Luminal Expression of PIK3CA Mutant H1047R in the Mammary Gland Induces Heterogeneous Tumors

Dominique S. Meyer¹, Heike Brinkhaus¹, Urs Müller¹, Matthias Müller², Robert D. Cardiff³ and Mohamed Bentires-Alj¹*

¹ Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
² Developmental and Molecular Pathways, Novartis Institutes for Biomedical Research, Basel, Switzerland
³ Center for Comparative Medicine and Department of Pathology, University of California Davis, Davis, USA

Running title: PIK3CA H1047R Induces Heterogeneous Mammary Carcinomas

Keywords: PIK3CA, PI3K, breast cancer

Abbreviations: PI3K: phosphatidylinositol 3-kinase

*Correspondence:
Mohamed Bentires-Alj
Friedrich Miescher Institute for Biomedical Research
Maulbeerstr. 66
4058 Basel, Switzerland
E-mail: bentires@fmi.ch
Abstract

The PI3K signaling cascade, a key mediator of cellular survival, growth and metabolism, is frequently altered in human cancer. Activating mutations in PIK3CA, which encodes the alpha catalytic subunit of PI3K, occur in ~30% of breast cancers. These mutations result in constitutive activity of the enzyme and are oncogenic but it was not known whether they are sufficient to induce mammary carcinomas in mice. Here we demonstrate that expression of mutant PIK3CA H1047R in the luminal mammary epithelium evokes heterogeneous tumors that express luminal and basal markers and are positive for the estrogen receptor. Our results suggest that the PIK3CA H1047R oncogene targets a multipotent progenitor cell and show that this model recapitulates features of human breast tumors with PIK3CA H1047R.

Introduction

Breast cancer is a complex and heterogeneous disease, probably due to the diversity of transforming events and mammary cells in which they occur, as well as to the cross-talk between the transformed epithelium and the surrounding stroma during breast tumorigenesis(1). Definition of the molecular and cellular alterations causing breast tumor heterogeneity should increase our understanding of breast pathogenesis and support the design of optimal therapeutic strategies.
The phosphatidylinositol 3-kinase (PI3K) pathway, a central regulator of diverse normal cellular functions, is often subverted during neoplastic transformation(2). Mechanisms of activation of the PI3K pathway in cancer include the mutation and/or amplification of PIK3CA, the gene encoding the alpha catalytic subunit of the kinase (p110α), the loss of expression of the PTEN phosphatase that reverses PI3K action, the activation downstream of oncogenic receptor tyrosine kinases, and the mutation/amplification of Akt. A hyperactive PI3K pathway results in cancer cells with a competitive advantage by decreasing cell death and increasing cell proliferation, migration, invasion, metabolism, angiogenesis, and resistance to chemotherapy.

PIK3CA is mutated in ~30% of human breast cancers with ~80% of the mutations occurring at three hotspots: E542K (~4% of human breast cancers) and E545K (~6%) within the helical domain and H1047R (~15%) within the kinase domain of p110α(3-5). These mutations result in a constitutively active enzyme that transforms cells in vitro and increases tumorigenicity in xenograft models(6-9). The increase in lipid kinase activity of the mutated p110α makes it a “druggable” target and several inhibitors have entered phase I/II clinical trials(10, 11). Mutations in PIK3CA are more frequent in estrogen receptor (ER)-positive and HER2-positive tumors than in basal-like breast cancers(12). Reported correlations between PIK3CA mutations and prognosis are contradictory; studies including large numbers of patients have shown a paradoxical correlation of PIK3CA mutations with good prognosis(12-15).

PIK3CA mutations have been reported in breast ductal carcinoma in situ (DCIS)(16) and the mutation frequencies in pure DCIS, DCIS adjacent to invasive ductal carcinoma (IDC) and IDC(17) are similar. Thus, mutations of PIK3CA appear to occur early in breast tumorigenesis. To test the hypothesis that PIK3CA mutation initiates mammary tumors, we generated a mouse
model conditionally expressing PIK3CA H1047R. We show here that expression of this mutation in the mammary gland induces carcinomas with different phenotypes composed of cells expressing luminal markers or basal markers or both, and a significant number expressing ER.

Materials and Methods

Transgenic Mice

We constructed a vector with a transcriptional STOP sequence flanked by loxP sites upstream of the 5’-terminally HA-tagged human PIK3CA cDNA (Addgene) and an IRES2-EGFP reporter element (pIRES2-EGFP vector, Clontech). The resulting loxP-STOP-loxP-HA-PIK3CA-IRES2-EGFP fragment was cloned into a recombination-mediated cassette exchange (RMCE) plasmid. The vector was introduced into the modified Rosa26 locus of Balb/c mouse embryonic stem (ES) cells by RMCE and the ES cells used for blastocyst injection(18). Chimeric mice were mated with Balb/c mice and transgenic mice identified by genotyping using the primers 5’-TGGCCAGTACCTCATGGATT-3’ and 5’-GCAATACATCTGGGCTACTTCAT-3’. FVB/N-Tg(MMTV-Cre) and FVB/N.B6-Tg(WAPiCre) mice were described previously(19, 20). Tg(MMTV-Cre) mice are in the FVB/N background and B6-Tg(WAPiCre) mice were backcrossed for five generations to an FVB/N background. MMTV-NeuNT (strain TG.NK) mice were purchased from Charles River (Wilmington, MA).
Immunohistochemistry

The following antibodies were used: K14 (Thermo Scientific, RB-9020, 1:1000), K18 (Fitzgerald, #GP11, 1:500), GFP (Invitrogen, A11122, 1:500), ER (Santa Cruz, SC-542, 1:1000), PR (Thermo Scientific, RM-9102, 1:200), α-SMA (Thermo Scientific, RB-9010, 1:500), cleaved caspase-3 (Cell Signaling, #9661, 1:100), and Ki-67 (Thermo Scientific, RM-9106, 1:1000).

Southern Blotting

Genomic DNA from mouse tails was digested with 8 U of AvrII enzyme (New England BioLabs (NEB)) and separated on a 1% agarose gel. A DIG-labeled DNA probe targeting the neomycin resistance cassette was amplified using the PCR DIG Probe Synthesis Kit (Roche) and the primers 5’-ATGGGATCGGCCATTGAACAAGAT-3’ and 5’-CGGCCATTTTCCACCATGATAT-3’.

RT-PCR

RNA was isolated from mouse tissue using TRIzol (Invitrogen). TaqMan polymerase and appropriate buffers were purchased from NEB. Human PIK3CA was detected using the primers 5’-CAGATCCCAGTGTGGTGGTACG-3’ and 5’-CCTCACGGAGGCATTCTAAAGT-3’ and endogenous gapdh detected using the primers 5’-CATCAAGAAGGTGGTGAAGC-3’ and 5’-GGGAGTTGCTGTGGAAGTGC-3’.
Results and Discussion

Expression of PIK3CA H1047R in luminal mammary epithelial cells induces carcinomas

To test whether PIK3CA H1047R evokes mammary carcinoma, we generated transgenic mice that conditionally expressed this mutation in mammary epithelium. The correct integration of the construct in ES cells conditionally expressing PIK3CA H1047R (Fig. 1A) was tested by Southern blotting and PCR (Fig. 1B left and data not shown). The ES cells were used to generate the H1047R line and the mutation was confirmed by DNA sequencing (Fig. 1B right). Next, H1047R animals were crossed to WAPiCre mice in which expression of recombinase Cre was driven by the whey acidic protein (WAP) promoter that is active in alveolar progenitor cells and differentiated secretory luminal cells(20-23). We also crossed H1047R animals to mice expressing Cre under the control of the mouse mammary tumor virus long terminal repeat (MMTV-Cre), which results in expression within luminal mammary epithelial cells(19).

Female bi-transgenic WAPiCre H1047R mice and littermate controls (WAPiCre) were generated. Mammary glands from WAPiCre H1047R virgin mice had GFP-positive areas indicating expression of the oncogene (Fig. 1C left). This is consistent with previous studies that reported activity of the WAP promoter in a fraction of mammary epithelial cells in virgin mice(21, 23). Examination of whole-mounts and hematoxylin and eosin (H&E)-stained sections revealed on average 5.7 (±2.2) neoplastic lesions in glands from 21- to 24–week-old virgin WAPiCre H1047R mice but not from up to 18–week-old WAPiCre H1047R virgin or age matched littermate controls (Fig. 1C right).
The WAPiCre H1047R and control mice were impregnated to achieve maximal Cre-mediated recombination and the pubs were removed the day after delivery. Whereas parous WAPiCre mice did not form tumors, WAPiCre H1047R mice developed mammary tumors on average 36.8 (±4.9) days after delivery of the pups, corresponding to an age of 140.3 (± 6.9) days (Fig. 2A). Bi-transgenic MMTV-Cre H1047R mice and littermate controls (MMTV-Cre) were generated and left as virgins. Surprisingly, ~75% of the MMTV-Cre H1047R animals died before the age of 4 months. Although we did not identify the cause of death, we consider that leakiness of the MMTV promoter causing deleterious H1047R expression in tissues other than the mammary gland was a likely cause (D.S.M. and M.B-A., unpublished observations). However, ~25% of the MMTV-Cre H1047R mice were viable and formed mammary carcinomas on average after 214 (±22.6) days, whereas no tumors were detected in MMTV-Cre control mice (Fig. 2B).

Since the average age of tumor onset between parous WAPiCre H1047R and virgin MMTV-Cre H1047R mice differs by ~75 days (140.3 vs. 214 days), we sought to investigate whether pregnancy accelerates PIK3CA H1047R-driven tumorigenesis. To address this question we compared tumor onset in nulliparous and parous WAPiCre H1047R mice and found tumor onset to occur significantly earlier in parous mice than in nulliparous mice (Fig. 2C). These data show that pregnancy accelerates tumor onset in WAPiCre H1047R mice.

We then assessed the mechanisms underlying the accelerated tumor onset seen in parous vs. nulliparous WAPiCre H1047R mice. Fluorescence images and Western Blot analysis showed enhanced GFP expression in glands from parous mice indicating an increase in the number of cells that underwent Cre-mediated recombination and thus expressed H1047R (Fig. S1). In
addition, whole mounts of the involuting glands revealed a dramatic delay in involution in mice expressing \( PIK3CA \) H1047R compared with control animals (Fig. S2A), which is in line with previous reports of a delayed involution when the PI3K pathway is hyperactivated (24, 25). Immunostaining for cleaved caspase-3 revealed a decrease in the number of apoptotic cells in involuting glands from WAPiCre H1047R mice compared with control mice, suggesting that reduced cell death is the cause of the delayed involution (Fig. S2B,C). Our results suggest, therefore, that the acceleration of tumor onset is most likely due to an increase in the number of cells expressing \( PIK3CA \) H1047R in parous glands and to impaired cell death in involuting glands with the H1047R mutation. Indeed, pregnancy-induced proliferation could facilitate the acquisition of further genomic alterations and therefore accelerates tumorigenesis.

Analysis of RNA and proteins from WAPiCre H1047R and MMTV-Cre H1047R-induced tumors confirmed that mutant \( PIK3CA \) was expressed in the bi-transgenic mice (Fig. 3A, B). In addition, tumors from both WAPiCre H1047R and MMTV-Cre H1047R mice showed threefold higher phospho-Akt levels than mammary tumors from the MMTV-NeuNT model. In contrast, activation of the Erk1/2 pathway in \( PIK3CA \) H1047R tumors tended to be weaker than in tumors from MMTV-NeuNT mice (Fig. 3C).

Our results show that luminal expression of \( PIK3CA \) H1047R induces mammary tumor formation. This is consistent with the observation that conditional expression of mutant \( PIK3CA \) H1047R in type II lung alveolar epithelial cells causes lung adenocarcinomas in transgenic mice(26) and suggests that this mutation plays a causal role in epithelial cancers.
WAPiCre H1047R and MMTV-Cre H1047R-evoked mammary tumors are heterogeneous

To gain insight into significant patho-physiological features, 22 WAPiCre H1047R and 21 MMTV-Cre H1047R-induced mammary tumors were characterized histologically. MMTV-Cre H1047R-caused tumors showed multiple adenomyoepitheliomas, with clusters of well-delineated polypoid tumors composed of a mixture of glandular epithelium and interstitial fusiform cells with abundant polar cytoplasm (Fig. 4A top row left). Similar tumors have been reported in MMTV-Cre/Pten^{fl/fl}/ErbB2^{Kl} mice, suggesting that an activated PI3K pathway mediates this histotype(27).

In contrast, the WAPiCre H1047R mice formed a more diverse spectrum of tumors with five distinct histotypes. The most prevalent tumor phenotypes found are adenosquamous carcinomas (54.6%) and adenomyoepitheliomas (22.7%). Adenocarcinomas with squamous metaplasia (13.6%) and adenocarcinomas (9.1%) were also observed albeit at lower frequencies (Fig. 4A,B). All the glands surrounding the tumors displayed diffuse adenocarcinomatosis with invasive periductal cords of neoplastic epithelial cells in dense connective tissue (Fig. 4A top row right).

To further characterize H1047R-induced carcinomas, tumors were stained for luminal cytokeratin 18 (K18), basal/myoepithelial cytokeratin 14 (K14), and myoepithelial α-smooth muscle actin (α-SMA) markers, as well as for ER and the progesterone receptor (PR). Notably, ~18 to ~26% of the tumor cells of the adenomyopetheliomas from both transgenic mice expressed ER and ~16% expressed PR in the luminal cells (Fig. 4A, Table S1 and Fig. S3A).
Other tumor histotypes also contained ER-positive cells but at lower frequencies (<5%) (Fig. 4A and Table S1). WAPiCre- and MMTV-Cre H1047R tumors were positive for both luminal K18 and basal K14. The relative tumor area positive for K14 was ~15% in adenomyoepitheliomas and adenocarcinomatosis, whereas in the other phenotypes it ranged between 26% and 43%. The percentage of K18-positive tumor area was 25% in adenocarcinomatosis and ranged between 36 to 45% in the other tumor histotypes (Fig. 4A and Table S1). Although the majority of tumor cells expressed either K18 or K14, some cells were positive for both K14 and K18 (Fig. 4C). As expected, the K14-positive cells within WAPiCre- and MMTV-Cre H1047R adenomyoepitheliomas were also α-SMA-positive (Fig. 4A). In contrast, the K14-positive cells within adenosquamous carcinomas observed in WAPiCre H1047R mice were largely negative for α-SMA, a characteristic of human metaplastic breast cancer in which PIK3CA is mutated in ~50% of cases(28). Interestingly, all tumors showed very low rates of apoptosis (0.2-1.4%) (Table S1 and Fig. S3A), most likely due to the anti-apoptotic effect of an activated PI3K pathway. We also found the percentage of Ki-67-positive cells to be lower in adenomyoepitheliomas and adenocarcinomatosis than in all other tumor phenotypes (Table S1 and Fig. S3A).

These data show that luminal expression of PIK3CA H1047R can induce mammary tumors expressing the basal marker K14. To exclude the possibility that luminal PIK3CA H1047R induces expression of paracrine factors that transform basal cells, we analyzed K14-positive cancer cells for GFP expression by immunostaining and FACS. There was a significant overlap between K14 and GFP expression (Fig. 4D and data not shown), suggesting that some K14-positive cancer cells within the WAPiCre H1047R and MMTV-Cre H1047R tumors
resulted from expression of the oncogene in luminal cells. This supports the emerging notion that some tumors with basal characteristics arise from luminal cells(29, 30).

Taken together, our results show that luminal expression of PIK3CA H1047R evokes mammary tumors, recapitulating the heterogeneity of human breast cancer. These results lead to major conclusions. The finding that PIK3CA H1047R causes ER- and PR-positive tumors suggests that PI3K activity expands ER-positive mammary cells, consistent with the presence of PIK3CA H1047R mutations in human ER-positive tumors(4).

The presence of cancer cells expressing luminal and basal markers in WAPiCre- and MMTV-Cre H1047R-evoked tumors suggests in both models that multipotent progenitor cells are the targets of H1047R-mediated transformation. The WAP promoter is active in a multipotent progenitor population, the parity-identified mammary epithelial cells (PI-MECs), which are present in nulliparous mice and are expanded after pregnancy(21, 23). Tumors that developed in WAPiCre H1047R nulliparous mice most likely derived from PI-MECs because this is the cell population that expresses WAP-driven Cre in glands from nulliparous mice(21). PI-MECs were shown recently to be the target of MMTV-NeuNT-driven carcinogenesis(21, 31). Therefore, it is plausible that PI-MECs are the cells-of-origin of cancer in both WAPiCre- and MMTV-Cre H1047R-evoked tumors but at this stage we cannot completely exclude that expression of PIK3CA H1047R in more differentiated cells also contributes to tumor formation in these models.

The observation of different histotypes between WAPiCre H1047R- and MMTV-Cre H1047R-derived tumors has several possible explanations. First, WAPiCre H1047R mice but not
MMTV-Cre H1047R mice went through pregnancy. Second, it is possible that the cellular targets of MMTV- and WAP- are overlapping but not congruent.

The fact that tumors from MMTV-Cre H1047R but not MMTV-NeuNT mice express K14 (Fig S3B) suggests a model in which PIK3CA H1047R transforms multipotent progenitors, allowing differentiation along the luminal and basal lineages. In contrast, the NeuNT oncogene favors luminal differentiation resulting in K18-positive but not K14-positive tumors. An alternative and more interesting explanation is that the MMTV promoter is active in differentiated luminal cells and H1047R causes their dedifferentiation to multipotent progenitor cells, which then give rise to K14- and/or K18-positive cancer cells. This would suggest a role for PIK3CA H1047R in cancer cell plasticity, a hypothesis that merits testing.

ACKNOWLEDGEMENTS

We thank N. Hynes (FMI) for helpful comments on the manuscript, W.J. Muller (McGill University) for the MMTV-Cre line, members of the Bentires-Alj lab for advice and discussions, Corinne Haller, Thierry Doll, and Marianne Lemaistre for excellent technical assistance, and various colleagues for reagents. Research in the lab of M.B-A. is supported by the Novartis Research Foundation, the European Research Council (ERC starting grant 243211-PTPsBDC), the Swiss Cancer League and the Krebsliga Beider Basel.
References


Figure Legends

Figure 1. Targeted expression of mutant p110α in luminal mammary epithelial cells. (A) Schematic of the construct used for generating transgenic mice conditionally expressing PIK3CA H1047R. The PIK3CA cDNA is flanked by a floxed STOP cassette upstream and an IRES2-EGFP reporter element downstream. Expression of PIK3CA H1047R is driven by a chicken β-actin (CAGS) promoter. (B) Southern blotting of genomic DNA from wild-type and PIK3CA H1047R mice (left) and sequencing of genomic DNA from H1047R transgenic mice harboring an A to G mutation at nucleotide 3140 (right). (C) Left panel: Fluorescence images of glands from virgin WAPiCre control and virgin WAPiCre H1047R mice showing GFP expression. Right panel: Representative images of mammary glands from WAPiCre control mice (left), WAPiCre H1047R virgin mice between 12 and 18 weeks old (center), and WAPiCre H1047R virgin mice between 21 and 24 weeks old (right). Images show whole-mount preparations (top) and H&E-stained sections (bottom). The red arrows indicate neoplastic lesions. Inserts show the indicated areas at higher magnification. Table shows quantification of neoplastic lesions. Scale bars = 1 mm (whole mounts, fluorescence images) and 100 μm (H&E images).

Figure 2. WAPiCre H1047R and MMTV-Cre H1047R mice develop mammary tumors. (A) Kaplan-Meier curves showing tumor onset in bi-transgenic WAPiCre H1047R mice (n=12) and WAPiCre littermate controls (n=7). The mice were impregnated and the pups removed from the mothers the day after delivery. Bi-transgenic animals developed palpable tumors on average 36.8 (±4.9) days after delivery, corresponding to age 140.3 (±6.9) days. (B) Kaplan-Meier curves
showing tumor onset in double transgenic MMTV-Cre H1047R mice (n=7) and MMTV-Cre littermate controls (n=8). MMTV-Cre H1047R mice developed palpable tumors on average within 214 (±22.6) days. (C) Kaplan-Meier curves showing tumor onset in virgin WAPiCre H1047R (n=7) and parous WAPiCre H1047R mice (n=12). Parous animals developed palpable tumors on average at 140.3 (±6.9) days and all animals had at least one tumor within 183 days of age. In contrast, by 170 days, only one out of seven virgin WAPiCre H1047R mice developed a tumor (at 141 days). The difference in tumor latency between parous and virgin animals is significant (P = 0.0006).

Figure 3. Tumors from WAPiCre H1047R and MMTV-Cre H1047R mice express mutant PIK3CA. (A) RT-PCR showing expression of PIK3CA H1047R in WAPiCre H1047R and MMTV-Cre H1047R mammary tumors but not in heart or kidney of a WAPiCre H1047R animal. (B) Expression of exogenous p110α as indicated by P110α-immunoprecipitation (IP) from MMTV-NeuNT, WAPiCre H1047R, and MMTV-Cre H1047R tumor lysates using anti-p110α (left) or anti-HA antibodies (right). (C) Immunoblotting of mammary tumor lysates from the indicated genotypes using the specified antibodies (left) and quantification of pAkt S473 and pErk1/2 signals (right). * not significant; **P <0.01; SN: supernatant.

Figure 4. WAPiCre H1047R and MMTV-Cre H1047R-evoked tumors express basal markers. (A) H&E-stained sections and immunostainings for ER, K18, K14, and α-SMA from MMTV-Cre H1047R adenomyoepithelioma and different WAPiCre H1047R tumor histotypes as
indicated. (B) Relative prevalence of adenosquamous carcinoma (blue), adenomyoepithelioma (red), adenocarcinoma with squamous metaplasia (green), and adenocarcinoma (purple) among MMTV-Cre H1047R and WAPiCre H1047R-evoked tumors. (C) Fluorescent image of DAPI staining (blue) and immunostaining of K18 (green) and K14 (red) in a WAPiCre H1047R tumor section. The arrow indicates K14/K18 double-positive cells. Asterisks indicate K14 and K18 single-positive cells. (D) Images of immunostaining for GFP and K14 in WAPiCre H1047R (left) and MMTV-Cre H1047R tumor sections (right). Arrows indicate areas of K14/GFP double-positive cells. Scale bars = 100 μm.
A. Schematic diagram of the transgenic mice used in the study. The CAGS promoter drives the expression of STOP, HA-PIK3CA H1047R, IRES2, and EGFP.

B. Image of a gel showing the electrophoresis of wild-type and H1047R sequences. The mutant sequence at position 3140 is highlighted.

C. Images of whole mounts and H&E stained sections comparing WAPiCre control and WAPiCre H1047R mice. The table below summarizes the number of neoplastic lesions:

<table>
<thead>
<tr>
<th></th>
<th>WAPiCre control virgins</th>
<th>WAPiCre H1047R virgins ≤ 4 months</th>
<th>WAPiCre H1047R virgins &gt; 4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td># neoplastic lesions</td>
<td>0</td>
<td>0</td>
<td>5.7 ± 2.2</td>
</tr>
</tbody>
</table>
Figure 2 Meyer et al

A

% Tumor-free Mice

Time after Delivery (Days)

- WAPiCre
- WAPiCre H1047R

B

% Tumor-free Mice

Age (Days)

- MMTV-Cre
- MMTV-Cre H1047R

C

% Tumor-free Mice

Age (days)

- WAPiCre H1047R virgin
- WAPiCre H1047R parous
Figure 3 Meyer et al

A

<table>
<thead>
<tr>
<th>WAPiCre H1047R tumors</th>
<th>MMTV-Cre H1047R tumors</th>
<th>heart</th>
<th>kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>MMTV-NeuNT</th>
<th>WAPiCre H1047R</th>
<th>MMTV-Cre H1047R</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP: p110α</td>
<td>HA</td>
<td>p110α</td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>Erk1/2</td>
</tr>
<tr>
<td></td>
<td>IP: HA</td>
<td>HA</td>
</tr>
<tr>
<td></td>
<td>p110α</td>
<td>p110α</td>
</tr>
</tbody>
</table>

C

<table>
<thead>
<tr>
<th>MMTV-NeuNT</th>
<th>WAPiCre H1047R</th>
<th>MMTV-Cre H1047R</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAkt S473</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pAkt T308</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pErk 1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erk 1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Luminal Expression of PIK3CA Mutant H1047R in the Mammary Gland Induces Heterogeneous Tumors

Dominique S Meyer, Heike Brinkhaus, Urs Müller, et al.

Cancer Res Published OnlineFirst April 11, 2011.