*Breast Cancer Online*, 2006, **9**(7), Page 1 of 4, ***e28***
doi:10.1017/S1470903106005591

ISSN 1470-9031

Breast Cancer Online
www.bco.org

*Journals Club*

# Functional characterization of PIK3CA as a breast cancer oncogene
# Review of:
# 1. Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells
# 2. The oncogenic properties of mutant p110 alpha and p110 beta phosphatidylinositol 3-kinases in human mammary epithelial cells

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**Citation of original article 1:**
S. J. Isakoff, J. A. Engelman, H. Y. Irie, J. Luo, S. M. Brachmann, R. V. Pearline, L. C. Cantley, J. S. Brugge. *Cancer Research* 2005; **65**: 10 992–11 000.

**Abstract of the original article 1:**
Activation of the phosphoinositide 3-kinase (PI3K) pathway has been implicated in the pathogenesis of a variety of cancers. Recently, mutations in the gene encoding the p110 catalytic subunit of PI3K (*PIK3CA*) have been identified in several human cancers. The mutations primarily result in single amino acid substitutions, with >85% of the mutations in either exon 9 or 20. Multiple studies have shown that these mutations are observed in 18% to 40% of breast cancers. However, the phenotypic effects of these *PIK3CA* mutations have not been examined in breast epithelial cells. Herein, we examine the activity of the two most common variants, E545K and H1047R, in the MCF-10A immortalized breast epithelial cell line. Both variants display higher PI3K activity than wild-type p110 yet remain sensitive to pharmacologic PI3K inhibition. In addition, expression of p110 mutants in mammary epithelial cells induces multiple phenotypic alterations characteristic of breast tumor cells, including anchorage-independent proliferation in soft agar, growth factor-independent proliferation, and protection from anoikis. Expression of these mutant p110 isoforms also confers increased resistance to paclitaxel and induces abnormal mammary acinar morphogenesis in three-dimensional basement membrane cultures. Together, these data support the notion that the cancer-associated mutations in *PIK3CA* may significantly contribute to breast cancer pathogenesis and represent attractive targets for therapeutic inhibition.

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Received: 31/03/06
Accepted: 06/06/06
First published online 06/07/06
BCO/559/2006/JC

**Citation of original article 2:**
J. J. Zhao, Z. N. Liu, L. Wang, E. Shin, M. F. Loda, T. M. Roberts. *Proceedings of the National Academy of Sciences of the United States of America* 2005; **102**: 18 443–18 448.

**Abstract of the original article 2:**
The *PIK3CA* gene encoding the p110α subunit of Class IA phosphatidylinositol 3-kinases (PI3Ks) is frequently mutated in human tumors. Mutations in the *PIK3CB* gene encoding p110β, the only other widely expressed Class IA PI3K, have not been reported. We compared the biochemical activity and transforming potential of mutant forms of p110α and p110β in a human mammary epithelial cell system. The two most common tumor-derived alleles of p110α, *H1047R* and *E545K*, potently activated PI3K signaling. Human mammary epithelial cells expressing these alleles grew efficiently in soft agar and as orthotopic tumors in nude mice. We also examined a third class of mutations in p110α, those in the p85-binding domain. A representative tumor-derived p85-binding-domain mutant R38H showed modestly reduced p85 binding and weakly activated PI3K/Akt signaling. In contrast, a deletion mutant lacking the entire p85-binding domain efficiently activated PI3K signaling. When we constructed in p110β a mutation homologous to the *E545K* allele of p110α, the resulting p110β mutant was only weakly activated and allowed minimal soft-agar growth. However, a gene fusion of p110β with the membrane anchor from c-Src was highly active and transforming in both soft-agar and orthotopic nude mouse assays. Thus, although introduction of activating mutations from p110α at the corresponding sites in p110β failed to render the enzyme oncogenic in human cells, the possibility remains that other mutations might activate the β isoform.

## Review

The phosphatidylinositol 3-kinase (PI3K) family of lipid kinases plays an important role in the transmission of pro-survival and proliferative signals in normal and cancer cells. The family is subdivided into three classes (I, II and III) based on modes of activation, substrate specificities and structure. Full activation of Class 1a PI3K requires SH2 domain interactions of the regulatory subunit p85 with phosphorylated tyrosine residues on receptor tyrosine kinases. This relieves inhibition of the catalytic subunit p110 by p85, leading to phosphorylation of the 3′-OH of specific phosphoinositides. Important downstream effectors of PI3K are the serine/threonine kinases AKT and target of rapamycin (TOR). Many human cancers including those of the breast, liver and colon exhibit deregulated Class 1a PI3K activity. Several contributory mechanisms have been identified. Silencing of the lipid phosphatase PTEN (phosphatase with tensin homology), a negative regulator of PI3K signalling, occurs in 15–35% of breast cancers, and germ-line mutations in PTEN found in syndromes such as Cowden disease lead to a predisposition to breast cancer [1]. In addition, somatic missense mutations of the PIK3CA gene occur in up to 40% of breast cancers [2]. This gene encodes the isoform p110α and mutations tend to be clustered at hotspots within the helical and catalytic domains [3]. As PTEN silencing and PIK3CA mutations are reported to be mutually exclusive, more than half of all breast cancers may exhibit increased PI3K signalling [4]. Furthermore, the pathway can also be perturbed at the level of AKT. For example, amplification of the gene encoding AKT2 has been detected in breast and other cancers [5]. Therefore, the PI3K pathway appears to play a major role in breast tumourigenesis.

The functional consequences of PIK3CA mutations in mammary epithelial cells have recently been determined by Isakoff *et al.* [6] and Zhao *et al.* [7]. Both groups focus on two of the most common mutations found in breast cancers, p110α H1047R, located in the kinase domain and E545K, found within the helical domain. Previously, Zhao and colleagues demonstrated that activation of PI3K in combination with expression of hTERT, inactivation of the p53 and pRb tumour suppressor pathways and c-Myc overexpression was sufficient to elicit transformation of primary human mammary epithelial cells (HMECs) [8]. Thus this model was ideal for the investigation of the potential transforming abilities of p110α hotspot mutants. In their current paper, they demonstrate that expression of H1047R or E545K in the genetically engineered HMECs leads to an increase in AKT phosphorylation and growth in soft agar, the latter an indication of cellular transformation. In addition, co-expression of the mutants with H-rasV12 confers on the cells the ability to form tumours in nude mice. Consequently, these hotspot mutations in PIK3CA are likely to play a critical role in breast tumourigenesis. The authors also investigate the mechanism whereby these mutations

activate PI3K activity. Since the mutations do not alter p85 binding or further enhance the activity of a p110α mutant with a deleted p85-binding domain, they propose that the mutants act to eliminate the negative constraints on p110α kinase activity exerted by p85. Of interest in this regard, mutations of p110α within the p85-binding domain, such as R38H, have been detected in cancer specimens, although at a low frequency. However, Zhao *et al.* determined that although this mutation led to a modest reduction in p85 binding and a small increase in activity, HMECs expressing R38H did not form colonies in soft agar. Conceivably, this class of mutant may co-operate with PTEN loss or overexpression of members of the erbB family to promote tumour progression.

In a complementary study, Isakoff and colleagues utilise the MCF-10A cell line, which is spontaneously immortalised and thus the underlying genetic alterations are not defined. Like Zhao *et al.*, this group demonstrates that expression of the hotspot mutants leads to enhanced AKT activation and colony formation in soft agar. They also show that mutant-expressing cells proliferate as a monolayer in an EGF-independent manner and can resist anoikis, a process of cell death due to matrix detachment. Furthermore, although MCF-10A cells grown on a reconstituted basement membrane form growth-arrested acini that model glandular architecture in vivo, cells expressing H1047R or E545K form large, hyperproliferative, abnormal structures. However, in contrast to the results obtained in monolayer culture, proliferation under these conditions is still dependent on the presence of exogenous EGF, indicating that autonomy from this growth factor requires one or more signals in addition to PI3K activation. This is supported by the observation that EGF-independence conferred by overexpression of the Gab2 docking protein in MCF-10A cells is associated with enhanced activation of not only PI3K but also ERK [9].

Importantly, the presence of PIK3CA mutations opens potential avenues for pharmacological intervention. Isakoff *et al.* show that the kinase activity of the hotspot mutants can be inhibited both in vitro and in vivo by the PI3K inhibitor LY294002, and that this compound also attenuates constitutive signalling by the mutants to p70s6k, a target of TOR. Also, rapamycin treatment of basement membrane cultures, and hence inhibition of TOR, prevented the formation of abnormal structures. This evidence implicates TOR as an important downstream effector of the p110α hotspot mutants, which is significant given that clinical trials of TOR inhibitors are currently underway [10]. Furthermore, the authors demonstrate increased resistance to paclitaxel in mutant-expressing cells. Consequently, PI3K/TOR inhibitors may be of clinical utility as components of combination therapies designed to overcome resistance to chemotherapeutics. It will also be of interest to examine the effects of these mutations on sensitivity of breast cancer cells to hormonal therapies, given that the presence of PIK3CA mutations has been positively correlated with oestrogen and progesterone receptor status [4], and AKT activation predicts decreased survival in tamoxifen-treated patients [11].

In summary, these two papers present compelling evidence for an important role of p110α mutations in driving human mammary oncogenesis and raise several important issues. First, the mechanism underlying activation of PI3K by these mutations needs to be clarified, for example by X-ray crystallography, which may enable the development of specifically targeted inhibitors. Second, characterization of the pathways downstream of PI3K that modulates chemosensitivity, and the targets of TOR that promote abnormal proliferation and morphogenesis, may lead to novel therapeutic strategies. Finally, it will be important to determine how PIK3CA mutations affect patient outcome and response to particular therapies, as screening for their presence may enable channelling of patients into optimal treatment regimens.

## Acknowledgements

Research in the authors' laboratory is supported by research grants from the National Health and Medical Research Council of Australia and the Association for International Cancer Research. H.L.B. is the recipient of an Australian Postgraduate Award.

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