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ErbB2-positive mammary tumors can escape PI3K-p110 α loss through downregulation of the Pten tumor suppressor

Alexandra M. Simond^{1,*}, Trisha Rao^{1,*}, Dongmei Zuo¹, Jean J. Zhao², and William J. Muller¹

¹Rosalind and Morris Goodman Cancer Research Centre, Department of Biochemistry, McGill University, Montreal, Quebec, Canada

²Departments of Cancer Biology, Dana–Farber Cancer Institute, Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA

Abstract

Breast cancer is the most common cancer among women and 30% will be diagnosed with an ErbB2-positive cancer. Forty percent of ErbB2-positive breast tumors have an activating mutation in p110 α , a catalytic subunit of phosphoinositide 3-kinase (PI3K). Clinical and experimental data show that breast tumors treated with a p110 α -specific inhibitor often circumvent inhibition and resume growth. To understand this mechanism of resistance, we crossed a p110 α conditional (p110 α flx/flx) mouse model with mice that overexpresses the ErbB2/Neu-IRES-Cre transgene (NIC) specifically in the mammary epithelium. Although mammary-specific deletion of p110 α dramatically delays tumor onset, tumors eventually arise and are dependent on p110 β . Through biochemical analyses we find that a proportion of p110 α -deficient tumors (23%) display downregulation of the Pten tumor suppressor. We further demonstrate that loss of one allele of PTEN is sufficient to shift isoform dependency from p110 α to p110 β in vivo. These results provide insight into the molecular mechanism by which ErbB2-positive breast cancer escapes p110 α inhibition.

Keywords

Breast cancer; ErbB2; p110 α ; p110 β ; PTEN; Therapy escape

Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide, and 70% of women with breast cancer have mutations in the phosphoinositide 3-kinase (PI3K) pathway [1]. PI3K is an essential lipid kinase whose downstream effects involve cell growth, proliferation and survival [2,3,4,5]. PI3K functions by phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP2) on its 3' hydroxyl group to generate

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Corresponding author: Dr. WJ Muller, Goodman Cancer Research Center, Room 516, 1160 Pine Avenue West, Montreal, Quebec H3A 1A3, Canada. william.muller@mcgill.ca, Phone: 514-398-5847, Fax: 514-398-8401.

*The authors equally contributed to this work

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phosphatidylinositol-3, 4,5-bisphosphate (PIP3) [6,7,8]. PIP3 is an important second messenger that recruits PI3K effectors to the membrane allowing subsequent activation of the pathway. Phosphatase and TENsin homolog (PTEN) is an essential lipid phosphatase that antagonizes PI3K by dephosphorylating PIP3 and has antagonistic functions to PI3K [2,3,4,5].

PI3K represents a large family of protein kinases that is divided into three classes, of which, class I is the most commonly studied in breast cancer. Class I is further subdivided into class Ia, which are activated mainly by Receptor Tyrosine Kinases (RTKs), such as the ErbB2/ ErbB3 heterodimer, and class Ib that are primarily driven by G protein-coupled receptors (GPCRs) [9,7]. Both subclasses are made up of a p110 catalytic subunit and a p85 regulatory subunit [2,10,11]. The catalytic subunit of Class I PI3K has four isoforms: p110 α , p110 β , p110 γ and p110 δ [12,13]. Both p110 α and p110 β are ubiquitously expressed, while p110 γ and p110 δ mainly expressed in leukocytes [14,15]. Today, the most studied isoform remains p110 α due to its 40% mutation frequency in breast cancer and 15% mutation rate across all cancers [16,17,18]. This makes p110 α the most mutated class Ia isoform [19]. However, p110 β has been increasingly in the spotlight due to its association with PTEN loss, an aberration associated with hereditary cancers and frequently observed in breast cancers [20,21,22,23]. Recent publications have suggested that PTEN-null breast tumors often depend on p110 β for PI3K signaling downstream of GPCRs, while PTEN wild-type tumors depend on p110 α downstream of RTKs [24,25]. Genetic context also seems to influence the choice between p110 α or p110 β dependency in the absence of PTEN. For example, in ErbB2-positive or KRAS mutated breast cancers, PTEN-null tumors are solely dependent on p110 α [26,27].

ErbB2 is an RTK that is found to be amplified and overexpressed in 20-30% of breast cancers, 40% of which have an activating mutation in p110 α [28,29]. A wide variety of pan and isoform-specific inhibitors have been developed against PI3K, some of which are currently in clinical trials [2]. Pan-PI3K inhibitors have been associated with toxicity, so there have been increasing numbers of clinical trials investigating isoform-specific inhibitors [28,2]. Unfortunately, inhibition of p110 α becomes ineffective over time both in vitro and in vivo, indicating the development of resistance mechanisms. [30,31]. Notably one group found that in response to a p110 α -specific inhibitor, luminal breast cancer cells rapidly compensate for p110 α through the engagement of p110 β [30]. Another group has found that continued treatment with a p110 α -specific inhibitor leads to a durable response in patients with a PIK3CA mutation, however, patients ultimately stop responding to therapy and develop lung metastasis that display PTEN-loss and p110 β dependency. [32]

We have previously shown that loss of p110 α in the Mouse Mammary Tumor Virus (MMTV)-ErbB2-IRES-Cre (NIC model) results in abrogation of mammary tumor development over an initial 8-month observation period [33]. Given that resistance to p110 α specific inhibitors occur with time, we decided to evaluate p110 α -deficient tumors over an extended period of 24-months. We find that although, ErbB2-driven mammary tumorigenesis is severely delayed in the absence of p110 α , the majority of animals eventually develop tumors. To understand the mechanism by which acquired resistance was occurring in our system, we performed detailed genetic and molecular analyses of the

resulting tumors. We show that one mechanism by which p110 α -loss is rescued is through spontaneous Pten downregulation. We further demonstrate that reduction in PTEN levels, through the loss of one allele, is sufficient to rescue the delay in tumor onset caused by the loss of p110 α , which was found to occur through the p110 β isoform. The isoform switch that occurs after p110 α loss raises the concern about using isoform-specific inhibitors as a way to reach durable remission in patients who have ErbB2-positive breast cancer, and allows us to suggest a therapeutic approach that is potentially more effective.

Results

Loss of p110 α significantly delays tumor onset, and impairs tumor growth and metastasis, with a subset of tumors exhibiting downregulation of the Pten tumor suppressor

To understand how ErbB2-positive tumors escape p110 α inhibition, we crossed a conditional p110 α mouse strain [34], to an activated ErbB2/Neu-IRES-Cre mouse model driven specifically to the mammary epithelium through the mouse mammary tumor virus promoter (MMTV) (NIC model) (supplemental figure 1a) [35]. The resulting mice have ErbB2 activation and Cre-mediated deletion of p110 α in one or both of the conditional alleles (supplemental figure 1b,c). Consistent with our previous report [33], mammary specific deletion of both alleles of p110 α significantly delayed tumor onset as compared to the parental strain, with an average of 351 days (n=44) versus 138 days (n=45) respectively. However, loss of one allele of p110 α has a significant but limited impact on tumor onset with an average of 150 days (n=38) (figure 1a). In the p110 α -deficient animals that eventually developed tumors, the number of tumors (n=35) and total tumor burden (n=43) was significantly lower than in the wild-type animals (n=43) (figure 1b). The p110 α -deficient tumors also exhibited reduced metastatic potential to the lungs : 72% (n=39) in the p110 α -deficient strain to 45% (n=20) in the heterozygous-p110 α mice versus 9% (n=23) in the wild-type animals (supplemental figure 2a). At a histological level, all tumors of each genotype displayed a classic adenocarcinoma phenotype that is typical of NIC model (supplemental figure 2b).

Given the known importance of p110 α in the PI3K/PTEN/AKT signaling axis, we next evaluated the levels of key downstream components of the PI3K pathway. We find that despite the loss of p110 α , tumors retain robust phosphorylation of Akt. In fact, a subset of tumors that tend to have higher levels of p-Akt are also found to downregulate Pten, ErbB2, p85 and total Akt. Of 39 p110 α -deficient tumors, 9 displayed a reduction in Pten levels (23%) (supplemental figure 3). However, most tumors that have high levels of p-Akt are not found to downregulate Pten, ErbB2, p85 or total Akt, suggesting the involvement of multiple mechanisms.

These observations indicate that regardless of p110 α status, tumors remain dependent on PI3K downstream signaling.

p110 α -deficient ErbB2 tumors remain dependent on PI3K

Given the observed downregulation of PTEN in a subset of p110 α -deficient/NIC tumors, we hypothesized that these tumors may still be dependent on PI3K signaling through another

p110 isoform. To test this hypothesis, we determined whether p110 α -deficient tumors remained sensitive to a pan-PI3K inhibitor (GDC-0941) [36]. Consistent with this contention, we found that potent inhibition of p-Akt occurred within 2 hours of treatment and is still apparent after 12 hours (figure 2a). Next, we established tumors in athymic nude mice (NCr) from two p110 α -deficient and two wild-type tumor lines (n=4-5 per line), and treated these mice with either a vehicle or GDC-0941. In contrast to animals treated with vehicle, the animals treated with GDC-0941 showed drastic reduction in tumor growth (figure 2b,c).

We further show that dependency on PI3K is observed regardless of Pten status, as tumor cells having wild-type or reduced levels of PTEN were equally responsive to GDC-0941 inhibition (figure 2d). These observations indicate that a p110 isoform switch may be dependent or independent of PTEN status, suggesting the existence of at least two distinct mechanisms by which tumors escape loss of p110 α . Taken together, these results indicate that, despite the loss of p110 α , NIC tumor cells remain dependent on PI3K enzymatic activity.

PTEN haploinsufficiency can compensate for loss of p110 α in ErbB2 mammary tumor progression

The data presented thus far argue that at least one of the molecular mechanisms by which p110 α -deficient tumors escape p110 α dependency may be through Pten downregulation. To directly address this hypothesis, we crossed a conditional PTEN strain to the NIC model to generate mice with heterozygous loss of PTEN and overexpression of activated ErbB2 in the mammary epithelium (supplemental figure 4a.b.c.d). This allowed us to produce mice that will express reduced levels of Pten, similar to what we observed in a subset of p110 α -deficient tumors. Consistent with the causal role of Pten downregulation in tumor escape of p110 α -loss, tumor onset in p110 α -deficient/NIC animals lacking an allele of PTEN was dramatically accelerated from 351 days (n=45) in the p110 α -deficient/NIC animals to 108 days (n=18) but only modestly accelerated tumor onset as compared to wild-type NIC mice that had tumors appear at 138 days (n= 44) (figure 3a). The number of tumors (n=17), total tumor burden (n=17), and the incidence of lung metastasis (n=17) was increased in p110 α -deficient/PTEN haploinsufficient animals as compared to the p110 α -deficient strain (n=36, n=36, n=23) (Figure 3b, supplemental figure 5a). Histologically, we observed an adenocarcinoma phenotype across all genotypes (supplemental figure 5b). Most strikingly, when analyzing protein levels of PI3K signaling pathway components, we observed that tumors that have lost p110 α and are PTEN-haploinsufficient have lower levels of ErbB2, p85, and total Akt, as well as higher levels of p-Akt. These results are consistent with what we observed in p110 α -deficient tumors that have spontaneous Pten downregulation (figure 3c). This suggests that loss of one allele of PTEN is sufficient to shift p110 isoform dependency away from p110 α in an ErbB2 mouse model, mimicking p110 α inhibition, and that PTEN is indeed the driver of the p110 isoform switch.

We have previously shown that PTEN loss in the NIC model accelerates tumorigenesis, but in the presence of wild type p110 α [37]. This report, along with our findings presented,

emphasize the importance of PTEN levels in p110 α -dependent and independent mammary tumorigenesis.

Tumors that have lost p110 α are p110 β dependent

To confirm that Pten deficiency is responsible for p110 isoform switch, we next investigated whether p110 α -deficient tumor cells with PTEN-haploinsufficiency remained sensitive to p110 β inhibition by treating them with a p110 β -specific inhibitor (TGX221) [38]. Using Akt phosphorylation as a readout for PI3K activity, we observed that both p110 α -deficient and p110 α /PTEN-deficient tumors cells displayed sensitivity to p110 β inhibition, whereas, wild-type tumor cells displayed constant levels of p-Akt. These results indicate that a 2-fold decrease in Pten levels can induce p110 β -driven tumors (figure 4a). The fact that these p110 α -deficient tumors are p110 β -dependent suggests that there are multiple mechanisms involved in p110 isoform switch. Furthermore, our results indicate that p110 β may be signaling downstream of receptors other than the ErbB2/ErbB3 heterodimer. Consistent with this expectation co-immunoprecipitation analyses of tumor lysates revealed that p110 β associates equally with the ErbB3 receptor in all the genotypes. Because the binding of p110 β to ErbB3 is not increased in response to p110 α -loss, p110 β may be signaling through another receptor (figure 4b). Consistent with this data, immunofluorescence analyses for p110 β and ErbB2 on tumor sections, revealed similar co-localization of p110 β and ErbB2 across genotypes, confirming that p110 β recruitment to the ErbB2/ErbB3 heterodimer is not increased in p110 α -deficient tumors (n=7 for each genotype) (figure 4c). Taken together, these data argue that PTEN downregulation is sufficient to drive the switch from p110 α to p110 β in an ErbB2 mouse model mimicking p110 α inhibition.

Discussion

One of the ongoing challenges in the treatment of breast cancer is the emergence of acquired resistance to therapies targeting oncogenic drivers such as PI3K and ErbB2. To uncover the molecular basis for resistance of ErbB2-positive breast cancer to p110 α -specific inhibition, we used an ErbB2 mouse model mimicking p110 α inhibition in the goal of understanding tumor therapy escape over time. We established that mammary epithelial-specific ablation of p110 α dramatically delays tumor onset, although, the majority of animals eventually develop tumors that are no longer dependent on p110 α . Using a pan-specific inhibitor we showed that all tumors remain dependent on PI3K. Interestingly, a proportion of p110 α -deficient tumors display downregulation of the Pten tumor suppressor. We found that transgenic loss of an allele of PTEN is sufficient to rescue the delay in tumor onset observed and induce tumors that are dependent on p110 β .

The observation that the subtle changes in Pten protein levels can have dramatic effects on p110 isoform dependency is critical as it concerns the design of targeted therapies for breast cancer. It is well known that Pten is a haploinsufficient tumor suppressor and that slight changes in its protein levels can be controlled by a variety of post-transcriptional and post-translational events [23].

In our mouse model, loss of p110 α has a significant impact on ErbB2-driven mammary tumorigenesis, which fits with the theory that ErbB2-positive tumors are p110 α addicted and

signal through p110 β as a last resort, as p110 β signals less efficiently through RTK's [39,40].

It still remains unclear why Pten has such an interconnected role with isoform specificity in ErbB2-positive breast cancer. One possible explanation is that Pten status can alter the levels of a variety of proteins some of which may be ligands to a receptor to which p110 β would have more affinity to than RTKs, such as GPCRs or Integrins. Alternatively, PTEN loss may also affect p110 β signaling through upregulation of GPCRs or Integrins [41,42,43]. (figure 5)

Evidence from our study that may support this point is the lower levels of p85 protein in p110 α -deficient tumors with spontaneous PTEN downregulation. p110 β has been found to not necessarily need p85 to bind to GPCR receptors, as it binds directly on the catalytic domain [43,44]. Consistent with this possibility, we also observed that increased p110 β dependency did not correlate with increased recruitment to the ErbB3 receptor or with increased co-localization with the ErbB2/ErbB3 heterodimer. However, another explanation for the results observed in figure 4 could be that p110 β is equally recruited to the ErbB2/ErbB3 heterodimer but only displays kinase activity in response to Pten downregulation.

Based on our genetic studies, we could predict that treatment of ErbB2-positive breast cancer with a p110 α -specific inhibitor would lead to the development of resistance through dependency on p110 β . Given that these tumors seem to solely escape through p110 β is reassuring and suggests that a combinational therapy between p110 α and p110 β specific inhibitors may be the best solution, to this date, for during remission. There are still many interesting avenues to be discovered in the molecular mechanisms by which p110 isoform switch occurs and future studies may permit us to develop better and less toxic therapeutic approaches for ErbB2-positive patients.

Methods

Animal husbandry

Our animals were housed at the animal facility in the Goodman Cancer Research Center and our experiments followed the approved animal use protocol. All strains used in this study were on an FVB/N background. The strains utilized for the study were : conditional p110 α [33], conditional PTEN (129/J, obtained from The Jackson Laboratory, Bar Harbor, ME) and NIC [35]. All mice used for experimental purposes were female and they were housed for a maximum of 600 days.

Genotyping and excision PCR

Genotyping on mouse tails was performed at weaning age and at sacrifice. Excision PCR was conducted at tumor endpoint. DNA was extracted from tails and tumors as outlined later. Primers for genotyping were: p110 α F: CTGTGTAGCCTAGTTTAGAGCAACCATCTA, R: CCTCTCTGAACAGTTCATGTTTGATGGTGA PTEN F: ACTCAAGGCAGGGATGAGC, R: GCCCCGATGCAATAAATATG Neu F: TTCCGGAACCCACATCAGGCC, R: GTTTCCTGCAGCAGCCTACGC Cre: F GCTTCTGTCCGTTTGCCG, R:

ACTGTGTCCAGACCAGGC Primers for excision PCR were: p110 α F: CTGTGTAGCCTAGTTT TAGAGCAACCATCTA, R: ACAGCCAAGGCTACACAGAGAAACCCTGTC PTEN P1: ACTCAAGGCAGGGATGAGC, P2: AATCTAGGGCCTCTTGTGCC, P3: GCTTGATATCGAATTCCTGCAGC. All primers were used at a concentration of 10 μ M.

Taq (20120X, Qiagen, Venlo, The Netherlands) was used for p110 α and PTEN genotyping and excision PCR's with twice the amount of recommended Taq was used for the excision PCR. Easy Taq (AP111, TransGen-EasyTaq, Beijing, China) was used for the Neu and the Cre genotyping PCR's

Mammary tumor monitoring

Female nulliparous mice were monitored weekly by mammary palpation and animals with tumors were sacrificed 5-7 weeks after tumor onset. Tumors were measured using a caliper and total volume was determined with the following formula: $(4/3 \times (3.14159) \times (\text{length}/2) \times (\text{width}/2)^2)$. For animals with multiple masses had the individual volumes of each tumor were added to determine total volume. Mice were sacrificed before the total total tumor volume reached 6cm³ or before a single tumor reached 2.5cm³

Tissue sample processing

Tissue samples were collected at necropsy and either flash frozen in liquid nitrogen and stored at -80°C until further use, or fixed immediately in 10% neutralized formalin for 24 hours. Fixed tissue was paraffin-embedded and sectioned at a thickness of 4 μ m by the Histological core facility in the Goodman Cancer Research Center at McGill university. H&E staining was performed by the Histology Core Facility.

Lung metastasis analysis—Lung metastasis was assessed by counting lesions in 5 \times 50 μ m step sections of lungs from tumor-bearing mice.

GDC-0941 in vivo assay

Tumor cells were isolated from Ncr tumor outgrowths (see supplemental methods for tumor dissociation protocol) and 5.0 \times 10⁵ tumor cells were injected into the mammary fat pad of Ncr mice (one side). Mice were treated with 125mg/kg of GDC-0941 or vehicle by oral gavage daily for 6 weeks. Tumor outgrowth was monitored bi-weekly until tumor burden endpoint (2.5 cm³)

Protein extraction

Tumour lysates were prepared from flash frozen tumor tissue, crushed with a mortar and pestle, and lysed in PLC γ or TNE lysis buffer (see supplemental methods). Protein concentration was determined by Bradford assay.

Immunoblotting/immunoprecipitation

For immunoblot, primary antibodies used were: p-ErbB2 (cell signaling# 2249, 1:1000), ErbB2 (Santa Cruz #284, 1:1000), p110 α (cell signaling #4249, 1:1000), p110 β (santacruz #602, 1:1000), p110 γ (cell signaling #5405, 1:1000), p85 (cell signaling #4257, 1:1000),

Pten (cell signaling #9559, 1:1000), p-Akt thr308 (cell signaling #4056, 1:500), p-Akt ser473 (cell signaling #9271, 1:1000), Akt1-2 (santa-cruz #1619, 1:1000), β -actin (Sigma A5441, 1:10000). Secondary antibodies used were conjugated to horseradish peroxidase (HRP) (Jackson Laboratory). For immunoprecipitation, a p110 β antibody (santa-cruz #602) was used. Primary antibodies for immunoblot were ErbB3 (santa-cruz #285 1:1000) and p110 β (santa-cruz #602 1:1000)

DNA/RNA extraction

tail DNA was extracted from tail pieces at weaning age and at sacrifice using salt precipitation (see supplementary methods). DNA was extracted from tumor tissue using phenol-chloroform (see supplemental methods) and RNA was extracted using the Qiashredder columns and the DNA/RNA mini kit from Qiagen

qRT-PCR

cDNA was generated from 1 μ g of tumor RNA using the M-MuLV Reverse Transcriptase (#M0253S, New England Biolabs, Ipswich, Massachusetts, United States) Oligo-dT(23VN) and a murine RNase inhibitor (New England Biolabs). Real-time PCR was performed using the Roche LightCycler master mix on a Roche LightCycler 480. Samples were always run in triplicates and normalized to Gapdh. Primers used for RT-PCR: Gapdh: F : CATCAAGAAGGTGGTGAAGC, R: GGGAGTTGCTGTTGAAGTCG, p110 α : F: TCCATCAGCTTCTGCAAGAC R: CTTCCCTTCTGCTTCTTGG, PTEN: F: CATTGCCTGTGTGGTGATA R: AGGTTTCCTCTGGTCCTGGTA

Immunofluorescence

Primary antibodies: p110 β (santa-cruz #602, 1:1000), ErbB2 (Dako A0485) Pten (cell signaling #9559, 1:1000). Secondary antibodies: Alexa fluor-488 TSA, Alexa fluor-456 TSA, Alexa fluor-455 from ThermoFisher. Colocalization of p110 β and ErbB2 was quantified using 6 different images from each sample. Images were obtained using the AxioScan slide scanner from Zeiss and analyzed with the Metamorph software. Slides stained for PTEN were imaged using the LSM 800 from Zeiss.

TGX-221 in vitro assay

Mammary tumor cells were isolated from transplanted outgrowths in NCr mice at the same time and transplanted in DMEM with 5% Fetal Bovine Serum (FBS). Cells were treated 1 day after plating with 100nM, 500nM or 1000nM of TGX-221 inhibitor (final DMSO concentration of 0.2%) or with DMSO for 6 hours. Cells were then harvested and lysed with PLC γ lysis buffer for immunoblot analysis.

Statistical analysis

All experiments on animals were done non-randomized and unblinded. For the Kaplan-Meier curves, cohorts of 18 mice and over were used to ensure enough statistical power. The variance within each group was assessed by F-test. In figure 1b the number of tumors in the wild-type NIC group as compared to the p110 α flx/flx/NIC and the p110 α flx/wt/NIC compared to the p110 α flx/flx/NIC had a significant P value. In Figure 2c, the F-test was

statistically significant when comparing vehicle and GDC-0941 treatment in sample 8665. Finally, in Figure 3b the variance of the number of tumors per group was significant for wild-type NIC mice compared to p110 α flx/flx/NIC and p110 α flx/flx/PTENflx/wt/NIC mice. The statistical analysis used throughout the manuscript was a two tailed Student-t-test, apart from the Kaplan-Meier survival analysis which was done using the log-rank (Mantel-Cox) test. For all statistical tests p-values smaller than 0.05 were considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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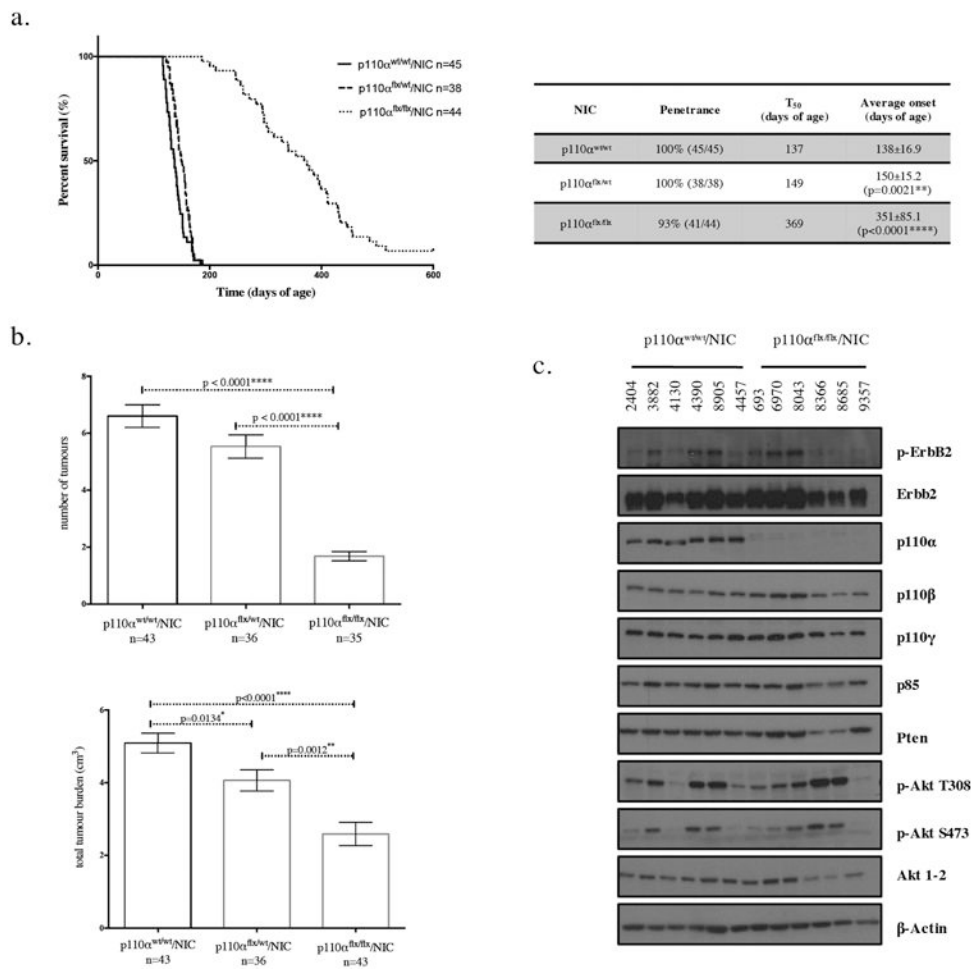


Figure 1. Loss of p110α significantly delays tumor onset, and impairs tumor growth and metastasis, with a subset of tumors exhibiting down regulation of the Pten tumor suppressor
 (a) Kaplan-Meier tumor onset curve for NIC mice that are wildtype, heterozygous, or homozygous for the p110α conditional allele. The table indicates for each genotype the penetrance (percentage of animals that developed tumours), T₅₀ (age when 50% of the animals have tumours), and average tumour onset with standard deviation for each of the curves shown on the graph. p values were calculated using a two-tailed student t-test. (b) The number of tumors and total tumor burden at endpoint (5-7 weeks post-palpation). The error bars represent the standard error of the mean and the p values were calculated using a two-tailed student t-test. (c) immunoblot analysis of tumor lysates (20μg) for the genotypes indicated.

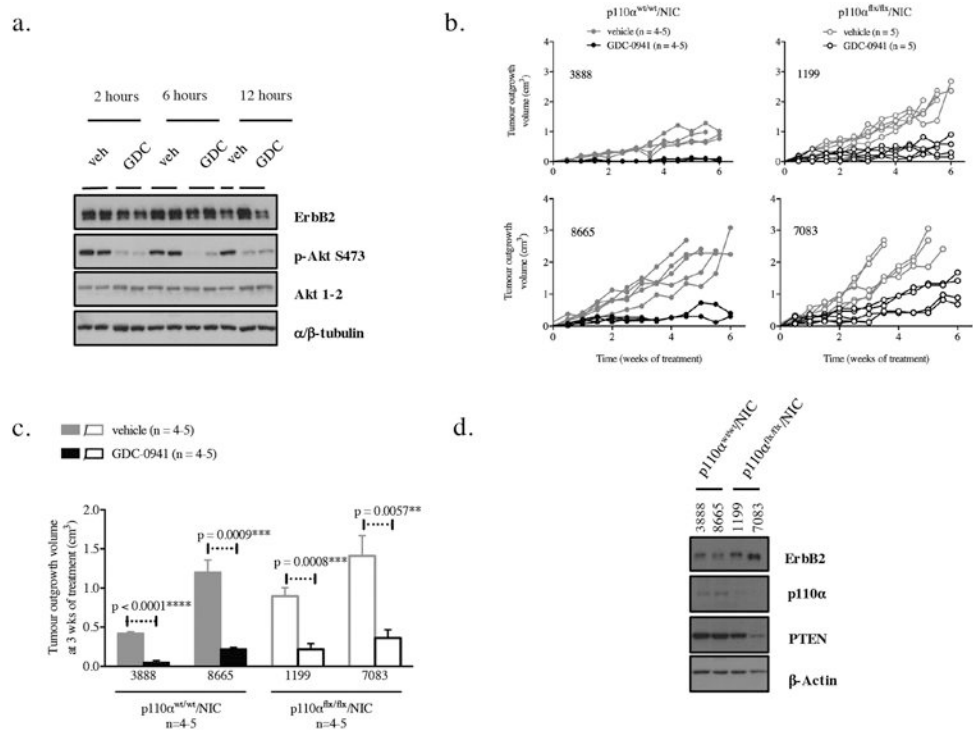


Figure 2. p110α-deficient ErbB2 tumors remain dependent on PI3K

(a) Immunoblot of p110α^{wt/wt}/NIC mammary tumor lysates (20 μg) from NCr mice injected with (500 000 tumor cells) after treatment with either a vehicle (veh) 125mg/kg of GDC-0941 by oral gavage for the indicated times, (b) Tumor outgrowth in NCr mice injected with tumor cells (500 000 tumor cells) of the indicated genotypes and treated with either vehicle or 125mg/kg of GDC-0941 by oral gavage daily for 6 weeks (c) Difference in tumor growth in between vehicle-treated and GDC-0941-treated tumors after 3 weeks of treatment. The p-values were calculated using a two-tailed Student t-test. (d) Immunoblot of NCr tumor lysates (20 μg) from the indicated genotypes.

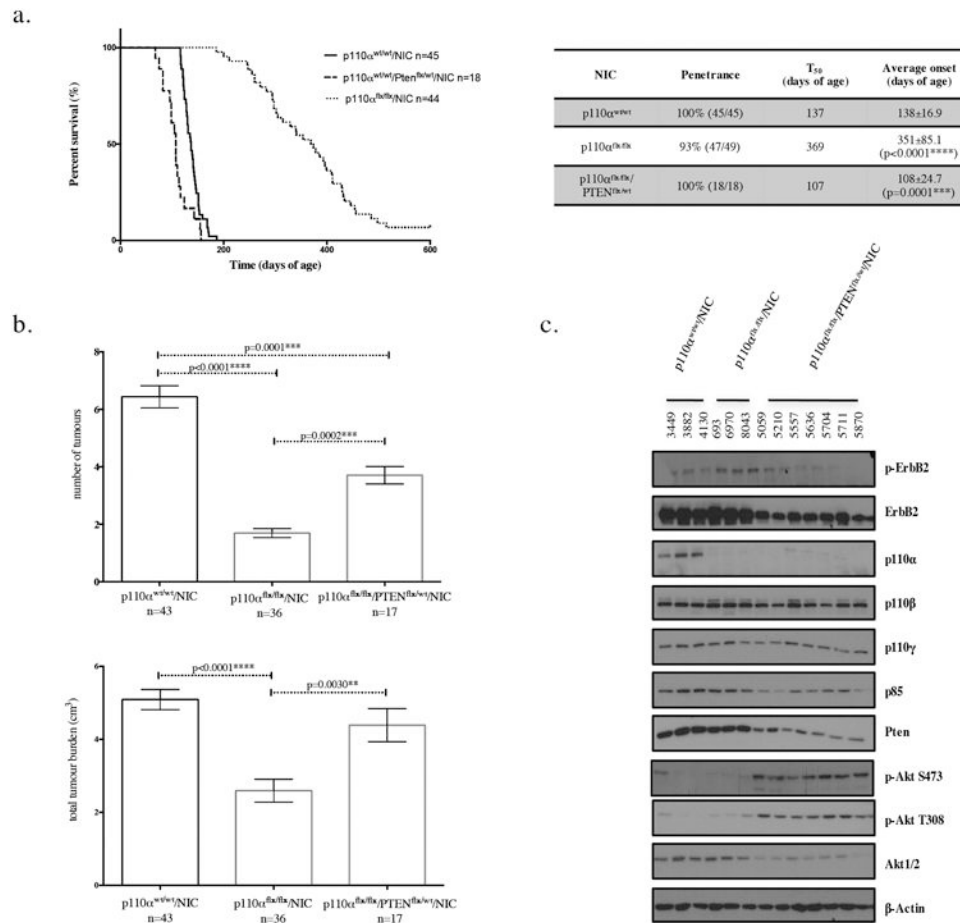


Figure 3. PTEN haploinsufficiency can compensate for loss of p110α in ErbB2 mammary tumor progression

(a) Kaplan-Meier tumor onset curve for NIC mice that are wild-type or homozygous for the p110α conditional allele and wild type or heterozygous for the PTEN conditional allele. The table indicates for each genotype the penetrance (percentage of animals that developed tumors), T₅₀ (age when 50% of the animals have tumors), and average tumor onset with standard deviation for each of the curves shown on the graph, p-values were calculated using the log-rank (Mantel-Cox) test, (b) The number of tumors and total tumor burden at endpoint (5-7 weeks post-palpation). The error bars represent the standard error of the mean and the p-values were calculated using a two-tailed student t-test (c) Immunoblot analysis of tumor lysates (20µg) for the genotypes indicated.

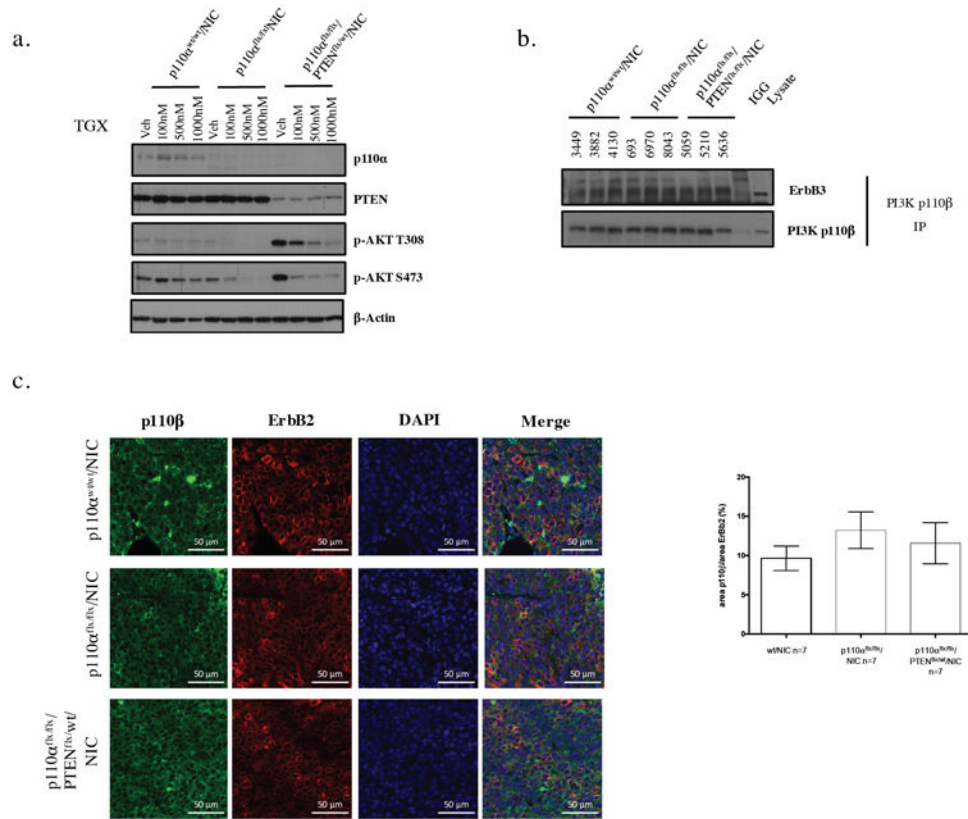


Figure 4. Tumors that have lost p110α are p110β dependent

(a) Immunoblot of NCr cells treated with vehicle or 100nM, 500nM or 1000nM of TGX-221 (p110β3 specific inhibitor) for 6 hours, (b) Co-immunoprecipitation of p110β and ErbB3, p110β and p-85 (500μg). (c) Immunofluorescence staining of p110β and DAPI on paraffin-embedded mammary tumor tissue, scale bar represents 50μm.

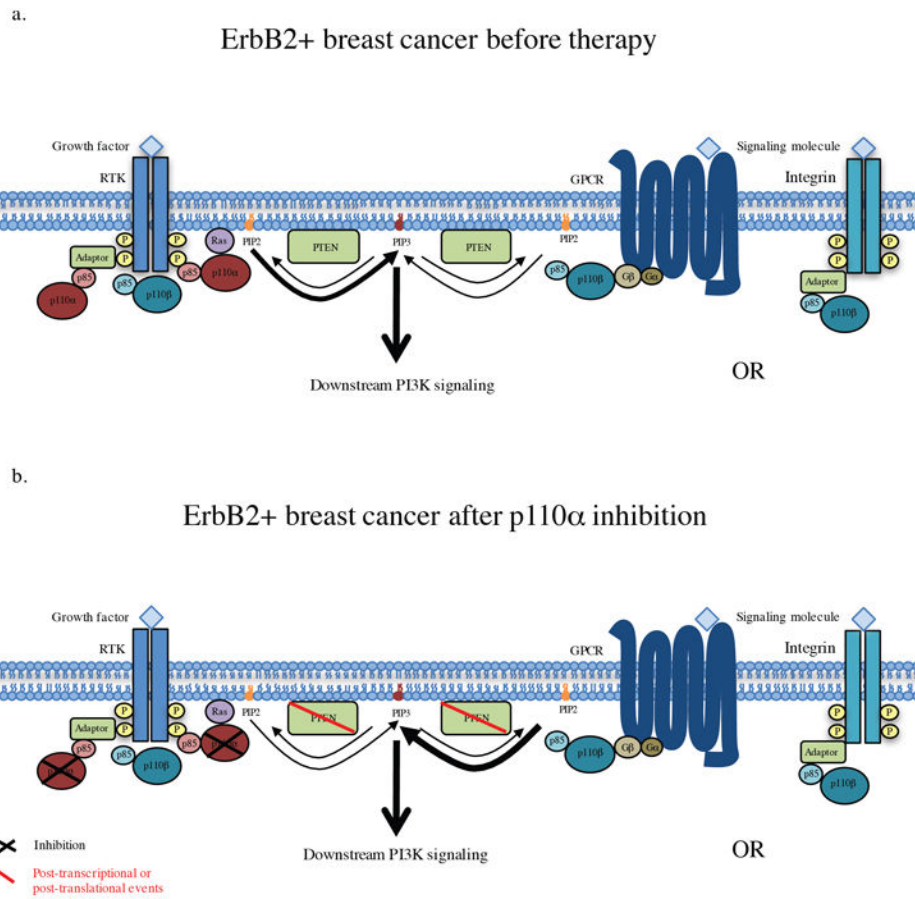


Figure 5. Summary diagram of PI3K signaling in response to p110 α targeted therapy
 (a) PI3K signaling in ErbB2 positive breast cancer before therapy, where the majority of PDK signaling occurs through p110 α binding to the ErbB3/ErbB2 heterodimer. (b) PI3K signaling after treatment with a p110 α -specific inhibitor. In response to p110 α a subset of tumors escape through p110 β in response to PTEN downregulation/heterozygous loss. In these tumors p110 β may signal through the ErbB2/ErbB3 heterodimer but is more likely to be signaling through another receptor, possibly GPCR's or Integrins.