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PI3K-p110 α mediates resistance to HER2-targeted therapy in HER2+, *PTEN*-deficient breast cancers

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Abstract

HER2 amplification/overexpression (HER2+) frequently co-occurs with PI3K pathway activation in breast tumors. PI3K signaling is most often activated by PIK3CA mutation or PTEN loss, which frequently results in sensitivity to p110 α or p110 β inhibitors, respectively. To examine the p110 isoform dependence in HER2+, PTEN-deficient tumors, we generated genetic mouse models of breast tumors driven by concurrent Her2-activation and Pten-loss coupled with deletion of p110 α or p110 β . Ablation of p110 α , but not p110 β , significantly impaired the development of Her2+/Pten-null tumors in mice. We further show that p110 α primarily mediates oncogenic signaling in HER2+/PTEN-deficient human cancers while p110 β conditionally mediates PI3K/AKT signaling only upon HER2 inhibition. Combined HER2 and p110 α inhibition effectively reduced PI3K/AKT signaling and growth of cancer cells both *in vitro* and *in vivo*. Addition of the p110 β inhibitor to dual HER2 and p110 α inhibition induced tumor regression in a xenograft model of HER2+/PTEN-deficient human cancers. Together, our data suggest that combined inhibition of HER2 and p110 α / β may serve as a potent and durable therapeutic regimen for the treatment of HER2+, PTEN-deficient breast tumors.

Keywords

PI3K; HER2; PTEN; genetic mouse models; breast cancer

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CONFLICT OF INTEREST

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INTRODUCTION

Human epidermal growth factor receptor-2 (HER2), also referred to as ERBB2 or *Neu*, is constitutively activated by overexpression or gene amplification in 15–20% of breast tumors (1). Multiple HER2-targeted therapies are available for patients with metastatic HER2-positive (HER2+) cancer, including the HER2-specific monoclonal antibodies trastuzumab and pertuzumab and the EGFR/HER2 small molecule inhibitor lapatinib. Although these therapies delay tumor progression and can prolong overall survival, resistance commonly occurs in metastatic HER2+ breast cancer treated with these agents (2, 3). These data suggest that recurring tumors may intrinsically possess or acquire escape mechanisms from HER2 inhibition (2).

Activation of PI3K signaling downstream from HER2 is one such mechanism that may explain the pro-growth and pro-survival signaling that is necessary to overcome HER2 inhibition in tumors. Class 1A PI3Ks are heterodimeric lipid kinases composed of a catalytic p110 subunit and a regulatory p85 subunit. Three homologous p110 isoforms (p110 α , p110 β , p110 δ) are encoded by *PIK3CA*, *PIK3CB* and *PIK3CD*, respectively. Active PI3Ks catalyze the phosphorylation of PIP₂ to form the second messenger PIP₃, which activates AKT and other effectors. PI3K function is antagonized by the lipid phosphatase and tumor suppressor PTEN, converting PIP₃ back into PIP₂. Recent studies have demonstrated that p110 α and p110 β maintain distinct functions in signaling and cellular transformation (4). While p110 α is the major effector downstream of receptor tyrosine kinases (RTKs) (5–7), p110 β is obligatory for signaling via GPCRs (8, 9).

PI3K signaling hyperactivation occurs via somatic *PIK3CA* point mutations or PTEN loss in more than 50% of breast cancers (10, 11). While these events are almost always mutually exclusive, one or the other is present in a substantial fraction of HER2+ tumors (11, 12). Expression of mutant *PIK3CA* or loss of PTEN in breast cancer cell lines is associated with resistance to HER2-targeted therapies and this resistance can be reversed with PI3K inhibition (13–15). Moreover, PI3K activation is associated with a poor clinical outcome in patients treated with trastuzumab or lapatinib (14, 16).

Currently, the majority of PI3K inhibitors in clinical testing target class IA PI3K isoforms. Pan-PI3K inhibition may result in excess toxicity as all PI3K isoforms are inhibited. Indeed, isoform-selective inhibitors are now emerging in the clinic. Early clinical studies have achieved remarkable results with a p110 δ -selective inhibitor in treating certain B cell malignancies (17). In breast cancer, p110 α -selective inhibitors have shown promise in early phase trials for patients with tumors bearing *PIK3CA* mutations (18).

We and others have demonstrated that p110 α is the primary PI3K isoform responsible for oncogenic HER2 signaling and tumorigenesis in the mammary epithelium (7), whereas p110 β is important for some PTEN-deficient tumors, including prostate and breast tumors (9, 19). It is critical to evaluate PI3K isoform dependency in HER2+ breast cancers with concurrent PTEN-loss, and to assess the efficacy of isoform-selective inhibitors in combination with HER2-targeted therapy.

Here we generated a genetic mouse model of breast tumorigenesis driven by *Her2/Neu* activation and *Pten* loss coupled with p110 α or p110 β deletion. Breast tumorigenesis was dependent on p110 α , and this finding was recapitulated with isoform-specific inhibitors. Combined treatment with a HER2 inhibitor and a p110 α -selective inhibitor inhibited proliferation of human HER2+/PTEN null breast cancer cell lines *in vitro* and *in vivo*. P110 β inhibition in combination with dual HER2 and p110 α inhibition induced tumor regression in xenograft models of HER2+/PTEN-deficient human cancers. These results suggest that combining HER2 inhibitors with both p110 α - and p110 β -selective inhibitors may be an effective therapy for HER2+, PTEN-null breast tumors.

RESULTS AND DISCUSSION

Mammary tumors driven by concurrent *Her2+* and *Pten* deletion are dependent on p110 α

To generate a mouse model of breast tumorigenesis with concomitant *Her2+* and *Pten*-loss, floxed *Pten* (*Pten*^{L/L}) mice were crossed with mice carrying MMTV-*Neu*-IRES-Cre (NIC) where the expression of *Her2* and Cre recombinase is driven by the same promoter within mammary epithelial cells (20). MMTV-NIC/*Pten*^{L/L} (NIC/P) virgin female mice develop mammary tumors indistinguishable from human HER2+, PTEN-deficient primary breast cancer. To determine which PI3K isoform is required for tumorigenesis, we crossed the MMTV-NIC/*Pten*^{L/L} mice with either *p110 α* ^{L/L} (5) or *p110 β* ^{L/L} (9) mice to generate mice with MMTV-NIC/*Pten*^{L/L};*p110 α* ^{L/L} (NIC/PA) or MMTV-NIC/*Pten*^{L/L};*p110 β* ^{L/L} (NIC/PB) genotypes. NIC/P virgin female mice developed multifocal mammary tumors with a median latency of 31 days (Figure 1A). Notably, *p110 α* ablation significantly delayed tumor onset (T_{50} =46 days) (Figure 1A), whereas *p110 β* loss had no effect (T_{50} =30.5 days) (Figure 1A). NIC/P and NIC/PB tumors grew at similar rates, whereas NIC/PA tumors remained significantly smaller even at later time points (Figure 1B). Tumor volume analyses revealed a significantly lower tumor burden at the end point (66d) in NIC/PA mice (Figure 1C). These results suggest that only p110 α is required for breast tumorigenesis in mouse models characterized by *Her2+* and *Pten* loss.

p110 α mediates PI3K/AKT signaling and anchorage-independent growth of human HER2+, PTEN-deficient breast cancer cells

We investigated whether human breast cancer cell lines harboring HER2+ and PTEN loss are also dependent on p110 α by using HCC1569 and BT474 cell culture models. The HCC1569 breast cancer cell line harbors HER2+ and PTEN loss. We evaluated PI3K signaling in HCC1569 cells after p110 α - or p110 β -selective inhibition (BYL719 and KIN193, respectively). BYL719 or pan-PI3K inhibition via BKM120, but not KIN193, reduced PI3K effector phosphorylation (Figure 1D). Moreover, inhibition of p110 α , but not p110 β , was sufficient to reduce the number of colonies formed in soft agar (Figure 1E).

To determine whether PTEN loss affects the p110 α dependency of HER2+ breast cancer cells, we stably depleted PTEN via shRNA in the BT474 HER2+ breast cancer cell line that harbors intact PTEN and is sensitive to HER2 inhibition. We confirmed that PTEN protein expression is suppressed in stable pLKO-sh*PTEN* expressing BT474 cells, and increases anchorage independent growth (Figure S1A). *In vivo* xenografts and *in vitro* dose response

curves demonstrated that loss of PTEN expression in BT474 decreases tumor-free survival and confers resistance to lapatinib (Figure S1B, 1C).

To determine if p110 α is the predominant isoform that mediates HER2 signaling, we treated BT474-*shPTEN* cells with BYL719, KIN193, or BKM120. Indeed, p110 α - and pan-PI3K inhibition decreased PI3K effector phosphorylation in both BT474 and BT474-*shPTEN* cells (Figure 1F, 1G). Moreover, BYL719 and BKM120, but not KIN193, effectively blocked anchorage-independent growth of BT474-*shPTEN* cells in soft agar (Figure 1H), demonstrating that p110 α is the major isoform that mediates oncogenic HER2 signaling and growth of HER2+ breast tumor cells in the absence of PTEN.

Inhibition of p110 α enhances sensitivity of HER2+, PTEN-deficient breast cancer cells to HER2-targeted therapy

HER2-targeted agents, such as trastuzumab and lapatinib, are commonly used in patients with HER2+ metastatic breast cancer. Because clinical data suggests PTEN-deficiency renders HER2+ breast tumors resistant to HER2-targeted therapy, we confirmed that HCC1569 and BT474-*shPTEN* cell lines are more resistant to lapatinib in comparison to PTEN wild-type, HER2+ breast cancer cell lines BT474 and SKBR3 (Figure 2, S1C). We then investigated whether inhibition of a single PI3K isoform can restore the sensitivity of these tumor cells to HER2 blockade. We assessed HER2/PI3K signaling in response to lapatinib, BYL719, or KIN193 alone or in combination in HCC1569 and BT474-*shPTEN* cell lines. While lapatinib reduced HER2 phosphorylation, it did not suppress PI3K/AKT signaling (Figure 2A). Combined BYL719 and lapatinib inhibited HER2 activation, and inhibited PI3K effector phosphorylation (Figure 2A). Combined HER2 and p110 β inhibition did not reduce PI3K/AKT signal transduction as effectively as dual HER2 and p110 α inhibition (Figure 2A).

To further investigate whether p110 α inhibition can overcome lapatinib resistance to block the proliferation of HER2+, PTEN-deficient breast cancer cells, we treated HCC1569 and BT474-*shPTEN* cells with lapatinib, BYL719, or KIN193 alone or in combination and measured cell viability. Combined BYL719 and lapatinib treatment more effectively inhibited cell viability compared to combined treatment with KIN193 with lapatinib (Figure 2B). Moreover, HER2 inhibition coupled with combined p110 α and p110 β inhibition maximally reduced cell viability (Figure S3). Taken together, these data suggest that p110 α is a major target that can overcome lapatinib resistance by suppressing HER2/PI3K/AKT signaling in HER2+, PTEN deficient cells lines.

Combined inhibition of p110 α / β and lapatinib blocks the growth of HER2+, PTEN-deficient breast tumors in vivo

To test whether p110 α inhibition can overcome lapatinib resistance in PTEN-deficient HER2+ cells *in vivo*, NCI nude mice bearing palpable BT474-*shPTEN* or HCC1569 xenografts were treated with lapatinib alone or in combination with BYL719 or KIN193. Lapatinib alone failed to inhibit tumor growth. Addition of KIN193 provided modest improvement over lapatinib monotherapy while addition of BYL719 significantly impaired tumor growth (Figure 3A, 3B). Moreover, durable tumor regression was achieved in the

HCC1569 xenograft tumor model following combined HER2/p110 α /p110 β inhibition (Figure 3B). Together, these data expand upon previous studies demonstrating p110 β lipid kinase activity increases following p110 α inhibition in breast cancers characterized by PIK3CA activation or PTEN-inactivated breast cancer (21, 22). Here we suggest that while p110 α is a primary target to overcome resistance to HER2-therapy in the context of HER2+ and PTEN-deficient breast cancers, combined HER2, p110 α and p110 β inhibition offers maximal PI3K inhibition and durable tumor regression.

To evaluate signaling and pharmacodynamic responses of HCC1569 and BT474-*shPTEN* xenografts during inhibitor treatment, tumors were isolated 72h post drug administration and molecular markers analyzed. Dual HER2 and p110 α inhibition decreased PI3K effector phosphorylation, blocked proliferation and induced apoptosis (Figure 3C, S4). Concomitant treatment with lapatinib, BYL719, and KIN193 significantly eliminated PI3K effector phosphorylation, blocked proliferation and induced apoptosis in HCC1569 xenografts (Figure 3C). These results establish that p110 α primarily mediates PI3K/AKT signaling in HER2+ PTEN deficient breast tumors whereas p110 β conditionally mediates HER2/PI3K signaling in the absence of p110 α .

Here we utilized a NIC-Pten^{-/-} mouse model of Her2 overexpressing, Pten-null breast cancer and showed that p110 α ablation, rather than p110 β loss, delayed the onset of tumor development. Pharmacological studies in which p110 isoform-selective inhibitors were used to treat human HER2+, *PTEN*-deficient breast cancer cells confirmed that specific targeting of p110 α was sufficient to inhibit AKT activity and tumor growth. This study suggests that the p110 α isoform of PI3K is essential for relaying RTK-mediated signal transduction in the context of HER2+, PTEN-deficient breast cancers, and p110 β can conditionally mediate PI3K/AKT signaling in this model. Our results suggest that patients with HER2+, PTEN-deficient breast cancers may durably respond to combined HER2 and p110 α /p110 β inhibition.

Our data support a model in which HER2 remains the oncogenic driver in the context of HER2+, PTEN-null breast cancers, as is shown in genetic experiments where HER2+, Pten-null mice depend on p110 α for tumor development (Figure 1). This model demonstrates that HER2 amplification is a dominant oncogenic event over Pten loss, and Pten null tumors that may otherwise be dependent on p110 β become dependent on p110 α in the presence of a potent tumorigenic event such as HER2 amplification, which has previously been shown to bias tumors to p110 α dependence. It is, however, also possible that the elevated p110 α expression observed in some breast tumors may contribute to enhanced and dominant HER2-associated signal transduction. We previously used mouse models in which mammary tumorigenesis driven by HER2 activation was complexed with ablation of p110 α and/or p110 β expression, to demonstrate that p110 α is the primary PI3K isoform responsible for AKT signaling and tumorigenesis in HER2+ mammary tumors (7). We observed similar effects in the ovary, where Kras activation biased ovarian cancer cell lines to p110 α dependence alone or in combination with Pten loss (23). In contrast, other previous studies suggested that signaling through p110 β is necessary in certain tumors driven by PTEN loss (9, 19). Thus, the data presented here fits into the context of previously published studies suggesting that tumors preferentially depend on either p110 α or p110 β , and that this dependence is based upon the identity of oncogenic driver(s) within the tumor. Our results

establish that tumors characterized by concurrent PTEN-loss and HER2-overexpression rely primarily on the p110 α isoform.

Our results provide concrete evidence that targeting p110 α is necessary to overcome the resistance of HER2+ PTEN-deficient breast tumors to HER2-directed therapies. In addition our data show that p110 β inhibition decreases PI3K/AKT signaling and tumor growth when combined with lapatinib, strongly supporting that PI3K isoform dependence of PTEN-deficient tumors can be altered by RTK activation. Combined Her2, p110 α and p110 β inhibition maximally abrogated AKT activation and minimized tumor growth. Of note, pan-PI3K inhibitors have not yielded durable or efficacious clinical results, but isoform-selective inhibitors that are currently emerging in the clinic may offer considerable promise of both increased efficacy and reduced toxicity in comparison to pan-PI3K inhibitors which target all class I PI3K isoforms (24, 25). Together, our work suggests that in the context of HER2+, PTEN-deficient breast cancers, a combination therapy targeting HER2 and p110 α / β should be considered for clinical application.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. p110 α ablation or inhibition inhibits mammary tumorigenesis and cell growth in NIC/*Pten*^{-/-} mice and HER2-positive, PTEN-deficient human breast cancer cells
 MMTV-NIC (26), *Pten*^{L/L} (27), *p110 α* ^{L/L} (5), and *p110 β* ^{L/L} (9) were crossed to obtain MMTV-NIC/*Pten*^{-/-} (NIC/P), MMTV-NIC/*Pten*^{-/-}/*p110*^{-/-} (NIC/PA), and MMTV-NIC/*Pten*^{-/-}/*p110 β* ^{-/-} (NIC/PB). **(A)** Kaplan-Meier (KM) curves in NIC/P (red), NIC/PA (green), and NIC/PB (blue). * $p < 0.001$, Log-rank test. **(B)** Average tumor volume in mice of the indicated genotypes over time. * $p < 0.01$, Two-way ANOVA. **(C)** Average volume of end-stage tumors in mice of the indicated genotypes. Data are shown as mean $\pm$ S.E.M (n=6 per genotype). * $p < 0.01$, Student's *t* test. **(D)** Immunoblot analyses in HCC1569 cells treated with BYL719 (Novartis), KIN193 (MedChemexpress) or BKM120 (Novartis) (μ M). **(E)** Anchorage-independent growth of 5×10^4 HCC1569 cells treated with BYL719, KIN193 or BKM120 (1 μ M). **(F, G)** Immunoblot analyses in BT474 and BT474-shPTEN cells treated as indicated in (D). **(H)** Anchorage-independent growth of BT474-shPTEN cells treated as described in (E). For (E) and (H), Mean $\pm$ S.E.M from three independent experiments are shown. ** $p < 0.001$, Student's *t* test.

Figure 2. The p110α inhibitor BYL719 sensitizes HER2+, *PTEN*-deficient cancer cells to lapatinib

(A) Immunoblot analyses in HCC1569 or BT474-*shPTEN* cells treated with BYL719 or KIN193 alone or in combination with lapatinib (MedChemexpress) (μM). (B) Cell viability of HCC1569 (Left) and BT474-*shPTEN* (Right) cells treated with lapatinib (1μM and 0.5 μM, respectively), 0.5μM BYL719 or 0.5μM KIN193 alone or in combination for 72h. Cell viability was measured by CellTiter 96® Assay. Mean ± S.E.M from three independent experiments. * $p < 0.01$, ** $p < 0.001$, Student's *t* test.

Figure 3. Treatment with a p110 α selective inhibitor reverses lapatinib resistance in orthotopic xenograft models

Mice bearing BT474-shPTEN (**A**) or HCC1569 xenografts (**B**) were treated with inhibitor combinations: Lapatinib (100mg/kg) was administered daily by oral gavage in 0.5% hydroxypropyl methylcellulose, 0.1% Tween80. BYL719 (45mg/kg) in 10% 2-hydroxypropyl- β -cyclodextrin (Sigma) was administered daily by (IP) injection. KIN193 (20mg/kg) in 7.5% NMP, 40% PEG400 was dosed twice a day by IP injection. BKM120 was reconstituted 1:9 in NMP and PEG300. Mice were dosed with this compound formulation at 45mg/kg daily by oral gavage (n=6 per treatment group). Tumor volume was measured every 3 days. *p<0.01, **p<0.001 and ***p<0.0001, Two-way ANOVA (**C**) Immunohistochemical analyses of FFPE HCC1569 xenograft sections treated with lapatinib

and/or BYL719, KIN193 for 72h. TUNEL assays were performed with the *In Situ* Cell Death Detection Kit (Roche). Representative images are shown, Scale=20µm (Left). Quantitative analyses of 6 images randomly obtained from 3 mice (Right). * $p < 0.01$, ** $p < 0.001$, Student's t test.

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