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The *PIK3CA* Gene as a Mutated Target for Cancer Therapy

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Abstract

The development of targeted therapies with true specificity for cancer relies upon exploiting differences between cancerous and normal cells. Genetic and genomic alterations including somatic mutations, translocations, and amplifications have served as recent examples of how such differences can be exploited as effective drug targets. Small molecule inhibitors and monoclonal antibodies directed against the protein products of these genetic anomalies have led to cancer therapies with high specificity and relatively low toxicity. Recently, our group and others have demonstrated that somatic mutations in the *PIK3CA* gene occur at high frequency in breast and other cancers. Moreover, the majority of mutations occur at three hotspots, making these ideal targets for therapeutic development. Here we review the literature on *PIK3CA* mutations in cancer, as well as existing data on *PIK3CA* inhibitors and inhibitors of downstream effectors for potential use as targeted cancer therapeutics.

Keywords

PIK3CA; mutation; oncogene; PI3 kinase; AKT; mTOR

INTRODUCTION

Insults to the genome are a hallmark of cancer and a driving force in carcinogenesis. These genomic alterations are an obvious difference between normal and cancerous cells, which provide an opportunity to exploit them as potential targets for therapeutics. Recently, successful clinical trials of imatinib, gefitinib/erlotinib, and trastuzumab, which are specific for *BCR-ABL* translocations [1], epidermal growth factor receptor (*EGFR*) mutations [2,3], and *HER2/neu* amplifications [4–6] respectively, have illustrated the ability to develop drugs that target genetic abnormalities and opened the possibility for future streamlined therapies based on the genomic landscape of an individual's cancer.

The phosphatidylinositol 3-kinase (PI3K) p110 α catalytic subunit, *PIK3CA*, is one the most highly mutated oncogenes in human cancers, and high mutational frequencies of *PIK3CA* have been reported in colorectal [7], breast [8] and liver cancers [9] while lower rates of mutation have been described in many other human malignancies including ovarian [10,11], lung [7,

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9], gastric [7,9,12,13], and brain cancers [7,9,14–21]. While a wide variety of *PIK3CA* mutations have been found, the vast majority of mutations occur in three hotspots, E542K, E545K, and H1047R, which will be the focus of this review (Figure 1). E542K and E545K are located within exon 9 in the helical domain of *PIK3CA* whereas H1047R is encoded by exon 20 within the kinase domain. Studying the effects of these mutations in colorectal cells [22–24], breast epithelial cells [25,26], and chicken embryos/fibroblasts [27,28] have illustrated a direct connection between these mutations and carcinogenesis. Through crystallographic and biochemical methods, it has been determined that the probable mechanism for the oncogenicity of the E545K mutation is the disruption of an inhibitory charge-charge interaction between *PIK3CA* and the N-terminal SH2 domain of the p85 regulatory subunit [29] (Figure 1). Additionally, it has been previously proposed that the oncogenic mechanism of the E542K mutation is a change in interaction with the p85 regulatory subunit, while the H1047R mutation increases binding affinity of *PIK3CA* for the negatively charged phosphatidylinositol substrate [30]. *PIK3CA* mutations have also been associated with paclitaxel resistance in breast epithelial cells [25], and PI3K signaling in general has been linked with resistance to a number of other cancer therapies. Clinically, the presence of *PIK3CA* mutations has been linked to both favorable [31,32] and unfavorable [33,34] patient prognosis, and it has also been reported that exon 9 mutations have a less favorable prognosis than exon 20 mutations in breast cancer [35]. The reasons for these conflicting data are not clear, but likely reflect limited sample sizes and difference in treatment regimens between the various studies.

TARGETING *PIK3CA* MUTATIONS

With the recent therapeutic successes of imatinib, erlotinib/gefitinib and trastuzumab, finding additional targeted therapies for high frequency oncogenic somatic genomic alterations is of great importance and interest. *PIK3CA* somatic mutations would be ideal for targeting due to their high rate of occurrence and the fact that 80% to 90% of these mutations are in one of three recurrent hotspot sequences. Below, we review several classes of targeted compounds that may have clinical utility for the treatment of cancers harboring *PIK3CA* mutations.

PI3K Inhibitors

The most direct method of targeting cancers that have *PIK3CA* mutations would be to develop inhibitors that have high specificity for mutant *PIK3CA* but not its wild type counterpart. The ability to create mutation specific small molecule inhibitors is exemplified by erlotinib and gefitinib, which were initially developed as EGFR inhibitors but were found to be most effective in patients whose tumors contained specific EGFR mutations [36,37]. This finding is attributed to oncogene addiction [36], which is the phenomenon whereby cancer cells become dependent on growth signals from aberrantly activated pathways by mutated oncogenes, and therefore removal of these signals leads to decreased cellular growth and apoptosis [38]. Although historical evidence suggests that mutant specific inhibitors may be feasible, there are currently no PI3K inhibitors with specificity for even the p110 α isoform, which initially suggested that the development of compounds specific for mutant *PIK3CA* would not be possible. However, recent structural data demonstrate that rational design of such inhibitors is feasible [39], and therefore the emergence of targeted mutant *PIK3CA* therapies is likely to be imminent.

Prior to the discovery of somatic *PIK3CA* mutations in human cancers, the PI3K enzyme was already recognized as being an important molecule in mediating carcinogenesis. As such, inhibitors of PI3K were developed with the hope that a therapeutic window could be achieved. The earliest and best characterized PI3K inhibitors are wortmannin and LY294002. Both of these compounds have been shown to be effective antitumor agents in *in vitro* cell culture models, as well as in *in vivo* animal models [40–42]. LY294002 has been shown to inhibit both *in vitro* PI3K activity and phosphorylation of downstream effectors of *PIK3CA* in breast

epithelial [25] and colorectal cancer cells [24]. However, due to their poor pharmacological properties and marked cytotoxicity, LY294002 and wortmannin do not have clinical utility as reviewed by Workman [43]. Additionally, they are not ideal for specifically targeting PIK3CA mutants because they can inhibit other kinases of the PI-3 kinase-like kinase (PIKK) family, and several other kinases such as the mammalian target of rapamycin (mTOR) [44–48]. Recently, a number of groups have reported developing PIK3CA selective inhibitors [49–55], and some have demonstrated efficacy *in vitro* and *in vivo* [49–51,53,54,56]. Within the past year, the PI3K inhibitors XL147 (Exilixis), BEZ235 (Novartis) and GDC-0941 (Genentech) have entered early phase clinical trials, and many more of these compounds will soon follow. Given that mutant PIK3CA results in constitutively active PI3K activity, it will be of interest to determine if the presence of *PIK3CA* mutations will allow for the selection of patients with a high likelihood of response.

Buttressed against these exciting developments, specific targeting of PIK3CA may be problematic, as PIK3CA is involved in a number of signaling pathways associated with normal cellular function, such as insulin signaling [55,57]. This may result in PIK3CA inhibitors that are prohibitively cytotoxic, thus limiting their clinical benefit. To illustrate this point, some PIK3CA inhibitors have been shown to abrogate the effects of insulin in mice [55]. Similarly, PIK3CA deficient mice have recently been shown to have an increased rate of heart failure in response to cardiac stress [58]. Thus, the possibility of a significant side effect profile has led to the development of compounds designed to target downstream effectors within the PIK3CA pathway with the hope that this may be a more effective strategy to target cancers containing *PIK3CA* mutations.

AKT Inhibitors

AKT, also known as protein kinase B, is a serine threonine kinase directly downstream of PI3K, and dysregulation of AKT is commonly associated with many different cancers [59]. More specifically, constitutive activation of AKT has been associated with *PIK3CA* mutations in several *in vitro* cell models [23–27]. Therefore, AKT inhibitors may prove to be useful in targeting cancers with *PIK3CA* mutations.

AKT was identified as an oncogene over two decades ago [60], and multiple AKT inhibitors have been developed and used successfully to inhibit AKT activation and cellular growth in *in vitro* and *in vivo* tumor models [61–88]. Perifosine is the most studied AKT inhibitor, proving efficacious at inhibiting the growth of various cancer cell types [85]. However as a single agent, Perifosine has not shown therapeutic benefit in several phase II trials. However, Perifosine may have some therapeutic potential when used in combination with radiation or other standard cytotoxic agents, as evidenced by *in vitro* studies [89–91]. Another AKT inhibitor, Miltefosine, has shown efficacy in clinical trials for the topical treatment of cutaneous lymphoma and breast cancer skin metastases [92–95]. Recently, it was discovered that API-2, a compound that had shown some efficacy in early phase trials but was abandoned due to excessive toxicity [96, 97], is an AKT inhibitor [82]. This finding opens the door to investigation of specific AKT inhibition at lower doses than those used previously, thus potentially avoiding the detrimental side effects. Currently, there are multiple ongoing early phase clinical trials of AKT inhibitors that should ascertain the effectiveness of this class of agents.

Additional studies need to be performed on the relationship between *PIK3CA* mutations and AKT as there are three distinct isoforms (AKT1, AKT2, and AKT3) and each may have different effects on tumor growth and/or cytotoxicity. To date, *PIK3CA* mutations have been most closely linked to AKT1 in *in vitro* cell models [23,25–27], with a single study examining all three isoforms revealing that AKT1 may be most affected by *PIK3CA* mutations [24]. However, isoform specific functions have been delineated through various laboratory studies. For example, RNA interference (RNAi) mediated gene knockdown of *AKT1* in breast cancer

cell lines has been shown to increase cell motility [98], and *AKT1* knockout mice are small in size and infertile [99]. In addition, *AKT2* knockout mice develop diabetes mellitus [100], while *AKT3* knockout mice exhibit abnormal brain development [101]. Selective AKT1 and 2 inhibitors have been developed and have shown some promise *in vitro* [77]. Further elucidation of the relative effects of inhibiting different AKT isoforms in cancers harboring *PIK3CA* mutations will be required, as the potential for significant toxicities remains an obstacle for using AKT inhibitors as effective targeted therapies for these cancers.

mTOR Inhibitors

mTOR is a downstream effector of *PIK3CA* and is very important for many cellular processes, including cell proliferation [102] and angiogenesis [103]. The mTOR pathway has been shown to be activated by *PIK3CA* mutations in both chicken embryo fibroblasts [27] and colorectal cancer cells [23]. Therefore, blocking the mTOR pathway may prove to be an effective strategy for targeting aberrant growth signaling in cancers with *PIK3CA* mutations.

Of the compounds that could potentially target cancers bearing *PIK3CA* mutations, mTOR inhibitors are the most mature in terms of their development and clinical use. Rapamycin, an mTOR inhibitor, was initially developed in the early 1970's as an antifungal agent [104] and was later FDA approved as an immunosuppressive therapy [105]. Rapamycin and its analogs have been used with variable success to treat a multitude of different cancers [106–115]. The best evidence of response thus far is in the treatment of advanced renal cancers for which temsirolimus [116], a rapamycin prodrug, has been FDA approved. Identifying biomarkers that predict for response to mTOR inhibitors is of great importance for the development of these agents, as response to mTOR inhibitors has been greatly variable. Theoretically, *PIK3CA* mutations may lend themselves as predictive biomarkers for response to mTOR inhibitors. For example, it has been shown that either rapamycin or its analogs can impede the transformation of chicken embryo fibroblasts expressing *PIK3CA* mutations [27], inhibit tumor growth induced by *PIK3CA* mutations in chicken embryos [28], and reduce the formation of abnormal human breast epithelial cell acini induced by mutant *PIK3CA* overexpression using a three-dimensional basement membrane morphogenesis assay [25].

Despite the clinical potential of mTOR inhibitors, significant hurdles for their further development still exist. mTOR actually forms two complexes within cells, mTORC1 and mTORC2 [117]. mTORC1 is known to mediate a negative feedback loop with PI3K/AKT signaling, and therefore inhibiting mTOR pharmacologically causes a paradoxical upregulation of PI3K/AKT growth promoting signaling [118]. Additionally, mTORC2 directly phosphorylates AKT [119] but is only rarely inhibited by rapamycin and its analogs in a cell/tissue type dependent manner [120]. Since the mTOR complexes are on both ends of the PI3K/AKT signaling pathway and mTORC2 inhibition is limited, more studies need to be completed to elucidate the mechanisms of sensitivity/resistance to mTOR inhibitors and to develop biomarkers to predict the efficacy of mTOR inhibition. Other effective strategies for targeting this complex signaling pathway in the future may include combining current mTOR inhibitors with either newer PI3K/AKT inhibitors or more traditional therapies, such as chemotherapy and endocrine therapy. In addition, it is possible that the development of new inhibitors capable of blocking mTORC2, or alternatively both mTORC1 and mTORC2, could provide a more effective therapeutic regimen.

Possibilities of Novel Dual Inhibition

Due to the genetic instability of most human cancers, it can be expected that any targeted therapy when used as a single agent will ultimately succumb to drug resistance. However, the development of targeted therapies with minimal side effects would hopefully enable the combinatorial use of multiple drugs with non-overlapping toxicities, to effectively treat and

eradicate the disease. Recently, it was demonstrated that the combination of PIK3CA and mTOR inhibitors could prevent the increase in AKT signaling caused by mTOR inhibition alone [121]. A single molecule, PI-103, was found to effectively inhibit both PIK3CA and mTOR, and its efficacy has been shown in glioma, ovarian cancer, and breast cancer in *in vitro* and *in vivo* models [121,122]. This class of molecules, capable of inhibiting multiple targets within the PIK3CA pathway, may prove to be an effective strategy for targeting cancer cells containing *PIK3CA* mutations.

In addition, combination therapies with existing anti-neoplastic drugs are also being explored. For example, *PIK3CA* mutations have been positively correlated with increased expression of both estrogen receptor alpha (ER α) and HER2/neu in the NCI 60 panel of cancer cell lines [123] as well breast tumor samples [124]. Hormonal therapies and trastuzumab are currently used to target ER α and HER2/neu respectively. However, it has been shown that increased AKT signaling is associated with resistance to both of these therapies [125–128], and it is therefore possible that *PIK3CA* mutations may actually confer resistance to these drugs. If this hypothesis proves to be true, then combining either of these therapies with a PIK3CA, AKT, or mTOR inhibitor may provide an effective strategy for abrogating drug resistance.

CONCLUSIONS

Due to their high frequency in many cancers, *PIK3CA* mutations are a prime candidate for targeted therapeutics. However given the fact that these mutations have only recently been accurately characterized, much work lies ahead to fully elucidate their biological and clinical significance. The process of discovery for developing targeted PIK3CA therapies remains in its infancy. However, the wealth of potential targets along the PIK3CA pathway is certainly enticing from a developmental therapeutics viewpoint, and the opportunity exists to significantly impact cancer care with future success in this arena.

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Abbreviations

EGFR	epidermal growth factor receptor
PI3K	phosphatidylinositol 3-kinase
mTOR	mammalian target of rapamycin
PIKK	PI-3 kinase-like kinase
RNAi	RNA interference
ER α	estrogen receptor alpha

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Figure 1.

A representation of the domains of the PI3K subunits p110 α and p85 α . The p110 α catalytic subunit has 5 domains including adaptor-binding domain (ABD), the Ras-binding domain (RBD), a calcium binding domain (C2), a helical domain and a kinase domain. The p85 α regulatory subunit contains 5 domains as well, which include a Src homology 3 domain (SH3), a GTPase activating protein domain (GAP), an N-terminal Src homology 2 domain (nSH2), an inter- Src homology 2 domain (iSH2), and a C-terminal Src homology 2 domain (cSH2). The exon 9 hotspot mutations, E542K and E545K, occur in the helical domain of the catalytic subunit p110 α , and the charge reversal caused by these mutations inhibits electrostatic interactions between those amino acids on the p110 α helical domain and R340 and K379 on the nSH2 domain of p85 α . The exon 20 hotspot mutation, H1047R, is in the kinase domain of p110 α , and this mutation has been proposed to form a hydrogen bond with L956 of p110 α , which in turn leads to catalytic activity of p110 α .