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The Crucial Role of Calcium/calmodulin-dependent Protein Kinase II in Wnt Signaling-induced Pathological Cardiac Remodeling

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Wnt signaling is not only involved in cardiac development but also involved in adult heart disease. Activation of Wnt signaling results in maladaptive cardiac remodeling and cardiomyopathy. Recently, growing lines of evidence document CaMKII as a pivotal participant in myocardial remodeling. Since CaMKII was suggested serving as a downstream target of non-canonical Wnt signaling, in this study we aimed to elucidate the role of CaMKII in Dvl-1-induced cardiomyopathy as well as the mechanisms underlying its function. Dvl-1-induced cardiomyopathy was reversed by deletion of neither CaMKIIδ nor CaMKIIγ. Therefore, DVL mouse were crossed with CaMKIIδγ double-knockout mouse. The Dvl-1-Tg with CaMKIIδγ-KO mice displayed a normal cardiac phenotype without cardiac hypertrophy, apoptosis, and fibrosis. Using echocardiography and *in vivo* hemodynamic measurements, we observed that LV dysfunction was completely reversed in the DWC mice.

On protein level, we detected, via western blot and HDAC4 kinase assay, robustly increased amount of phosphorylated HDAC4, a class II HDAC, which was inhibited by CaMKIIδγ-KO in DVL animals. In order to further confirm these observations, adenoviral Dvl-1 overexpression was applied to stimulate cultured NRVMs, causing up to 40% increase of cardiamyocyte size and 2-fold upregulation of ANF-expression. Addition of KN93, a CaMKII inhibitor, prevented myocyte hypertrophy and blocked the increase of ANF-expression. Consistent with the data from the crossed animals, CaMKII antagonism also suppressed the phosphorylation of HDAC4 *in vitro*. Moreover, when NRVMs were treated with adenovirus encoding a phosphorylation-resistant HDAC4 mutant--HDAC4-3S/A which localized predominantly to the nucleus, Dvl-1 forced overexpression evoked enhancement of ANF and cardiomyocyte size

were completely reversed, implicating HDAC4 functioning downstream of CaMKII in Dvl-1-induced myocardial hypertrophy. In addition, we found, in NRVMs, both conditional Wnt5a medium and overexpression of Dvl-1 resulted in a significant augmentation of MEF2 activity determined by MEF2 luciferase assay. Interestingly, CaMKII antagonism and HDAC4-3S/A overexpression remarkably blocked both Wnt5a and Dvl-1-induced increase of MEF2 activity, reflecting Wnt5a-Dvl-CaMKII-HDAC4 axis converges at MEF2. These observations unveiled that CaMKIIδγ couples non-canonical Wnt signaling to the HDAC4/MEF2 complex, playing a key role in the process of development of cardiac remodeling and LV dysfunction. Hence, we conclude that the Wnt5a-Dvl-CaMKII-HDAC4-MEF2 axis comprises series of attractive therapeutic targets for prevention of cardiac remodeling and its progression to HF.