IN THE SPOTLIGHT

New Connections between Old Pathways: PDK1 Signaling Promotes Cellular Transformation through PLK1-Dependent MYC Stabilization

John T. Cunningham and Davide Ruggero

Summary: Limited understanding of the functional link between multiple oncogenic pathways is a major barrier in the ongoing effort of cancer biologists to design an effective therapeutic approach to treat malignancies characterized by driver oncogenic network signals. In this issue of *Cancer Discovery*, Tan and colleagues elucidate a novel PDK1–PLK1–MYC signaling pathway connecting two fundamental oncogenic programs, phosphoinositide 3-kinase and MYC. They define the functional role for PDK1–PLK1–MYC signaling in cancer cell survival and tumor formation and show the therapeutic benefit of inhibiting PDK1 and PLK1 pharmacologically in cancer, tackling the most undruggable tumors defined by elevated levels of the MYC oncoprotein. *Cancer Discov; 3(10); 1099–1102. ©2013 AACR.*

See related article by Tan et al., p. 1156 (3).

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The phosphoinositide 3-kinase (PI3K) signaling pathway is one of the most frequently deregulated pathways in human cancers. Genetic lesions that result in increased PI3K pathway signaling, such as deletion of the *PTEN* gene (encoding the phosphatase and tensin homolog), activating mutations in the catalytic subunit of PI3K, or genomic amplifications of upstream receptor tyrosine kinases, are a hallmark of a staggering number of malignancies emanating from myriad tissue types (1). Given the central role of PI3K signaling as an oncogenic driver of tumorigenesis, there is a significant ongoing effort to generate and use inhibitors of PI3K pathway components for the treatment of various human cancers. Indeed, a host of these kinase inhibitors that target key pathway components such as PI3K, AKT, and mTOR are in various stages of clinical development (2). However, to date, the efficacy of these agents in treating human cancers has been limited, perhaps in part due to the presence of multiple feedback loops and/or cross-talk with alternative oncogenic signaling pathways. Therefore, a more complete characterization of the interconnections between PI3K pathway components and different oncogenic pathways is necessary to identify underlying mechanisms of tumor formation that may be exploited therapeutically. The discovery of these critical nodes that lie at the intersection of PI3K pathway components and other oncogenes will be vital to understanding the precise mechanisms governing sensitivity and resistance to PI3K pathway inhibition. Translating these key findings into novel therapeutic strategies in the clinic will significantly improve patient outcomes.

In this regard, a new study by Tan and colleagues (3) sheds light on this outstanding aspect of cancer biology by illuminating a novel connection between the PI3K pathway and the *MYC* oncogene. Specifically, in the current issue of *Cancer Discovery*, Tan and colleagues (3) provide important findings that elucidate a role for one of the most critical components of the PI3K signaling network in tumorigenesis, 3-phosphoinositide–dependent protein kinase-1 (PDK1). PDK1 lies directly downstream of PI3K by virtue of its activation and membrane recruitment by the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP_3), and serves as a master regulator of the AGC family of protein kinases, which includes AKT, S6K1, SGK, and PKC. Currently, AKT is considered the main effector of PDK1 in cancer. However, recent studies suggest that oncogenic functions of PDK1 may be mediated through the activities of other substrates. For example, Vasudevan and colleagues (4) showed that many *PIK3CA*-mutant cancer cell lines and human breast tumors display minimal AKT activation and diminished reliance on AKT activity for anchorage-independent cell growth. In this context, their findings suggest that the robust activation of PDK1 signaling in these cells engages a signaling cascade that relies on PDK1-dependent SGK3 phosphorylation to sustain cancer cell viability. These findings point toward a more nuanced view of PDK1 signaling in cancer that relies on substrates other than AKT. However, the mechanism by which PDK1 signaling interfaces with other oncogenic pathways to drive cancer formation remains an open question.

Tan and colleagues (3) address this outstanding question by investigating how hyperactive PDK1 functionally intersects with MYC oncogenic activity. Using a cell culture model, they observed that upon induced PDK1 overexpression, transformed cells displayed significantly increased levels of the MYC oncoprotein. Interestingly, manipulation of upstream

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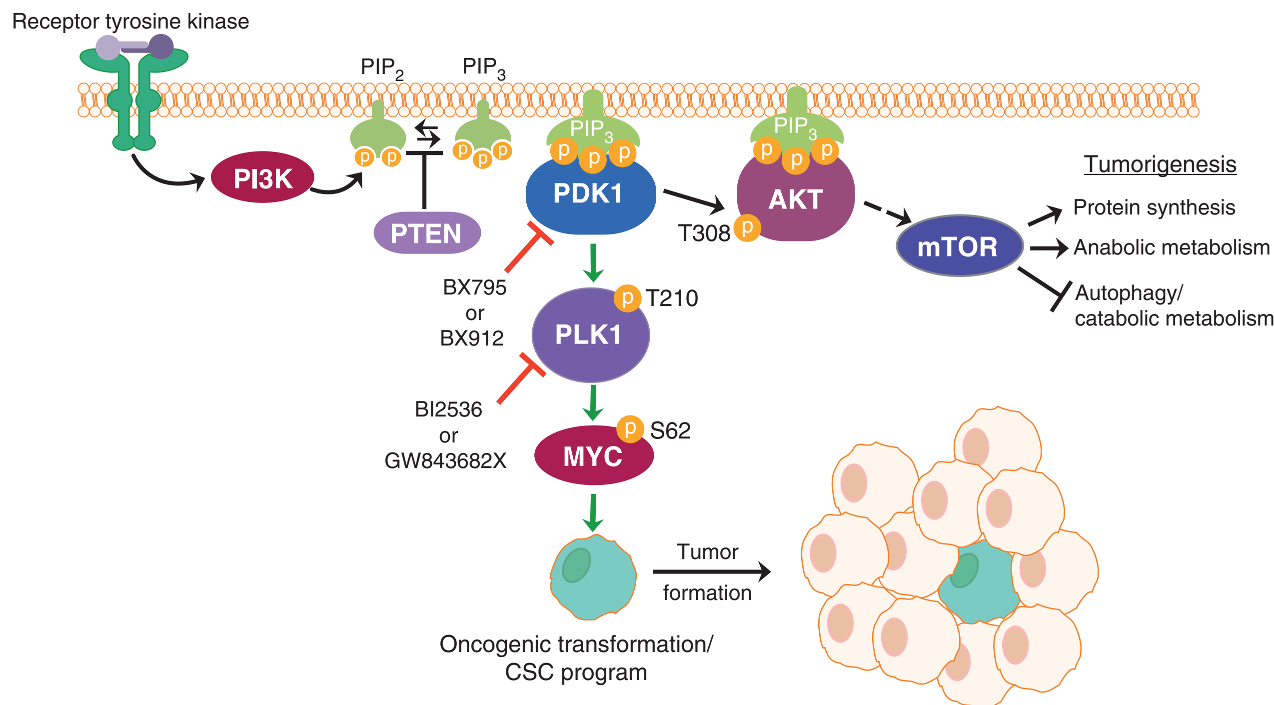

Figure 1. A basic depiction of the classical view of the PI3K signaling network (black arrows) as well as the novel signaling pathway revealed in the study by Tan and colleagues (green arrows; ref. 3). Tumors with hyperactive PDK1 signaling, in addition to signaling through the classical downstream effector AKT, induce a novel signaling pathway through phosphorylation of PLK1. Phosphorylation and activation of PLK1 results in phosphorylation of serine 62 of MYC, leading to stabilization of MYC protein, which promotes cellular transformation, in part through transcriptional upregulation of a cancer stem cell (CSC)-like gene signature. As reported by Tan and colleagues (3), therapeutic targeting of this novel signaling pathway with inhibitors of PDK1 (BX795 or BX912) or PLK1 (BI2536 or GW843682X) promotes programmed cell death of cancer cells that rely on MYC hyperactivation.

activation of PDK1 in the same cells, through expression of mutant PI3K or loss of function of PTEN, did not increase MYC levels. Although the reason activation of upstream signals that ultimately converge on PDK1 fails to increase MYC protein levels is unclear, one possible explanation may be that increased PIP_3 concentrations that are produced upon activation of PI3K are not sufficient to fully activate PDK1. This differential effect on MYC protein levels between PDK1- and PI3K-transformed cells provides evidence of a divergent signaling network downstream of PDK1 that promotes cellular transformation through the elevation of MYC protein levels. To identify critical components of the PDK1 signaling network required for cancer cell viability, the authors conducted an unbiased synthetic lethal screen using a panel of 60 small-molecule kinase inhibitors. The authors identified numerous compounds that showed preferential decreases in viability of PDK1-transformed cells versus vector-transduced HEK cells, including inhibitors of MAP–ERK kinase (PD0325901), ALK (NVP-TAE684), BCR–ABL (PD180970), and tyrosine kinases (sunitinib). Of particular interest, the authors identified two inhibitors of PLK1 (BI2536 and GW843682X) that revealed a previously unappreciated role for PLK1 in PDK1-dependent cellular transformation, which they chose to further explore.

PLK1 hyperactivation in cancer cells leads to deregulation of multiple steps during the G_2–M phase of the cell cycle, including mitotic entry, centrosome maturation, microtubule dynamics, spindle assembly checkpoint, and mitotic exit (5). However, results presented in the study by Tan and colleagues (3) suggest a new role for PLK1 in promoting cancer downstream of PDK1 hyperactivation that is independent of its role in cell-cycle progression. The authors showed that PDK1 directly phosphorylates and activates PLK1, which in turn directly phosphorylates serine 62 of MYC, leading to its stabilization (Fig. 1). MYC protein stability is controlled via a series of independent events involving sequential phosphorylations of serine 62 and threonine 58 that lie within a phosphodegron motif. Serine 62 phosphorylation interferes with, whereas threonine 58 phosphorylation promotes, the phosphodegron-mediated ubiquitylation and degradation of MYC by the FBW7 E3 ubiquitin ligase. Of functional significance, Wang and colleagues (6) recently generated mouse models that overexpress wild-type MYC or an alanine substitution mutant of MYC that specifically blocks its phosphodegron-mediated turnover. This study shows that the mutant MYC protein is more efficient at promoting breast cancer than the wild-type MYC counterpart (6). In addition, oncogenic lesions that promote MYC stability, such as inactivating missense mutations in the FBW7 E3 ubiquitin ligase, have been found in human tumors, highlighting the importance of this mode of regulating MYC levels in cancer (7). Furthermore, numerous immunohistologic studies have shown that many cancers display high levels of MYC protein in the absence of genomic amplification or translocation events at the *MYC* locus. For example, one study conducted in a cohort of patients with prostate cancer found MYC protein

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levels to be elevated in 70% of cases during the earliest stage of disease, prostate intraepithelial neoplasia, whereas the *MYC* gene was found to be amplified in fewer than 15% of tissues analyzed (8). Importantly, this study also showed that not all tissues with elevated levels of MYC protein displayed increases in *MYC* mRNA, suggesting a role for posttranscriptional regulation of MYC in prostate cancer. It would be interesting to evaluate whether the PLK1–MYC axis acts as a mechanism to promote tumorigenesis in tumors harboring *PDK1* amplification, such as breast cancer. Despite Tan and colleagues' (3) findings that upregulation of MYC levels does not occur in response to overexpression of activated PI3K or loss of PTEN function, it is important to emphasize that genomic alterations of these two components of the PI3K signaling pathway are often observed in cancers concomitant with amplifications or translocations in the *MYC* locus. In addition, several recent studies have shown cooperation between PI3K pathway activation and MYC overexpression in mouse models of lymphoma and prostate cancer, suggestive of a synergistic relationship between these two pathways in tumor formation (9, 10). However, to what extent PDK1–PLK1–MYC signaling could provide a mechanistic basis to explain the oncogenic cooperation between the PI3K pathway and MYC remains unknown.

The identification of the PDK1–PLK1–MYC signaling axis raised several important questions that the authors sought to address: (i) Is the mechanism of cellular transformation by PDK1 overexpression a direct consequence of PLK1-dependent elevation in MYC levels? (ii) Does the PDK1–PLK1–MYC pathway affect a specific subset of cancer cells by modulating a distinct gene expression signature? and (iii) Which human cancers depend on the PDK1–PLK1–MYC pathway? The authors provide some clues that begin to address these outstanding questions about the role of PDK1–PLK1–MYC signaling in cancer development. Using genetic and pharmacologic approaches to inhibit PLK1, they first showed that PLK1-dependent stabilization of MYC is required for the survival of various cancer cell lines characterized by PDK1 hyperactivation, suggesting that cancers with hyperactive PDK1 signaling or genomic amplifications of the *PDK1* gene may be susceptible to therapies targeting the PDK1–PLK1–MYC axis. Reciprocally, the authors showed that PDK1 inhibition was able to reduce cell viability in a panel of MYC-dependent human breast cancer cell lines, indicating a functional relationship between PDK1 and MYC in cancer cell survival. To address whether PDK1–PLK1–MYC signaling affects a particular subset of cells, the authors used sphere-forming assays and limiting dilution experiments, showing that PDK1- and MYC-transformed cells possessed an innate self-renewal capacity resembling that of cancer stem cells (CSC). Furthermore, to identify the classes of genes underlying CSC features downstream of PDK1 and MYC hyperactivation, they performed gene expression profiling and discovered that these cancer cells are enriched in genes involving embryonic stem cell and CSC function, such as *SOX2*, *LIN28B*, *SALL4*, and *EZH2*, and *EPCAM*, *ALDH1A*, and *S100A4*, respectively. Importantly, they also showed that the expression of these genes is enriched in tumors from patients with lung, colon, and aggressive breast cancers. The authors then showed that pharmacologic inhibition of PDK1 or PLK1 signaling decreased the survival of a CSC-like population of cells, characterized by $CD44^{+}/CD24^{-/low}$ marker expression present in MDA-MB-231 cells, an aggressive metastatic human breast cancer cell line. Pharmacologic inhibition of PDK1 or PLK1 markedly reduced tumorsphere formation, which recapitulates *in vitro* the development of human breast cancer tumors. These findings suggest that PDK1 or PLK1 inhibition may lead to the selective elimination of CSCs, which represent a therapeutically resistant reservoir of cancer cells known to be capable of tumor regeneration. Although further experiments are required to fully characterize the role of PDK1–PLK1–MYC signaling in human cancers, this study provides a foundational road map for the treatment of several aggressive cancers using either PDK1- or PLK1-directed therapies. Notably, at least six PLK1 inhibitors have already completed phase I clinical trials, and several PDK1 inhibitors have progressed from the preclinical phase into phase I clinical trials. Even if PDK1 or PLK1 inhibitor monotherapy proves to have limited effectiveness in treating cancer, the current study suggests that a combinatorial approach to treating PI3K-driven cancers using PDK1 or PLK1 inhibitors in conjunction with mTOR inhibitors may be efficacious. Indeed, the authors showed that colon cancer cells that upregulate the PDK1–PLK1–MYC signal as a mechanism of resistance in response to mTOR inhibition are rendered sensitive to combined treatment with BEZ235 (dual PI3K/mTOR inhibitor) and BI2536 (PLK1 inhibitor). In addition, this study suggests a novel alternative means to therapeutically target the currently undruggable *MYC* oncogene that could be relevant for a broad spectrum of human cancers.

Given the critical role of MYC overexpression in promoting a multitude of human cancers, inhibiting the function of this protein is of utmost clinical importance. Multiple approaches are currently being used to target both the *MYC* oncogene directly, as well as specific cellular/molecular targets of MYC that contribute to tumor formation upon MYC hyperactivation. For example, in the first scenario, the small-molecule bromodomain inhibitor JQ1, which decreases expression of *MYC*, has shown some efficacy in inhibiting cancer cell viability in culture as well as *in vivo* in xenograft and genetically engineered mouse models of MYC-driven hematologic malignancies such as multiple myeloma (11–13). However, some evidence suggests that JQ1 treatment may not reduce MYC expression below the threshold required to have an effect on viability in some cancer cells (13). In addition, the human proteome encompasses more than 40 bromodomain-containing proteins and, therefore, use of inhibitors that nonselectively target this functional class may pose significant off-target risks to patients. Implementing inhibitors of PDK1 or PLK1, as supported by this work from Tan and colleagues (3), may provide a potent new strategy for directly reducing MYC protein levels. Alternatively, in addition to its role in regulating transcription, the ability of MYC to direct ribosome biogenesis and translation control has been shown to be an important driver of MYC-mediated tumorigenesis, representing a powerful approach to limiting the oncogenic potential of MYC (14–16). In addition, MYC-dependent protein synthesis triggers a coping mechanism, referred to as the unfolded protein response, that plays a prosurvival role that, when inhibited, confers synthetic lethality to MYC-overexpressing cells (17). Taken together, combined efforts are necessary to target the multifaceted oncogenic programs that characterize cancer cells.

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In this respect, the study from Tan and colleagues (3) provides a new valuable tool to add to the arsenal in the ongoing strategy to render the currently "undruggable" oncogene *MYC* druggable.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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