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Somatic Mutations in p85α Promote Tumorigenesis through Class IA PI3K Activation

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SUMMARY

Members of the mammalian phosphoinositide-3-OH kinase (PI3K) family of proteins are critical regulators of various cellular process including cell survival, growth, proliferation, and motility. Oncogenic activating mutations in the p110α catalytic subunit of the heterodimeric p110/p85 PI3K enzyme are frequent in human cancers. Here we show the presence of frequent mutations in p85α in colon cancer, a majority of which occurs in the inter-Src homology-2 (iSH2) domain. These mutations uncouple and retain p85α's p110-stabilizing activity, while abrogating its p110-inhibitory activity. The p85α mutants promote cell survival, AKT activation, anchorage-independent cell growth, and oncogenesis in a p110-dependent manner.

INTRODUCTION

Phosphoinositide 3-kinase (PI3K) family of lipid kinases are divided into three major classes based on primary sequence, substrate preference, and regulation (Cantley, 2002; Engelman et al., 2006; Fruman et al., 1998; Hawkins et al., 2006). While class IA PI3Ks are heterodimeric enzymes composed of a catalytic subunit (p110α, p110β, or p110δ) complexed with one of five regulatory subunits (p85α, p55α, p50α, p85β, or p55γ), the class IB enzyme is a dimer made of p110γ catalytic subunit and p101 or p84 regulatory subunit (Cantley, 2002; Hawkins et al., 2006; Vanhaesebroeck et al., 2005). The class I catalytic subunit polypeptides p110α, p110β, p110δ, and p110γ are encoded by *PIK3CA*, *PIK3CB*, *PIK3CD*, and *PIK3CG*, respectively (Cantley, 2002; Vanhaesebroeck and Waterfield, 1999). The regulatory subunits are encoded by five genes: *PIK3R1* codes p85α, p55α, and p50α; *PIK3R2* codes p85β; *PIK3R3* codes p55γ; *PIK3R5* codes p101; and *PIK3R6* codes p84 (Cantley, 2002; Vanhaesebroeck and Waterfield, 1999).

The p110 catalytic subunits of PI3K share a common domain architecture consisting of an N-terminal adaptor binding domain that binds to p85 regulatory subunits, a Ras binding domain, a putative membrane binding C2 domain, a helical region that makes a regulatory contact with the p85 nSH2 domain (Miled et al., 2007), and a C-terminal kinase domain. Similarly, the p85 regulatory subunits have in common an N-terminal SH3 domain, a domain homologous to the Rho GTPase-activating protein domain of the BCR gene product (BCR domain), and two SH2 domains (nSH2 and cSH2) that flank an intervening antiparallel coiled-coil (iSH2) required for binding to the adaptor binding domain in p110 (Holt et al., 1994). Besides its role in inhibiting the catalytic activity of p110, in the basal state, the

SIGNIFICANCE

Somatic mutations in the catalytic p110α subunit of PI3K are common in cancers. In this study, we show the occurrence of frequent mutations in the regulatory p85α subunit of PI3K in human cancers. Our data demonstrate an alternate mechanism for PI3K pathway activation and oncogenesis resulting from the impaired regulation of p110 activity by mutant p85α. Further, p85α mutations are likely to be useful as diagnostic markers for identification of p110-dependent tumors that may not carry an activating p110α mutation, but are candidates for targeted treatment with PI3K pathway inhibitors that are in development.

p85 regulatory subunit is required to stabilize the catalytic p110 subunit (Kodaki et al., 1994; Yu et al., 1998). Upon growth factor stimulation, the nSH2 and cSH2 domains of p85 bind to phosphorylated tyrosines (YXXM motif) in activated receptors and adaptors that activate catalytic p110 (Backer et al., 1992; Carpenter et al., 1993; Otsu et al., 1991). Once activated, PI3Ks phosphorylate phosphoinositide 4,5-bisphosphate leading to the production of phosphoinositide 3,4,5-triphosphate, which serves as an important second messenger that regulates cell survival, growth, proliferation, and motility through a variety of downstream effectors (Cantley, 2002; Carpenter et al., 1993; Engelman et al., 2006; Jimenez et al., 2002; Vanhaesebroeck and Waterfield, 1999; Yu et al., 1998).

Several studies have identified common somatic mutations in *PIK3CA* in cancers of colon, rectum, breast, ovary, brain, and liver (Bader et al., 2005; Samuels et al., 2004). Several *PIK3CA* mutants, including hotspot mutations in the helical and kinase domain, show elevated lipid kinase activity in vitro and induce oncogenic transformation in vivo (Gymnopoulos et al., 2007; Ikenoue et al., 2005; Isakoff et al., 2005; Kang et al., 2005; Samuels et al., 2004; Zhao and Vogt, 2008). While oncogenic p110α mutations are common in cancers (Bader et al., 2005; Samuels et al., 2004), such mutations in the regulatory p85 subunit are not as common (Bader et al., 2005; Hennessy et al., 2005). Previously, a truncated form of p85α containing residues 1–571 fused to a fragment of Eph (p65) was identified in an X-ray-irradiated mouse lymphoma model (Borlado et al., 2000; Chan et al., 2002; Jimenez et al., 1998). However, this mutation has so far not been found in human cancers. Although, a p85α truncation mutant was described in a human lymphoma cell line (Jucker et al., 2002), its relevance in oncogenesis is not clear (Horn et al., 2008). A low prevalence of p85α mutation in breast (Wood et al., 2007), colon (Philp et al., 2001), and ovarian (Philp et al., 2001) tumors has been reported, but the functional role of these mutations in tumorigenesis is not known. Recently, frequent occurrence of p85α mutations in glioblastoma was reported (Parsons et al., 2008; TCGA, 2008). However, the ability and role of these mutations in promoting oncogenesis remains to be studied.

In this study we have systematically sequenced a large number of tumors and found mutations in p85α that uncouple its p110-inhibitory effects from the stabilization activity, leading to p110-mediated survival signaling and oncogenesis.

RESULTS

Identification of PI3K Regulatory Subunit Mutations

We sequenced coding exons and an ~50 bp region flanking the exons of *PIK3R1*, *PIK3R2*, *PIK3R3*, *PIK3R4*, and *PIK3R5* regulatory subunits of PI3K in primary human cancers. A total of 672 human primary tumor samples consisting of 213 non-small-cell lung, 108 colorectal, 62 breast, 87 renal cell, 46 ovary, 40 skin (melanomas), 37 gastric, 21 small-cell lung, 16 esophageal, 13 bladder, 12 chronic lymphocytic leukemia, 11 hepatocellular, and 6 pancreatic cancers were analyzed (see Table S1 available online). We found that *PIK3R1*, which codes for p85α, was mutated in 9 of 108 colorectal (8%), 1 of 62 breast (2%), and 1 of 6 pancreatic (17%) tumor samples (Figure 1, Figure S1, and Tables S1 and S2). All the mutations were confirmed to be somatic by their presence in the original tumor DNA and absence in the matched adjacent normal tissue through additional sequencing or mass spectrometric analysis (Figures S1 and S2). A majority of the mutations identified were amino acid substitutions that clustered in the iSH2 domain. Interestingly, one of the mutated residues, N564, was within hydrogen bonding distance of p110α C2 domain residue N345 (Huang et al., 2007; Figures 1A and 1C). Substitutions at residue N345 of p110α are oncogenic (Gymnopoulos et al., 2007), probably due to destabilization of iSH2-C2 inhibitory interactions. Therefore, it is likely that the p85α N564D mutation mimics the effect of N345 substitutions in p110α. Interestingly, both N564 and an adjacent residue, D560 of p85α, which is also within hydrogen bonding distance of N345 of p110α, were recently reported to be mutated in glioblastoma (TCGA, 2008). Though the potential effect of these substitutions on relieving the inhibitory contacts have been proposed (TCGA, 2008), the actual functional consequence of the mutation and its role in oncogenesis have not been explored. The regulatory p85α subunit, besides regulating p110α, also regulates p110β and p110δ (Engelman et al., 2006; Vanhaesebroeck et al., 2005). A similar set of regulatory contacts, involving residue N344 in p110β and residue N334 p110δ, analogous to p110α residue N345, and p85α iSH2 residues N564 and D560, has been previously proposed (Amzel et al., 2008). Our modeling of the C2 domain of p110β and p110δ supports these regulatory interactions (Figure 1C), suggesting that the p85α mutations may lead to general activation of all class IA catalytic subunits.

In addition to mutations that clustered in iSH2, we identified mutations in the cSH2 domain, specifically a mutation in residue R649, which is part of the conserved FLVERS motif required for phosphotyrosine engagement (Hoedemaeker et al., 1999). The R649 mutation is likely to alter the activity of the protein, given that analogous conserved Arg residues within SH2 domains of other proteins are mutated in multiple human diseases (Figure S3). Mutations in the nSH2 and BCR domains and in the spacer between those domains were also identified (Figure 1A). It is noteworthy that the majority of the mutated residues identified within nSH2, iSH2, and cSH2 were conserved across the p85 family (Figure 1 and Figure S4). Meta-analysis of mutations in p85α (Jimenez et al., 1998; Jucker et al., 2002; Parsons et al., 2008; Philp et al., 2001; TCGA, 2008; Wood et al., 2007) revealed several recurrent mutation sites that include R348, G376, L380, K459, D560, N564, and R574. Interestingly, these mutated sites are conserved across the three closely related p85 family members (p85α/p55α/p50α, p85β, and p55γ), suggesting that these mutations are likely to have a functional role in oncogenesis. We also detected mutations in *PIK3R2* (p85β) and two additional regulatory subunits, *PIK3R4* (p150) and *PIK3R5* (p101), albeit at much lower frequencies (Figure S5 and Tables S2 and S3).

In addition to sequencing PI3K regulatory subunit genes, we tested the tumor samples for somatic mutations in *PIK3CA*, *KRAS*, and *PTEN*. In colon tumor bearing p85α mutations, we rarely found *KRAS* or *PTEN* mutations. However, ~45% of p85α mutant colon tumor samples had *PIK3CA* mutations, suggesting that the p110α and p85α mutations are not always mutually exclusive in this tumor type (Figure 1D, Figure S6, and Tables S2 and S3). Unlike in colon cancer, published *PIK3R1* mutation