

MINI REVIEW

What a Tangled Web We Weave: Emerging Resistance Mechanisms to Inhibition of the Phosphoinositide 3-Kinase Pathway

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ABSTRACT

The phosphoinositide 3-kinase (PI3K) pathway is one of the most frequently mutated pathways in cancer, and is actively being pursued as a therapeutic target. Despite the importance of the PI3K pathway in cancer, durable responses to PI3K pathway-targeted therapies are uncommon with monotherapy. Several *in vitro* and xenograft models have elucidated compensatory signaling and genomic changes which may limit the therapeutic effectiveness of PI3K inhibitors in the clinic. Future clinical trials with prospective evaluation of tumor signaling and genomic changes are likely to identify novel resistance mechanisms as well as subsets of patients who may derive maximal benefit from PI3K pathway inhibitors.

Significance: There are multiple ongoing clinical trials targeting the PI3K pathway members in several malignancies. This review summarizes the known mechanisms of resistance to targeting the PI3K pathway. Understanding of resistance mechanisms will help to inform more rational clinical trial design to optimize the clinical impact of targeting the PI3K pathway in cancer. *Cancer Discov*; 3(12); 1345–54. ©2013 AACR.

INTRODUCTION

Sustained proliferative signaling is a major hallmark of malignancy and commonly occurs through transmembrane receptor tyrosine kinases (RTK) and their downstream effectors (1). One of the most important effectors of RTK signaling is phosphoinositide 3-kinase (PI3K). PI3K was initially implicated in cancer through identification of its physical association with the Rous sarcoma (*Src*) oncogene-encoded protein tyrosine kinase, pp60^{v-src}, and with the polyoma virus middle t protein (2, 3). Subsequent studies revealed that the PI3K pathway was central to multiple malignant hallmarks, including survival, growth, metabolism, motility, and progression. Activating mutations in key components of the PI3K pathway are common in numerous tumor types, and loss of the negative PI3K regulator phosphatase and tensin homolog (PTEN) is the second most common event in can-

cer after p53 mutations (4). The central importance of PI3K signaling and its downstream effects is now well established and previously reviewed (5–7). However, despite scientific rationale and *in vitro* and tumor xenograft data, PI3K/AKT/mTOR inhibitors have yet to achieve maximal clinical impact.

CANONICAL CLASS IA PI3K SIGNALING

To date, mainly the class IA PI3Ks are implicated in human cancers; therefore, this review will be restricted to the class IA PI3K isoforms. The class IA PI3Ks are composed of heterodimers of regulatory subunits (p85 α , p85 β , p50 α , p55 α , and p55 γ) and catalytic subunits (p110 α , p110 β , p110 δ). Regulatory subunits of PI3K are encoded by three genes; *PIK3R1* encodes p85 α (and its alternative transcripts, p55 α and p50 α), and *PIK3R2* and *PIK3R3* encode the p85 β and p55 γ isoforms of the p85 regulatory subunit, respectively (5). Three genes, *PIK3CA*, *PIK3CB*, and *PIK3CD*, encode the highly homologous p110 catalytic subunit isoforms p110 α , p110 β , and p110 δ , and all share a similar five-domain structure (7). The association of the regulatory p85 subunit with various RTKs occurs via physical interaction between the SH domains of p85 and phosphotyrosines in the consensus YxxM sequence, which becomes autophosphorylated by RTK dimerization. RTKs can also phosphorylate YxxM motifs in adaptors such as insulin receptor substrate 1 and 2 (IRS1 and IRS2; ref. 7). Through binding of the regulatory subunit to RTKs or adaptors, PI3K is recruited to the plasma membrane where it phosphorylates the 3' position of its preferred *in vivo*

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doi: 10.1158/2159-8290.CD-13-0063

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substrate phosphatidylinositol-4,5-bisphosphate (PI-4,5-P₂) to generate the second messenger phosphatidylinositol (3,4,5) trisphosphate (PIP₃). PIP₃ generation recruits cytosolic proteins with PIP₃-binding pleckstrin homology (PH) domains and thereby localizes them to the plasma membrane. The serine/threonine kinase protein kinase B (AKT) is the most notable of the recruited proteins and a major effector of PI3K signaling (8). The extent and duration of PIP₃ elevation at the plasma membrane is primarily regulated by the tumor suppressor PTEN, whose lipid phosphatase activity converts PIP₃ back to PI-4,5-P₂.

The direct interaction between the PH domain of AKT and PIP₃ induces conformational changes, exposing the two important residues, threonine 308 (T308) and serine 473 (S473), for phosphorylation (9). A second PIP₃-binding protein kinase named phosphoinositide-dependent kinase 1 (PDK1) phosphorylates T308, which is located in the AKT activation loop. Full activation of AKT requires phosphorylation of S473 in the hydrophobic AKT motif by mTOR complex 2 (mTORC2). Fully active AKT phosphorylates multiple targets containing the consensus RxRxxS/TΨ sequence (where Ψ is a hydrophobic residue; ref. 10). In turn, fully active AKT turns on mTOR complex 1 (mTORC1) activity via two main mechanisms; turning off the inhibitory protein, Tuberin [the protein product of the tuberous sclerosis complex 2 (TSC2) gene] by phosphorylation, and directly phosphorylating proline-rich AKT substrate of 40 kDa (PRAS40; refs. 11, 12). Active AKT, and extracellular regulated kinase 1 and 2 (ERK1/2), phosphorylate TSC2 which inhibits the GTPase activating protein (GAP) activity of the TSC1-TSC2 complex toward Ras homolog enriched in brain (RHEB). GTP-loaded RHEB is then able to activate mTORC1 (13–15). Concomitantly, active AKT phosphorylates PRAS40, causing it to release and bind to 14-3-3 proteins which prevent PRAS40 from inhibiting mTORC1 (16, 17).

Following activation, mTORC1 plays a major role in stimulating 5'cap-dependent protein translation through its substrates, ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1; refs. 7, 11). Phosphorylated 4EBP1 is no longer capable of inhibiting the translation initiation factor eukaryotic initiation factor 4E (eIF4E), thereby allowing the translation of capped messages (18). AKT also phosphorylates the Forkhead box (FOXO) family of transcription factors, which influence the expression of multiple proapoptotic proteins (19, 20). Researchers have attempted to exploit each component of the PI3K signaling cascade with approaches ranging from pan-PI3K inhibition, isoform-specific PI3K inhibitors, mTOR inhibitors, AKT inhibitors, and combination PI3K-mTOR inhibitors. Currently, there are more than 20 compounds targeting nodes in the PI3K pathway in various phases of clinical development (21–24). Biologically based advances have led to increased interest in isoform-specific inhibitors and combinatorial strategies to maximize efficacy (25). Although there is significant clinical experience with the mTORC1 inhibitor rapamycin in several malignancies, we await results from multiple ongoing clinical trials to learn more about newer compounds targeting the PI3K pathway.

Although the clinical experience with PI3K/AKT/mTOR inhibitors is limited, one might anticipate conserved resistance mechanisms as observed with other targeted therapies

(26, 27). Resistance to the prototypic targeted therapy imatinib mesylate, an inhibitor of BCR-ABL, has been observed through acquisition of second mutations, specifically the T315I mutation in BCR-ABL that develops in the presence of chronic imatinib treatment (28). A similar phenomenon involving the acquisition of the gatekeeper T790M mutation in the EGF receptor (EGFR) confers resistance to the small-molecule EGFR inhibitor erlotinib, and occurs at a median of 6 to 12 months in most patients on the EGFR tyrosine kinase inhibitor (TKI) erlotinib (29, 30). Secondary mutations have yet to be observed with PI3K inhibitors; rather, compensatory signaling changes are more likely drivers of acquired resistance. To date, the main mediators of resistance to PI3K pathway inhibition have been changes in control of feedback loops, compensatory pathway activation, and genomic alterations activating targets downstream of pharmacologic inhibition. Here, we review the known resistance mechanisms and discuss studies aimed at abrogating resistance.

IRS-1 AND IGF1R

The mitogen-activated protein kinase (MAPK) and PI3K pathways converge at the regulation of protein synthesis through mTORC1 function, specifically, via modulation of TSC2 (31–33). Thereby, TSC2 can act as a sensor of both RAS-MAPK and PI3K-AKT pathway activation. Inhibition of mTORC1 with rapamycin has been known to increase insulin receptor substrate 1 (IRS-1) levels and induce AKT phosphorylation and downstream signaling (34). Inhibition of mTORC1, and the subsequent decrease in p70/S6 kinase, decreases the basal inhibition (via p70/S6K-mediated S312, S636/639 phosphorylation) of IRS-1 and results in IRS-1 stabilization (Fig. 1A; refs. 35, 36). Furthermore, although the rapamycin analogs (referred to as rapalogs) initially inhibit both mTORC1 substrates, S6K and 4EBP1, phosphorylation of 4EBP1, and thus cap-dependent translation, reemerges 6 hours after rapamycin treatment and mediates rapalog resistance (Fig. 1B; ref. 37). The exact mechanism by which phosphorylated 4EBP1, but not S6K, reappears is not known and may involve differential mTORC1-binding affinity for S6K and 4EBP1, binding of adaptor protein, and/or phosphorylation by a yet-to-be-identified kinase (38). Clinically, these findings raise the issue of biomarker readouts and suggest that S6 phosphorylation may not be the optimal biomarker of mTORC1 inhibition, as loss of S6 phosphorylation does not necessarily correlate with inhibition of all mTORC1 substrates. In fact, 4EBP1 phosphorylation status may correlate more tightly with cancer aggressiveness (39). The catalytic mTOR inhibitors, though improved over the rapalogs, are also subject to resistance via IRS-1-mediated feedback activation, particularly at suboptimal dosing (40, 41). These mTOR catalytic inhibitors also activate the RTK-PI3K-PDK1 cascade leading to AKT phosphorylation at T308 which can drive AKT signaling in the absence of S473 phosphorylation by mTORC2 (42). In addition, a recent report suggested that combined inhibition of both PI3K and mTOR may lead to feedback upregulation of JAK2 and its downstream target STAT5 via IRS-1 and insulin-like growth factor receptor 1 (IGF1R)-mediated activation, thereby decreasing sensitivity to p110-mTOR inhibitors (43). Combined targeting of

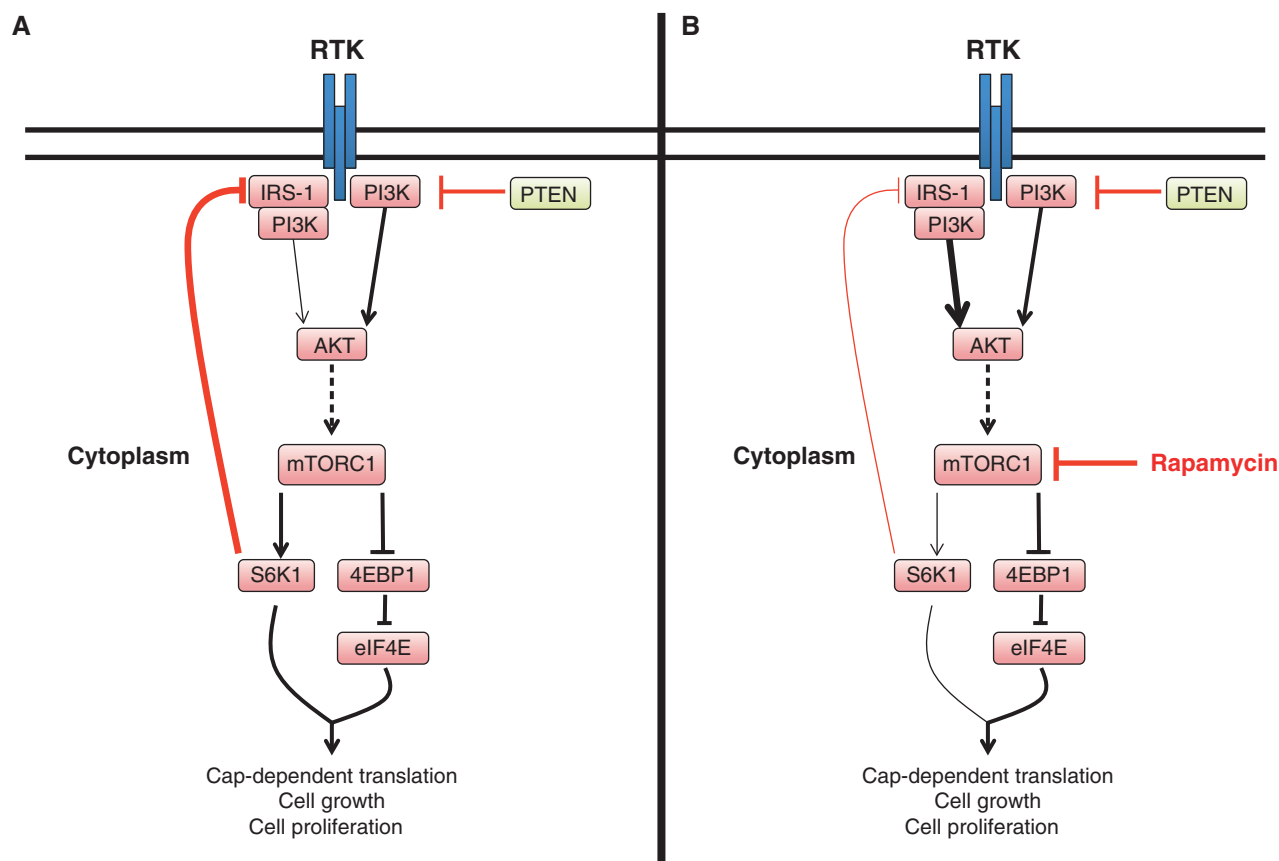


Figure 1. Inhibition of mTORC1 results in feedback PI3K activation. **A**, in the presence of increased PI3K activity, p70/S6K provides basal negative feedback to PI3K signaling by phosphorylating IRS-1 and targeting it for degradation, thus decreasing positive input into the PI3K pathway. **B**, in the presence of mTORC1 inhibition there is a decrease in p70/S6K-mediated IRS-1 phosphorylation, thereby stabilizing the protein and allowing for positive input into the PI3K pathway. Dotted lines represent multiple steps not shown graphically, line strength represents relative activation. S6K1, ribosomal protein S6 kinase.

PI3K/mTOR and JAK/STAT signaling was able to abrogate the resistance, although this has not been shown clinically (43). Newer reports have also suggested a non-feedback inherent resistance pathway in which PI-4,5-P₂ can directly activate signaling by the serine/threonine p21-activating kinase p21 protein (Cdc42/Rac)-activated kinase 1 (PAK1) to drive cell survival in lymphoma cell lines, and that PAK1 inhibition increases PI3K inhibitor sensitivity (44, 45).

An S6K-mediated feedback loop can also lead to MAPK activation reflected in ERK phosphorylation at threonine 202 and tyrosine 204, and this feedback is clinically relevant, occurring in up to 50% of everolimus-treated patients with breast cancer (46). The biologic and tumor-specific importance of feedback MAPK activation in response to PI3K inhibition has been highlighted in multiple studies. Our lab showed the importance of PI3K signaling in *Kras*^{G12D}-driven murine lung cancers in which the concomitant inhibition of mitogen-activated protein kinase kinase (MEK) and PI3K had synergistic effects on inhibition of tumor growth (47). IRS-1-mediated feedback activation of the PI3K, MAPK, and JAK/STAT pathways has led to the initiation of early-phase combination trials. However, emerging clinical data from combination trials such as MEK inhibitors combined with

PI3K inhibitors suggest narrow therapeutic indices for combination therapy. Achieving combination targeting strategies may call for alternative dosing schedules such as sequential or alternating drugs to mitigate toxicity while minimizing the development of resistance.

FOXO-MEDIATED MECHANISMS

The FOXO family of transcription factors influences the expression of multiple proapoptotic proteins (19, 20). Activated AKT phosphorylates FOXO3a, thereby preventing its translocation to the nucleus and inhibiting the transcription of proapoptotic target genes. Several studies have now shown the importance of FOXO-mediated feedback loops in the development of resistance to PI3K inhibitors.

A subset of breast cancers are defined by *HER2* (*ErbB2*) amplification, and the HER2/HER3 heterodimer potently activates PI3K signaling via six docking sites for the p85 subunit of PI3K (48–50). The success of HER2-directed therapies relies partly on their ability to inhibit downstream PI3K signaling, and inhibition of HER2 results in feedback HER3 upregulation, abrogating the effect of HER2 inhibition (51–53). The feedback HER3 upregulation seems to be

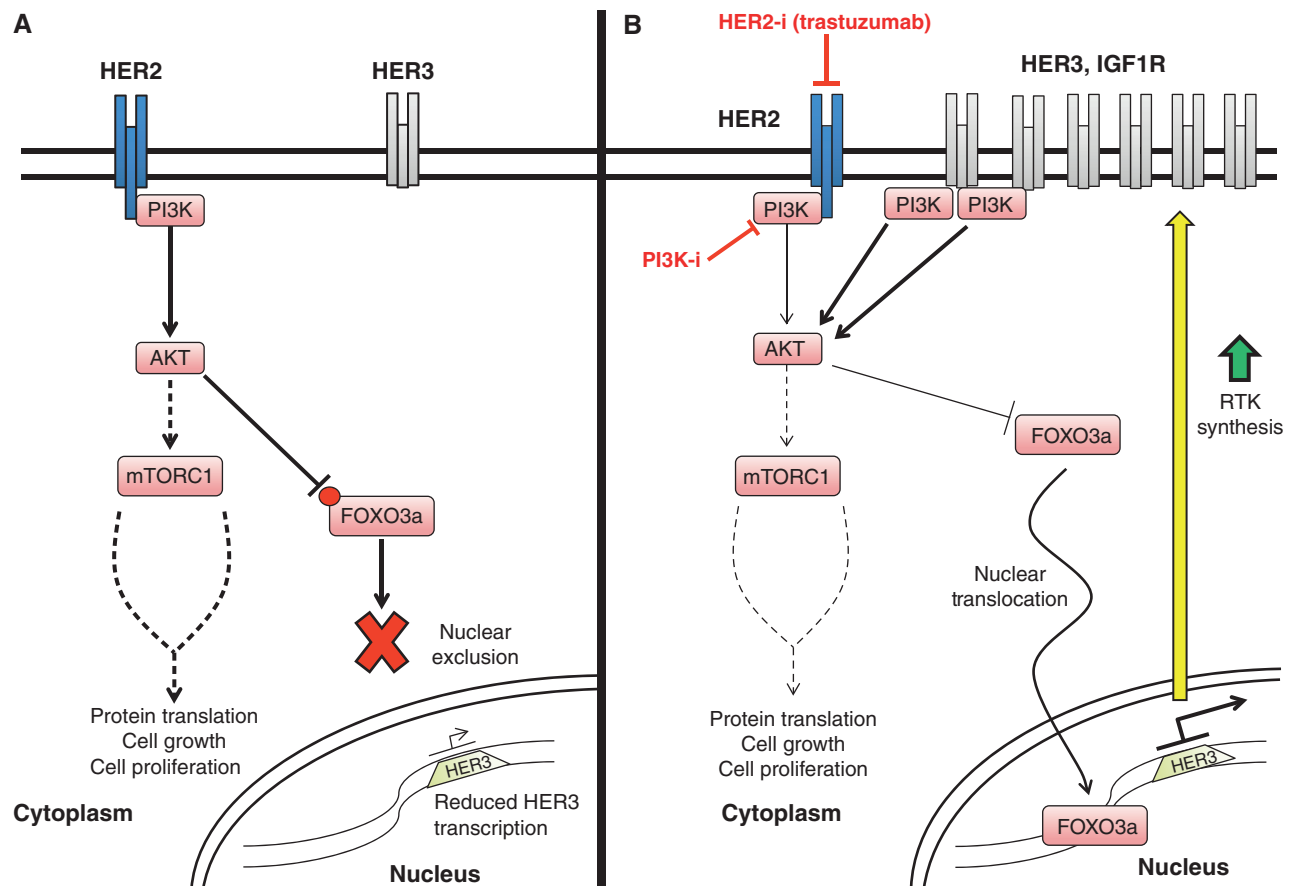


Figure 2. HER2 and PI3K-targeted therapies result in FOXO3a-mediated feedback upregulation of HER3 and IGF1R and provide an escape from PI3K pathway inhibition. **A**, in the basal state, AKT-mediated FOXO3a phosphorylation inhibits translocation of FOXO3a to the nucleus and provides a basal inhibition of RTK synthesis. **B**, in the presence of PI3K inhibition, via either upstream RTK blockade or small molecule inhibitors, decreased AKT activity allows FOXO3a to translocate to the nucleus and effect transcription of FOXO3a target genes, including *HER3* and *IGF1R*. The increased RTK expression mediates resistance to PI3K inhibition by increasing input into the PI3K pathway and alternate growth pathways, including MAPK. Dotted lines represent multiple steps not shown graphically; line strength represents relative activation. HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; FOXO3a, forkhead box O3a.

clinically relevant and has been shown in tumor samples from patients treated with the HER2 inhibitor lapatinib, and is the basis for the recent U.S. Food and Drug Administration approval of the HER3-directed antibody pertuzumab. Clinically, the combination of pertuzumab and the HER2 inhibitor trastuzumab is significantly better than trastuzumab alone (54, 55). HER3 upregulation is mediated by PI3K-AKT-dependent phosphorylation of the AKT substrate FOXO3a in which HER2-induced PI3K-AKT signaling normally represses HER3 transcription (Fig. 2A; ref. 52). Thus, inhibition of either HER2 or PI3K-AKT results in decreased FOXO3a phosphorylation and increased nuclear FOXO3a where FOXO3a is free to bind the FOXO3a-binding sites (ATAAACA) upstream of the *HER3* gene and induce mRNA and ultimately protein synthesis (Fig. 2B; refs. 52, 56, 57). The importance of FOXO-mediated feedback has also been shown in 3-dimensional ovarian cancer tumor spheroids where treatment with BEZ-235, a dual PI3K/mTOR inhibitor, led to increased levels of Bcl-2, IGF-IRb, p-STAT3, p-STAT6, p-c-Jun, p-SMAD3, p-p90RSK, EGFR, and p-HER2 (58). Furthermore, Muranen and colleagues (58) showed

that inhibition of the compensatory pathways abrogates the resistance to BEZ-235.

β -CATENIN

The wingless related MMTV integration site (Wnt)/ β -catenin pathway regulates limb development in embryogenesis and has been implicated in several cancers. In colon cancer, increased β -catenin pathway activation through loss of function of adenomatosis polyposis coli (APC) is a common event in early tumorigenesis, and increased nuclear β -catenin has been well documented (59–61). At the convergence of the Wnt/ β -catenin pathway and the PI3K signaling pathway is the AKT substrate FOXO3a, which is normally considered a tumor suppressor (62). In fact, the antitumoral effects of AKT inhibition are largely related to the resultant proapoptotic program induced by nuclear FOXO accumulation (63). Tenbaum and colleagues (64) recently highlighted the tumor-specific importance of AKT inhibition by showing that in the presence of a high nuclear level of β -catenin, AKT inhibition and the associated nuclear FOXO3a

retention resulted in increased metastatic potential (Fig. 3A and B). Mechanistically, the authors suggest that high nuclear β -catenin and increased nuclear FOXO3a (due to AKT inhibition) cooperate to regulate a prometastatic genetic program including IQ motif containing GTPase activating protein 2 (IQGAP2), which weakens E-cadherin junctions, resulting in increased cell scattering and motility (Fig. 3B; ref. 64). Moreover, nuclear β -catenin content conferred inherent resistance to AKT inhibition, possibly via reduction in the proapoptotic FOXO3a target genes *BCL2L11* and *TNFSF10*. The authors also suggest that high nuclear β -catenin results in nuclear translocation of FAS-associated adaptor protein (FADD) as well as inhibition of PARP1 cleavage normally induced by FOXO3a. Thus, a high nuclear β -catenin level creates an environment which is inherently less sensitive to a nuclear FOXO-mediated antitumoral effect. Exposure to the Wnt inhibitor XAV-939, and subsequent reduction in nuclear β -catenin, was able to reverse AKT inhibitor resistance (Fig. 3C; ref. 64). The work by Tenbaum and colleagues (64) suggests the importance of identifying tumor biomarkers that may select patients less likely to respond to PI3K/AKT/mTOR inhibitors. It remains to be determined whether or not the AKT/ β -catenin interaction is limited to colon cancer or whether this can be extended to other tumor types.

ANDROGEN RECEPTOR FEEDBACK

PI3K pathway activation through PTEN loss is common to metastatic prostate cancer, with a frequency of up to 70%, and PTEN loss promotes castrate resistance in cell line and xenograft models (65, 66). Despite the increase in PI3K pathway readouts in prostate cancer, a phase II trial of the mTOR inhibitor everolimus showed limited activity with only 2 of 36 patients showing a more than 50% decrease in serum prostate-specific antigen (PSA; ref. 67). Carver and colleagues (68) have recently shown that in PTEN-negative prostate cancers PI3K inhibition leads to androgen receptor (AR) pathway activation via relieving basal AR inhibition through HER kinases, specifically HER2/3. Conversely, AR inhibition led to a feedback increase in AKT phosphorylation via a decrease in the AR-dependent immunophilin FKBP5, which is a chaperone for the AKT phosphatase PH domain and leucine-rich repeat protein phosphatases (PHLPP; ref. 68). Thus, PTEN-deficient prostate cancers are able to bypass the growth-suppressive effects of PI3K inhibition via increased AR signaling. The compensatory increase in AR signaling likely underlies the poor clinical response rates to everolimus in combination with the weak anti-androgen bicalutamide (67). This observation argues that combined PI3K blockade with effective AR inhibition with abiraterone or enzalutamide may improve responses in metastatic prostate cancer (68). This combination is currently being studied in early-phase clinical trials (NCT01634061).

C-MYC AND CAP-DEPENDENT TRANSLATION

Up to 25% of breast cancers contain *PIK3CA* mutations in the two oncogenic hotspot domains (E545 helical domain and H1047R kinase domain), with H1047R being the most

common mutation (6, 69–72). Liu and colleagues have shown that *Pik3ca* induction can lead to mammary tumors in mice and that sustained PI3K signaling was required to maintain malignant mammary tumors (69). However, following withdrawal of inducible *Pik3ca*^{H1047R}, a significant proportion of mammary tumors resumed growth in PIK3CA-independent manner and a single-nucleotide polymorphism array revealed amplification of *c-Myc* and *Mdm2* in the recurrent tumors (69). Similarly, forced *c-MYC* expression confers resistance to otherwise sensitive *Pik3ca*^{H1047R}-mutant mammary tumors (69). As further validation, there are several human breast cancer databases confirming increased *c-MYC* expression in *PIK3CA*-mutant breast cancers with frequencies ranging from 27% to 47% (73–75). The importance of *c-MYC* as a regulator of PI3K/AKT/mTOR resistance has been highlighted in several other models.

NOTCH signaling is altered in a significant portion of adult T-cell acute lymphoblastic leukemia (ALL; ref. 76). *c-MYC* is a direct transcriptional target of NOTCH signaling, and NOTCH inhibition results in reduced *c-MYC* levels (76–79). *c-MYC* is also downstream of the PI3K–AKT pathway in that AKT-mediated phosphorylation and inhibition of GSK3 prevents the phosphorylation and degradation of *c-MYC* protein (80, 81). In T-cell ALL lines the dual PI3K/mTOR inhibitor PI-103 showed a predictable initial decrease in *c-MYC*. However, subsequently *c-MYC* levels were rescued by compensatory increased NOTCH signaling potentially mediated by a decrease in SGK-enhanced FBXW7-mediated degradation of active intracellular NOTCH (NICD; refs. 82–84). Using a chemical genetic screen, Muellner and colleagues (85) also identified NOTCH pathway alterations and downstream MYC activation as a mechanism to override to PI3K/AKT/mTOR-dependent proliferation in multiple breast cancer cell lines.

The eukaryotic initiation factor 4E is a major mediator of cap-dependent translation and tightly regulated by the PI3K–AKT–mTOR pathway. Upon activation, mTORC1 phosphorylates 4EBP1 (PHAS-1), thereby disrupting its interaction with eIF4E and allowing eIF4E to dissociate from 4EBP1 and participate in the initiation of translation after binding to the mRNA 5' cap structure (86–88). Ilic and colleagues (89) recently showed that *eIF4E* gene amplification and protein increase developed as a compensatory resistance mechanism in cells initially sensitive to the combined PI3K/mTOR inhibitor BEZ-235. Considering that the gene encoding eIF4E is a known MYC-regulated target, cooperation between MYC and eIF4E in regulating resistance is likely (89–92).

The internal ribosome entry site (IRES) is a conserved nucleotide sequence targeting mRNA for 5' cap-independent translation (93–96). IRES-mediated cap-independent translation is seen in response to nutrient deprivation (analogous to PI3K/mTOR inhibition) and oxidative stress in several organisms, and serves as a method for producing proteins required for cell survival (97–99). Several FOXO targets can be translated via IRES-mediated mechanisms and the ability of tumor cells to maintain translation in the face of PI3K/AKT/mTOR-mediated inhibition of cap-dependent translation may serve as a conserved resistance mechanism.

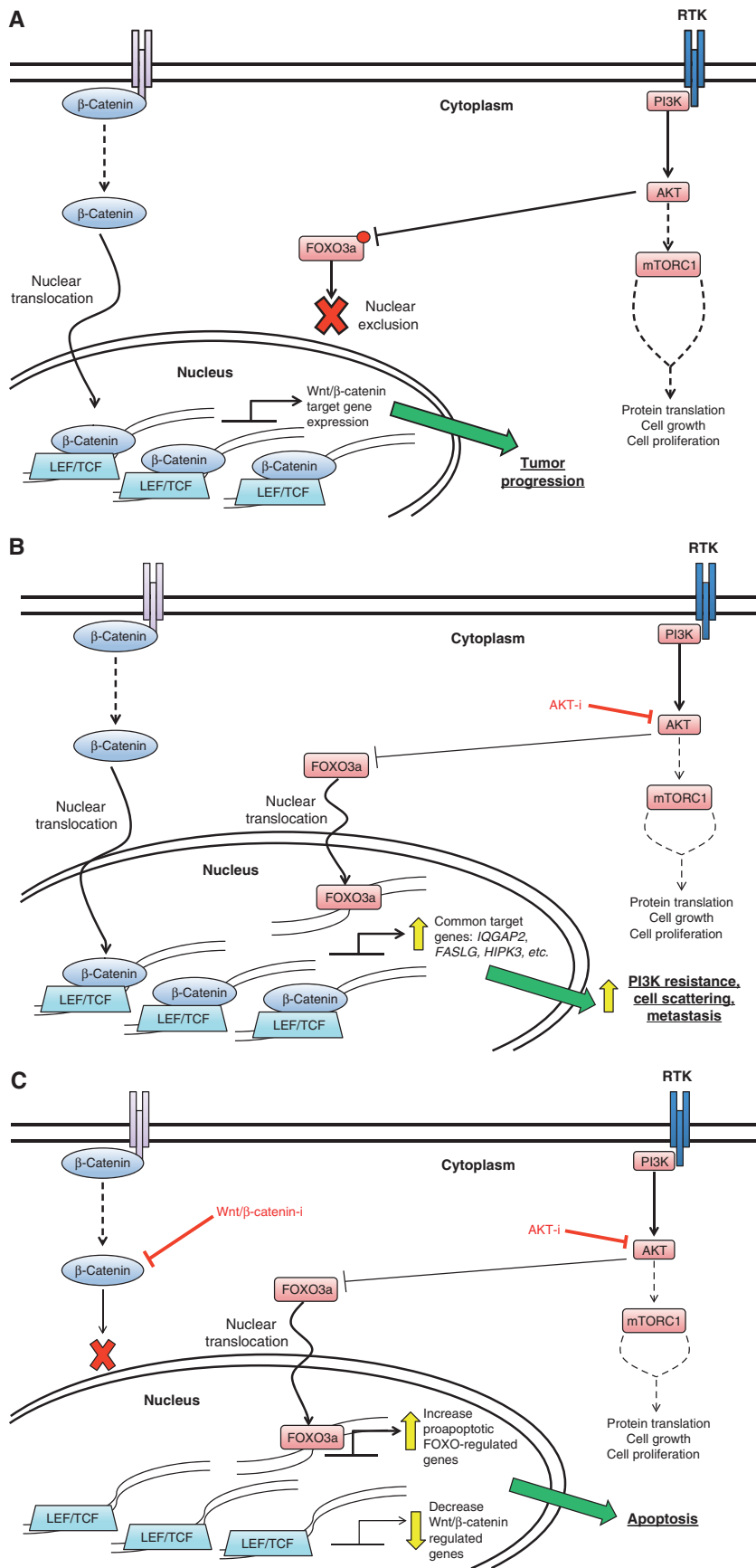


Figure 3. High nuclear β -catenin levels confer resistance to AKT inhibition and coordinates with increased nuclear FOXO3a to promote metastasis in colon cancer. **A**, activation of the Wnt/ β -catenin leads to nuclear accumulation of β -catenin, and activation of the PI3K-AKT pathway inhibits nuclear translocation of FOXO3a. **B**, the presence of high nuclear β -catenin and increased nuclear FOXO3a results in resistance to PI3K pathway inhibitors and promotes cell scattering and metastasis. **C**, reducing nuclear β -catenin through Wnt/ β -catenin pathway inhibition reverses the metastatic potential and resistance to PI3K/AKT inhibitors, resulting in increased apoptosis. Dotted lines represent multiple steps not shown graphically, and line strength represents relative activation. LEF, lymphoid enhancer factor; TCF, T-cell factor.

The common theme to c-MYC- and eIF4E-related resistance mechanisms is the uncoupling of proliferation from dependence on the PI3K-AKT-mTOR pathway. Both c-MYC and eIF4E are likely able to bypass clinically available inhibitors via exerting their action downstream of the commonly targeted signaling nodes (PI3K, AKT, and mTOR). Although it appears that c-MYC activation, either through increased NOTCH signaling or genomic alterations, may represent a conserved resistance mechanism, it is likely that multiple pathways capable of activating PI3K-AKT-mTOR pathway components downstream of the commonly pharmacologically inhibited targets will confer varying degrees of resistance. As it will be difficult to inhibit redundantly regulated distal nodes (e.g., 5' cap-dependent translation) in the PI3K-AKT-mTOR pathway, improved responses to PI3K pathway-targeted therapies are more likely to result from improved patient selection based on patient tumor testing.

CLINICAL IMPLICATIONS

Several retrospective clinical studies have attempted to analyze the predictive and prognostic significance of mutations, amplifications, and protein loss in the PI3K/AKT/mTOR axis with varying results (100-103). Large genomic datasets coupled to phosphoproteomic and small-molecule screens will inform the interactions of oncogenic aberrations in the PI3K/AKT/mTOR axis with other commonly altered pathways in cancer. Simply incorporating genomic data into the selection of therapeutic options may be improving the response to PI3K/AKT/mTOR inhibitors in early-phase clinical trials (104-106). Although emerging preclinical work continues to inform resistance mechanisms, it will be important to obtain patient tumor samples during therapy ("on-treatment biopsies") to validate preclinical observations. The importance of on-treatment biopsies is already being highlighted in preclinical models and informing treatment strategies such as discontinuous therapy to delay the emergence of resistance (26, 107). The incorporation of tumor signaling data using proteomic techniques such as reverse-phase protein array with genomic studies may further identify both patients likely to be resistant to PI3K pathway inhibitors as well as patients who may derive maximal benefit. Importantly, more work is needed to separate resistance mechanisms which develop under the selective pressure of drug exposure from those which are inherent prior to PI3K/AKT/mTOR-targeted therapy. Ultimately, prospective human trials are needed to determine whether or not knowledge of resistance mechanisms can affect hard clinical outcomes such as progression-free and overall survival.

CONCLUSIONS

Here, we have highlighted the central importance of the PI3K pathway in regulating multiple hallmarks of cancer, and known escape mechanisms that bypass PI3K/AKT/mTOR blockade. The ongoing observations of emerging resistance mechanisms highlight the paradigm for translational research. The flow of information obtained from bench studies to early-phase clinical trials is paramount, as is flow of patient-derived samples back to the bench. As more resistance mechanisms are elucidated, conserved patterns address-

ing compensatory signaling changes and genomic changes affecting targets downstream of pharmacologic inhibition are likely to emerge. Although the importance of the PI3K-AKT-mTOR pathway is well established in cancer, much remains to be determined about potential inherent and adaptive resistance mechanisms and their therapeutic potential.

Disclosure of Potential Conflicts of Interest

A.P. Myers is a Clinical Program Leader at Novartis and a consultant/advisory board member of Sanofi Aventis. L.C. Cantley has received a commercial research grant from GlaxoSmithKline, is a consultant/advisory board member of Novartis, Genentech, and Gilead, and has an immediate family member who is a consultant/advisory board member of Piramal. No potential conflicts of interest were disclosed by the other author.

The Editor-in-Chief of *Cancer Discovery* (L.C. Cantley) is an author of this article. In keeping with the AACR's Editorial Policy, the paper was peer reviewed and a member of the AACR's Publications Committee rendered the decision concerning acceptability.

Authors' Contributions

Conception and design: S.J. Klemptner, L.C. Cantley

Development of methodology: S.J. Klemptner

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.J. Klemptner

Writing, review, and/or revision of the manuscript: S.J. Klemptner, A.P. Myers, L.C. Cantley

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.J. Klemptner

Study supervision: S.J. Klemptner, L.C. Cantley

Acknowledgments

The authors apologize to colleagues whose important contributions to the field could not be cited because of space limitations.

Grant Support

L.C. Cantley is supported by NIH Grant GM041890, Stand Up To Cancer Dream Team Translational Research Grant, a program of the Entertainment Industry Foundation (SU2C-AACR-DT0209), and the American Association for Cancer Research.

Received February 8, 2013; revised August 21, 2013; accepted August 22, 2013; published OnlineFirst November 21, 2013.

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Cancer Discovery 2013;3:1345-1354. Published OnlineFirst November 21, 2013.

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