Priority Report

Cancer Research

PIK3CA Mutations in *In situ* and Invasive Breast Carcinomas

Alexander Miron[1,5], Maria Varadi[2], Daniel Carrasco[2], Hailun Li[3], Lauren Luongo[4], Hee Jung Kim[2,5], So Yeon Park[8], Eun Yoon Cho[9], Gretchen Lewis[1], Sarah Kehoe[4], J. Dirk Iglehart[1,5], Deborah Dillon[5,6], D. Craig Allred[10], Laura Macconaill[4], Rebecca Gelman[3,5,7], and Kornelia Polyak[1,2,4]

Abstract

The PIK3 signaling pathway has been identified as one of the most important and most frequently mutated pathways in breast cancer. Somatic mutations in the catalytic subunit of *PIK3CA* have been found in a significant fraction of breast carcinomas, and it has been proposed that mutant *PIK3CA* plays a role in tumor initiation. However, the majority of primary human tumors analyzed for genetic alterations in *PIK3CA* have been invasive breast carcinomas and the frequency of *PIK3CA* mutations in preinvasive lesions has not been explored. To investigate this, we sequenced exons 9 and 20 of *PIK3CA* in pure ductal carcinoma *in situ* (DCIS), DCIS adjacent to invasive carcinoma, and invasive ductal breast carcinomas. In a subset of cases, both *in situ* and invasive areas were analyzed from the same tumor. We found that the frequency of *PIK3CA* mutations was essentially the same (~30%) in all three histologic groups. In some cases, *in situ* and invasive areas of the same tumor were discordant for PIK3CA status, and in two cases in which multiple invasive and adjacent *in situ* areas within the same tumor were analyzed independently, we detected intratumor heterogeneity for *PIK3CA* mutations. Our results suggest that mutation of *PIK3CA* is an early event in breast cancer that is more likely to play a role in breast tumor initiation than in invasive progression, although a potential role for exon 9 mutations in the progression of a subset of DCIS cases cannot be excluded. *Cancer Res; 70(14); 5674–8. ©2010 AACR.*

Introduction

The phosphatidylinositol 3 kinase (PIK3) signaling pathway is an important regulator of cell growth, metabolism, proliferation, survival, motility, and invasion (1, 2). Highlighting the importance of PIK3 signaling in human cancer, genetic alterations have been reported in several components of the pathway in various tumor types including deletion of *PTEN* (3), amplification of *AKT1* and *PIK3CA*, and somatic mutations of *PIK3CA* (2) and *AKT1* (4). In human breast cancer, mutations in *PIK3CA* have been reported to occur in 8% to 40% of tumors, making it one of the most frequently mutated genes in this tumor type (5, 6). The majority of mutations have been identified in the helical domain (exon 9) and in the kinase domain (exon 20) of PIK3CA.

Authors' Affiliations: Departments of [1]Cancer Biology, [2]Medical Oncology, and [3]Biostatistic and Computational Biology, and [4]Center for Cancer Genome Discovery, Dana-Farber Cancer Institute, [5]Harvard Medical School, [6]Department of Pathology, Brigham and Women's Hospital, [7]Harvard School of Public Health, Boston, Massachusetts; [8]Department of Pathology, Seoul National University College of Medicine and Bundang Hospital, Seongnam, Gyeonggi, South Korea; [9]Department of Pathology, Samsung Medical Center, Seoul, South Korea; and [10]Department of Pathology, Washington University School of Medicine, St. Louis, Missouri

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Corresponding Author: Kornelia Polyak, Dana-Farber Cancer Institute, 44 Binney Street D740C, Boston, MA 02115. Phone: 617-632-2106; Fax: 617-582-8490; E-mail: Kornelia_Polyak@dfci.harvard.edu.

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Expression of cancer-derived *PIK3CA* mutants in cultured cells increases kinase activity, invasion, resistance to apoptosis, and in immortalized human mammary epithelial cells, it is sufficient to induce soft agar growth and tumorigenicity, suggesting that mutant PIK3CA might play a role in the initiating steps of breast tumorigenesis (5, 6). In contrast, somatic mutations in *PIK3CA* have been reported to occur at a higher frequency in advanced stage invasive tumors, suggesting a role in tumor progression (7, 8). Analysis of preinvasive tumors including colon adenomas and Barrett's esophagus have shown a paucity of mutations in *PIK3CA* compared with invasive carcinomas (7, 8). Similarly, the frequency of *PIK3CA* mutations was significantly higher in advanced gastric carcinomas compared with early stage tumors (9), and gain of *PIK3CA* was more frequent in high-grade dysplasias and carcinomas than in low-grade dysplasias in head and neck cancer (10). Each of these studies analyzed only a few cases of *in situ* tumors and did not test adjacent *in situ* and invasive areas from the same patient.

In human breast cancer, only a few published studies on a small number of samples have analyzed *PIK3CA* mutations in preinvasive tumors including ductal carcinoma *in situ* (DCIS; refs. 9, 11–13). Campbell and colleagues performed single-strand conformational polymorphism analysis of *PIK3CA* exons 1 to 20 in 70 breast tumors, including 3 DCIS, and detected mutations in 28 of 67 invasive tumors and 0 of 3 DCIS (11), whereas Lee and colleagues analyzed exons 9 and 20 by single-strand conformational polymorphism in 93 breast tumors and identified mutations in 24 of 78 invasive breast tumors and only 2 of 15 DCIS (9). Neither report provided information on the type of DCIS analyzed (i.e., pure DCIS

or *in situ* areas adjacent to invasive tumor), but nonetheless, they implicated PIK3CA in the *in situ* to invasive carcinoma progression. A third study analyzed 11 DCIS adjacent to invasive ductal breast carcinoma (IDC) and found concordant mutations in all cases (12).

Based on these data, we hypothesized that mutational activation of the PIK3CA pathway might play a role in the progression of *in situ* carcinomas to invasive disease. To test this hypothesis, we analyzed the sequence of the two mutational hotspot regions of *PIK3CA* (exons 9 and 20) in pure DCIS and in invasive breast carcinomas and adjacent DCIS regions.

Materials and Methods

Tissue specimens

Fresh, frozen, or formalin-fixed and paraffin-embedded tumor specimens were obtained for the pilot cohort from Harvard-affiliated hospitals (Boston, MA), Duke University (Durham, NC), University Hospital Zagreb (Zagreb, Croatia), Baylor College of Medicine (Houston, TX), and the National Disease Research Interchange (Philadelphia, PA); the larger cohort's specimens were obtained from Bundang Hospital of Seoul National University College of Medicine and Samsung Medical Center (Seoul, South Korea). All human tissue was collected without patient identifiers using protocols approved by the Institutional Review Boards.

Manual and laser capture microdissection

Six-micron-thick paraffin sections were deparaffinized in xylene and rehydrated in graded alcohols. For manual dissection, slides were stained with H&E, and tumor cell–enriched areas collected with a razor blade. For laser capture microdissection, slides were stained with 0.5% methyl green for 30 seconds, rinsed in distilled water, and left to air-dry. Stained slides were microdissected with an IR laser–enabled microscope (Arcturus Engineering) in the Dana-Farber Cancer Institute Pathology Core facility. A 30-μm laser spot size was used to isolate a minimum of 2,000 selected cells onto CapSure laser capture microdissection caps (Molecular Devices).

DNA extraction, mutation, and statistical analyses

DNA preparation and mutation analyses were performed essentially as described (14), detailed procedures are available from the authors upon request. Statistical analysis used Fisher's exact test and exact power calculations for it, as well as logistic regression (with likelihood ratio tests) and exact binomial confidence intervals; all *P* values were two-sided and no correction for multiple comparisons were done. Each case with matched IDC and DCIS lesions analyzed for mutations could only be used in a logistic regression (or a Fisher test) once (to avoid violating the assumption of independence), so analyses were done twice (once using the DCIS mutation status and categorizing them as DCIS adjacent to IDC and once using the IDC mutation status).

Results and Discussion

We determined the frequency of *PIK3CA* mutations in pure DCIS, DCIS adjacent to invasive cancer, and in IDCs. All samples were microdissected to ensure the proper identification and selection of *in situ* and invasive areas (Fig. 1). Due to the limited amount of DNA recovered, our mutational analysis was limited to two previously characterized mutational hotspots: exons 9 and 20.

First, we analyzed mutations using Sanger sequencing in a smaller cohort: specimens from 83 patients, including 31 patients with matched DCIS and IDC lesions; two of the cases had several IDC and DCIS areas isolated for analysis (Supplementary Table S1). *PIK3CA* mutations were found in only 5% of pure DCIS (95% confidence interval, 1–16%) but in ~16% of DCIS adjacent to IDC and 9% of IDC. Of the 29 matched cases with a single DCIS and a single IDC lesion evaluated, only 2 (7%) had discordant results (95% confidence interval, 1–25%); when both IDC and adjacent DCIS were mutant, they had identical mutations confirming their clonal relatedness

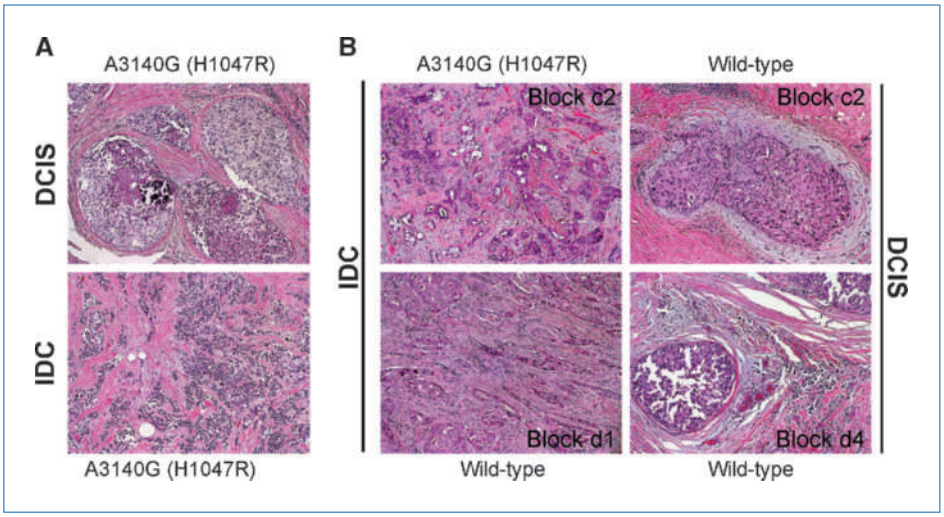

Figure 1. Examples of DCIS and invasive tumors used for microdissection. H&E-stained sections of *in situ* and invasive areas selected for microdissection from two independent cases in which DCIS and IDC had the same mutation (A) or showed intratumor heterogeneity (B).

(Fig. 2A). In the two cases with multiple DCIS and IDC regions analyzed, in one case, the IDC was wild-type but two out of three DCIS areas had a mutation, whereas in the other case, one invasive area had a mutation and the remaining invasive and DCIS areas were all wild-type (Fig. 2B).

Because the frequency of *PIK3CA* mutations in the initial cohort was low and the 95% confidence intervals were so large, we expanded our study to 374 Korean patients (including 48 patients with matched DCIS and IDC lesions). In addition, due to the intratumor heterogeneity of PIK3CA mutations we observed in our pilot study, we analyzed the most frequent exon 9 (E542K) and exon 20 (H1047R and H1047L) mutations by mass spectrometry (ref. 14; Supplementary Fig. S1), which is more sensitive for mutation detection than Sanger sequencing (14, 15). Using this approach, the frequency of any *PIK3CA* mutation was between 28% and 31% in each histologic subgroup, and was not significantly different (Table 1; Supplementary Table S2). The higher frequency of mutations detected by mass spectrometry is unlikely to be due to ethnic or other differences between the pilot and extended cohorts, but highlights the advantage of using this method for the testing of known mutations in cancer genes that may be present only in a subset of cancer cells (14, 15).

To determine if the sample size used had enough power to detect significant differences in the three histology groups, we performed power calculations. We found that when the matched cases were analyzed as DCIS, there was 80% power to detect as significantly different true mutation frequencies of 38% and 22% in pure DCIS and DCIS adjacent to IDC, and there was 80% power to detect true mutation frequencies of 41% and 20% in DCIS adjacent to IDC and IDC. Of the 45 matched cases, 15 (33%; 95% confidence interval, 20–49%) had discordant results, 8 with a mutation only in IDC, and 7 with a mutation only in DCIS (Supplementary Table S3). These results indicate that divergence for *PIK3CA* mutations occur in a significant fraction of breast tumors during *in situ* to invasive progression. Furthermore, the equal frequency of the two possible discordant patterns (i.e., mutant DCIS and wild-type IDC and vice versa) implies the lack of selective advantage of cells with mutant PIK3CA during invasive progression.

We also explored associations between any *PIK3CA* mutation and age or pathologic variables (when histologic group was forced into the model), as well as interactions between histologic group and other variables (Supplementary Table S4). Neither histologic group nor any other variable was significantly associated with the frequency of any mutation (or specific H1047R, H1047L, E542K mutations). When matched cases were analyzed as DCIS, the only significant interaction was between mutant p53 (based on positivity by immunohistochemistry) and pure DCIS ($P = 0.03$); mutant p53 was associated with a greater percentage of patients having a *PIK3CA* mutation among pure DCIS cases (Supplementary Table S5). When matched cases were analyzed as IDC, the p53 interaction with DCIS remained significant ($P = 0.01$), and the interaction of grade and pure DCIS was also significant ($P = 0.02$); high grade was associated with a lower percentage of patients having any *PIK3CA* mutation in pure DCIS (Supplementary Table S5). The interaction of Her2 and histology was close to significant ($P = 0.07$), with the greatest mutation frequency in tumors with an immunohistochemical staining score of 3+ among DCIS adjacent to IDC (Supplementary Table S5). Models for H1047R mutations were similar to models of any mutation (Supplementary Table S6) and there were too few H1047L mutations to analyze separately.

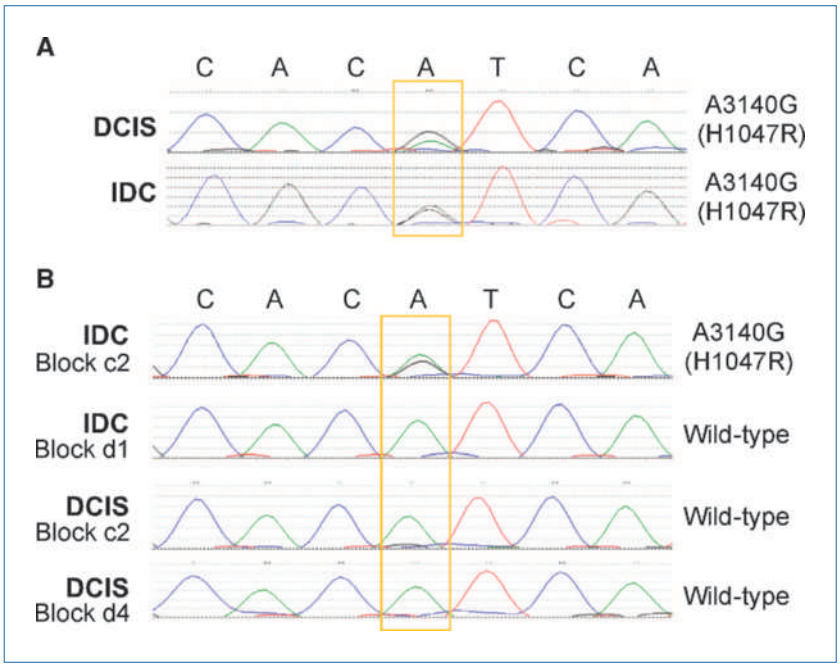

Figure 2. Examples of PIK3CA Sanger sequencing results. A, concordant somatic *PIK3CA* mutation (A3140G) in IDC and adjacent DCIS. B, intratumor heterogeneity for *PIK3CA* mutation. One of the invasive areas of this tumor had an exon 20 A3140G (H1047R) mutation whereas other invasive and adjacent DCIS were wild-type.

Table 1. Summary of PIK3CA mutations by histology

	PIK3CA mutation	No. of cases with specific mutations*		
		E542K	H1047L	H1047R
Pure DCIS	61/202 (30%)	5	5	52
DCIS adjacent to IDC (not matched)	15/49 (31%)	2	0	14
Matched				
DCIS	14/48 (29%)	2	0	13
IDC†	14/45 (31%)	0	0	14
IDC alone	21/75 (28%)	4	0	17
DCIS adjacent to IDC (matched or not)	29/97 (30%)	4	0	27
IDC (matched or not)	35/120 (29%)	4	0	31
P value of difference between histologic groups (DCIS used for matched cases)	0.94			
P value of difference between histologic groups (IDC used for matched cases)	0.97			

*Three cases (one pure DCIS and two DCIS adjacent to IDC) had more than one mutation.
†Three cases had matched DCIS and IDC specimens but mutation analyses for IDC lesions were inconclusive.

The H1047L mutation was only seen in pure DCIS, but the frequencies of the mutation did not differ significantly between histologic groups (Supplementary Table S4; $P = 0.21$ and 0.17 depending on whether we counted the matched cases as DCIS or IDC). There were too few E542K mutations to estimate interactions. However, this mutation was not detected among patients diagnosed with pure DCIS who were older than 46 ($P = 0.03$) or had high-grade tumors ($P = 0.02$; Supplementary Table S4), implying that E542K mutation might increase the risk of DCIS progression in these subsets. Correlating with our results, previous studies have found exon 9 mutations to be more frequent in IDCs of older women (16), and that they were associated with shorter disease-free and overall survival (17).

In summary, this is the first study to show that *PIK3CA* mutation is a relatively early event in breast tumorigenesis preceding invasion because the frequency of *PIK3CA* mutations was the same in pure DCIS as in DCIS adjacent to IDC and in IDC. Furthermore, we also showed that invasive and *in situ* areas of the same tumor had identical *PIK3CA* sequences in the majority of cases, although clonal divergence and intratumor heterogeneity could also be observed in a significant fraction of tumors. Our results support the hypothesis that mutational activation of the *PIK3CA* pathway might play a role in breast tumor initiation and it is not likely to be involved in promoting invasive progression. However, the association between exon 9 mutations and the risk of DCIS progression merits further investigation.

Disclosure of Potential Conflicts of Interest

K. Polyak: commercial research grant and consultant, Novartis. The other authors disclosed no potential conflicts of interest.

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