

3-Phosphoinositide-dependent protein kinase-1 as an emerging target in the management of breast cancer

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Abstract: It should be noted that 3-phosphoinositide-dependent protein kinase-1 (PDK1) is a protein encoded by the *PDPK1* gene, which plays a key role in the signaling pathways activated by several growth factors and hormones. PDK1 is a crucial kinase that functions downstream of phosphoinositide 3-kinase activation and activates members of the AGC family of protein kinases, such as protein kinase B (Akt), protein kinase C (PKC), p70 ribosomal protein S6 kinases, and serum glucocorticoid-dependent kinase, by phosphorylating serine/threonine residues in the activation loop. AGC kinases are known to play crucial roles in regulating physiological processes relevant to metabolism, growth, proliferation, and survival. Changes in the expression and activity of PDK1 and several AGC kinases have been linked to human diseases including cancer. Recent data have revealed that the alteration of PDK1 is a critical component of oncogenic phosphoinositide 3-kinase signaling in breast cancer, suggesting that inhibition of PDK1 can inhibit breast cancer progression. Indeed, PDK1 is highly expressed in a majority of human breast cancer cell lines and both PDK1 protein and messenger ribonucleic acid are overexpressed in a majority of human breast cancers. Furthermore, overexpression of PDK1 is sufficient to transform mammary epithelial cells. PDK1 plays an essential role in regulating cell migration, especially in the context of phosphatase and tensin homologue deficiency. More importantly, downregulation of PDK1 levels inhibits migration and experimental metastasis of human breast cancer cells. Thus, targeting PDK1 may be a valuable anticancer strategy that may improve the efficacy of chemotherapeutic strategies in breast cancer patients. In this review, we summarize the evidence that has been reported to support the idea that PDK1 may be a key target in breast cancer management.

Keywords: 3-phosphoinositide-dependent protein kinase-1, phosphoinositide 3-kinase, AGC protein kinases, oncogenic kinase, cell signaling, breast cancer therapy

Introduction

Breast cancer is the second most common cancer worldwide and, although treatments have improved greatly over the last decade, this cancer remains the leading cause of cancer-related death in women.¹ Therefore, there is a huge need to identify novel molecular targets in breast cancer. 3-Phosphoinositide-dependent kinase 1 (PDK1), encoded by the gene *PDPK1*, is a molecular kinase belonging to the phosphoinositide-3-kinase (PI3K) signaling pathway. Since their discovery over 20 years ago, the enzymes PI3Ks have been established as major signaling molecules implicated in a variety of different cellular functions such as glucose metabolism,²⁻⁴ cellular proliferation, cellular survival, and angiogenesis.⁵⁻⁸ Their discovery was also paralleled by the observation that genetic lesions to the PI3K pathway are frequently observed in cancer; abnormal PI3K signaling

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has been estimated to occur in as many as 50% of all human malignancies.⁹ The PI3K family contains eight mammalian isoforms grouped into three classes. Class 1A consists of a catalytic subunit and a regulatory subunit. The catalytic subunits include p110 α , p110 β , and p110 δ , while the regulatory subunit consists of p85 α , p85 β , and p55 γ . Class 1B consists of only a p110 γ catalytic unit and two regulatory units, p87 and p101.⁸ Class 2 PI3Ks are monomeric proteins that comprise three isoforms, PI3KC2 α , PI3KC2 β , and PI3KC2 γ ,⁹ whilst class 3 comprises only one isoform (hVps34). PI3Ks catalyze the phosphorylation of position 3 of the inositol head group of phosphoinositides. The best characterized PI3K product is the phospholipid phosphatidylinositol-3,4,5-trisphosphate (PtdIns[3,4,5]P₃) derived from the phosphorylation of the 3' position of phosphatidylinositol-4,5-bisphosphate (PtdIns[4,5]P₂).⁸ PtdIns(3,4,5)P₃ is synthesized by class 1 PI3K in response to either receptor tyrosine kinases, or G-protein-coupled receptor activation, and it acts as a second messenger by inducing the translocation of proteins to the membrane and subsequent activation.

The discovery of modular domains, such as the pleckstrin homology (PH) domains, able to interact with 3'-phosphorylated lipids marked a significant step forward in understanding the PI3K pathway.^{10–12} The most significant example of PtdIns(3,4,5)P₃-dependent protein activation is that of serine/threonine-specific protein kinase B (Akt). Akt binds to PtdIns(3,4,5)P₃ via its PH domain, and it is subsequently phosphorylated at its residue threonine (Thr)308 and activated by PDK1, which itself is also associated to the membrane via PH domain-dependent binding to PtdIns(3,4,5)P₃. A second Akt residue is phosphorylated by distinct kinases, mainly the mammalian target of rapamycin (mTOR) complex 2.^{13–14} In its fully active phosphorylation state, Akt phosphorylates several downstream targets, including glucose synthase kinase, transcription factors such as Foxo-1 and cyclic adenosine monophosphate (cAMP) response element-binding (CREB).¹⁶ This pathway is negatively regulated by the phosphatase and tensin homologue (PTEN), which directly dephosphorylates PtdIns(3,4,5)P₃ at the 3' position, hence attenuating the PI3K signal.¹⁷

Indeed, the oncogenicity of abnormal PI3K signaling is emphasized by the observation that deactivating mutations to the gene encoding PTEN are among the most frequently occurring in human malignancy.¹⁷ Similarly, activating mutations are also frequently seen in the class 1 p110 α subunit including ~8% of breast cancers.^{18–23} Due to the importance of this pathway in cancer cell signaling, it is of little surprise that molecular inhibitors of the PI3K pathway are often investigated

for their potential therapeutic benefit in human malignancy. There are currently vast arrays of PI3K-targeted molecular inhibitors in production, some of which are pan inhibitors designed to target all isoforms, and others which are isoform specific. Whilst the former of the two have raised concerns regarding toxicity, there is growing momentum in the research to suggest that targeting specific isoforms may be sufficient to attenuate the PI3K signal in specific cancer settings.^{23–25} For example, CAL-101 a molecular inhibitor specific for the class 1 catalytic subunit p110 δ , is currently entering Phase III trials for the treatment of chronic lymphocytic leukemia.^{26–30}

While strategies aimed at attenuating the PI3K signal have been mainly focused on inhibiting the PI3K catalytic subunits, there is currently growing evidence suggesting that PDK1 itself may be a viable target. Indeed evidence is beginning to accumulate suggesting that in particular cancer settings, PDK1 is overexpressed and it activates cancer cell growth and survival in a mechanism that is independent of Akt signaling.^{31,32} This, therefore, detracts from the conventional notion that PDK1 is merely an Akt-activating stepping stone, but rather it highlights the notion that PDK1 may be an important oncogenic regulator and a potential therapeutic target in cancer.³³ In this review, we will discuss the role of PI3K/PDK1 signaling in cancer and more specifically assess the role of PDK1 in breast cancer.

PDK1, mechanisms of activation, and physiological functions

PDK1 belongs to the family of AGC kinases (serine and threonine kinases) that show a sequence homology in their catalytic domain to cAMP-dependent protein kinase 1, cyclic guanosine monophosphate-dependent protein kinase, and protein kinase C (PKC).³⁴ The amino-terminal small lobe and the carboxy-terminal large lobe “sandwich” one adenosine triphosphate molecule essential for the subsequent substrate phosphorylation.³⁴ Many AGC kinases possess two phosphorylation sites that regulate their activation: one in the activation loop, which is located within the kinase domain; and another in the hydrophobic motif, which is located in a region adjacent to the catalytic domain.¹³ Phosphorylation of these sites increases kinase activity and leads to enzymatic full activation. PDK1 was originally discovered in 1997 as the kinase responsible for the phosphorylation of the Akt activation loop, at residue Thr308, which is essential for enzyme activation.¹³ Furthermore Akt phosphorylation at Thr308 was dependent on PtdIns(3,4,5)P₃ concentration in vitro, linking PDK1 to the upstream activation of PI3K. PDK1 kinase is a protein of 556 amino acids that possesses an N-terminal cata-

lytic domain, a C-terminal PH domain, and a nuclear export sequence (Figure 1). The nuclear export sequence is a short sequence of four amino acids that are essential for exporting PDK1 from the cell nucleus to the cytoplasm through the nuclear pore complex using nuclear transport.

Similar to other AGC kinases, PDK1 possesses a phosphorylation site within the activation loop (S241), which is phosphorylated in resting cells and is not affected by growth factor stimulation. The phosphorylation of PDK1 in S241 is catalyzed by an autophosphorylation reaction in trans.³⁵ PDK1 kinase activity is therefore constitutively active, and the regulation of PDK1-activated signaling involves different mechanisms. The first mechanism was discovered investigating the steps involved in Akt T-loop phosphorylation in living cells. PDK1 is localized at the plasma membrane due to the interaction of its PH domain with the phosphoinositides PtdIns(3,4,5)P₃, phosphatidylinositol-3,4-bisphosphate [PtdIns(3,4)P₂], and PtdIns(4,5)P₂, with the highest affinity toward the PI3K lipid products.^{33,36} Although PDK1 membrane localization has been largely investigated and the affinity of the PDK1-PH domain for the PI3K products suggested a potential PI3K-dependent PDK1 membrane translocation, this localization is still controversial. Indeed, the jury is still out on whether PDK1 translocates to the plasma membrane following growth factor stimulation, or it is constitutively localized to the plasma membrane. Nevertheless, it is well established that PDK1 membrane localization is essential for Akt phosphorylation in Thr308 (Figure 2). PDK1 is constitutively associated in a homodimeric complex through PH domain interaction of two PDK1 monomers, and this interaction is important in the regulation of Akt phosphorylation.³⁷

Many other kinases are known to be downstream of PDK1 and are attracting an increasing interest. Among these, serum glucocorticoid-dependent kinase (SGK), p70 ribosomal protein S6 kinases (S6K), p90 ribosomal protein S6 kinase (RSK), and atypical PKC isoforms are known to be direct targets of PDK1, which phosphorylates specific serine/

threonine residues of their activation loop (Figure 2).³⁸ The mechanism of activation of these kinases differs from the Akt activation mechanism. PDK1 possesses a hydrophobic pocket, which is termed PDK1 interacting fragment pocket, and is essential for PDK1 interaction with the hydrophobic motif of the targeted protein kinases. Mutations within the PDK1 interacting fragment pocket abolish the binding of their subsequent phosphorylation and activation of PDK1 to PKC, S6K, and SGK1. The physiological role of PDK1 has been investigated in vivo in yeast, *Drosophila melanogaster*, and mice. These studies have shown that deletion of PDK1 is lethal, indicating that PDK1 is required for normal embryo development. PDK1^{-/-} mice lack branchial arches, and have problems in neural crest specification and forebrain development, as well as several disruptions in the development of a functional circulatory system, which eventually causes death at the E9.5 embryonic stage. In order to study the role of PDK1 in development, hypomorphic mice for PDK1 were generated, in which the neomycin resistance gene is inserted between exons 2 and 3 of the *PDK1* gene in order to reduce the expression of PDK1 by 80%–90% in all tissues.

These mice showed a decreased body size of 40%–50% compared to the wild type littermate, but no significant differences in the activation of AKT, S6K, and RSK were induced by insulin. Analysis of organs revealed that the difference in size is due to a decreased cell size rather than a reduction in cell number.

Specific function of PI3K/PDK1 in cancer

The PI3K pathway is one of the most frequently deregulated pathways in human malignancy; indeed, there are a variety of genetic abnormalities observed in this pathway in cancer, including activating and deactivating mutations, copy number changes, and posttranscriptional epigenetic irregularities. Among these, deactivating mutations in the gene encoding the tumor suppressor, PTEN, are among the most common.

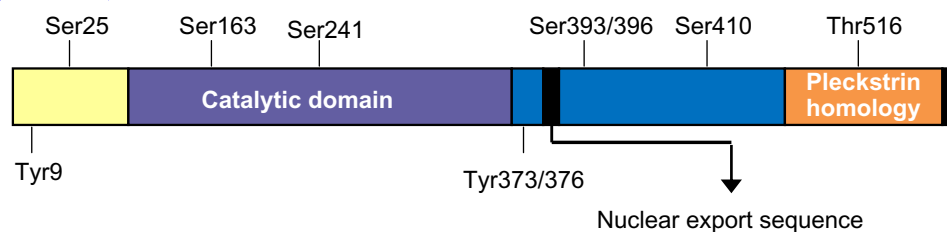


Figure 1 Schematic representation of PDK1 structure.

Abbreviations: PDK1, 3-phosphoinositide-dependent protein kinase-I; Thr, threonine; Tyr, tyrosine; Ser, serine.

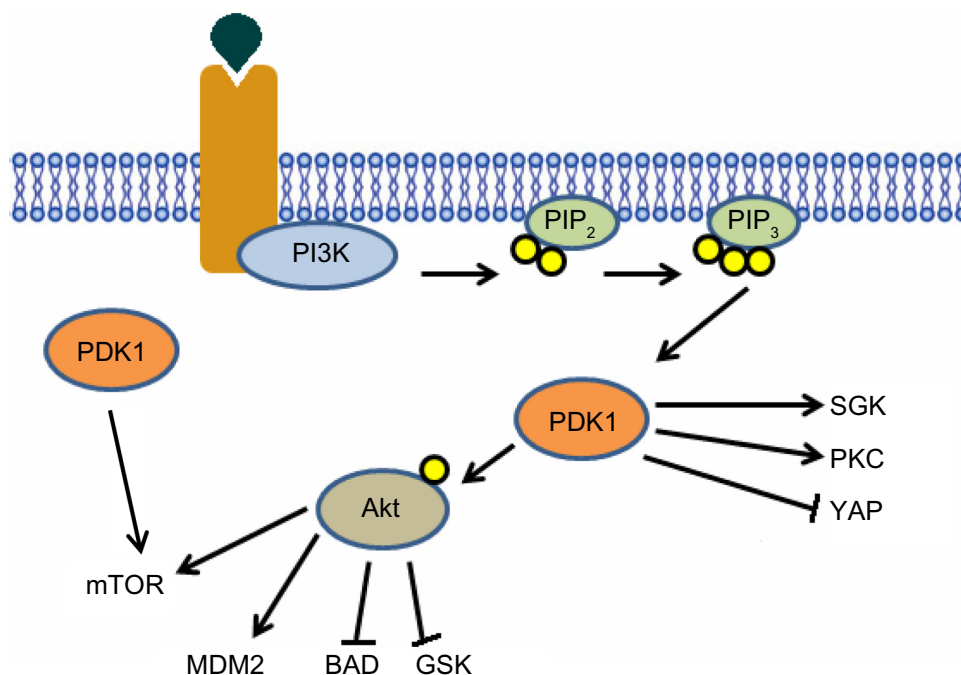


Figure 2 Activated receptor provides a docking site for PI3K, once bound to the receptor PI3K becomes active and phosphorylates PIP₂, forming PIP₃. PIP₃ acts as a membranous second messenger, providing a docking site for downstream proteins such as Akt and PDK1. Upon binding PIP₃, Akt undergoes a conformational change, facilitating PDK1-dependent phosphorylation of Akt at threonine 308. Once phosphorylated, Akt becomes active and dissociates from the membrane and phosphorylates a variety of downstream targets involved in growth and survival pathways, such as mTOR, MDM2 and BAD. PDK1 is also known to phosphorylate a variety of other downstream proteins, which have also been implicated in cancer cell signaling, such as SGK and YAP. There is also evidence to suggest PI3K-independent PDK1-dependent activation of mTOR.

Abbreviations: PDK1, 3-phosphoinositide-dependent protein kinase-1; PI3K, phosphoinositide-3-kinase; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol (3,4,5)-trisphosphate; SGK, serum glucocorticoid-dependent kinase; PKC, protein kinase C; YAP, Yes-associated protein kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; MDM2, mouse double minute 2 homologue; BAD, Bcl-2-associated death promoter; GSK, glycogen synthase kinase.

Evidence for the role of PTEN as a tumor suppressor was first suggested in 1997, where it was seen that *PTEN* was frequently mutated in patients with the cancer predisposition syndrome, Cowden disease.^{39,40} Studies in *PTEN* knockout mice further confirmed PTEN as an important tumor suppressor in a number of tissue types including 38% of endometrial, 14% of prostate, and 20% of central nervous system cancers.^{41,42} This, together with reports of homozygous deletion of the PTEN allele in cancer and suggestions of epigenetic and micro ribonucleic acid-based mechanism of regulation, have resulted in PTEN being considered one of the most frequently deregulated tumor suppressors in human malignancy.¹⁷ Similarly important, *PIK3CA* – the gene encoding the p110 α catalytic subunit – is frequently mutated in human cancer. Indeed, the PI3K sequencing of human tumor tissues samples revealed *PIK3CA* somatic mutations in different cancer types such as lung (4%), breast (8%), gastric (25%), brain (27%), and colon (32%).^{19,21} Over 90 different somatic mutations have been identified in *PIK3CA*,⁹ with 47% of these found in the helical domain and 33% found in the kinase domain, indicating that these mutations increase p110 α lipid kinase activity. Statistical analysis has since revealed three “hot spot” *PIK3CA* mutations: *H1047R*, *E542K*, and *E545K*. To date, *PIK3CA*-activating

mutations have been observed in a variety of other cancers such as endometrial cancer, head and neck cancer, ovarian cancer, bladder cancer, and skin cancer.^{18,43} In addition, p110 α is not the only isoform known to contain mutations; mutations have been found in all PI3K isoforms, although their prevalence and functional relevance in disease is considered limited. In particular, *PIK3CD* was seen to be mutated in 6.5% of human neuroblastoma tumor samples compared to 0% in normal tissue, and significant recurrent mutations are also seen in both *PIK3CG* (9.7%) and *PIK3C2B* (12.9%) in lung cancer.^{44,45} There is also evidence to suggest that mutations to the PI3K class 1 regulatory subunit p85 α may play a key role in tumorigenicity; mutations have been found in the p85 α SH2 domain, which diminish regulatory function of p85 α on p110 α , thereby increasing p110 α activity.

While less frequently mutated, evidence is beginning to accumulate to suggest a role for the other class 1 PI3K isoforms in cancer. In particular, we reported that p110 γ is over-expressed in both human pancreatic ductal adenocarcinoma and human hepatocellular carcinoma, where it regulates cell proliferation.^{25,46} In addition, more recent data indicated that upregulated p110 γ conveys the metastatic signal initiated by G-protein coupled receptors in breast cancer cells.⁴⁷

Expression of physiological levels of p110 β , p110 δ , and p110 γ isoforms in chicken embryo fibroblasts was sufficient to induce oncogenic transformation, whereas the induction of physiological levels of p110 α did not induce transformation.⁴⁸ These data in particular indicate that a variety of class 1 isoforms may be involved in tumorigenesis and tumor maintenance in a cancer-specific setting.

The first major indication that PDK1 itself may be a viable target in cancer came in 2005 when Bayascas et al⁴⁹ generated transgenic mice that were hypomorphic for PDK1. These mice were crossed with tumorigenic heterozygous PTEN^{+/-} mice, and data showed that the prevalence of tumor development was reduced in mice with deficient PDK1 levels.⁴⁹ Since then, various groups have investigated the role of PDK1 in a variety of different cancers, where in particular PDK1 is seen to play a key role in the development of breast cancer, as will be discussed in more detail. Increased levels of PDK1 expression have been reported in 45% of patients with acute myeloid leukemia, and PDK1 has also been suggested as a viable target in head and neck cancer, multiple myeloma, pancreatic cancer, and colorectal cancer.⁵⁰⁻⁵⁴ With regards to the latter, Tan et al⁵⁴ observed that PDK1 induced resistance to rapamycin inhibition via Myc activation; this pathway was seen to be negatively regulated by the B55 β -associated protein phosphatase 2A complex. While the mechanism of Myc activation was seen to be PDK1-dependent, it was also suggested to be PI3K/Akt independent, thereby adding to the evidence that in a cancer-specific manner, PDK1 may be a viable target that can act independently of Akt.⁵⁴ Similarly, Vasudevan et al³² observed that a subset of *PIK3CA* mutated breast cancer cell lines displayed reduced dependence on Akt for tumorigenicity, but rather relied on PDK1-dependent activation of another AGC kinase and PDK1 target, SGK-3.

While there has been some doubt regarding the effectiveness of PDK1-directed inhibition on in vitro two-dimensional proliferation, both genetic ablation and pharmacological inhibition of PDK1 was sufficient to reduce soft agar colony formation of a panel of cancer cell lines, suggesting a role for PDK1 in anchorage-independent cell growth.^{55,56} Indeed, perhaps the most convincing evidence to suggest PDK1 as a potential therapeutic target in cancer has come from the investigation of the role of PDK1 in cell migration, invasion, and metastasis. In particular, PDK1 has been shown to play an essential role in the regulation of cellular migration in the context of PTEN deficiency.⁵⁷ The mechanistic role of PDK1 in the motility of cancer cells was investigated in 2008, when Pinner and Sahai⁵⁸ reported a PDK1-regulated Rho-associated, coiled-coil

containing protein kinase 1 (ROCK1)-dependent contraction of actin–myosin. Interestingly, ROCK1 activation was not dependent on PDK1 kinase activity, but activation involved the direct binding of PDK1 to ROCK1 at the plasma membrane.⁵⁸ This result was observed both on deformable gels and also in vivo, and further suggests that PDK1 may have important regulatory functions in cancer, which are distinct from Akt activation. This particular observation also suggests that PDK1 may have regulatory functions that are completely distinct from its kinase activity, relying rather on allosteric interactions.⁵⁸ In this respect, we recently demonstrated that PDK1 plays a role in phospholipase C (PLC) γ 1 activation in a mechanism which requires association of the two proteins.⁵⁹ This novel PDK1-PLC γ 1 pathway was shown to regulate the invasion of breast and melanoma cancer cell lines.⁵⁹

Overview of the evidence suggesting PDK1 as a potential target in breast cancer

As previously mentioned, perhaps the most compelling evidence to suggest PDK1 as a molecular target in human malignancy comes from investigations into the role of PDK1 in breast cancer. The *PDPK1* gene maps on chromosome 16 at 16p13.3. The 16p13.3 gain was found to be associated with poor survival of breast cancer patients.⁶⁰ A similar copy number gain of chromosome 16p13.3 was found in prostate cancer and lung cancer.⁶¹ Mapping of the focal 16p13.3 genomic gain has identified *PDPK1* as the driver of the gain. Indeed, phosphorylation of PDK1 at serine (SER)241 is frequently elevated in breast cancer, with concomitantly increased phosphorylation of downstream kinases, including Akt mTOR, p70S6K, S6, and signal transducer and activator of transcription (Stat)3.⁵⁵ Moderate to high levels of PDK1 phosphorylation were found in 86% of high-grade metastasized breast tumors. In addition, PDK1 protein and messenger ribonucleic acid were found to be overexpressed in a majority of human breast cancers, with 21% of tumors having five or more copies of the PDK1 encoding gene *PDPK1*.⁵⁵ This copy number variation was seen to correlate with upstream lesions in the PI3K pathway such as *PIK3CA* mutation, *ERBB2* amplification, and PTEN loss, and increased PDK1 expression was suggested to increase AKT activation.⁵⁵ Several groups have investigated the role of PDK1 in breast cancer in vitro, and both genetic ablation and pharmacological inhibition of PDK1 have proved to be critical for anchorage-independent growth, cellular proliferation, migration, and invasion.^{56,63,64} In

addition to these *in vitro* findings, the role of PDK1 *in vivo* has also been explored, in most instances using severe combined immunodeficient mice.⁶⁵ In these instances, genetic ablation of PDK1 reduced lung colonization of human breast cancer cells and also reduced growth of xenograft tumors. In addition, PDK1 overexpressing mammary epithelial cells readily formed invasive tumors when injected into the inferior mammary fat pad of severe combined immunodeficient mice.⁵⁵ Notably PDK1 overexpression has been found oncogenic only in the COMMA-1D murine mammary cell model. On the contrary, in breast-derived cell lines, PDK1 overexpression is not tumorigenic, but it is able to potentiate the oncogenic effects of upstream lesions.⁵⁵

The mechanistic function of PDK1 in breast cancer has also been investigated. PDK1 is integral to the PI3K/AKT pathway, as it phosphorylates the activating segment of AKT, a potent proto-oncogene involved in a variety of cellular functions such as proliferation and survival. With this, the role of PDK1-dependent AKT activation in breast cancer has been explored, where indeed both genetic ablation and pharmacological inhibition of PDK1 in breast cancer cells have been shown to reduce AKT Thr308 phosphorylation.^{64,65} Similarly, overexpression of PDK1 in the human breast epithelial cell line MCF10A potentiates AKT signaling, indeed suggesting that PDK1 does have important AKT-dependent activity in breast cancer.⁵⁵ However, as previously mentioned, while PDK1 is integral to the PI3K/AKT pathway, there is a growing indication that in specific cancer settings, PDK1 may have important functions that are AKT independent.⁶³ Results obtained with cancer cell lines and the involvement of PDK1 in resistance mechanisms to several anticancer drugs suggest that PDK1 regulates different oncogenic signaling pathways.^{71,73} Other suggested downstream targets of PDK1 in breast cancer include SGK-3 (which was required for tumorigenicity in a subset of *PIK3CA* mutated breast cancer cells), p70 ribosomal kinase (an AGC kinase that can be directly phosphorylated by PDK1), and PLC γ 1 (which directly binds to PDK1 and regulates cellular invasion in a PDK1-dependent manner.^{32,59,64}

Recently, it has been shown that PDK1 regulates anchorage-independent growth, resistance to and tumor formation in breast cancer cells not only harboring *PIK3CA* mutations, but also in the absence of these genetic alterations.⁵⁵ The effect of PDK1 inhibition using short hairpin ribonucleic acid or chemical inhibitors when apoptosis is induced by absence of anchorage, involves antiapoptotic signaling rather than mitogenic signaling.⁶³ This is in agreement with other studies reporting a specific role for PDK1 in

cell migration and invasion, but not in proliferation.⁵⁷ More recently, the PI3K/PDK1 pathway was also shown to regulate the Hippo pathway in the mammary epithelial cell line MCF10A, and as previously discussed, PDK1 was shown to regulate cancer cell motility by antagonizing inhibition of ROCK1 in highly invasive breast adenocarcinoma cells.^{58,66} Interestingly, a specific role for PDK1 downstream of mutant *KRAS* is emerging. It has been found that somatic cell knock-down of both *KRAS* G12V and oncogenic *PIK3CA* mutations in human breast epithelial cells results in cooperative activation of the PI3K and mitogen-activated protein (MAP) kinase pathways *in vitro* and *in vivo*.⁶⁷ This oncogene cooperativity seen with concurrent mutations in *KRAS* and *PIK3CA* is mediated by Ras/p110 α interaction and signaling through PDK1 that directly mediates p90RSK activity. Further evidence that PDK1 is a downstream effector of mutant *KRAS* has been recently found in models of pancreatic cancer.⁵³ Interestingly, MDA-MB-231 cells, carrying *KRAS* and *p53* mutations, are more sensitive to PDK1 inhibitors than breast cancer cells, such as T47D, harboring *PIK3CA* mutation.⁶³ Consistently, Akt inhibitors are not able to inhibit anchorage-independent growth of MDA-MB-231 cells, whereas they are highly effective in blocking T47D cell growth.

Interestingly, PDK1 seems to also be involved in the molecular mechanisms by which diurnal and circadian rhythms regulate cell proliferation in human breast cancer xenografts.⁶⁸ Indeed, tumor growth in nude rats bearing MCF7 breast tumors can be significantly accelerated by exposing the rats to light at night through a PDK1-dependent mechanism.

Molecular targeting of PDK1 in breast cancer

In order to evaluate the clinical potential of PDK1-directed therapy in breast cancer, it is important to determine whether pharmaceutical targeting of PDK1 can be achieved in a safe and specific manner. Indeed with the emerging role of PDK1 in cancer, there has been a plethora of PDK1 targeted molecular inhibitors entering production with varying specificity and structure.⁶⁹ The first generation of PDK1 inhibitors, reviewed in 2008 by Peifer and Alessi,⁷⁰ lacked high selectivity for PDK1. In recent years, a few highly selective PDK1 inhibitors have been described.^{69,70} The most notable include compounds able to bind the PDK1 active site disclosed by GlaxoSmithKline (Benford, UK), Pfizer (Pfizer, Inc, New York, NY, USA), Sunesis (Sunesis Pharmaceuticals, Inc., San Francisco, CA, USA), and Biogen Idec (Biogen Idec Inc., Weston, MA, USA). GlaxoSmithKline and Pfizer compounds bind to the active form of the PDK1 kinase, whereas the Sunesis compound,

perhaps the most selective PDK1 kinase inhibitor, binds to the inactive form of PDK1.⁶⁹ We have developed a selective compound (2-O-Bn-InsP₅, a derivative of the natural compound inositol 1,3,4,5,6-pentakisphosphate [InsP₅]), which possesses enhanced proapoptotic and antitumor activity compared to the parent molecule, InsP₅.⁷¹ Kinase profiling assays on almost 60 different kinases revealed a remarkable specificity of this compound for PDK1, inhibiting PDK1-dependent phosphorylation of Akt Thr308 *in vitro* and *in vivo*. These results are particularly important considering that 2-O-Bn-InsP₅ is among the first compounds to be confirmed as highly specific and also be well tolerated *in vivo*.⁷¹ Similarly, the GlaxoSmithKline compound GSK2334470 has recently been characterized as a highly specific adenosine triphosphate-competitive PDK1 inhibitor.⁷² Notably, this compound inhibited S6K and SGK more potently than AKT.⁷² While these compounds do show promise for potential therapeutic activity, there is currently a lack of *in vivo* characterization, which will be required for progression of these compounds into preclinical testing. The availability of these potent and selective PDK1 inhibitors provides the opportunity to test the pharmacological consequences of PDK1 inhibition. Growing evidence suggests that PDK1 is required for experimental tumor formation in a mechanism that could be PI3K- and Akt-independent. This suggests that the choice of tumor to be treated with a PDK1 inhibitor should not be based solely on the basis of PI3K pathway deregulation. Taking into consideration the data suggesting that PDK1 plays a specific role in cell migration and invasion in breast cancer cells, the best models to test the efficacy of PDK1 inhibitors are certainly the metastatic models.

Combination therapy using PDK1 inhibitors with chemotherapy and other targeted therapies

Acquired drug resistance represents the major problem in the clinical success of targeted therapy. There are indications that PDK1 inhibition represents a valuable tool for tackling drug resistance in breast cancer. We have recently showed that 2-O-Bn-InsP₅ increases the effect of tamoxifen and paclitaxel in MCF7 and MDA-MB-468 human breast cancer cell lines, respectively.⁷¹ This is consistent with the reported role of PDK1 inhibition in tamoxifen sensitization.⁷³ Many lines of evidence indicate that the components of the PI3K pathway may decisively contribute to the resistant phenotype, and therefore it is a major target for anticancer drug development.⁷⁴ Crosstalk between mTOR and PI3K/Akt signaling pathways has been recently identified in clinical settings.⁷⁵

This has prompted the development of a novel targeted strategy aiming at the combination of mTOR and PI3K inhibitors. Interestingly, mTOR inhibitors, such as everolimus and temsirolimus, have recently attracted strong interest due to a reported clinical success combined with endocrine therapies in patients with metastatic breast cancer.⁷⁶ In addition, several clinical trials are currently evaluating the activity of mTOR, PI3K, Akt, and dual PI3K/mTOR inhibitors in combination with various targeted agents.^{75,76} Despite some early promising results, toxicity associated with these targeted therapies, especially mTOR inhibitors, represents a major problem for breast cancer patients. Therefore, novel combinations and agents are being actively investigated. Recent data from Najafov et al⁷⁷ demonstrate that Akt Ser(473) phosphorylation using mTOR inhibitors sensitizes Akt to PDK1 inhibitors.⁷⁷ Consequently, a combination of PDK1 and mTOR inhibitors reduced Akt activation to below basal levels and markedly inhibited proliferation of the different cell lines being tested, including breast cancer cells. This result marks the utility of combining PDK1 and mTOR inhibitors as a therapeutic strategy for the treatment of cancers that harbor mutations that elevate Akt activity. Interestingly, even though 2-O-Bn-InsP₅ is highly selective for PDK1 (inhibitory concentration 50 = 26 nM) it possesses weaker but promising activity toward mTOR (inhibitory concentration 50 = 1.3 micro-M). Therefore, 2-O-Bn-InsP₅ may represent the lead compound in developing novel mTOR/PDK1 dual inhibitors. Studies in transgenic mice have also shown that PDK1 and other kinases in the PI3K pathway, such as p110 α , Akt, and mTOR, are important for protecting the heart from ischemia reperfusion, and from aortic stenosis-induced cardiac dysfunction.⁷⁸ This may suggest that cancer therapies targeting this pathway will inevitably have a significant impact on the heart. Nevertheless, it should be noted that these results are confined to mouse models and not to clinical trials. Finally, it is worth stating that the existence of PDK1 pathways that can be PI3K- and Akt-independent is emerging and therefore targeting PDK1 has this unique feature when compared to PI3K/Akt targeting.^{32,79} This conclusion is further supported by the ability of PDK1 inhibition, but not PI3K and Akt, to sensitize therapeutic response of rapamycin in colorectal cancer cells, as discussed above.⁵⁴

Conclusion

Emerging evidence indicates that PDK1 plays a critical role in the context of PI3K activation. Indeed, PDK1 has been found to be overexpressed and hyperactivated in several cancers including breast carcinomas. The recently discovered

potent and selective, small molecule PDK1 inhibitors provide a unique opportunity to exploit the consequences of PDK1 inhibition in cancer therapy. Key issues that remain to be addressed before proceeding down the long road that leads to clinical testing include the advantage of PDK1 targeting over specific PI3K inhibition, and which particular clinical settings will benefit from specific PDK1 inhibition. Accumulating evidence suggests that PDK1 plays a specific and distinct role from the canonical PI3K/Akt pathway, and that PDK1 may specifically activate signal propagation in tumor progression, as well as in cell migration and invasion. Therefore, breast cancer progression and metastasis represents a major challenge for the future development of PDK1 inhibitors.

Disclosure

The authors report no conflicts of interest in this work.

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