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<td>doi:10.12703/P7-13</td>
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The phosphoinositide 3-kinase pathway and therapy resistance in cancer

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F1000Prime Reports 2015, 7:13 (doi:10.12703/P7-13)

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Abstract
The phosphoinositide 3-kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) signaling network is a master regulator of processes that contribute to tumorigenesis and tumor maintenance. The PI3K pathway also plays a critical role in driving resistance to diverse anti-cancer therapies. This review article focuses on mechanisms by which the PI3K pathway contributes to therapy resistance in cancer, and highlights potential combination therapy strategies to circumvent resistance driven by PI3K signaling. In addition, resistance mechanisms that limit the clinical efficacy of small molecule inhibitors of the PI3K pathway are discussed.

Introduction
The PI3K/Akt/mTOR signaling pathway
Signaling via the PI3K/Akt/mTOR pathway has been the subject of a number of in-depth reviews and will only be briefly summarized here. In response to the activation of receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs), the lipid kinase PI3K phosphorylates phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂) to synthesize the second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP₃). PIP₃ recruits Akt to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK-1) and mTOR complex 2 (mTORC2). Activated Akt subsequently phosphorylates numerous substrates that promote tumorigenesis, including tuberous sclerosis complex 2 (TSC2), which in turn activates mTOR complex 1 (mTORC1). Signal termination of the PI3K/Akt/mTOR pathway is primarily accomplished by the tumor suppressor phosphatase and tensin homolog (PTEN), which catalyzes the dephosphorylation of PIP₃ back to PI(4,5)P₂.

The PI3K pathway in cancer
Dysregulated signaling through the PI3K pathway is implicated in virtually all human cancers. Amplification and gain-of-function mutations of the PIK3CA gene encoding the catalytic p110α subunit of PI3K are extremely prevalent in cancer, and promote increased signaling through the PI3K pathway. Indeed, PIK3CA is one of the most frequently mutated oncogenes in human tumors [1-4]. Loss-of-function mutations, deletion, and decreased expression levels of PTEN are also frequently observed in human tumors [5]. Even in the absence of alterations in PI3K or PTEN, disruption of the PI3K pathway can occur as a consequence of a multitude of lesions, including amplification or mutation of upstream RTKs, Akt amplification or mutation [6], and loss of inositol polyphosphate-4-phosphatase (INPP4B) that converts phosphatidylinositol (3,4)-bisphosphate (PI[3,4]P₂) to PI(3)P [7].

Signaling through the PI3K/Akt/mTOR pathway contributes to tumorigenesis through effects on a myriad of cellular processes, including apoptosis, proliferation, motility, and metabolism. The PI3K pathway is now also recognized as a major driver of resistance to diverse anti-cancer therapeutics. The frequency of PI3K/Akt/mTOR hyperactivation in cancer, coupled with the critical role of PI3K signaling in tumorigenesis and anti-cancer therapy
resistance, has culminated in significant efforts to generate small molecule inhibitors to target this pathway.

**Contribution of the PI3K pathway to therapy resistance**

**The PI3K pathway and resistance to chemotherapy**

Despite significant advances in the development of molecularly targeted therapies, traditional cytotoxic chemotherapy drugs remain an integral component of treatment regimens for most types of cancer. However, the clinical efficacy of diverse chemotherapy agents is undermined by resistance mechanisms. The PI3K pathway has been recognized for some time as a major contributor to chemotherapy resistance, with activation of the pathway being associated with reduced sensitivity to chemotherapy drugs including the topoisomerase II inhibitor doxorubicin and the mitotic inhibitor paclitaxel [8-10]. The mechanisms that contribute to chemotherapy resistance downstream of PI3K signaling are diverse; however, the elevated expression of anti-apoptotic proteins, including myeloid cell leukemia 1 (Mcl-1), is frequently observed [11,12]. It has also become apparent that DNA damage triggered by some chemotherapy drugs, including doxorubicin, can trigger the phosphorylation of Akt to promote survival and therapy resistance [13-15]. The critical role of the PI3K pathway in chemotherapy resistance has been confirmed by studies showing significant synergy between PI3K pathway inhibitors and chemotherapy in preclinical models [15,16]. Clinical trials examining the efficacy of combination treatment with chemotherapy and PI3K pathway inhibitors are in progress (https://clinicaltrials.gov).

**The PI3K pathway and resistance to endocrine therapy**

Endocrine therapies, which interfere with estrogen receptor or androgen receptor signaling, are frequently used in the management of breast and prostate cancers. Hyper-activation of the PI3K pathway has been associated with resistance to endocrine therapies, including the estrogen receptor antagonist tamoxifen [8,17]. Furthermore, breast and prostate cancer cells that have adapted to endocrine therapy rely on PI3K signaling for survival, and preclinical studies indicate that inhibition of the PI3K pathway can circumvent resistance to endocrine therapy [17,18]. The phase III BOLERO-2 trial highlighted the contribution of PI3K signaling to endocrine resistance. Resistance to PI3K inhibition in breast cancer [20]. While breast cancer patient data have primarily associated the activation of the PI3K pathway with poor outcome following endocrine therapy, it should be noted that mutations in PIK3CA have been associated with favorable prognosis in several studies [21-23]. These apparently contradictory findings are suggestive of a dual role for the PI3K pathway in estrogen receptor-positive breast cancer. Indeed, Mayer and Arteaga hypothesize that, in early estrogen receptor-positive breast cancers, PIK3CA mutations may be a marker of highly hormone-dependent, indolent tumors, whereas in late estrogen receptor-positive breast cancers (selected by primary endocrine therapy), PIK3CA mutations provide a mechanism of endocrine therapy resistance, and are therefore associated with poor outcome [24].

**The PI3K pathway and resistance to RTK inhibitors**

Overexpression or mutational activation of RTKs is frequently observed in cancer and thus has rendered RTKs important therapeutic targets for cancer therapy. PI3K pathway activity has been shown to predict a response to RTK inhibitors, and to contribute to resistance to RTK inhibitors (including the epidermal growth factor receptor inhibitor gefitinib and the anti-HER2 antibody trastuzumab) [25-27]. Indeed, most models of acquired resistance to RTK inhibitors demonstrate persistent PI3K signaling. In some cancers, multiple RTKs drive the activation of the PI3K pathway, and these cancers are therefore resistant to RTK inhibitor monotherapy [28,29]. Combination therapy with agents targeting multiple RTKs, or RTKs in combination with PI3K pathway inhibitors, may circumvent RTK inhibitor resistance [30]. Indeed, early signs of clinical activity have recently been observed in a phase Ib study investigating combination therapy with the PI3K inhibitor NVP-BKM120 and trastuzumab in patients with HER2-positive advanced/metastatic breast cancer resistant to trastuzumab monotherapy [31].

**The PI3K pathway and resistance to agents targeting the MAPK pathway**

Aberrant signaling through the mitogen-activated protein kinase (MAPK) pathway plays a critical role in cancer development and progression, and significant effort has been made to develop MAPK pathway inhibitors. Extensive crosstalk exists between MAPK and PI3K signaling pathways and therefore, not surprisingly, enhanced PI3K signaling has been associated with BRAF inhibitor resistance in cell lines and human tumors [32]. Interestingly, the MEK inhibitor PD-0325901 has been proposed to enhance PI3K signaling by disrupting the membrane localization of PTEN [33]. Synergy between MAPK inhibitors and PI3K pathway inhibitors has been observed in many reports [32,34,35].
The PI3K pathway and resistance to anti-angiogenic therapy
Anti-angiogenic therapies target vessels that grow to provide oxygen and nutrients to actively proliferating tumors. The most established approach for disrupting tumor angiogenesis is the inhibition of vascular endothelial growth factor (VEGF) signaling. Upregulation of PI3K pathway activity, particularly mTOR signaling, has been observed in breast cancer xenografts exposed to the anti-VEGF-A antibody bevacizumab and, as a consequence, combination therapy with bevacizumab and the PI3K/mTOR inhibitor NVP-BEZ235 enhances anti-tumor effects in preclinical models [36]. In addition, a recent study has revealed that disruption of the interaction between Ras and the p110α subunit of PI3K can reduce tumor-induced angiogenesis, at least in part by inhibiting VEGF-A signaling [37].

The PI3K pathway and resistance to immunotherapy
In recent years, there has been an emerging interest in modulating the immune system for cancer therapy, and strategies that stimulate the immune system to recognize and attack cancer cells have been developed. The ability of the PI3K pathway to mediate resistance to immunotherapy has been associated with the increased expression of anti-apoptotic proteins including Mcl-1 [38,39]. In addition, PI3K pathway hyperactivity induced by loss of PTEN is associated with the elevated expression of programmed death-ligand 1 (PD-L1), which plays a major role in suppressing the immune system [40,41]. There is some evidence that PI3K inhibitors can dramatically heighten the response to cancer immunotherapy [42] and a recent study demonstrated that Pl-3065, a small molecule inhibitor of PI3Kδ, disrupts tumor-induced immune tolerance and promotes anti-tumor immunity [43].

The PI3K pathway and resistance to hedgehog pathway inhibitors
Aberrant activation of the hedgehog pathway is associated with cancer, especially basal cell carcinoma and medulloblastoma. The hedgehog pathway is activated by the binding of the hedgehog ligand to the transmembrane receptor Patched, which prevents Patched from repressing the GPCR-like molecule Smoothened (SMO). A recent study demonstrated that PI3K target genes are enriched in mouse medulloblastoma tumors resistant to the SMO antagonist NVP-LDE225, and combination treatment with NVP-LDE225 and the PI3K inhibitor NVP-BKM120, or with NVP-LDE225 and the PI3K/mTOR inhibitor NVP-BEZ235, can suppress the emergence of drug resistance [44].

Resistance to PI3K pathway inhibitors
There are currently more than 30 drugs and five classes of PI3K/Akt/mTOR pathway inhibitors (PI3K inhibitors, PI3K/mTOR dual inhibitors, Akt inhibitors, mTORC catalytic inhibitors and rapamycin analogues) in clinical trials for cancer therapy [45]. Despite the enormous promise of targeting the PI3K pathway for cancer therapy, to date, the responses of solid tumors to PI3K pathway inhibitor monotherapy have been disappointing and drug resistance mechanisms have rapidly emerged. Broadly speaking, mechanisms of resistance to PI3K pathway inhibitors involve the incomplete inhibition of PI3K pathway activity, reactivation of the PI3K pathway, or activation of alternate pro-survival pathways (Figure 1).

Insufficient inhibition of the PI3K pathway promotes resistance
PI3K resistance mechanisms can arise through insufficient blockade of PI3K pathway activity. Indeed, it has been proposed that poor responses to PI3K inhibition in clinical trials could result from the inability to adequately inhibit PI3K signaling due to dose-limiting toxicities. For example, the concentrations of NVP-BKM120 required to fully inhibit PI3K activity are toxic, due to off-target effects on tubulin [46]. Studies have also revealed that considerable Akt and mTORC1 activity can be maintained despite inhibition of PI3K [47,48]. Therefore, simultaneous inhibition of PI3K and mTORC1, or of PI3K and CDK4/6-Cyclin D complex (which is regulated by mTORC1) has been shown to be highly efficacious in vitro and in xenograft models [47,48].

Reactivation of the PI3K pathway promotes resistance
PI3K pathway inhibitor resistance can arise via mechanisms that promote the reactivation of PI3K signaling. For example, inhibition of RTKs, PI3K or Akt induces the expression and activation of multiple RTKs by relieving FOXO-dependent activation of RTK expression [49-51]. Similarly, inhibition of mTOR induces PI3K signaling through the induction of RTKs [52]. Dual inhibition of PI3K and mTOR also increases activation of JAK2/STAT5 to promote the reactivation of Akt [53]. As a consequence, combination therapy with the PI3K/mTOR inhibitor NVP-BEZ235 and the JAK2 inhibitor NVP-BSK805 reduces tumor growth and enhances survival. Furthermore, the amplification of a mutant PIK3CA allele was observed in a breast cancer cell line chronically exposed to the PI3K inhibitor GDC-0941 [54]. In this study, PIK3CA amplification resulted in the significant upregulation of PI3K signaling and restored proliferative capacity at clinically relevant concentrations of GDC-0941. Whether or not PI3K copy number variation occurs in patients treated with PI3K inhibitors remains to be determined. Recently, resistance to the mTOR inhibitor everolimus was attributed to the acquisition of mTOR mutations that confer resistance to allosteric
More than 30 small molecule inhibitors targeting the phosphoinositide 3-kinase (PI3K) pathway are currently in clinical trials for cancer therapy. PI3K inhibition can promote anti-tumor responses, but drug resistance mechanisms frequently limit the efficacy of PI3K pathway inhibition. Mechanisms of resistance to the PI3K pathway inhibitors involve the incomplete inhibition of pathway activity, reactivation of the PI3K pathway or activation of alternative pro-survival pathways.

GF, growth factor; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PDK-1, phosphoinositide-dependent kinase-1; PIP$_3$, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; RTK, receptor tyrosine kinase.
mTOR inhibition [55,56]. Interestingly, the mTOR mutants identified in both studies retain sensitivity to mTOR kinase inhibitors.

Activation of alternate pro-survival pathways promotes resistance

Inhibition of the PI3K pathway frequently leads to enhanced signaling through complementary pro-survival signaling pathways. For example, the inhibition of the PI3K pathway activates the MAPK pathway and promotes MAPK dependency in mouse models of cancer and in human tumors [57,58]. Consequently, pharmacological inhibition of the MAPK pathway enhances the anti-tumor effects of PI3K pathway inhibition. Ribosomal S6-kinase (RSK) also promotes survival under conditions of PI3K/mTOR inhibition and RSK-specific inhibitors can overcome resistance phenotypes in patient-derived xenograft models [59]. In addition, mouse mammary tumors driven by mutant PIK3CA recur after the inactivation of the mutant kinase and are associated with the amplification of Myc, which promotes tumor survival independently of the PI3K pathway [60]. Indeed, Myc amplification has been shown to confer resistance to PI3K pathway inhibitors in multiple studies [61,62].

Additional strategies to circumvent resistance mechanisms driven by the PI3K pathway and enhance the clinical efficacy of PI3K pathway inhibitors

A number of combination therapy strategies have been identified in preclinical models of therapy resistance driven by the PI3K pathway, and additional combinations have been proposed. For example, an obvious approach to sensitize cancer cells to PI3K pathway inhibitors is to disrupt pathways that prevent cell death. Indeed, the combined inhibition of PI3K/mTOR and the anti-apoptotic protein Bcl-2 has been shown to prevent resistance to PI3K/mTOR inhibitors [63]. Another potential combination therapy approach relies on the contribution of the PI3K pathway in maintaining genomic stability, with recent studies demonstrating that PI3K inhibitors can increase DNA damage and sensitize cells to inhibitors of poly(ADP-ribose) polymerase (PARP), which plays a critical role in regulating the cellular response to DNA damage [64-66].

Conclusions

Therapy resistance is critical to tumor maintenance and severely limits cancer patient survival. As outlined here, the PI3K pathway has emerged as a major driver of resistance to diverse anti-cancer agents. PI3K pathway inhibitor combination therapy strategies have been devised to circumvent resistance driven by PI3K signaling and many show promise in both the pre-clinical and clinical setting. The therapeutic efficacy of numerous PI3K pathway inhibitors has also been limited by resistance mechanisms. It is therefore essential that mechanisms contributing to the ability of the PI3K pathway to promote therapy resistance, as well as mechanisms that drive PI3K pathway inhibitor resistance, continue to be elucidated. This information will enable rational combination therapies to be developed and will also identify biomarkers for patient selection.

Abbreviations

GPCR, G protein-coupled receptor; INPP4B, inositol polyphosphate-4-phosphatase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; PARP, poly (ADP-ribose) polymerase; PDK-1, phosphoinositide-dependent kinase-1; PD-L1, programmed death-ligand 1; PI(3,4)P_2, phosphatidylinositol (3,4)-bisphosphate; PI3K, phosphoinositide 3-kinase; PI3P, phosphatidylinositol 3-phosphate; PI(4,5)P_2, phosphatidylinositol (4,5)-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog; RSK, ribosomal S6 kinase; RTK, receptor tyrosine kinase; TSC2, tuberous sclerosis complex 2; VEGF, vascular endothelial growth factor.

Disclosures

The authors declare that they have no disclosures.

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F1000Prime Reports 2015, 7:13
http://f1000.com/prime/reports/b/7/13

(page number not for citation purposes)


