activity assay kit (Abcam). Cardiomyocytes were cultured on 6 cm dishes. 48 h after Dpr1 siRNA transfection cells were stimulated with Wnt5a conditioned medium for 5 h, washed three times with ice cold PBS and lysed with 200 μL extraction buffer. Cleared lysates were obtained for immunoprecipitaion with JNK specific antibodies combined with protein A coated agarose beads. After washing precipitated JNK was incubated together with its substrate c-jun and ATP's for 4 h in 30 °C. Supernatant of the mix was analyzed by Western blot.

2.8. Immunofluorescence

For immunofluorescence analysis, cardiomyocytes were fixed with 3.7% formaldehyde, permeabilized with 0.1% Triton-X followed by incubation steps with the primary and secondary antibody (anti-Vangl2 antibody and anti-mouse FITC labeled antibody, Santa Cruz). Co-immunostaining was performed with antibodies against Dpr1 (Abcam; secondary antibody: goat anti-rabbit FITC-labeled antibody, Abcam) and Vangl2 (Santa Cruz; secondary antibody: sheep anti-mouse Cy3 labeled antibody, Sigma). For counterstaining of DNA we used Hoechst 33342 probes (Invitrogen). The fluorescence signals were detected and visualized by using a Nikon A1Rsi 32 channel spectral imaging confocal laser scanning system on a Nikon Ti inverted microscope and an Olympus BX51 fluorescence microscope.

2.9. Statistical analysis

All data are reported as the mean ± S.D. Difference between the groups was compared by a Wilcoxon-test or a 2-tailed Student *t* test, when applicable. Significance was accepted at the $P < 0.05$ level.

3. Results

3.1. Dpr1 depletion inhibits Wnt5a induced cardiomyocyte hypertrophy

In primary cultures of isolated cardiomyocytes we evaluated whether onset of non-canonical Wnt signaling induces features of hypertrophy by stimulation with Wnt5a conditioned medium. Compared to control cells we determined an increase of myocyte surface area and protein synthesis in Wnt5a treated cells indicating

that Wnt5a is sufficient to trigger cardiomyocyte growth (Fig. 1A and B). In order to examine to which extent Dpr1 contributes to cardiomyocyte hypertrophy mediated by non-canonical Wnt signaling we knocked down Dpr1. In Dpr1 depleted cells the hypertrophic effect of Wnt5a stimulation was inhibited indicating that Dpr1 is required for Wnt5a induced hypertrophic growth which links Dpr1 to the non-canonical Wnt signaling pathway in cardiomyocytes (Fig. 1A and B).

3.2. Dpr1 is essential to activate the Wnt/PCP pathway in cardiomyocytes

To gain more insight to the role of Dpr1 in non-canonical Wnt transduction we assayed protein and phosphorylation levels of JNK, PKC and CamK2 by Western blot upon overexpression or knockdown of Dpr1 in isolated cardiomyocytes. Adenoviral overexpression of Dpr1 had no consequence on JNK, PKC and CamK2 expression or phosphorylation (Fig. 2A and B). However, we observed a strong reduction of JNK phosphorylation in Dpr1 depleted cells (Fig. 2C and D). Of note, PKC and CamK2 expression and phosphorylation was not effected (Fig. 2A–D). By means of a JNK activity assay we examined whether transmission of Wnt/PCP signals was blocked in Dpr1 depleted cells. Stimulation with Wnt5a conditioned medium induced strong JNK activation in control cells but failed to activate this kinase in cells lacking Dpr1 all indicating that Dpr1 is essential to maintain and to induce Wnt/PCP signaling (Fig. 2E and F).

3.3. Wnt5a induced cardiomyocyte hypertrophy requires JNK activity

The fact that Wnt/PCP pathway is blocked, in conjunction with inhibition of Wnt5a induced hypertrophy in Dpr1 depleted cells points to an essential role of JNK in non-canonical Wnt signaling mediated cardiomyocyte growth. To prove this hypothesis we incubated isolated cardiomyocytes with either DMSO or with JNK inhibitor SP600125 [10 μM], stimulated the cells with Wnt5a conditioned medium and assayed cell surface area and protein synthesis. Both features of cardiomyocyte hypertrophy were blocked in SP600125 treated cells indicating that JNK activity is required for hypertrophic growth mediated by Wnt5a (Fig. 3A and B).

3.4. Dpr1 depletion leads to accumulation of Vangl2 in perinuclear located vesicles

To map the molecular mechanism of Wnt/PCP pathway inhibition in Dpr1 depleted cells we focused on the role of the transmembrane receptors ROR2 and Vangl2. Binding of Wnt ligand Wnt5a to ROR induces the phosphorylation of Vangl2 and mediates transduction of Wnt/PCP signals through JNK [11]. Adenoviral over-expression of Dpr1 had no effect on ROR2 and Vangl2 levels (Fig. 4A and B). Unexpectedly, we observed increased protein levels of Vangl2 receptor in Dpr1 deficient cells while expression of ROR2 remained unaltered (Fig. 4C and D). Unchanged Vangl2 mRNA transcripts suggest a mechanism of stabilization which occurs posttranscriptionally (Fig. 4E). To address this question we immunostained Vangl2 in Dpr1 knockdown cardiomyocytes. While Vangl2 localization in control cells was mainly in the membrane and in the cytoplasm the situation in Dpr1 depleted cells was different. We observed a conspicuous accumulation of Vangl2 in vesicles located in perinuclear regions indicating that stabilization of Vangl2 protein levels is a result of vesicular enrichment induced by Dpr1 depletion (Fig. 4F). Moreover, co-immunostaining of Dpr1 and Vangl2 in untreated, isolated cardiomyocytes revealed a co-localization in perinuclear areas but not in the cytoplasm and in regions of the cell membrane (Fig. 4G).

3.5. Members of the Wnt/PCP pathway are upregulated in a rat model of myocardial infarction

In a recently published study we have shown a robust overexpression of Dpr1 in the remote area (vital area) of left ventricular tissue obtained from a rat myocardial infarction (MI) model, 1 week after induction of MI [12]. By immunoblotting the same protein lysates we observed increased protein levels of the ligand Wnt5a and the Wnt/PCP membrane receptors ROR2 and Vangl2 (Fig. 5A and B). Moreover, mRNA copy numbers of these receptors were upregulated compared to sham operated mice suggesting that the ROR2/Vangl2 axis plays an important role in the remodeling process after MI and underlining the relevance of the findings above in a cardio-pathological context. Interestingly, mRNA expression of the Wnt ligand Wnt5a remained stable indicating that Wnt5a secretion occurs in another area of the infarcted heart (Fig. 5C).

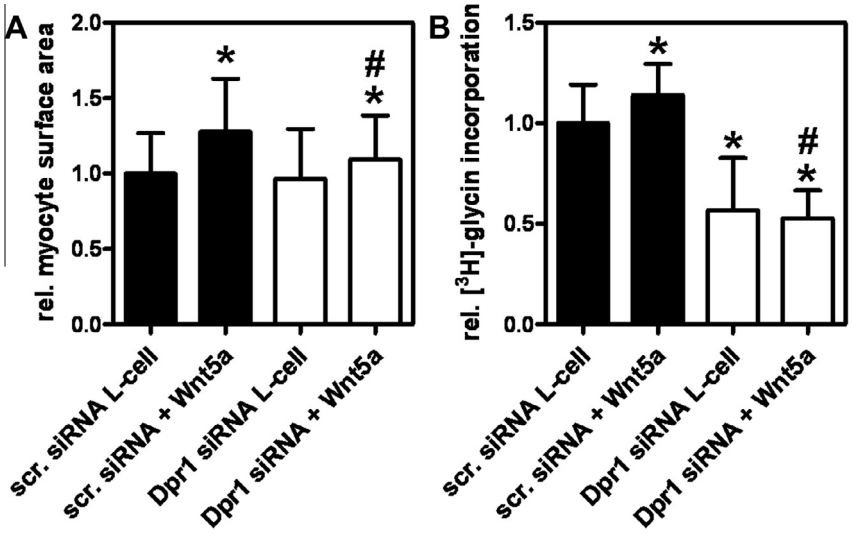

Fig. 1. Dpr1 depletion inhibits Wnt5a induced cardiomyocyte hypertrophy. (A) Bar graph shows an increase in myocyte surface area following Wnt5a treatment and inhibition of cardiomyocyte growth in Dpr1 knockdown cells. (B) Bar graph indicates Wnt5a induced increase of protein synthesis in cardiomyocytes. Protein synthesis is blocked in Dpr1 depleted cells. ($^{*}P < 0.05$ vs. unstimulated control; $^{\#}P < 0.05$ vs. Wnt5a stimulated control; all experiment were performed at least three times in triplicates; $n \geqslant 300$ cells per group).

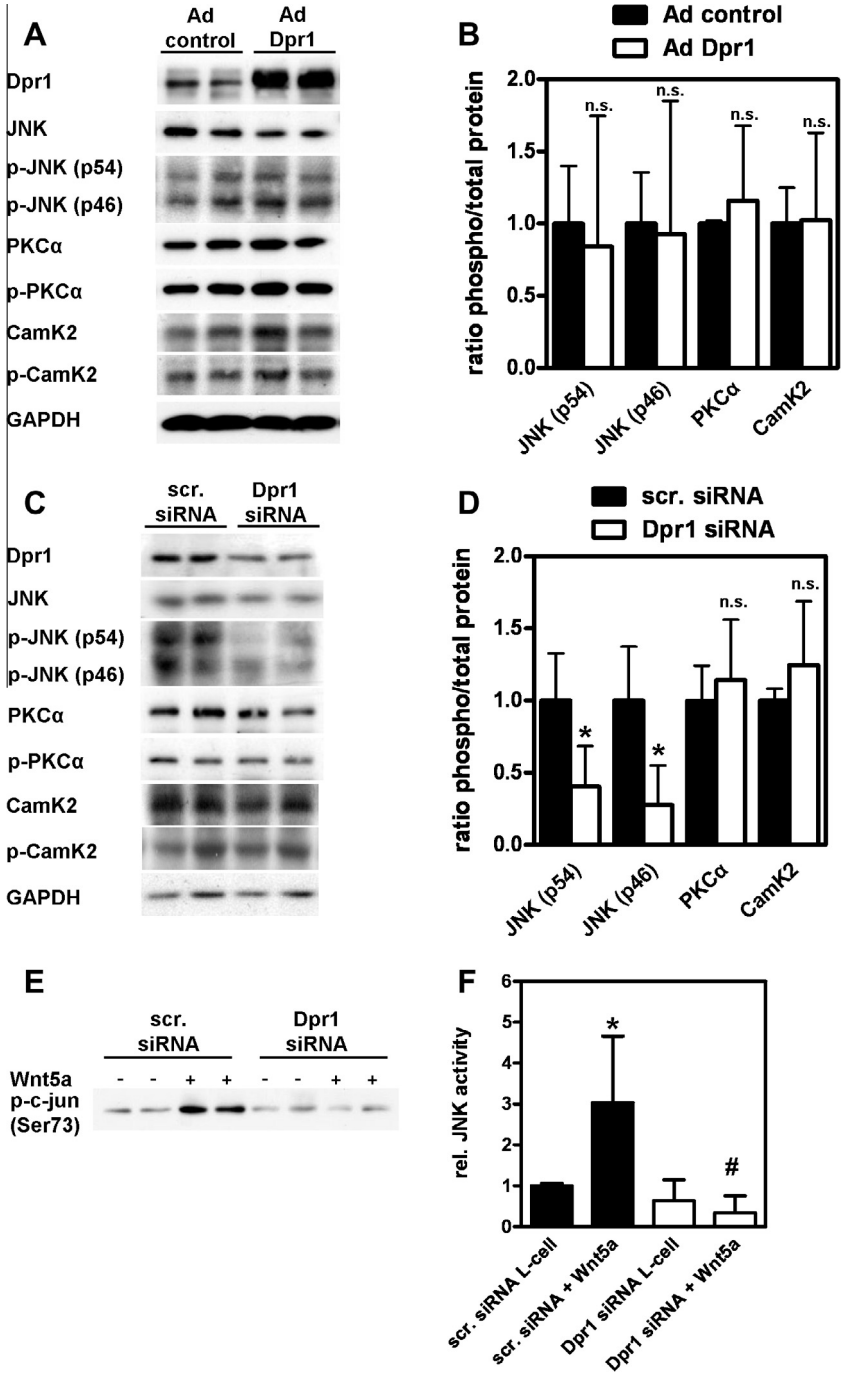

Fig. 2. Dpr1 is essential to activate Wnt/PCP pathway in cardiomyocytes. (A and B) Representative Western blots of cardiomyocyte protein lysates reveals no difference in total- and phospho-JNK, -PKCα and -CamK2 abundance between control cells and Dpr1 overexpressed cells. (C and D) Representative Western blots of cardiomyocyte protein lysates show no difference in levels of total- and phophorylated PKCα- and CamK2 between control and Dpr1 depleted cells. Phosphorylation of JNK decreases dramatically following Dpr1 knockout. (E and F) Western blot result of a JNK activity assay. Wnt5a induced activation of JNK increases levels of phosphorylated c-jun^{Ser73}. Activation of JNK is completely blocked in Dpr1 depleted cardiomyocytes. ($^{*}P < 0.05$ vs. unstimulated control; $^{\#}P < 0.05$ vs. Wnt5a stimulated control; experiments were performed three times in duplicates).

4. Discussion

Here, we provide evidence that Wnt5a induced cardiomyocyte hypertrophy is inhibited in Dpr1 depleted cells, coupling Dpr1 to non-canonical Wnt signaling. Wnt5a is the most investigated non-canonical Wnt ligand and is implicated in a variety of different Wnt aspects [15]. Since Wnt5a activates not only Wnt/PCP signaling it is not unambiguous which of the non-canonical Wnt branch is responsible for cardiomyocyte growth. However, given the result of inhibited Wnt/PCP pathway in Dpr1 knockdown cells and the inhibition of Wnt5a induced cardiomyocyte hypertrophy in cells treated with the JNK inhibitor SP600125 we propose that reduced cell surface area and protein synthesis is a result of the blocked Vangl2/JNK axis. The implication of JNK in cardiac hypertrophy was first demonstrated by Wang et al. [16]. Unlike our experiments they induced JNK activation by over-expression of dual-specific-mitogen-activated-protein-kinase-kinase-7 (MKK-7) which is located upstream of JNK. In agreement with our findings they

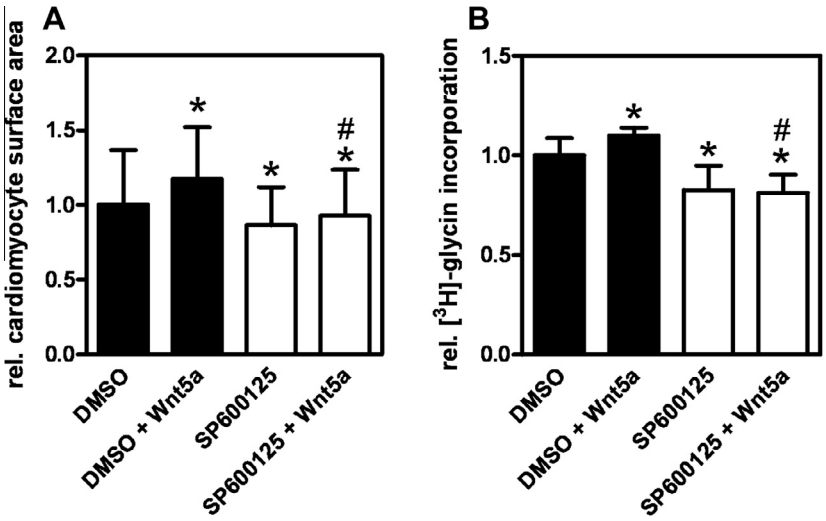

Fig. 3. JNK inhibition blocks Wnt5a induced cardiomyocyte hypertrophy. (A) Bar graph shows an increase in myocyte surface area following Wnt5a treatment and inhibition of cardiomyocyte growth in SP600125 treated cells. (B) Bar graph indicates Wnt5a induced increase of protein synthesis in cardiomyocytes. Protein synthesis is blocked in SP600125 treated cells. ($^{*}P < 0.05$ vs. unstimulated control; $^{\#}P < 0.05$ vs. Wnt5a stimulated control; all experiment were performed at least three times in triplicates; $n \geqslant 300$ cells per group).

observed several features of cardiomyocyte hypertrophy such as increased cell surface area, protein synthesis and ANF expression. However, the effect of JNK inhibition on suppression of hypertrophy was not investigated in their study. Subsequent publications revealed a more controversial and complex role of JNK in the context of cardiac diseases. Chronic treatment of cardiomyopathic Bio14.6 hamsters with JNK inhibitor SP600125 aggravates LV chamber dilation and LV dysfunction [17]. In contrast, but consistently with our study, the group of Wu et al. observed improvement of cardiac function and decreased fibrosis in cardiomyopathy caused by mutation in the lamin A/C gene by using the same JNK inhibitor [18].

Moreover, by performing knockdown experiments in isolated cardiomyocytes we identified Dpr1 as an essential regulator to maintain and to trigger non-canonical Wnt/PCP signaling. In Dpr1 depleted cells Wnt5a induced activation of JNK was completely blocked. The contribution of Dpr1 in Wnt/PCP signaling was described in earlier studies. Wen et al. established a global homozygote Dpr1 knockout mouse model. These animal exhibit neural tube defects accompanied by severe posterior malformations all indicating deregulation of the Wnt/PCP pathway which is considered to organize neural tube development [19]. JNK and the Ras homologue guanosine triphosphatase (Rho GTPase) are two among several Wnt/PCP effectors [20,21]. Contradictory to our observations, in $Dpr1^{-/-}$ mouse embryonic fibroblasts (MEF) Wnt/PCP deregulation was associated with higher JNK activity but lower activity of Rho GTPase. An explanation could be cell and context dependent functions of Dpr1. It is possible that activation of JNK substitutes Rho GTPase activity in Dpr1 depleted MEF's. Suriben et al. discovered an association between Dpr1, Dvl2 and the four-span-trans-membrane receptor Vangl2 in regulating the Wnt/PCP pathway. Similar to the study of Wen et al. they established a mouse model with homozygous mutation for Dpr1. The group observed the same phenotypes accompanied by deregulation of the Wnt/PCP pathway. They provide evidence that Dpr1 is a crucial regulator of Vangl2 in the development of the primitive streak. On molecular levels they discovered genetic interaction of Dpr1 and Vangl2 [22]. In agreement with our study they identified post-translational stabilization of Vangl2 in the primitive streak of their $Dpr1^{-/-}$ mouse model. Furthermore, they showed that Dpr1 forms complexes with Vangl2 and Dvl2 in HEK293 cells. This interaction occurs with different binding domains of Dpr1 and binding of Dpr1 to Vangl2 is independent of Dvl2. Remarkably and congruent to the study of Wen et al. deregulation of Wnt/PCP signaling in $Dpr1^{-/-}$ was associated with higher JNK activity. In our study, Dpr1 depletion led to decreased phosphorylation of JNK under basal conditions and complete inhibition of Wnt5a induced JNK activation. Gao et al. demonstrated that binding of Wnt5a to ROR2 induces a complex formation of Ror2 and Vangl2 followed by phosphorylation of Vangl2 on multiple Serine/Threonin residues. Increased phosphorylation leads to higher activity which is subsequently followed by activation of JNK [11].

In Dpr1 deficient cardiomyocytes we observed stabilized Vangl2 proteins enriched in perinuclear vesicles. We hypothesize that depletion of Dpr1 blocks Vangl2 carriage to the membrane. As a result, Vangl2 cannot be recruited for ROR2 mediated phosphorylation and therefore Wnt5a induced activation of the Wnt/PCP pathway is blocked (Fig. 6). However, the present study does not elucidate in detail how Dpr1 depletion impairs Vangl2 membrane trafficking mechanistically. Since co-localization of Dpr1 and Vangl2 was observed only in perinuclear regions but not in areas near the cell membrane, it is likely that Vangl2 requires another molecular player for membrane trafficking and it can be excluded that this process occurs through direct interaction with Dpr1 alone.

A further limitation of the present study is the lack of siRNA or commercial available inhibitors which successfully target Vangl2 to investigate the precise role of Vangl2 in Wnt5a signaling transduction and concomitant cardiomyocyte hypertrophy. However, upregulation of Wnt5a and the membrane receptors ROR2 and Vangl2 in the remote area of left ventricular tissue obtained from a rat MI model suggests an involvement of the Wnt/PCP pathway in a cardio-pathological context. This finding supports our in vitro hypothesis since both receptors are activated by binding of the Wnt ligand Wnt5a to ROR2 [11]. In addition, we have previously described an upregulation of Dpr-1 protein and mRNA levels in the same protein lysates [12]. It is still an open question where the Wnt ligands come from. Although we have observed more Wnt5a proteins in the remote area of left ventricular MI tissue, levels of Wnt5a mRNA copy numbers remained stable, indicating that expression and secretion of this ligand occurs in another area of the infarcted heart.

In conclusion, our data suggest a novel role for Dpr1 and the Wnt/PCP pathway in cardiomyocyte hypertrophy mediated by

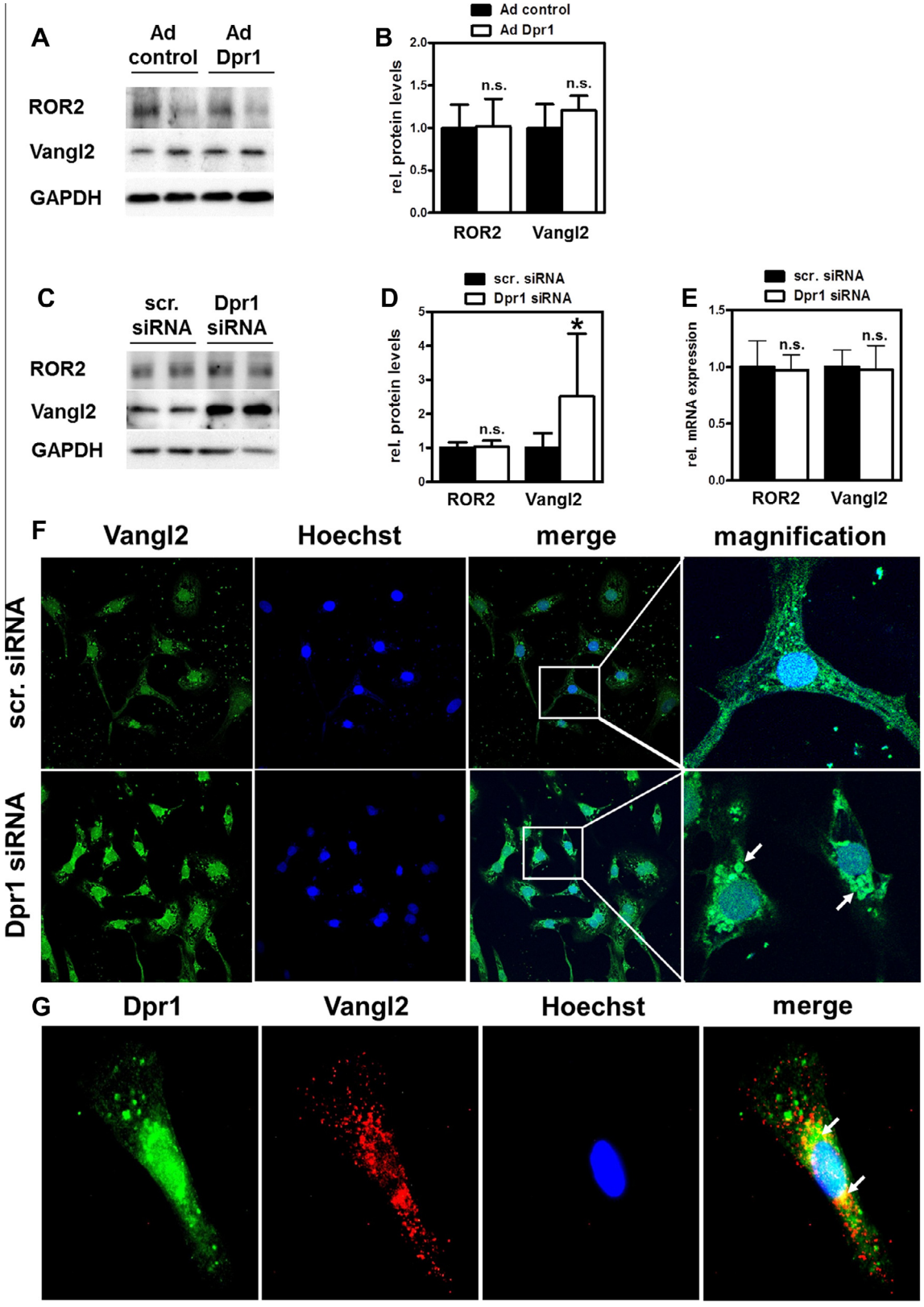

Fig. 4. Dpr1 depletion leads to accumulation of Vangl2 in perinuclear located vesicles. (A and B) Representative Western blot of cardiomyocyte lysates shows no difference in protein amount of ROR2 and Vangl2 between control and Dpr1 overexpressed cells. (C and D) Representative Western blots of cardiomyocyte lysates with ROR2 and Vangl2 specific antibodies. A robust increase of Vangl2 protein amount in Dpr1 depleted cells was observed while expression of ROR2 was unaffected. (E) ROR2 and Vangl2 mRNA copy numbers remained stable in Dpr1 deficient cells. (F) Confocal microscope image of cardiomyocytes transfected with unspecific or Dpr1 siRNA. Cells were immunostained with Vangl2 specific antibodies (green) and counterstained with Hoechst 33342. Dpr1 depleted cells display accumulation of Vangl2 in perinuclear regions (arrows). (G) Co-immunostaining of cardiomyocyte with specific antibodies against Dpr1 (green) and Vangl2 (red) reveals co-localization in perinuclear regions (arrows) but not in membrane areas. Cells were counterstained with Hoechst 33342. (*$P < 0.05$ vs. control; n.s. not significant, experiments were done three times in duplicates).

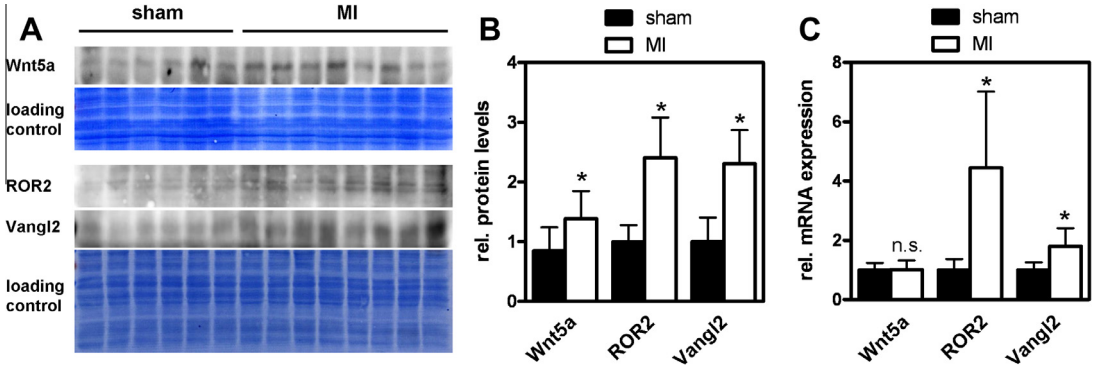

Fig. 5. Members of the Wnt/PCP pathway are upregulated in a rat model of myocardial infarction. (A and B) Immunoblots and quantification of tissue lysates prepared from the remote area of the left ventricle of a rat MI model shows upregulation of the Wnt/PCP-pathway members Wnt5a, ROR2 and Vangl2. Coomassie staining of the membranes used was performed as a loading control. (C) mRNA levels of ROR2 and Vangl2 but not of Wnt5a were increased in the remote area of the left ventricular tissue of a rat MI model. (*$P < 0.05$ vs. control; n.s. not significant, $n = 6$ in sham operated rats, $n = 8$ in MI rats).

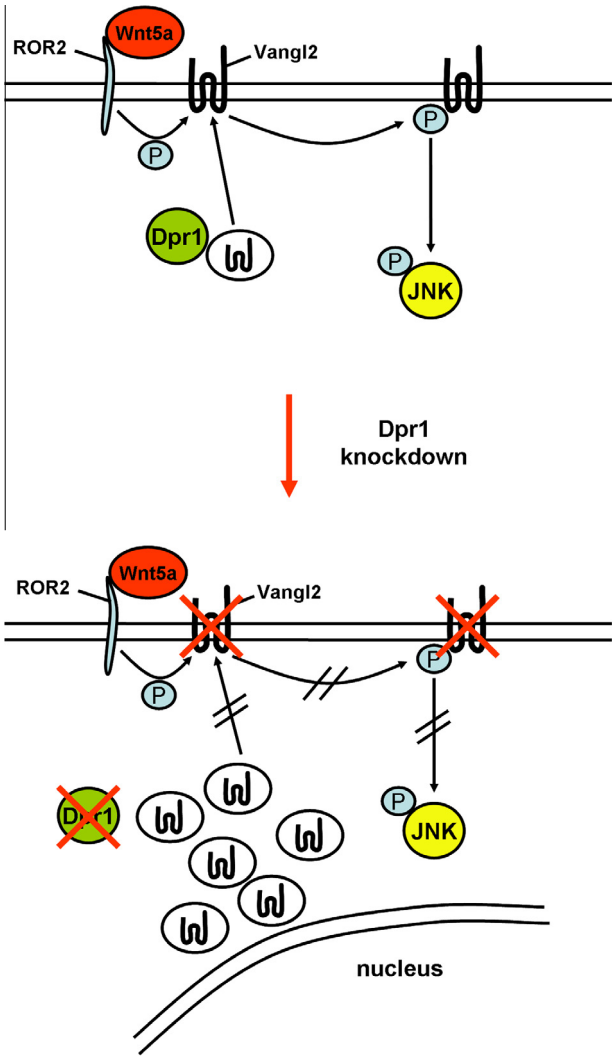

Fig. 6. Suggested role of Dpr1 in Wnt/PCP-pathway in cardiomyocytes. Binding of Wnt5a to ROR induces the phosphorylation of Vangl2. In turn Vangl2 mediates the activation of JNK-pathway. Here, Dpr1 is involved in Vangl2 membrane transport. Following Dpr1 depletion Vangl2 membrane transport is blocked. As a result Vangl2 is physically not present for Wnt5a/ROR2 induced phosphorylation and JNK cannot be activated.

Wnt5a and provide a promising starting point for a potential novel treatment option.

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Conflict of interest

None.

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