High Frequency of Mutations of the *PIK3CA* Gene in Human Cancers

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Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that regulate signaling pathways important for neoplasia, including cell proliferation, adhesion, survival, and motility (*1*–*3*). To determine if PI3Ks are genetically altered in tumorigenesis, we sequenced PI3K genes in human cancers and corresponding normal tissue.

Hidden Markov models were used to identify eight PI3K and eight PI3K-like genes, including two uncharacterized genes, in the human genome (*3*) (table S1). We initially examined the sequences of 117 exons that encode the predicted kinase domains of these genes in 35 colorectal cancers (*3*). PIK3CA, which encodes the p110α catalytic subunit, was the only gene with somatic (i.e., tumor-specific) mutations. Subsequent sequence analysis of all coding exons of *PIK3CA* in 199 additional colorectal cancers revealed mutations in a total of 74 tumors (32%) (Fig. 1, fig. S1, and table S2). We also evaluated 76 premalignant colorectal tumors; only two mutations were found, both in very advanced tubulovillous adenomas greater than 5 cm in diameter. Thus, *PIK3CA* mutations generally arise late in tumorigenesis, just before or coincident with invasion.

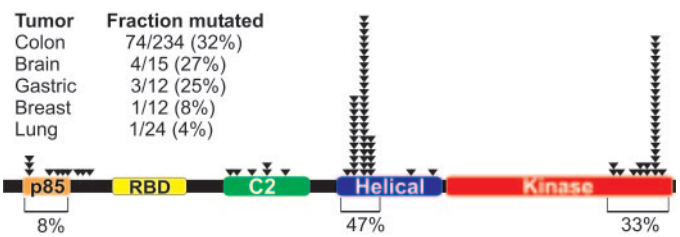

Fig. 1. Mutations in *PIK3CA*. Arrowheads indicate the location of missense mutations, and boxes represent functional domains (the p85 binding domain, Ras binding domain, C2 domain, helical domain, and kinase domain). The percentage of mutations detected within each region is indicated below, and the fraction of tumors with mutations is noted above. Nucleotide positions of the mutations are listed in table S2.

Mutations in *PIK3CA* were also identified in 4 of 15 glioblastomas (27%), 3 of 12 gastric cancers (25%), 1 of 12 breast cancers (8%), and 1 of 24 lung cancers (4%) (table S2). No mutations were observed in 11 pancreatic cancers or 12 medulloblastomas. In total, 92 mutations were observed, all of which were determined to be somatic in the cancers that could be assessed.

The sheer number of mutations observed in this gene strongly suggests that they are functionally important. This hypothesis is buttressed by two lines of evidence. First, analysis of the ratio of nonsynonymous (NS) to synonymous (S) mutations is a good measure of selection during tumor progression, as silent alterations are unlikely to exert a growth advantage. The ratio of NS to S mutations in *PIK3CA* was 92 to 3, far higher than the 2 to 1 ratio expected by chance ($P < 0.0001$). Second, the prevalence of NS changes located in the PI3K helical and kinase domains was ~120 per Mb of tumor DNA, more than 100 times higher than the background mutation frequency of nonfunctional alterations observed in cancer cells ($P < 0.001$) (*4*).

The positions of the mutations within *PIK3CA* imply that they are likely to increase kinase activity. No truncating mutations were observed and >75% of alterations occurred in two small clusters in the helical and kinase domains (Fig. 1 and table S2). The affected residues within these clusters are highly conserved evolutionarily. The clustering of somatic missense mutations in specific domains is similar to that observed for activating mutations in other oncogenes, such as *RAS*, *BRAF*, *CTNNB1*, and members of the tyrosine kinome (*3*). To directly test this hypothesis, we expressed the wild-type p110α or a "hot-spot" mutant (H1047R) in NIH3T3 cells and assessed lipid kinase activities in immunoprecipitated PI3K complexes. Expression of mutant p110α conferred more lipid kinase activity than expression of wild-type protein (fig. S2).

Our data suggest that mutant *PIK3CA* is likely to function as an oncogene in human cancers. This idea is consistent with previously reported alterations of members of the PI3K pathway, particularly inactivation of the *PTEN* tumor suppressor, whose function is to reverse the phosphorylation mediated by PI3Ks (*1*, *3*). Reduplication or amplification of the chromosomal regions containing *PIK3CA* has been reported in some human cancers (*1*, *5*). We found no evidence of *PIK3CA* gene amplification in 96 colorectal cancers, suggesting that amplification is not a common mechanism of activation in this tumor type.

These data imply that *PIK3CA* may prove useful for diagnostic and therapeutic purposes. The clustering of mutations within *PIK3CA* could make the gene an excellent marker for early detection of cancers or for monitoring tumor progression. If future experiments verify that mutational activation of *PIK3CA* is essential for tumor growth, specific inhibitors of PIK3CA could be developed for treatment of the large number of patients with these mutations.

References and Notes

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Supporting Online Material
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Materials and Methods
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