Frequent Mutation of the *PIK3CA* Gene in Ovarian and Breast Cancers

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Abstract

Purpose: Activation of the phosphatidylinositol 3-kinase (PI3K)-AKT pathway, resulting in increased cell proliferation, survival, and motility, is believed to play an oncogenic role in many cancer types. The *PIK3CA* gene encodes the p110α catalytic subunit of PI3K, and is amplified in some ovarian cancers, whereas the *AKT2* gene is amplified in some ovarian, breast, and pancreatic cancers. Recently, in a mutational screen of eight *PI3K* genes and eight *PI3K*-like genes, *PIK3CA* was found to be the only gene affected by somatic mutations, which were observed frequently in gastrointestinal and brain cancers. Here, we test whether *PIK3CA* is subject to mutation in ovarian and breast cancers.

Experimental Design: Exons 9 and 20, encoding the highly conserved helical and kinase domains of *PIK3CA*, were subjected to sequence analysis in 198 advanced stage epithelial ovarian carcinomas and 72 invasive breast carcinomas (48 of ductal histology and 24 of lobular histology).

Results: Somatic missense mutations were observed in 24 of 198 (12%) ovarian carcinomas, and in 13 of 72 (18%) breast carcinomas.

Conclusions: These data indicate that mutations of *PIK3CA* play an oncogenic role in substantial fractions of ovarian and breast carcinomas, and in consideration of mutation of other components of the PI3K-AKT pathway in both tumor types, confirm the major oncogenic role of this pathway in ovarian and breast carcinomas.

Increased mitogenic signaling through receptor tyrosine kinases has been proven to play a major role in human tumorigenesis. One of the major downstream mediators of signaling initiated by these receptors is the phosphatidylinositol 3-kinase (PI3K)-AKT pathway. Several components of this signaling system are dysregulated in a wide variety of cancer types, including amplification and/or overactivation of *AKT2* and *PIK3CA* (encoding the p110 catalytic subunit of PI3K), mutation of *PIK3R1* (encoding the p85 regulatory subunit of PI3K), and mutational inactivation or silencing of the *PTEN* gene, a tumor suppressor that negatively regulates this pathway (1). Such deregulation of this pathway promotes a number of phenotypic properties associated with tumorigenesis, including increased cell proliferation, glucose transport and catabolism, cytoskeletal rearrangements, cell adhesion and migration, and decreased apoptosis. With respect to ovarian cancer, aberrations in components of this pathway include elevated AKT1 kinase activity (2), *AKT2* amplification (3), *PIK3CA* amplification (4), *PIK3R1* mutation (5), and allelic imbalance and mutation of the *PTEN* gene (6). In breast cancers, *AKT2* amplification (3) and elevated kinase activities of AKT1 (2) and PI3K (7) are observed.

Recently, a large-scale sequence analysis of 16 *PI3K* or *PI3K*-like genes revealed tumor-specific somatic mutations in only one of these genes, *PIK3CA* (8). All were apparent activating missense mutations, occurring primarily in the p85, C2, helical, or kinase functional domains. The great majority of mutations (85%) were present in exons 9 and 20, partially encoding the helical and kinase domains, respectively. Of the seven tumor sites studied, mutations were most common in colon (32%), glioblastoma (27%), and gastric (25%) cancers. Mutations were observed in 1 of 12 (8%) breast cancers; ovarian cancers were not examined. The purpose of this study was to use relatively large sample sizes to test whether activating mutations of the *PIK3CA* gene play a significant role in ovarian and breast tumorigenesis.

Materials and Methods

This study was approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center. Flash-frozen primary tumor specimens and corresponding normal tissues or blood samples were retrieved from a tissue bank maintained by the Gynecology and Breast Research Laboratory of the Department of Surgery at this institution. Following pathologic review to confirm diagnosis, tumor tissue was microdissected and genomic DNA was isolated from these tissues using

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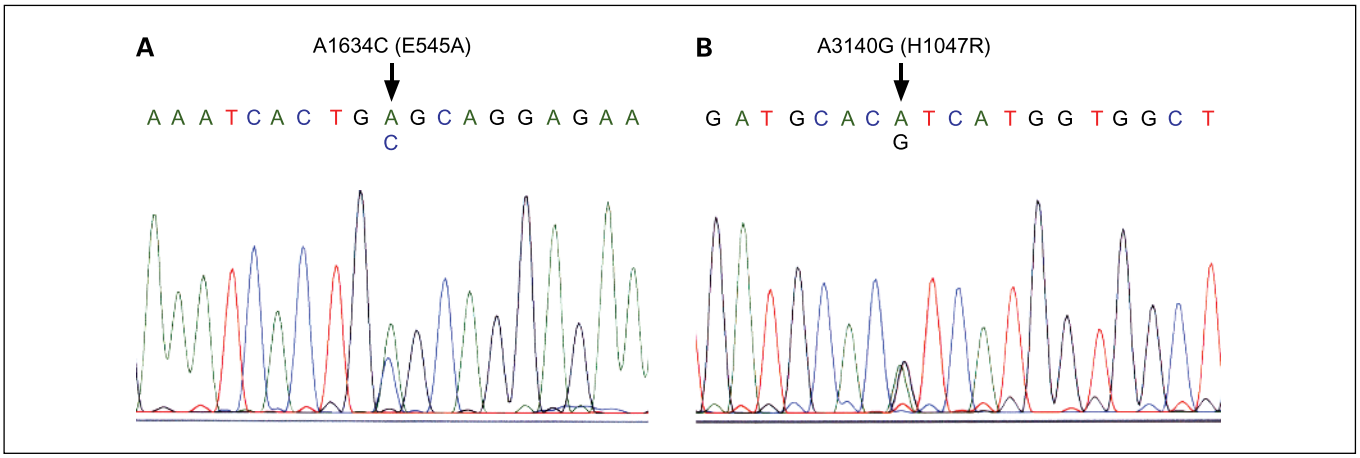

Fig. 1. Examples of *PIK3CA* mutations as determined by automated sequence analysis. *A*, an E545A (A1634C) missense mutation in exon 9 in an ovarian cancer. *B*, an H1047R (A3140G) missense mutation in exon 20 in a breast cancer.

standard techniques. A total of 198 unselected, advanced surgical stage (III or IV), moderately or poorly differentiated (Federation Internationale des Gynaecologistes et Obstetristes grades 2 or 3) invasive epithelial ovarian carcinomas were analyzed. A total of 72 unselected, invasive breast carcinomas were analyzed, 48 of ductal histology and 24 of lobular histology. The majority (78%) of the breast carcinomas were of early stage. Using PCR primers previously specified (8), exons 9 and 20 of *PIK3CA* were sequenced directly using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI 3730 automated capillary sequencer. Analyses of all PCR products with sequence variants were repeated for confirmation. Genomic DNA from corresponding normal tissue or blood samples was isolated and subjected to sequence analysis to confirm the somatic nature of tumor mutations. A representative example of sequencing data is shown in Fig. 1. The VMD molecular visualization program (http://www.ks.uiuc.edu/Research/vmd/) was used to prepare Fig. 2.

Results and Discussion

Somatic missense mutations in the *PIK3CA* gene were identified in 24 of 198 (12%) ovarian carcinomas and 13 of 72 (18%) breast carcinomas (Table 1). Since mutation analysis was limited to the exons encoding the helical and kinase domains of *PIK3CA*, where 85% of previous mutations were reported (8), these figures may represent underestimates of the true mutation frequencies in these tumor types. For ovarian cancers, 22 of 24 (92%) mutations occurred at codon 545 within the helical domain, a codon affected by 26% of all previously reported mutations (when the entire gene is analyzed). The other two ovarian cancers with mutations were affected at codon 1043 in the kinase domain, a mutation not previously reported, and at codon 1047, the second most common mutation previously reported. In addition, four synonymous mutations, three at codon 1025 and one at codon 1049, were also observed in ovarian cancers. For breast cancers, the mutations were somewhat less clustered than for ovarian cancers; 5 of 13 (38%) mutations occurred at codon 545, whereas 4 of 13 (31%) occurred at codon 542, and a similar number occurred at codon 1047. Mutations at all of these *PIK3CA* codons are frequently seen in other tumor types (8). Three breast tumors also contained the same synonymous mutation at codon 1025. The functional significance of the H1047R mutation has been described (8). Furthermore, it was recently reported that somatic mutations of *PIK3CA* in ovarian cancers occur almost to the exclusivity of gene amplification (9).

To gain further insight into the potential structural and functional implications of the mutations identified in this study, we attempted to model the PIK3CA protein. The crystal structure of PIK3CA has not yet been described, but the structure of the highly homologous PIK3CG protein is known (Protein Data Bank, 1E8Z). In the helical domain, the E542K mutation changes the charge from negative to strongly positive. At codon 545, where most of the ovarian carcinoma mutations occurred, the mutation resulted in a charge change from negative to highly positive (E545K), or caused it to become hydrophobic (E545G and E545A). As shown in Fig. 2, both of these residues are on the exposed surface of the molecule. These changes in charge may

Table 1. Summary of *PIK3CA* mutations in ovarian and breast cancers

Exon	Nucleotide	Codon	Domain	Ovarian*	Breast†
9	G1624A	E542K	Helical	0	4
9	G1633A	E545K	Helical	1	4
9	A1634G	E545G	Helical	0	1
9	A1634C	E545A	Helical	21	0
20	C3075T	T1025T‡	Kinase	3	3
20	A3127G	M1043V	Kinase	1	0
20	A3140T	H1047L	Kinase	0	2
20	A3140G	H1047R	Kinase	1	2
20	T3147G	G1049G‡	Kinase	1	0
Total tumors with missense mutations				24	13
Number of tumors screened				198	72
Percentage of tumors with missense mutations				12%	18%

*Number of ovarian cancers with the specified mutation.
†Number of breast cancers with the specified mutation.
‡Synonymous mutation.

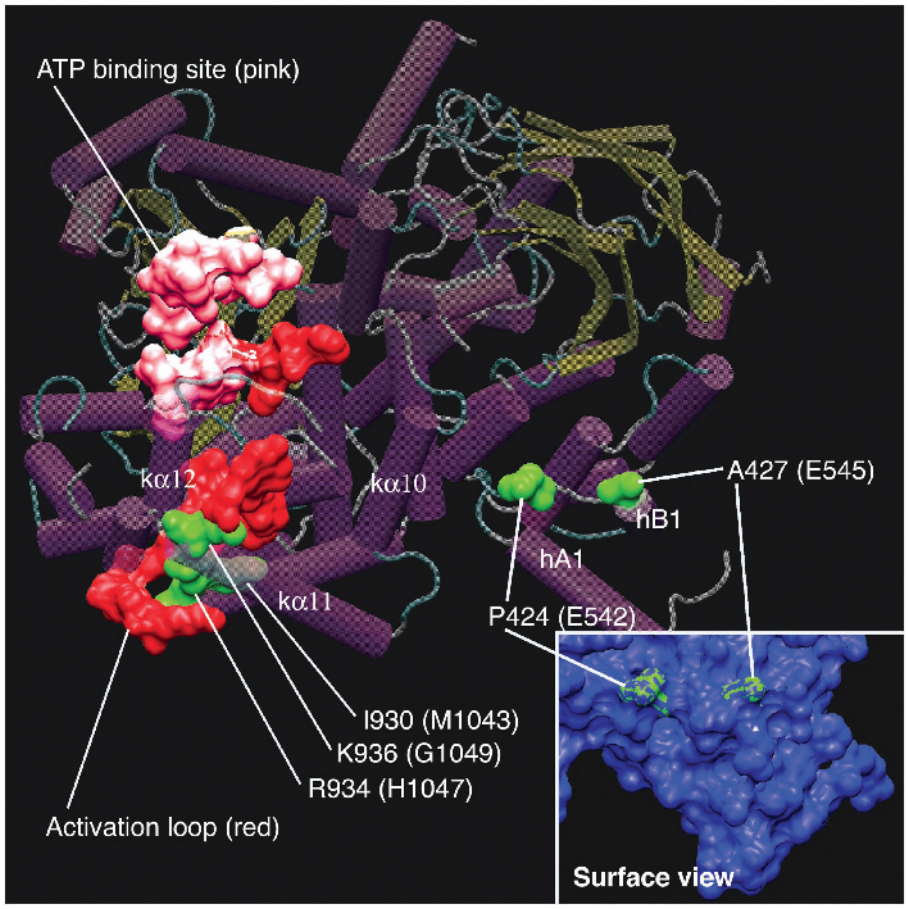

Fig. 2. Overall related structure of PIK3CG with corresponding PIK3CA mutated residues identified in parentheses. Inset, surface view of the helical domain. The E542 and E545 residues are located in an A/B pair between the hA1 and hB1 loops and have a surface location (*inset*). The M1043 and H1047 residues are located in kα11 and are intimately juxtaposed with the activation loop.

therefore affect protein-protein or other intermolecular interactions. This region of the PIK3CG surface is believed to interact with other proteins, and it is reasonable to assume that similar interactions occur in the highly homologous PIK3CA (10). This helical domain of PIK3CG consists of five A/B pairs of antiparallel helices. Mutations in similar motifs of the PP2A PR65/A subunit have been shown to prevent intramolecular binding with PP2Ac and disrupt protein conformation (11).

The mutations in the kinase domain at codon 1047 change the amino acid charge from positive to hydrophobic (H1047L) or to strongly positive (H1047R). These two mutations and the M1043V mutation are located within the kα11 helix (Fig. 2). This helix is found between kα10 and kα12, which lie on two sides of the activation loop. Mutations in the activation loop have been shown to affect the specificity of lipid substrates and access to the catalytic core of PIK3CA (12). Mutations in the kα11 helix shown in this study may affect the conformation of the activation loop and substrate specificity. As mentioned above, the H1047L mutation may also affect function of the kinase domain, although biochemical data to support this hypothesis are lacking.

There were no correlations observed between the presence of *PIK3CA* mutation and any clinicopathologic feature of the ovarian cancers, such as stage, grade, histologic type, patient age, or survival. These data add to a growing body of literature implicating the PI3K-AKT pathway as playing a major oncogenic role in ovarian tumorigenesis, with a substantial proportion of these tumors having sustained amplification or mutation of *PIK3CA*, mutation of *PIK3R1*, amplification of *AKT2*, or mutational inactivation of *PTEN*. Notably, *PIK3CA* represents one of very few established human oncogenes that is commonly activated through either amplification or point mutation. Clinically, the clustering of mutations at codon 545 provides a convenient opportunity for early detection or monitoring for recurrence in the fraction of ovarian cancers with this mutation, and the common activation of the PI3K-AKT pathway in ovarian cancers generally suggests a possible therapeutic opportunity for pharmacologic intervention.

In breast cancers, the presence of a *PIK3CA* mutation did not correlate with clinicopathologic findings such as age at diagnosis, hormone receptor status, or histologic subtype (data not shown). Activating mutation of *PIK3CA* represents one of the most common oncogenic mutations thus far described in breast carcinoma. Unlike ovarian carcinoma, *PIK3CA* is not subject to amplification in breast tumorigenesis, but the observation of *AKT2* amplification and *PIK3CA* mutation in breast cancers again implicates the PI3K-AKT pathway as among the most important yet described in sporadic breast tumorigenesis.

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