Akt activation by PHLPP1 ablation prevents pathological hypertrophy by promoting angiogenesis

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This editorial refers to ‘Physiological activation of Akt by PHLPP1 deletion protects against pathological hypertrophy’ by C. Moc et al., pp. 160–170, this issue.

Akt/PKB (protein kinase B) plays a central role in regulating cardiac hypertrophy and contractile function. Appropriate activation of Akt in the heart is known to protect against cardiac remodelling, but abnormal long-term Akt stimulation leads to heart failure. Although Akt modulation could, in principle, be exploited to promote adaptive cardiac remodelling, an efficient way to safely activate Akt has so far never been reported. Moc et al. rescue pressure overload-induced cardiac hypertrophy through a safe way to activate Akt by ablation of the protein phosphatase PHLPP1 (Figure 1).

In the heart, Akt regulates multiple cardiac functions, such as cardiomyocyte growth, apoptosis, angiogenesis, and contractility. Akt is at the centre of a crossroad involving a large plethora of different signalling molecules. When cells receive multiple stimulations from the environment, receptors in the cell membrane, like RTK (receptor tyrosine kinase) and GPCR (G-protein-coupled receptor), activate Class I PI3K (phosphatidylinositol 3-kinase) to catalyse the production of PIP3 [phosphatidylinositol (3,4,5)-trisphosphate], which, in turn, recruits Akt to the membrane and promotes its activation. This process is mediated by two crucial phosphorylation events at Thr308 and Ser473, both of which are important for full Akt activation. PDK1 (phosphoinositide-dependent kinase 1) phosphorylates Akt on Thr308, whereas mTORC2 (mammalian target of rapamycin complex 2) phosphorylates it on Ser473. The major antagonist of these events is PIP3 dephosphorylation by the lipid phosphatase PTEN (phosphatase and tensin homologue). However, a new player, the protein phosphatase PHLPP (PH domain leucine-rich repeat protein phosphatase), recently emerged as a key negative regulator of Akt activity. PHLPP is a protein phosphatase first discovered in 2005 by Gao et al. and proved to promote apoptosis and suppress tumour growth. Three isoforms, namely PHLPP1α, PHLPP1β, and PHLPP2, were identified to inactivate Akt by selectively dephosphorylating the Ser473 site. Considering the critical role of Akt in cardiac function, understanding the potential of PHLPP inhibition appears a critical issue in cardiac biology.

This is particularly true when considering that ‘appropriate’ Akt activation protects hearts from pathological hypertrophy and maladaptive remodelling, evoked, for example, in rodent models by aortic constriction (TAC) and consequent pressure overload. While this condition is associated with cardiac fibrosis, cardiomyocyte apoptosis, induction of fetal genes expression, and contractile dysfunction, Akt overexpression or hyperactivation of its upstream signalling IGF1R attenuates fibrosis and apoptotic cell death and improves left ventricle contractility. In contrast, down-regulation of Akt activity by Akt1 knockout deteriorates pressure overload-induced hypertrophy, fetal genes expression, and heart failure. Consistently, Moc et al. demonstrate that pressure overload-induced hypertrophy is significantly attenuated in their PHLPP1 knockout (KO) model. Two-week TAC induces enlargement of cardiomyocytes as well as increases expression of heart failure-related genes in wild-type mice, while this up-regulation is blunted in PHLPP1 KO mice. Unlike physiological hypertrophy, pressure overload-induced hypertrophy is associated with increased fibrosis and cardiomyocyte apoptosis. However, in PHLPP1 KO mice, the development of fibrosis and expression of its related genes remain low even 2 weeks after TAC. Similarly, apoptosis in KO mice is significantly reduced compared with wild-type mice following TAC. As a proof for a specific involvement of Akt in this protection, loss of PHLPP1 in murine hearts increases Akt phosphorylation and kinase activity, but does not affect phosphorylation of other potential PHLPP1 targets like PKC.

Moc et al. extend their study to show that the protection against pressure overload-induced pathological hypertrophy by inactivation of PHLPP1 is due to enhanced angiogenesis. At basal level, PHLPP1 KO mice show increased capillary density in the heart and higher expression of VEGFa, a protein which stimulates angiogenesis. After 2 weeks of TAC, angiogenesis is elevated in wild-type mice, but this increase is enhanced in PHLPP1 KO mice, thus demonstrating the contribution of capillaries growth in rescuing pressure overload-induced remodelling. Furthermore, co-culture of neonatal cardiomyocytes with bovine aortic endothelial cells (BAECs) shows that knockdown of PHLPP1 in cardiomyocytes with siRNA significantly increases tube formation. Accordingly, Akt activation promotes the expression of angiogenesis-related genes, such as VEGF and Ang-2, and significantly increases the amount of capillaries in the heart. Conversely, coronary angiogenesis inhibition results in impaired cardiac growth and contractile dysfunction. Although the role of angiogenesis in cardiac health needs to be comprehensively studied, Moc et al. hypothesize that, in their PHLPP1...
KO mice, enhanced angiogenesis supplies more oxygen to meet the demands under stress and avoids cardiac enlargement. Regrettably, the authors did not deeply investigate whether PHLPP1 deficiency also protects the heart against contractile dysfunction and heart failure induced by long-term pressure overload. A significant contractile dysfunction might develop only 8 weeks after TAC, but the study by Moc et al. has been concluded after a 2-week follow-up. Therefore, it would be interesting to study the effects of the loss of PHLPP1 for longer periods, before reaching a conclusive statement on the therapeutic potentials of PHLPP1 inhibition.

Although appropriate activation of Akt shows beneficial outcomes, the effect of long-term Akt overexpression is still controversial. Shiojima et al. demonstrate that 2 weeks of Akt1 overexpression, through an inducible myrAkt1 transgene, triggers reversible cardiac hypertrophy. However, sustained myrAkt1 activation for 6 weeks causes irreversible hypertrophy and heart failure that cannot be dampened even after shutting down Akt1 overexpression. Nonetheless, other constitutively active Akt alleles, like the E40K Akt mutant, appeared to exert only the beneficial effect. This implies that Akt level must be strictly controlled and whether this has to be achieved through either spatial or temporal regulation is still unclear. Moc et al. suggest that PHLPP1 can be part of the molecular mechanisms discriminating these two apparently paradoxical effects of Akt activation in the heart. Excitingly, in response to pressure overload, loss of PHLPP1 does not cause hypertrophy, does not impact on cardiomyocyte contractility but promotes angiogenesis, and ultimately sustains adaptive cardiac remodelling. Given that PHLPP1 is an enzyme amenable to inhibition by small molecule drugs, these findings suggest an interesting opportunity to safely activate Akt by PHLPP1 inhibition.

Recently, PHLPP inhibitors have been identified by chemical and virtual screening, opening the way to strategies aimed at PHLPP targeting in therapy. Future studies will likely extend these initial observations and provide a solid proof of concept for pursuing PHLPP1 inhibition in heart failure prevention/treatment.

References


Figure 1 Involvement of PHLPP1 in Akt-related cardiac processes. (Left) PHLPP1 dephosphorylates Akt on Ser473 and inhibits its activity. The pro-angiogenic and pro-survival Akt-dependent signalling remains low. (Right) Blocking of PHLPP1 releases the inhibition of Akt activity, enhances angiogenesis, and reduces apoptotic cell death.