Molecular Pathways: Targeting the PI3K Pathway in Cancer— BET Inhibitors to the Rescue

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Abstract

The phosphoinositide 3-kinase (PI3K) signaling pathway is a complex and tightly regulated network that is critical for many physiological processes such as cell growth, proliferation, metabolism and survival. Aberrant activation of this pathway can occur through mutation of almost any of its major nodes and has been implicated in a number of human diseases including cancer. The high frequency of mutations in this pathway in multiple types of cancer has led to the development of small molecule inhibitors of PI3K, several of which are currently in clinical trials. However, several feedback mechanisms either within the PI3K pathway or in compensatory pathways can render tumor cells resistant to therapy. Recently, targeting proteins of the bromodomain and extra-terminal (BET) family of epigenetic readers of histone acetylation has been shown to effectively block adaptive signaling response of cancer cells to inhibitors of the PI3K pathway, which at least in some cases can restore sensitivity. BET inhibitors also enforce blockade of the MAPK, JAK/STAT and ER pathways suggesting they may be a rational combinatorial partner for divergent oncogenic signals that are subject to homeostatic regulation. Here, we review the PI3K pathway as a target for cancer therapy and discuss the potential use of BET inhibition to enhance clinical efficacy of PI3K inhibitors.

Background

PI3Ks are a family of lipid kinases that phosphorylate the 3-hydroxyl group on phosphoinositides, generating second messengers that regulate several downstream pathways that are central in both normal physiology and disease (1, 2). In mammals, there are three classes of PI3Ks that differ in structure and substrate specificity but to date, mainly class IA PI3Ks has been implicated in the etiology of various diseases including cancer (3). Class IA PI3Ks are heterodimers composed of a p110 catalytic subunit (α, β and δ) and a p85/55 regulatory subunit (encoded by three different genes that are subject to alternative splicing) that can be activated downstream of Receptor tyrosine kinases (RTKs), G protein-coupled receptors (GPCRs) and small GTPases (4). Although PI3K was first linked to cancer almost thirty years ago when it was associated with the transforming activity of viral oncogenes (5), it wasn't until the early 2000's that PI3Ks were brought to the forefront of

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cancer research when *PIK3R1* (6) and *PIK3CA* (7), the genes encoding p85α and p110α respectively, were found to be frequently mutated in several types of solid tumors. Since then, multiple studies have established that *PIK3CA* is one of the most, if not the most, frequently mutated oncogenes in human cancer. Mutations are mainly clustered in two hot-spots of the enzyme and can increase p110α activity through a variety of mechanisms (8–10).

In quiescent cells, p85 binds to p110, stabilizing it and inactivating its kinase activity (Fig. 1). Following growth factor stimulation, the PI3K complex is activated after binding to phosphotyrosines on receptors and adaptor proteins. The primary consequence of PI3K activation is the conversion of phosphatidylinositol-4,5-bisphosphate (PIP_2) into the short-lived second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3) on the inner leaflet of the plasma membrane. PIP_3 recruits proteins to the membrane that contain a pleckstrin-homology (PH) domain, including AKT and its upstream activators PDK1 and mTORC2. At the membrane, PDK1 phosphorylates AKT at T308 (11) and mTORC2 phosphorylates it at S473 (12), which results in full activation of the enzyme. AKT acts as a major mediator of PI3K signaling by phosphorylating a wide range of substrates that regulate cell cycle entry, survival, protein synthesis, RNA translation, glucose metabolism and migration. PI3K activity is tightly controlled in cells and can be attenuated by lipid phosphatases, such as PTEN (13), INPP4B (14), and SHIP2 (15) that dephosphorylate phospholipids in positions 3, 4 and 5, respectively, on the inositol ring. PIP_3 is also an important signal upstream of several pro-oncogenic signals including SGK3 (16, 17) and PREX1/ PREX2 (18, 19).

Overactivation of the PI3K signal is one of the most frequent events in human cancers and can be achieved through alterations in most of the major nodes of the pathway (4). Activating mutations and/or amplification of the genes encoding the PI3K catalytic subunits p110α (7) and less frequently p110β (20), mutations in the p85α regulatory subunit that abrogate its p110-inhibitory activity (6, 21) as well as loss of function of the lipid phosphatases PTEN (22, 23) and INPP4B (14) can all promote PI3K activity and cellular transformation. Mutations in genes acting both upstream of PI3Ks such as RTKs (for example *EGFR* and *HER2*) and the PI3K activator *K-RAS* as well as the downstream PI3K effectors *AKT1–3*, *PDK1*, *TSC1/2* and *mTOR* are variably present in human tumors and should also result in aberrant activation of the PI3K pathway.

Clinical-Translational Advances

Given the high frequency of PI3K pathway activation in human cancers, a significant amount of effort has been put into the development of drugs targeting several kinases throughout the pathway, especially PI3Ks. Several inhibitors targeting all PI3K isoforms (Pan-PI3K) are currently in clinical trials but although they have turned out to be reasonably well tolerated, they have shown limited efficacy as single agents (24). Partial inhibition of the signal due to limitations in the dosing, compensatory feedback mechanisms as well as off-target effects could all account for the limited clinical responses that were observed in patients treated with Pan-PI3K inhibitors. Recent preclinical studies have highlighted the divergent roles of class I PI3K catalytic isoforms depending on the genetic context. For example, HER2/Neu- and KRAS-driven tumors have been shown to rely mainly on p110α

(25, 26) whereas p110β has been shown to be important in certain PTEN-deficient tumors (27–[29]). In light of these findings PI3K isoform-selective inhibitors have been developed and are being tested in clinical trials with the hope to maximize target-inhibitory doses while sparing the adverse toxic effects of Pan-PI3K inhibitors. In 2014 Idelalisib (CAL-101) became the first p110 isoform-specific inhibitor (against p110δ) that has been approved by the FDA for the treatment of chronic lymphocytic leukemias (30) and B cell lymphomas (31). Alpelisib (BYL719) is a p110α-specific PI3K inhibitor that has shown some encouraging results in early phase clinical trials. As a single agent it resulted in some tumor regressions and prolonged disease control in heavily pretreated patients with various tumor types carrying a *PIK3CA* mutation (32). Preliminary results from the combination of BYL719 and Fulvestrant, an estrogen receptor antagonist, also indicate an encouraging activity in patients with PIK3CA-mutant breast cancer, with 3 patients achieving confirmed partial responses (33). GDC-0032, a β isoform-sparing PI3K inhibitor targeting PI3Kα/δ/γ, is another example of next generation PI3K inhibitors that has shown promising preliminary clinical activity in PIK3CA mutant cancers (34) and is currently being evaluated in solid tumors both as a single agent and in combination with endocrine therapies and other anticancer therapies. Given these early signals of clinical activity more PI3K inhibitors could be FDA approved for the use in human cancer, especially as part of combination therapies. As PI3K inhibitors are making their way into trials, it is critical to identify biomarkers that will help future selection of patients that are most likely to benefit from these targeted therapies.

While Herceptin (Trastuzumab), a monoclonal antibody against HER2, has shown remarkable activity in HER2-postitive breast cancer patients, the efficacy of kinase-based therapeutics that act downstream has been limited due to a variety of adaptive and compensatory responses to the drugs within the cancer cell. Early preclinical studies with inhibitors against mTOR identified that blockade of the mTOR-mediated activation of S6 kinase released inhibition of IRS1, resulting in aberrant PI3K/AKT activation (35). To deal with unleashing of this natural break in the pathway several dual PI3K/mTOR inhibitors have been developed and are being tested in early phase clinical trials. As these compounds exhibit a broad activity profile and significantly higher toxicity, they might be more suitable for the treatment of patients with more than one alteration in the PI3K pathway. Multiple studies have also shown that feedback activation of the PI3K pathway in response to PI3K inhibitors can also be achieved by suppression of the FOXO-dependent activation of expression of RTKs (36–[38]). Blocking PI3K, along with different upstream RTKs has been shown to block this adaptive response and resensitize cancer cells (36, 39–[41]).

PI3K isoforms are able to compensate for each other as well. For example, preclinical studies have shown that cancer cells can induce expression of p110β to counteract the blockade of p110α and vice versa (42, 43). In an ER+/PIK3CA-mutant breast cancer patient treated with the p110α-specific inhibitor BYL719, loss of PTEN expression (which could likely switch dependence on the p110β isoform) was present in multiple metastatic lesions (44). Markedly, treatment of xenografts derived from the BYL719-resistant lesions with the p110β-selective inhibitor AZD6482 was able to restore sensitivity. Concurrent inhibition of multiple PI3K isoforms or the use of dual-isoform PI3K inhibitors might be necessary for the treatment of tumors that have become refractory to isoform-specific inhibitors.

Compounds that would be active against only the mutant PIK3CA should minimize increased systemic insulin production that is seen in the clinic upon treatment with p110α inhibitors and are highly anticipated in the field. Finally, activation of several compensatory pathways has been documented to drive resistance to PI3K inhibitors and could therefore inform combinatorial treatments. Clinical trials are currently evaluating preclinical findings of synergism between PI3Ki and antiestrogen therapy (45) as well as inhibitors against poly-ADP-ribose polymerase (PARP) (46), Cyclin-dependent kinases 4 and 6 (CDK4/6) (47) and MEK (37, 48).

Within the past year inhibition of BET proteins (BETi) has emerged as a potential therapeutic approach to restore sensitivity to kinase inhibitors of the PI3K pathway. The BET family of proteins consists of 4 members, BRD2–4 which are ubiquitously expressed and BRDT that is only expressed in germ cells. They contain two tandem bromodomains (BRDs) located at the N-terminus that recognize and bind acetylated-lysine residues in nucleosomal histones to facilitate the recruitment of transcription factors and chromatin organizers required in transcription initiation and elongation. Initially, the concept of targeting a bromodomain was thought to be challenging because it would involve inhibiting a protein-peptide interaction. Moreover there was a lot of skepticism around the concept of epigenetic therapy as inhibition of BET proteins which are global regulators of gene transcription was expected to impact on the transcriptional activity across all active genes and be highly toxic. In 2010 two selective and potent BET protein inhibitors, JQ1 (49) and I-BET762 (50) were reported to have activity in a NUT midline carcinoma and an inflammatory disease model respectively. Mechanistically, BET inhibition was shown to result in significant reduction of the transcript levels of only a small number of key genes in a cell- and context-specific manner (51, 52). This recent discovery of small molecules capable of blocking their lysine-binding pocket is the first successful example of pharmaceutical inhibition of epigenetic readers and has sparked intense efforts to develop novel BRD antagonists. Several compounds are currently tested in early phase clinical trials (Table 1) for solid tumors and hematological malignancies where for example deregulated c-MYC, a major target of BET proteins in this context, is an important driver of tumorigenesis. Most of them share a similar thieno-diazepine scaffold whereas for some of them the exact chemical structure has not yet been disclosed (53). Notably, multiple studies have recently documented that BET inhibition can effectively block adaptive responses to inhibitors of the PI3K pathway that help cancer cells to evade the effects of the drug and develop resistance. For example, our group has shown that BET inhibitors can block BRD4 from binding to regulatory regions of genes encoding several RTKs that are frequently induced by treatment with Pictilisib (GDC-0941), a Pan-PI3K inhibitor (Fig. 1). As a result, combined PI3K and BET inhibition can prolong blockade of the PI3K signal and induce cell death in multiple tumor cell lines (54). BET proteins have also been shown to regulate sensitivity of breast cancer cells to treatment with Lapatinib, an EGFR/HER2 inhibitor, by blocking the expression/phosphorylation of kinases (55) and/or activation of the FOXO/c-MYC axis (56). Finally, inhibitors of BET proteins have shown synergism with mTOR inhibitors in multiple tumor models (57–59) suggesting that blockade of the epigenetic regulation of the PI3K pathway may present an opportunity to overcome resistance to kinase inhibitor therapy.

Several groups have investigated BETi as a potential therapeutic approach to potentiate the effect of targeted therapy against oncogenic drivers in other signaling pathways. For example, combinations of JQ1 and tyrosine kinase inhibitors such as Imatinib and JAK inhibitor I were shown to synergize and induce apoptosis in leukemias and lymphomas driven by constitutive STAT5 activation (60). Using an anti-cancer drug library containing 180 small molecule inhibitors, Jing et al identified that combined BETi/MEKi suppressed both cell proliferation and survival in an ovarian cancer model (61). In an ER+/tamoxifen-resistant breast cancer model, JQ1 induced a strong growth inhibitory effect when it was combined with fulvestrant (62). These studies highlight the ability of BETi to synergize with inhibition of diverse oncogenic signals which could be due to their unique ability to interfere with the adaptive feedback expression of a few relevant genes to restore signaling homeostasis depending on the genetic context (Fig. 1). However, it remains to be seen whether there will be a therapeutic window for any of the above combinatorial therapies in the clinic.

A striking development in BRDi research is the recent finding that BET proteins are unanticipated targets of certain widely used kinase inhibitors. The first such report was by Martin et al. who discovered that the potent CDK inhibitor Dinaciclib interacts with the acetyl-lysine recognition site of the bromodomain testis-specific protein BRDT (63). Soon after, two new studies (64, 65) identified more than a dozen kinase inhibitors that possess cross-reactivity towards BRD4 with nanomolar potencies, including the JAK2 inhibitor TG101209 and the PLK1 inhibitor BI2536 which is in Phase I/II Clinical trials for acute myeloid leukemia (AML) and non-small cell lung cancer (NSCLC). This finding has triggered the development of more potent dual BET/PLK1 inhibitors using a structure–activity relationship (SAR) study (66). Notably, LY94002 which has been routinely used to block PI3K activity was also recently shown to interact with BRD2–4 (67). The above studies provide the framework for the rational design of next-generation dual BET-Kinase inhibitors. The simultaneous inhibition of two structurally and functionally unrelated proteins by a single drug may provide a new opportunity when the application of combination therapies poses significant clinical challenges.

Conclusions

The concept of oncogene addiction described by the dependence of certain tumors for their growth and survival on a limited number of mutational events has fueled the development of targeted therapies. The PI3K signaling pathway has attracted much interest due to its involvement in a large fraction of human tumors and several inhibitors targeting oncogenic kinases throughout the pathway are currently being tested in clinical trials. However, preclinical studies and past experience with other kinase-based therapies suggest that escape mechanisms and drug resistance may eventually limit the efficacy of these compounds as monotherapies. Concurrent inhibition of PI3K and BET proteins may provide an alternative for durable inhibition of the oncogenic signal and clinical trials could test the safety and efficacy of such combinations.

Acknowledgments

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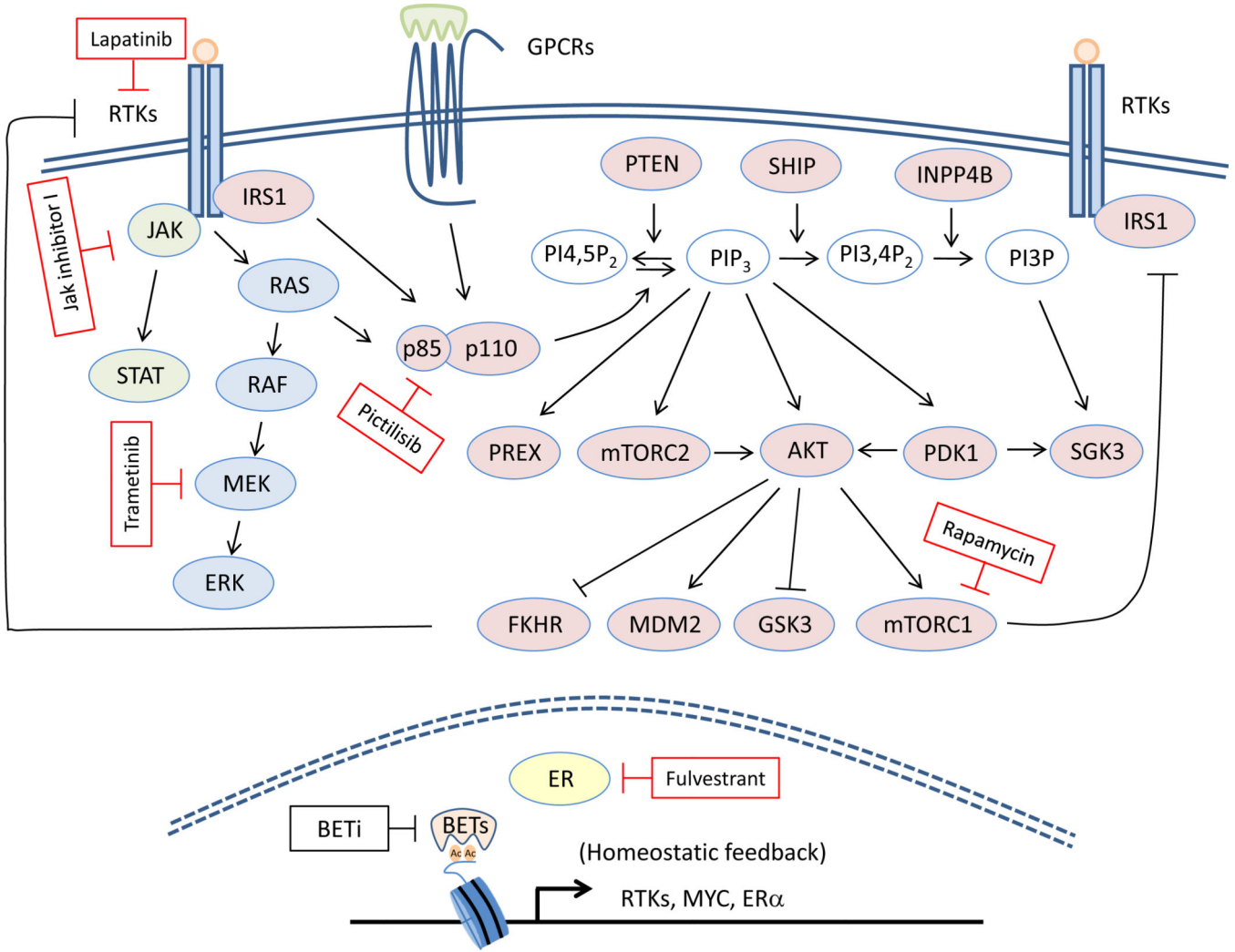
Figure 1.
Schematic representation of nodes of therapeutic blockade in the PI3K and other signaling pathways that synergize with BET inhibition. PI3Ks are a family of heterodimeric proteins that can be activated downstream of RTKs, GPCRs and small-GTPases. They catalyze the conversion of PIP_2 to the second messenger PIP_3 which helps recruit to the membrane proteins that contain a PH-domain such as AKT, PDK1, mTORC2 and PREX1/2. PI3K activity can be attenuated by several phosphatases including PTEN, SHIP1/2 and INPP4B. AKT acts as a major mediator of PI3K signaling by phosphorylating a wide range of substrates that regulate cell growth, proliferation, metabolism and survival. Given the high frequency of PI3K pathway activation in human cancers several inhibitors targeting kinases throughout the pathway are currently being evaluated in clinical trials. However their efficacy as monotherapies can be limited due to a variety of mechanisms including the unleashing of FOXO- and mTORC1-mediated feedback loops that reactivate the pathway. Inhibition of BET proteins has recently been shown to effectively block adaptive signaling response of cancer cells to inhibitors of the PI3K pathway and other signaling pathways

(shown in red rectangles) suggesting they may be rational combinatorial partners for multiple oncogenic signals.

Table 1

BET inhibitors currently in clinical trials for human cancers

Drug	Sponsor	Solid tumors or hematological malignancies	Most advanced clinical phase
I-BET762	GlaxoSmithKline	Both	I/II
GSK2820151	GlaxoSmithKline	Solid tumors	I
TEN-010	Tensha	Both	I
OTX015	Oncoethix/Merck	Both	II
BAY 1238097	Bayer	Both	I
CPI-0610	Constellation Pharmaceuticals	Hematological malignancies	I
GS-5829	Gilead	Both	I
INCB054329	Incyte	Both	I
ABBV-075	Abbvie	Both	I
BMS-986158	Bristol-Myers Squibb	Solid tumors	I/IIa
FT-1101	Forma Therapeutics, Inc	Hematological malignancies	I

Data taken from http://clinicaltrials.gov/.