BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-21-095

Title: Emerging roles of PHLPP phosphatases in metabolism

Article Type: Mini Review

Keywords: PHLPP1; PHLPP2; substrates; metabolic diseases; metabolism

Corresponding Author: KyeongJin Kim

Authors: Jong-Ho Cha[1,2], Yelin Jeong[1,2,3], Ah-Reum Oh[1,2,3], Sang Bae Lee[4,5], Soon-Sun Hong[1,2,3], KyeongJin Kim[1,2,3,*]

Institution: [1]Department of Biomedical Sciences, College of Medicine, Inha University, Incheon 22212, South Korea,
[2]Program in Biomedical Science & Engineering, Inha University, Incheon 22212, South Korea, South Korea,
[3]Research Center for Controlling Intercellular Communication (RCIC), College of Medicine, Inha University, Incheon 22212, South Korea,
[4]Division of Life Sciences, Jeonbuk National University, Jeonju 54896, South Korea,
[5]Sarcopenia Total Solution Center, onkwang University School of Medicine, Iksan, Jeonbuk 54538, South Korea,

Manuscript Type: Mini Review

Title: Emerging roles of PHLPP phosphatases in metabolism

Author's name: Jong-Ho Cha[1,2,#], Yelin Jeong[1,2,3,#], Ah-Reum Oh[1,2,3], Sang Bae Lee[4,5], Soon-Sun Hong[1,2,3] & KyeongJin Kim[1,2,3*]

Affiliation: [1]Department of Biomedical Sciences, College of Medicine, Inha University, Incheon 22212, [2]Program in Biomedical Science & Engineering, Inha University, Incheon 22212, South Korea, [3]Research Center for Controlling Intercellular Communication (RCIC), College of Medicine, Inha University, Incheon 22212, [4]Division of Life Sciences, Jeonbuk National University, Jeonju 54896, South Korea, [5]Sarcopenia Total Solution Center, Wonkwang University School of Medicine, Iksan, Jeonbuk 54538, South Korea.

Running Title: PHLPPs: their roles in metabolism

Keywords: PHLPP1, PHLPP2, substrates, metabolic diseases

[#]These authors contributed equally

***Corresponding Author's Information:** KyeongJin Kim PhD, Tel: +82-32-860-9870; E-mail: kimkj@inha.ac.kr

ABSTRACT

Over the last decades, research has focused on the role of pleckstrin homology (PH) domain leucine-rich repeat protein phosphatases (PHLPPs) in regulating cellular signaling via PI3K/Akt inhibition. The PKB/Akt signaling imbalances are associated with a variety of illnesses, including various types of cancer, inflammatory response, insulin resistance, and diabetes, demonstrating the relevance of PHLPPs in the prevention of diseases. Furthermore, identification of novel substrates of PHLPPs unveils their role as a critical mediator in various cellular processes. Recently, researchers have explored the increasing complexity of signaling networks involving PHLPPs whereby relevant information of PHLPPs in metabolic diseases were obtained. In this review, we discuss the current knowledge of PHLPPs on the well-known substrates and metabolic regulation, especially in liver, pancreatic beta cell, adipose tissue, and skeletal muscle in relation with the stated diseases. Understanding the context-dependent functions of PHLPPs can lead to a promising treatment strategy for several kinds of metabolic diseases.

INTRODUCTION

The pleckstrin homology (PH) domain leucine-rich repeat protein phosphatase (PHLPP) was discovered in the suprachiasmatic nucleus (SCN) of the hypothalamus of a rat as a protein whose mRNA expression levels oscillated in a circadian rhythm-dependent fashion and was therefore named as SCN circadian oscillatory protein (SCOP) to represent its behavior (1). Several years later, the ability of SCOP to act as a serine/threonine kinase Akt-specific phosphatase was identified (2). Years after the discovery of Akt-specific phosphatase, more evidence has been accumulated that PHLPP family has different substrates which possess different biology in managing the activity and stability of kinases with respect to cellular processes including cell growth, survival, or metabolism. This review provides an overview of PHLPPs by highlighting recent findings on their roles as novel regulators in cellular metabolism.

PHLPP: GENE AND PROTEIN ORGANIZATION

The PHLPP family of phosphatases composes of PHLPP1α (1205 amino acids), PHLPP1β (1717 amino acids, corresponding to SCOP), and PHLPP2 (1323 amino acids, also referred as PHLPPL). PHLPP1α and PHLPP1β are produced by two splice variants from the same gene located at chromosome 18q21.33, and have different sizes because of a 56 kDa N-terminal extension (3), while the PHLPP2 gene resides at the chromosomal location 16q22.3 (4).

The PHLPP family composes the same domain including N-terminal domain, PH domain, leucine-rich repeat (LRR) region, protein phosphatase 2C (PP2C) phosphatase domain, and C-terminal postsynaptic density protein PSD95, *Drosophila* disc large tumor

suppressor DLG1, and zonula occludens-1 protein zo-1 (PDZ)-binding motif (2, 5) (**Figure 1**). In addition, PHLPP1β and PHLPP2 comprises a Ras association domain (RA domain) preceding the PH domain, and PHLPP1 and PHLPP2 share 58% and 63% amino acid identify in the PP2C domain and PH domain, respectively (2). Despite the three isoforms namely PHLPP1α, PHLPP1β, and PHLPP2 have similar domain structures, they have a certain degree of substrate specificity. Additionally, although the PHLPP isoforms are ubiquitously expressed, their levels vary within different tissues and are broadly associated with its scaffolding proteins in the cytoplasm, nucleus, plasma membrane and mitochondria (6-10). The substrates of the PHLPP isoforms will be discussed briefly in the following section.

PHLPP SUBSTRATES AND SIGNALING NETWORK

Akt

PHLPP was identified in a rational research for a phosphatase that dephosphorylated Akt (2). Three Akt isoforms in mammals, Akt1, Akt2, and Akt3, require phosphorylation at the hydrophobic motif (Ser473) and activation loop (Thr308) to acquire full catalytic activity, which further characterize the downstream substrates of Akt (11). PHLPPs specifically regulate dephosphorylation on the hydrophobic motif of Akt in cells, resulting in decreased activity of Akt (2). Interestingly, isoforms of PHLPPs have substrate specificity in regulating three Akt isoforms. Genetic depletion study elucidated that PHLPP1 regulated the Akt2 and Akt3 phosphorylation, while PHLPP2 affected the Akt1 and Akt2 phosphorylation (4). Specificity of PHLPPs in regulation of Akt isoforms could rewire the differential regulation of specific Akt substrates. For example, the PHLPP1-Akt2 pathway acts on both HDM2 and glycogen synthase 3α (GSK3α) to prevent p53 degradation, whereas the PHLPP2-Akt1 plays

the activity of p27 to inhibit cell cycle progression (3, 4). Both isoforms dephosphorylate Akt2, modulating the GSK3β and tuberous sclerosis complex 2 (TSC2) phosphorylation to restrain cell survival. (4). As the Akt signaling contributes to the expanding repertoire of metabolic regulation, especially in the insulin-responsive tissues, we will further discuss its tissue-specific function in disease contexts in the following section.

PKC

Further study demonstrated that both PHLPP1 and PHLPP2 modulate dephosphorylation of the hydrophobic motif site Ser660 on PKC βII (3, 12), which is one of the stable and priming phosphorylation occurring during initial translation, maintaining the protein in a stable, autoinhibited state (13). PKC is unique among the PHLPP1 hydrophobic motif substrates as that phosphate stabilizes the kinase, while dephosphorylation of other substrates, such as Akt and S6K1, attenuates catalytic activities without affecting their stability (2, 14). Thus, total PKC expression levels are negatively correlated with PHLPP1 expression, showing that PKC in tumor is phosphorylated and dephosphorylated PKC is degraded (15). Whereas PKC is reframed as having a tumor suppressive function (16, 17), development of novel approaches to block the dampening of PKC by PHLPP1 may open a new therapeutic strategy for cancer progression.

Mst1

Both PHLPP1 and PHLPP2 manifest their tumor-suppressing roles to induce apoptosis irrespective of the well-known targets of PHLPPs. A member of the STE kinase family, mammalian sterile 20-like kinase 1 (Mst1), is dephosphorylated on the Thr387 inhibitory site, which in turn activates Mst1 and its downstream targets p38 and JNK to impose apoptosis.

Similar to Thr387 that is found to be phosphorylated by Akt, the PHLPP-Akt-Mst1 axis constitutes an inhibitory triangle that regulates apoptosis and proliferation, probably in a cell type-dependent fashion (18).

S6K1

Ribosomal protein S6 Kinase 1 (S6K1) is a closely related cousin of Akt and PKC in the AGC kinase family. The S6K1 activation is governed by signaling inputs from growth factor, nutrient, and energy balance directed by downstream of mechanistic target of rapamycin (mTOR), a phosphoinositide 3-kinase-like serine/threonine protein kinases (19, 20). S6K1 activation positively directs protein translation by phosphorylating several downstream components, which is required for protein translation initiation, as S6K1 acts as one of the major substrates of mTOR (21). The study suggested that PHLPP-mediated S6K1 dephosphorylation is independent of its ability to induce Akt dephosphorylation. PHLPP negatively contributes to regulation of both protein translation and cell growth via managing the S6K1 activity directly (14).

RAF1

Hyperactivation of the RAS-RAF signaling in various cancer types is associated with metastasis and poor survival of patients. Both PHLPPs dephosphorylate RAF1 at Ser338, which is downstream of EGFR and Ras (22), inhibiting its kinase activity *in vitro*. The knockdown of PHLPP1 or PHLPP2 increases the invasiveness of colorectal cancer (CRC) cells by inducing duration of RAF-MEK-ERK signaling, epithelial-mesenchymal transition (EMT), which expands properties of tumor progression (23).

Myc

Myc is an oncogenic driver of many types of cancer, including human prostate cancer (PC) and classic genetically engineered mice (GEMs) of the disease (24, 25). Recent study showed that PHLPP2 induces direct dephosphorylation on the Thr58 site of Myc, leading to an increased in its stability (26). Interestingly, the recurrent mutation on T58A was found in patient with Burkitt's lymphoma to cause increased transformation both *in vitro* and *in vivo* (27, 28). The T58A mutant is constitutively dephosphorylated, which constantly mimic PHLPP2 activity. Therefore, PHLPP2 can be an unexpected, druggable target on PC and its progression driven by myc.

HSL

Hormone-sensitive lipase (HSL) is a critical enzyme in mobilizing fatty acids from stored triacylglycerols (TAGs) (29). Its activity is regulated by phosphorylation of at least four serine. In rat HSL, the Ser563, Ser569 and Ser660 were phosphorylated by protein kinase A (PKA). It is reported that Ser659 and Ser660 are the activity regulating sites *in vitro*. However, the precise molecular events of PKA-mediated activation and dephosphorylation were not yet to be determined. Recent study showed that PHLPP2 directly dephosphorylates HSL on Ser563 and Ser660, which leads to a decreased HSL activity and alters its localization in cytoplasm or at the peripheries of the lipid droplets (30). The PHLPP2-HSL axis is further associated with systemic lipid and glucose homeostasis as well as hepatic lipid accumulation as discussed in the following section.

PHLPPs: IMPLICATIONS IN METABOLIC DISEASES

Since PHLPPs are a negative regulator of key processes and signaling pathways, they have critical roles in several pathologies. The most well-known examples of their roles are in

cancer progression, as PHLPPs have been identified as tumor suppressors in many types of cancers (31-34). Since maintaining balanced levels of PHLPP expression is critical for preventing cancer progression, the loss of PHLPP increases cell proliferation, migration, metastasis, and cell motility by activating Akt phosphorylation in the diverse cancer cells, such as pancreatic cancer, colon cancer, prostate cancer, leukemia and glioblastoma, breast cancer and melanoma (8, 26, 35-37). On the other hand, an overexpression of PHLPP leads to inhibition of tumor formation and increases apoptotic cell death decreasing Akt phosphorylation on Ser473 in pancreatic, lung, colon and breast cancer cells (5). Apart from the progression of cancer, growing evidences revealed promising functions of PHLPPs in metabolic diseases, as dysregulation of Akt pathway is related with obesity, insulin resistance, and type 2 diabetes. In addition, identification of novel substrates is associated with cellular metabolic disturbances, emphasizing the significance of PHLPPs in the progression of metabolic diseases, highlighting recent findings on their functions in metabolic regulation.

PHLPPs and regulation of hepatic lipids

With the increased prevalence of obesity and its metabolic consequences, nonalcoholic fatty liver disease (NAFLD), defined by excess liver fat, is becoming the most common chronic liver disease (38-40). Although the molecular mechanisms underlying hepatic lipid homeostasis in NAFLD are not clearly defined, an increase in *de novo* lipogenesis (DNL), a process to synthesize new fatty acids from acetyl coenzyme A (acetyl-CoA), could contribute to the development of NAFLD (41, 42). Obesity-associated insulin resistance and compensatory hyperinsulinemia increases DNL, exacerbating hepatic lipid accumulation in NAFLD (43). Identification of molecular regulator of DNL associated with insulin resistance and hyperinsulinemia is expanding to develop novel therapeutics to improve public health problems including obesity-induced type 2 diabetes and NAFLD. One of the promising

targets of DNL is the mTOR that comprises of the catalytic core of two distinct protein complexes namely mTOR complex 1 (mTORC1) and 2 (mTORC2) (44-46). Previous studies suggested that mTORC1-independent Raptor (free Raptor) stabilizes PHLPP2, but not PHLPP1, to reduce signaling through Akt (47, 48). In aged or obese mice, hepatic PHLPP2 levels were lower with decreased free Raptor levels, resulting to prolonged Akt signaling. This allows increased Akt-mediated DNL, that exacerbates NAFLD. These data explain how insulin-mediated Akt action is permissive for increased DNL in obesity-induced insulin resistance.

A recent study suggested more defined mechanisms underlying PHLPP2 degradation in obesity-induced fatty liver. PHLPP2 is rapidly phosphorylated by glucagon/PKA signaling to trigger PHLPP2 degradation. However, its phosphorylation is necessary but not sufficient to induced its degradation. The authors further suggested that obesity-mediated increased potassium channel tetramerization domain containing 17 (KCTD17) in hepatocytes is critical to link PHLPP2 phosphorylation with proteasomal degradation, which elevated Akt signaling and hepatic lipid accumulation (49). Therefore, normalized PHLPP2 levels in the context of NAFLD could provide therapeutic benefits.

PHLPPs, regulation of insulin resistance and pancreatic beta cell dysfunction

Pancreatic beta cell failure, which is characterized by the impaired insulin action or the intrinsic susceptibility of the beta cell to functional exhaustion, is critical to develop insulin resistance and type 2 diabetes (50). While the impaired insulin action in peripheral tissues remains constant as diabetes progresses, beta cell function worsens continuously with disease progression, resulting from the persisting exposure to damaging factors, such as high glucose concentrations (glucotoxicity), increased levels of circulating free fatty acid (lipotoxicity), and chronic inflammation (51-53), which therefore necessitates further studies in beta cell

failure. Since Akt contributes to the regulation of beta cell homeostasis (54), modulation of Akt should be actively sought to restore a healthy beta cell. The observations showed that the altered pancreatic beta cell homeostasis upon the chronic high glucose exposure is accompanied by an increased PHLPP1 and PHLPP2 expression both at mRNA and protein levels with a consequent reduction of the phosphorylation levels of Akt. Further knockdown of PHLPPs is able to curtail a pro-survival profile in INS-1 cells chronically exposed to high glucose concentrations as well as increased Akt phosphorylation and mTOR activation (55). These findings trigger the need for further studies in order to identify pharmacological PHLPPs modulators, raising the possibility of new treatments for beta cell dysfunction.

PHLPPs, insulin resistance, and lipolysis on adipose tissue

Obesity and type 2 diabetes are closely associated with increased adiposity, and insulin resistance is a fundamental characteristic of both diseases (56). As stated above, PHLPPs' substrates specificity on Akt isoforms raised the intriguing possibility of tissue-specific functions of PHLPP family in the context of insulin-responsive or nonresponsive tissues. A report highlighted that the protein levels of PHLPP1 are greatly induced in adipose tissue of morbidly obese participants as compared to non-obese participants and are negatively associated to Ser473 phosphorylation of Akt (57). Interestingly, increased level of PHLPP1 is positively associated with body mass index (BMI), fasting insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR). However, it is observed that PHLPP1 is not further induced in obese participants with impaired fasting glucose or type 2 diabetes (57), showing that enhanced PHLPP1 levels may be related with a state of insulin resistance and compensatory hyperinsulinemia, but not with hyperglycemia.

The function of adipose PHLPP2 in normal or obese states is not well documented. A recent discovery sheds light on a unique role of PHLPP2 in obese adipocytes. The authors

revealed that adipocyte PHLPP2 levels are higher in obese mice than in lean animals (30). Interestingly, a decrease in adipocyte PHLPP2 increases adipose lipolysis due to prolonged hormone-sensitive lipase (HSL) phosphorylation, which allows to improve glucose homeostasis, increase peroxisome proliferator-activated receptor alpha (PPARα)-dependent adiponectin secretion, and hepatic fatty acid oxidation to alleviate obesity-induced fatty liver. These findings suggested that blocking excess PHLPP2 in adipocyte may be a therapeutic strategy to improve obesity-induced metabolic comorbidities.

Accumulated evidences showed an association of PHLPP2 with insulin resistance and glucose intolerance (57-61). However, mechanisms underlying increased adipose PHLPP2 expression in patients associated with obesity or diabetes are far less understood. A recent report suggested that hepatic miR-130a-3p targets PHLPP2 to retard dephosphorylation of Akt to change self-stability, which in turn reduced PHLPP2 to activate Akt signaling in adipose cells (62). These data supported new molecular mechanisms by which the crosstalk between liver and adipose tissues improve glucose metabolism, further providing therapeutic options for insulin resistance.

PHLPPs and insulin action on skeletal muscle

Skeletal muscle is also a sub-optimal response of peripheral tissues in insulin resistance to the insulin action (63). Several studies speculated the relevance of PHLPPs during pathogenesis of insulin resistant in skeletal muscle. A study showed that PHLPP1 levels were greater in primary myoblasts derived from 9 obese type 2 diabetes patients than in cells taken from lean healthy participants (64). Furthermore, it has confirmed by showing higher PHLPP1 level in skeletal muscle biopsies from 12 obese insulin-resistant individuals (57). Although it is evident that elevated levels of PHLPPs, probably PHLPP1, might be associated with hampering insulin resistance in skeletal muscle, the mechanisms underlying increased

PHLPP1 in insulin-resistant skeletal muscle are not clear. Over-nutrition provokes low-grade chronic inflammation, dyslipidemia, and dysbiosis incrementally affecting in endoplasmic reticulum (ER) stress, a physiologically changed condition of the ER (65). A study showed that ER stress enhanced the PHLPP1 expression as well as its ERK1/2-mediated phosphorylation. Additionally, the study identified that PHLPP1 is associated with and dephosphorylated AMPK, a key mediator in insulin-independent glucose utilization (66), supporting that PHLPP1 as a novel therapeutic option for the management of ER stress-mediated insulin resistance and type 2 diabetes.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Years after the discovery of Akt-specific phosphatases, there was growing evidence demonstrating that PHLPPs have several substrates and the majority are engaged in the control of cellular growth and survival (67). Recent accumulated evidences suggested PHLPPs as critical players in the regulation of metabolism, which unveiled their different expressions and novel substrates in a tissue-specific or disease-specific manner. Studies concerning PHLPPs in metabolic diseases are being studied to identify their substrates and upstream regulators. It would be greatly impressive to ascertain various new targets and mechanisms underlying functions in different pathophysiologies in the tissue-specific or disease-specific context. For now, it is clear that PHLPPs perform multifaceted and complex functions in metabolic diseases (**Figure 2**). Collectively, our understanding of PHLPP regulation in normal and pathophysiological conditions will uncouple the development of desirable therapeutic options to ameliorate specific metabolic diseases in which PHLPPs are involved.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation (NRF) grant funded by the Korea government (MSIT) (No. 2020R1C1C1005631 to JHC, 2020R1C1C1014281 to SBL, 2021R1A5A8029876 to SBL, 2020R1C1C1004015 to KK and 2021R1A5A2031612 to SSH and KK) and INHA UNIVERSITY Research Grant (JHC and KK).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Domain architecture of PHLPP isoforms

PHLPP family retains the Ras association domain (RA), pleckstrin homology (PH) domain, leucine rich repeat region (LRR), PP2C domain and PDZ binding motif. Black arrow head denotes the splice site for PHLPP1β.

Figure 2. The roles of PHLPPs in the regulation of tissue metabolism in pathophysiological state

(A) PHLPP2 suppresses *de novo* lipogenesis in the liver via interfering with prolonged Akt activation. Aging or obesity reduces the level of PHLPP2, resulting in the sustenance of the Akt signaling and hepatic steatosis. (B) In the pancreas, both PHLPP1 and PHLPP2 regulate pancreatic beta cell survival and proliferation. Insulin resistance drives pancreatic beta cell failure partially by upregulation of both PHLPP1 and PHLPP2 in response to high glucose exposure, which favors progression toward type 2 diabetes. (C) In adipose tissue, PHLPP1 or PHLPP2 controls insulin action and lipolysis. Obesity promotes PHLPP2 levels, which dephosphorylates HSL and causes glucose and lipid dysregulation. (D) In skeletal muscle, PHLPP1 plays crucial role in regulating insulin action. High circulating nutrient or ER stress potentiates increased PHLPP1 expression, contributing to insulin resistance.

REFERENCES

1. Shimizu K, Okada M, Takano A and Nagai K (1999) SCOP, a novel gene product expressed in a circadian manner in rat suprachiasmatic nucleus. FEBS Lett 458, 363-369
2. Gao T, Furnari F and Newton AC (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol Cell 18, 13-24
3. Brognard J and Newton AC (2008) PHLiPPing the switch on Akt and protein kinase C signaling. Trends Endocrinol Metab 19, 223-230
4. Brognard J, Sierecki E, Gao T and Newton AC (2007) PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. Mol Cell 25, 917-931
5. Baffi TR, Cohen-Katsenelson K and Newton AC (2021) PHLPPing the Script: Emerging Roles of PHLPP Phosphatases in Cell Signaling. Annu Rev Pharmacol Toxicol 61, 723-743
6. Mendoza MC and Blenis J (2007) PHLPPing it off: phosphatases get in the Akt. Mol Cell 25, 798-800
7. Molina JR, Agarwal NK, Morales FC et al (2012) PTEN, NHERF1 and PHLPP form a tumor suppressor network that is disabled in glioblastoma. Oncogene 31, 1264-1274
8. Reyes G, Niederst M, Cohen-Katsenelson K et al (2014) Pleckstrin homology domain leucine-rich repeat protein phosphatases set the amplitude of receptor tyrosine kinase output. Proc Natl Acad Sci U S A 111, E3957-3965
9. Ohwada W, Tanno M, Yano T et al (2020) Distinct intra-mitochondrial localizations of pro-survival kinases and regulation of their functions by DUSP5 and PHLPP-1. Biochim Biophys Acta Mol Basis Dis 1866, 165851
10. Aviv Y and Kirshenbaum LA (2010) Novel phosphatase PHLPP-1 regulates mitochondrial Akt activity and cardiac cell survival. Circ Res 107, 448-450
11. Brazil DP and Hemmings BA (2001) Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem Sci 26, 657-664
12. Gao T, Brognard J and Newton AC (2008) The phosphatase PHLPP controls the cellular levels of protein kinase C. J Biol Chem 283, 6300-6311
13. Tovell H and Newton AC (2021) PHLPPing the balance: restoration of protein kinase

C in cancer. Biochem J 478, 341-355

14. Liu J, Stevens PD, Li X, Schmidt MD and Gao T (2011) PHLPP-mediated dephosphorylation of S6K1 inhibits protein translation and cell growth. Mol Cell Biol 31, 4917-4927
15. Baffi TR, Van AN, Zhao W, Mills GB and Newton AC (2019) Protein Kinase C Quality Control by Phosphatase PHLPP1 Unveils Loss-of-Function Mechanism in Cancer. Mol Cell 74, 378-392 e375
16. Zhang LL, Cao FF, Wang Y et al (2015) The protein kinase C (PKC) inhibitors combined with chemotherapy in the treatment of advanced non-small cell lung cancer: meta-analysis of randomized controlled trials. Clin Transl Oncol 17, 371-377
17. Hsu AH, Lum MA, Shim KS et al (2018) Crosstalk between PKCalpha and PI3K/AKT Signaling Is Tumor Suppressive in the Endometrium. Cell Rep 24, 655-669
18. Qiao M, Wang Y, Xu X et al (2010) Mst1 is an interacting protein that mediates PHLPPs' induced apoptosis. Mol Cell 38, 512-523
19. Burnett PE, Barrow RK, Cohen NA, Snyder SH and Sabatini DM (1998) RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. Proc Natl Acad Sci U S A 95, 1432-1437
20. Isotani S, Hara K, Tokunaga C, Inoue H, Avruch J and Yonezawa K (1999) Immunopurified mammalian target of rapamycin phosphorylates and activates p70 S6 kinase alpha in vitro. J Biol Chem 274, 34493-34498
21. Ma XM and Blenis J (2009) Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 10, 307-318
22. Wellbrock C, Karasarides M and Marais R (2004) The RAF proteins take centre stage. Nat Rev Mol Cell Biol 5, 875-885
23. Li X, Stevens PD, Liu J et al (2014) PHLPP is a negative regulator of RAF1, which reduces colorectal cancer cell motility and prevents tumor progression in mice. Gastroenterology 146, 1301-1312 e1301-1310
24. Ellwood-Yen K, Graeber TG, Wongvipat J et al (2003) Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell 4, 223-238
25. McKeown MR and Bradner JE (2014) Therapeutic strategies to inhibit MYC. Cold Spring Harb Perspect Med 4
26. Nowak DG, Katsenelson KC, Watrud KE et al (2019) The PHLPP2 phosphatase is a

druggable driver of prostate cancer progression. J Cell Biol 218, 1943-1957

27. Chang DW, Claassen GF, Hann SR and Cole MD (2000) The c-Myc transactivation domain is a direct modulator of apoptotic versus proliferative signals. Mol Cell Biol 20, 4309-4319
28. Hemann MT, Bric A, Teruya-Feldstein J et al (2005) Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature 436, 807-811
29. Schwartz JP and Jungas RL (1971) Studies on the hormone-sensitive lipase of adipose tissue. J Lipid Res 12, 553-562
30. Kim K, Kang JK, Jung YH et al (2021) Adipocyte PHLPP2 inhibition prevents obesity-induced fatty liver. Nat Commun 12, 1822
31. Chang RM, Yang H, Fang F, Xu JF and Yang LY (2014) MicroRNA-331-3p promotes proliferation and metastasis of hepatocellular carcinoma by targeting PH domain and leucine-rich repeat protein phosphatase. Hepatology 60, 1251-1263
32. Cai J, Fang L, Huang Y et al (2013) miR-205 targets PTEN and PHLPP2 to augment AKT signaling and drive malignant phenotypes in non-small cell lung cancer. Cancer Res 73, 5402-5415
33. Gao G, Kun T, Sheng Y et al (2013) SGT1 regulates Akt signaling by promoting beta-TrCP-dependent PHLPP1 degradation in gastric cancer cells. Mol Biol Rep 40, 2947-2953
34. Li X, Stevens PD, Yang H et al (2013) The deubiquitination enzyme USP46 functions as a tumor suppressor by controlling PHLPP-dependent attenuation of Akt signaling in colon cancer. Oncogene 32, 471-478
35. Yu Y, Dai M, Lu A, Yu E and Merlino G (2018) PHLPP1 mediates melanoma metastasis suppression through repressing AKT2 activation. Oncogene 37, 2225-2236
36. O'Hayre M, Niederst M, Fecteau JF et al (2012) Mechanisms and consequences of the loss of PHLPP1 phosphatase in chronic lymphocytic leukemia (CLL). Leukemia 26, 1689-1692
37. Smith AJ, Wen YA, Stevens PD, Liu J, Wang C and Gao T (2016) PHLPP negatively regulates cell motility through inhibition of Akt activity and integrin expression in pancreatic cancer cells. Oncotarget 7, 7801-7815
38. Araujo AR, Rosso N, Bedogni G, Tiribelli C and Bellentani S (2018) Global epidemiology of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: What we need in the future. Liver Int 38 Suppl 1, 47-51

39. Loomba R and Sanyal AJ (2013) The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol 10, 686-690
40. Younossi Z, Anstee QM, Marietti M et al (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 15, 11-20
41. Postic C and Girard J (2008) Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest 118, 829-838
42. Kim K and Kim KH (2020) Targeting of Secretory Proteins as a Therapeutic Strategy for Treatment of Nonalcoholic Steatohepatitis (NASH). Int J Mol Sci 21
43. Savage DB and Semple RK (2010) Recent insights into fatty liver, metabolic dyslipidaemia and their links to insulin resistance. Curr Opin Lipidol 21, 329-336
44. Sarbassov DD, Ali SM, Kim DH et al (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14, 1296-1302
45. Jacinto E, Loewith R, Schmidt A et al (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 6, 1122-1128
46. Laplante M and Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149, 274-293
47. Kim K, Qiang L, Hayden MS, Sparling DP, Purcell NH and Pajvani UB (2016) mTORC1-independent Raptor prevents hepatic steatosis by stabilizing PHLPP2. Nat Commun 7, 10255
48. Kim K and Pajvani UB (2016) "Free" Raptor - a novel regulator of metabolism. Cell Cycle 15, 1174-1175
49. Kim K, Ryu D, Dongiovanni P et al (2017) Degradation of PHLPP2 by KCTD17, via a Glucagon-Dependent Pathway, Promotes Hepatic Steatosis. Gastroenterology 153, 1568-1580 e1510
50. Weyer C, Bogardus C, Mott DM and Pratley RE (1999) The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 104, 787-794
51. Christensen AA and Gannon M (2019) The Beta Cell in Type 2 Diabetes. Curr Diab Rep 19, 81
52. Hribal ML, Perego L, Lovari S et al (2003) Chronic hyperglycemia impairs insulin

secretion by affecting insulin receptor expression, splicing, and signaling in RIN beta cell line and human islets of Langerhans. FASEB J 17, 1340-1342

53. Ye R, Onodera T and Scherer PE (2019) Lipotoxicity and beta Cell Maintenance in Obesity and Type 2 Diabetes. J Endocr Soc 3, 617-631
54. Elghazi L, Balcazar N and Bernal-Mizrachi E (2006) Emerging role of protein kinase B/Akt signaling in pancreatic beta-cell mass and function. Int J Biochem Cell Biol 38, 157-163
55. Hribal ML, Mancuso E, Arcidiacono GP et al (2020) The Phosphatase PHLPP2 Plays a Key Role in the Regulation of Pancreatic Beta-Cell Survival. Int J Endocrinol 2020, 1027386
56. Sesti G, Federici M, Lauro D, Sbraccia P and Lauro R (2001) Molecular mechanism of insulin resistance in type 2 diabetes mellitus: role of the insulin receptor variant forms. Diabetes Metab Res Rev 17, 363-373
57. Andreozzi F, Procopio C, Greco A et al (2011) Increased levels of the Akt-specific phosphatase PH domain leucine-rich repeat protein phosphatase (PHLPP)-1 in obese participants are associated with insulin resistance. Diabetologia 54, 1879-1887
58. Nigro C, Mirra P, Prevenzano I et al (2018) miR-214-Dependent Increase of PHLPP2 Levels Mediates the Impairment of Insulin-Stimulated Akt Activation in Mouse Aortic Endothelial Cells Exposed to Methylglyoxal. Int J Mol Sci 19
59. Sun X, Lin J, Zhang Y et al (2016) MicroRNA-181b Improves Glucose Homeostasis and Insulin Sensitivity by Regulating Endothelial Function in White Adipose Tissue. Circ Res 118, 810-821
60. Mathur A, Pandey VK and Kakkar P (2017) PHLPP: a putative cellular target during insulin resistance and type 2 diabetes. J Endocrinol 233, R185-R198
61. Xiong X, Wen YA, Mitov MI, M CO, Miyamoto S and Gao T (2017) PHLPP regulates hexokinase 2-dependent glucose metabolism in colon cancer cells. Cell Death Discov 3, 16103
62. Wu J, Dong T, Chen T et al (2020) Hepatic exosome-derived miR-130a-3p attenuates glucose intolerance via suppressing PHLPP2 gene in adipocyte. Metabolism 103, 154006
63. Goodyear LJ and Kahn BB (1998) Exercise, glucose transport, and insulin sensitivity. Annu Rev Med 49, 235-261
64. Cozzone D, Frojdo S, Disse E et al (2008) Isoform-specific defects of insulin

stimulation of Akt/protein kinase B (PKB) in skeletal muscle cells from type 2 diabetic patients. Diabetologia 51, 512-521

65. Johnson AM and Olefsky JM (2013) The origins and drivers of insulin resistance. Cell 152, 673-684

66. Behera S, Kapadia B, Kain V et al (2018) ERK1/2 activated PHLPP1 induces skeletal muscle ER stress through the inhibition of a novel substrate AMPK. Biochim Biophys Acta Mol Basis Dis 1864, 1702-1716

67. Newton AC and Trotman LC (2014) Turning off AKT: PHLPP as a drug target. Annu Rev Pharmacol Toxicol 54, 537-558

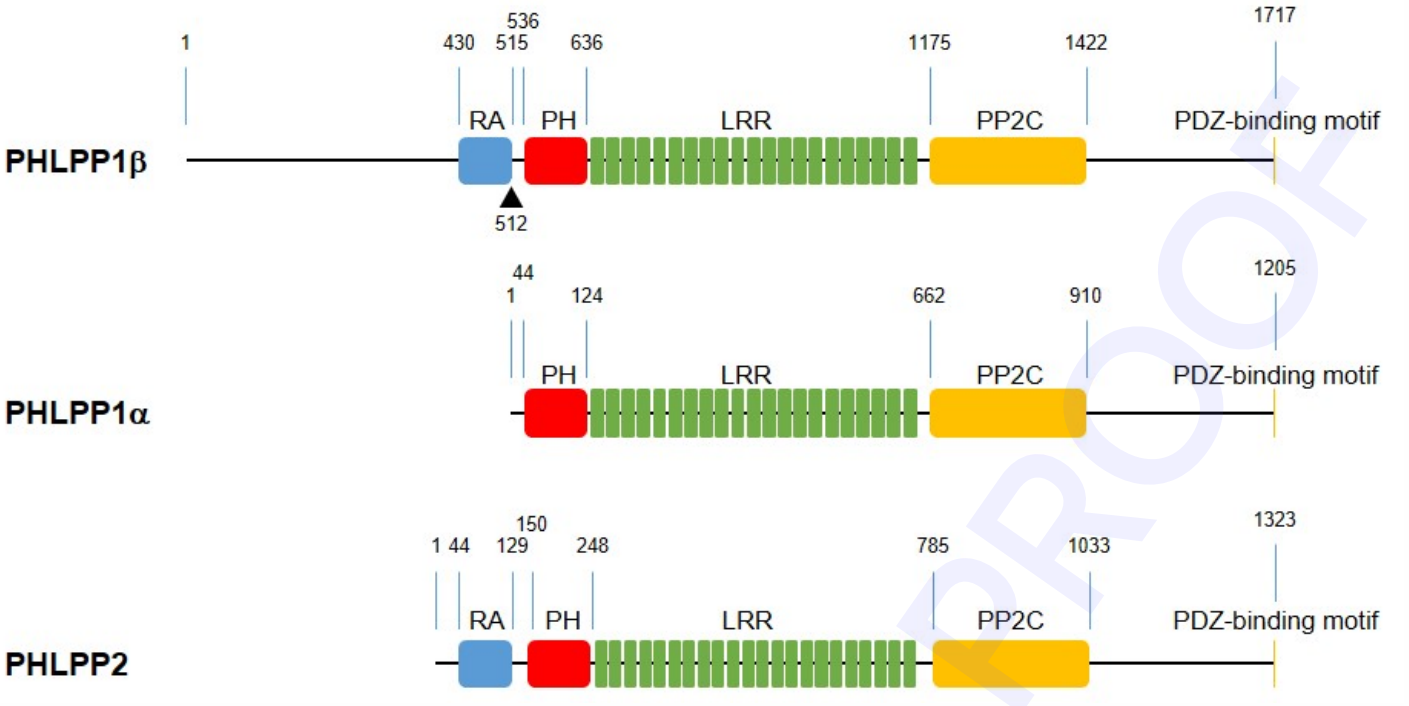

Fig. 1.

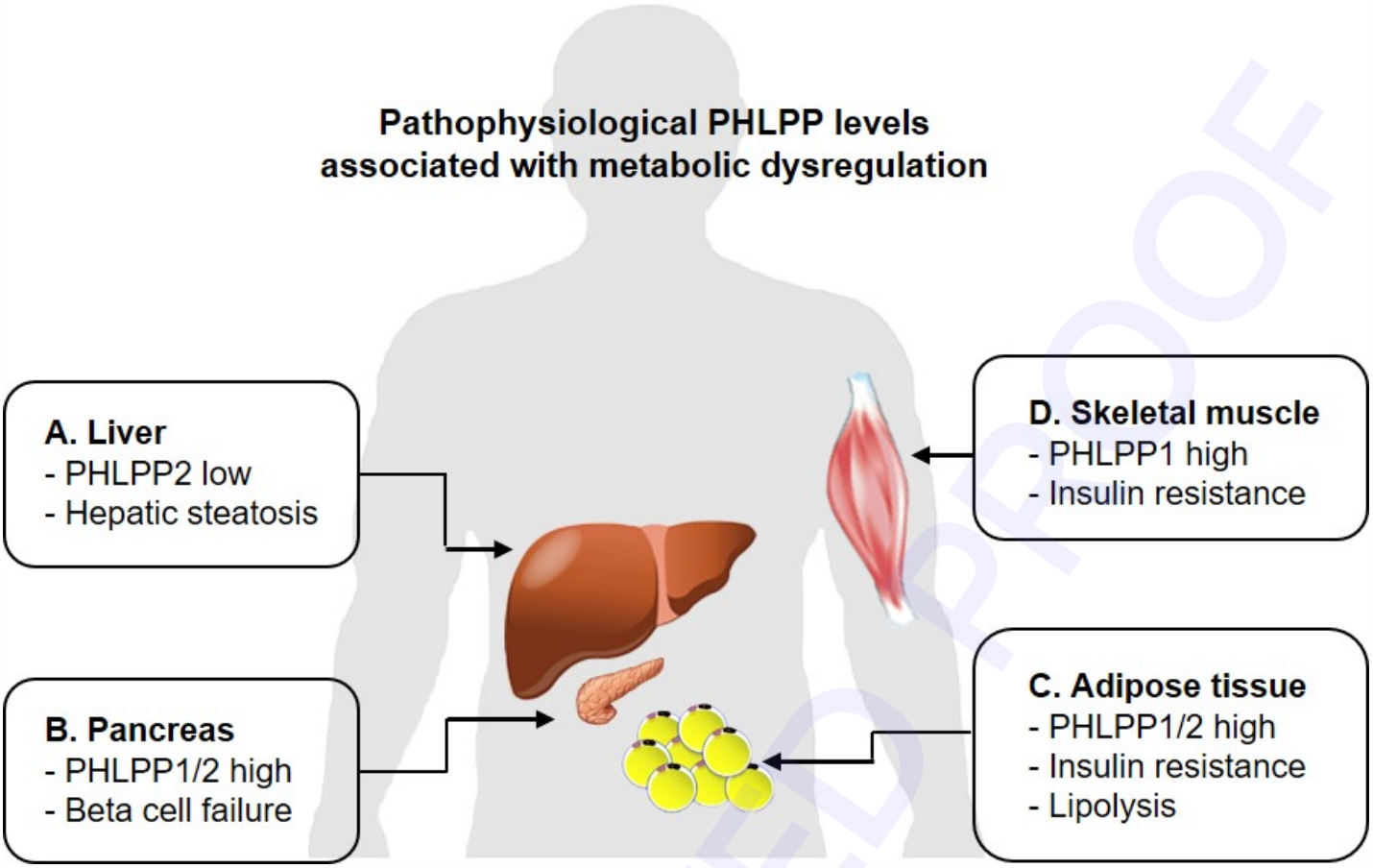

Fig. 2.