The serine-threonine kinase Akt/PKB regulates a variety of diverse cellular functions, including cardiac growth, glucose uptake, energy metabolism, gene expression, and, most recently, mitochondrial integrity and survival. In mammalian cells, 3 isoforms of Akt (Akt1, Akt2, and Akt3) have been identified, exhibiting similar structure and moderate overlap for substrate specificity.1 All 3 Akt isoforms are expressed in the myocardium, with the Akt1 and Akt2 isoforms most abundant.1 Following growth factor stimulation, Akt is phosphorylated at 2 critical regulatory phosphorylation sites, Ser473/Thr308 in Akt1, Ser474/Thr309 in Akt2, and Ser472/Thr305 in Akt3.1,2 Threonine phosphorylation is vital and sufficient for Akt activation, and Ser473 phosphorylation is required for maximal Akt activity.1 Based on Akt gene knockout studies, Akt1 and Akt2 seem to have partially redundant functions. Disruption of the Akt1 gene results in mild growth retardation and spontaneous apoptosis in restricted cell types.2,3 Notably, in the heart, Akt1-dependent signaling pathways have been implicated in myocyte survival and cardiac growth.2,4

Arguably, among the best proven and well studied posttranslational events for regulating cellular Akt activity is phosphorylation. Akt contains an N-terminal PH domain, a central catalytic kinase domain, and a C-terminal regulatory domain. Cellular Akt is regulated differentially by phosphatidylinositol 3-kinase respectively via modulation of intracellular phosphatidylinositol (3,4,5)-triphosphate levels.5,6 In cells, Akt is activated following its recruitment to the cell membrane via its PH domain, and sequential phosphorylation of Thr308 in the catalytic domain by PDK1 (3-phosphoinositide–dependent kinase 1) and Ser473 in the C-terminal regulatory domain by the TORC2 complex.1 Following activation, Akt exerts multicompartamental effects in the nucleus, cytoplasm, and mitochondria. Moreover, Akt is activated by tyrosine kinase receptors/cytokines and G protein–coupled receptors. These, in turn, phosphorylate several downstream targets, including glycogen synthase kinase (GSK)-3β, mTOR (mammalian target of rapamycin), endothelial nitric oxide synthetase, PFK2 (6-phospho-fructo-2-kinase), c-Raf, and several other antiapoptotic effectors in the cytoplasmic, mitochondrial, and nuclear compartments.7–10

Despite the wealth of available information regarding the activation by Akt, there remains a paucity of information regarding the cellular mechanisms that lead to Akt inactivation. In general, the serine/threonine phosphatases, protein phosphatase (PP)2A or calcineurin (PP2B) and PTEN (phosphatase and tensin homolog) indiscriminately dephosphorylate Ser473 and Thr308, diminishing Akt activity.6,11 Given the central role of Akt for vital cellular processes including cell growth and survival, a better understanding of how cellular Akt activity is regulated under normal or disease conditions would be of significant clinical importance.

In this issue of Circulation Research, Miyamoto et al12 provide novel insight into the regulation of Akt by demonstrating in cardiac myocytes a novel PH domain leucine-rich repeat protein phosphatase-1 (PHLPP-1) that selectively dephosphorylates Akt on Ser473 (Figure). As a step toward characterizing the specificity and selectivity of PHLPP-1 for Ser473-Akt versus Thr308-Akt, the authors demonstrated higher levels phospho–Akt-Ser473 following siRNA knockdown of PHLPP-1 in rat ventricular myocytes stimulated with the Akt agonists leukemia inhibitory factor (LIF), insulin-like growth factor-1, or sphingosine 1-phosphate. Interestingly, no apparent change in phospho–Akt-Thr308 was observed in agonist-stimulated cells following PHLPP-1 knockdown. In addition, Miyamoto et al showed expression levels of Akt or the gp130 LIF receptor were unaffected by PHLPP-1 expression. Depletion of PHLPP-1 in colon cancer and breast epithelial cells had been previously shown to increase levels of PKC isofoms.13,14 Surprisingly, no changes in PKC levels were observed following PHLPP-1 knockdown in ventricular myocytes, for which the authors attribute to differences in model systems (ie, cancer cells versus nontransformed cardiac myocytes). For instance, PHLPP-1 was found to have greater selectivity for Akt2 versus Akt1 in cancer cells, whereas PHLPP-1 knockdown in ventricular myocytes dramatically increased activities of both Akt1 and Akt2 along with phospho–GSK-3α and –GSK-3β.15

Consistent with these findings, Miyamoto et al similarly showed that PHLPP-1 knockdown enhanced LIF-mediated protection against doxorubicin-induced apoptosis, indicat-
ing that enhanced and selective Akt phosphorylation of Ser473, but not of Thr308, is crucial for cardiomyocyte survival. Importantly, Miyamoto et al established that agonist-induced mitochondrial targeting of Akt enhanced phosphorylation of hexokinase II, thereby inhibiting Ca\(^{2+}\)-induced cytochrome c release and cell death during ischemia/reperfusion (I/R). These findings mechanistically link mitochondrial perturbations to PHLPP-1 for cell survival. Interestingly, PHLPP-1 had no apparent effect on nuclear targeting or expression of phospho-Akt in LIF-stimulated cells. Lastly, Miyamoto et al found a 45% reduction in myocardial infarct size following conditional PHLPP-1 gene knockout, demonstrating the importance of PHLPP-1 in phospho–Ser473-Akt regulation and cell survival. Collectively, the data provide important new evidence that site-specific regulation of phospho–Ser473-Akt by PHLPP-1 is an obligate step in regulating Akt and cardiomyocyte survival during I/R.

Although the report by Miyamoto et al provides additional insight into the regulation of Akt-dependent survival pathways by PHLPP-1, several questions remain unanswered. Given that PHLPP-1 expression or activity was not appreciably changed during I/R or following growth stimulation yet it impaired Akt activity, raises the following question: What are the upstream signaling mechanisms that regulate PHLPP-1 activity under basal conditions or during I/R? Moreover, what is the relationship between PHLPP-1 and other phosphatases such as PTEN or calcineurin for regulating Akt activity? Do these phosphatases work in concert with or parallel to PHLPP-1 during stress-induced I/R? If so, would the combined inhibition of PHLPP-1 and PTEN further enhance Akt activity and cardioprotection such as seen in cancer cells? Another unanswered question relates to why mitochondrial-targeted but not nuclear-localized Akt was affected by PHLPP-1 or, for that matter, whether PHLPP-1 regulates other mitochondrial proteins, including the voltage-dependent ion channel or cyclophilin D in addition to hexokinase II for cell survival? To date, the majority of the studies on PHLPP-1 have been conducted in cancer cells, with little information regarding the role of PHLPP-1 in the heart or skeletal muscle. Given the importance of coordinated site-specific phosphorylation of Akt for maximal activation by LIF or IGF-1 signaling pathways, it remains to be established whether PHLPP-1 plays an equivalent role in regulating Akt-dependent hypertrophy or whether these are independent events separate from PHLPP-1’s effects on cell survival. Nevertheless, the work by Miyamoto et al provides additional insight into the site-specific regulation of Ser473-Akt activity by PHLPP-1 and identifies PHLPP-1 as a novel therapeutic target for enhancing Akt activity and cell survival during cardiac stress.
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References

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