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AKT-Independent Signaling Downstream of Oncogenic *PIK3CA* Mutations in Human Cancer

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SUMMARY

Dysregulation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway occurs frequently in human cancer. *PTEN* tumor suppressor or *PIK3CA* oncogene mutations both direct PI3K-dependent tumorigenesis largely through activation of the AKT/PKB kinase. However, here we show through phosphoprotein profiling and functional genomic studies that many *PIK3CA* mutant cancer cell lines and human breast tumors exhibit only minimal AKT activation and a diminished reliance on AKT for anchorage-independent growth. Instead, these cells retain robust PDK1 activation and membrane localization and exhibit dependency on the PDK1 substrate SGK3. SGK3 undergoes PI3K- and PDK1-dependent activation in *PIK3CA* mutant cancer cells. Thus, PI3K may promote cancer through both AKT-dependent and AKT-independent mechanisms. Knowledge of differential PI3K/PDK1 signaling could inform rational therapeutics in cancers harboring *PIK3CA* mutations.

INTRODUCTION

Aberrant phosphatidylinositol 3-kinase (PI3K) signaling occurs commonly in cancer (Vivanco and Sawyers, 2002). Upon activation at the plasma membrane by receptor tyrosine kinases or RAS proteins (Engelman et al., 2006), PI3Ks phosphorylate the D3 position on membrane phosphatidylinositides, thereby recruiting and activating proteins that contain a pleckstrin homology (PH) or

SIGNIFICANCE

Genetic alterations targeting the PI3K pathway are highly prevalent in many human cancers. For example, gain-of-function mutations in *PIK3CA*, which encodes a key enzymatic subunit of PI3K, occur frequently in breast, colon, and endometrial cancers, among others. Downstream activation of the AKT kinase is regarded as the dominant tumor-promoting mechanism enacted by PI3K signaling. However, this study shows that AKT signaling is markedly diminished in many cancer cell lines and human breast tumors harboring *PIK3CA* mutations. Instead, these cells elaborate a signaling pathway involving the PI3K effector PDK1 and its downstream substrate SGK3. These findings may have important implications for PI3K signaling and the development of rational therapeutics against this key cancer pathway.

other lipid-binding domain. This activity is antagonized by the PTEN tumor suppressor protein. The serine/threonine kinase AKT/PKB (AKT), upon activation by PDK1 and the TORC2 complex (Alessi et al., 1997; Sarbassov et al., 2005; Stephens et al., 1998), is believed to transduce the major downstream PI3K signal in cancer. AKT regulates cell growth and survival pathways by phosphorylating substrates such as GSK3, Forkhead transcription factors, and the TSC2 tumor suppressor protein (Vivanco and Sawyers, 2002).

Both *PTEN* and *PIK3CA*, which encodes the catalytic (p110α) subunit of PI3K, are frequently mutated across many human cancers. The most common tumor-associated *PIK3CA* mutations (>80% of cases) involve either the helical domain (exon 9; e.g., E542K and E545K) or the kinase domain (exon 20; e.g., H1047R) of p110α (Samuels et al., 2004, 2005). Inactivating *PTEN* mutations occur commonly in prostate cancer, endometrial cancer, and glioblastoma, among others (Vivanco and Sawyers, 2002). Rare activating somatic mutations of *AKT1* have also been described in cancer (Carpten et al., 2007).

Although inactivating *PTEN* mutations and activating *PIK3CA* mutations both augment AKT signaling in several experimental systems (Kang et al., 2005; Nakamura et al., 2000), it is not clear whether such genetic alterations are functionally redundant in vivo. For example, in endometrial cancers *PIK3CA* and *PTEN* mutations often co-occur (Oda et al., 2005), suggesting that they may have distinct roles. Similarly, *PIK3CA* mutations may be seen in breast cancers with low PTEN levels, and AKT phosphorylation correlates poorly with *PIK3CA* mutation in this malignancy (Stemke-Hale et al., 2008). In addition, while PTEN loss has been associated with adverse clinical outcome in breast cancer (Depowski et al., 2001), the prognosis associated with *PIK3CA* alterations may depend on the type of mutation. In one study, for example, helical mutations correlated with poorer prognosis than kinase-domain mutations (Barbareschi et al., 2007). Thus, as observed for RAS and RAF oncoproteins in the MAP kinase cascade (Solit et al., 2006), the position of somatic alterations within the PI3K pathway (or *PIK3CA* itself) may influence the mechanisms and, by extension, the functional output of oncogenic pathway deregulation. Here, we used a phosphoprotein profiling and functional genetic approach to characterize signaling mechanisms downstream of PI3K in *PIK3CA* mutant cancer cells.

RESULTS

PIK3CA Mutant Cancer Cells Frequently Show Diminished AKT Signaling

To determine whether somatic PTEN loss and *PIK3CA* activation lead to the same signaling consequences in cancer, we interrogated phosphoprotein profiles associated with distinct alterations affecting the PI3K pathway by reverse-phase protein array (RPPA) analysis (Tibes et al., 2006). Analysis of the quantitative protein expression signal from PTEN and phosphorylated AKT (p-AKT) in the NCI60 cancer cell line collection (Stinson et al., 1992) identified 12 lines with low or absent PTEN protein (Figure 1A). As expected (Nakamura et al., 2000), all cell lines with low PTEN (PTEN null) exhibited enhanced AKT phosphorylation at both serine 473 and threonine 308 (Figures 1B and 1C; $p < 0.001$ for both p-AKT sites).

We then analyzed the relationship between the *PIK3CA* mutations and levels of p-AKT. Previous sequencing studies identified seven NCI60 cell lines (spanning four tumor types) that harbor *PIK3CA* mutations; three lines with kinase-domain mutations (SK-OV-3, HCT-116, and T-47D), and four with helical mutations (HT-29, HCT-15, MCF-7, and NCI-H460) (http://www.sanger.ac.uk/genetics/CGP/cosmic/ and confirmed with the lines used here). In contrast to the PTEN null setting, NCI60 lines with activating *PIK3CA* mutations contained much lower p-AKT RPPA signals when compared to PTEN null cell lines, irrespective of tumor type ($p < 0.001$ for Ser473 and $p = 0.002$ for Thr308; Figures 1B and 1C).

As *PIK3CA* mutations were relatively uncommon in the NCI60 panel, we confirmed this observation in 51 human breast cancer cell lines (Neve et al., 2006) (see Figure S1 available online). We also observed similar RPPA patterns by hierarchical clustering of PTEN and p-AKT RPPA signals in 64 hormone receptor-positive breast tumor samples (Figure S2). Whereas elevated p-AKT at Ser473 and Thr308 correlated inversely with PTEN levels in all cases, many *PIK3CA* mutant cell lines and breast tumors contained low p-AKT levels (Figures S1 and S2). In these experiments, reduced p-AKT expression was particularly apparent (though not universal) in the setting of helical (e.g., E542K/E545K) *PIK3CA* mutations (*PIK3CA*^{helical}) (Figures S1B, S1C, and S2B), although multiple cell lines and tumors with kinase domain (e.g., H1047R) mutations (*PIK3CA*^{kinase}) also showed low p-AKT (Figures 1B and 1C and Figures S1B, S1C, and S2A). These findings, together with previous studies of human breast tumors (Stemke-Hale et al., 2008), raised the possibility that PTEN loss and *PIK3CA* mutation might have different effects on AKT signaling.

To examine AKT pathway activation in more detail, we performed immunoblot analyses on selected cancer cell lines that lack *PTEN* or express activating *PIK3CA* alleles. Strikingly, p-AKT at both Ser473 and Thr308 was markedly diminished in the four *PIK3CA*^{helical} cell lines examined, as shown in Figure 1D. AKT phosphorylation in *PIK3CA*^{kinase} cells was more variable, approaching the levels observed in PTEN null cells in some cases (e.g., BT-20, MDA-MB-453, and HCC1954; Figure 1D), while virtually undetectable in others (e.g., HCT-116; Figure S3A). For most ensuing experiments, we considered MCF-7, HCT-15, and SW948 as representative *PIK3CA* mutant cells with low p-AKT; T47D and HCC1954 as representative *PIK3CA* mutant cell lines with elevated p-AKT; and 786-0 as a representative PTEN null cell line. Additional studies under serum-starved conditions showed that the level of AKT phosphorylation in several *PIK3CA* mutant cancer cell lines was comparable to a nontransformed setting (e.g., MCF-10A and MCF-12A cells; Figure S3B). Thus, many *PIK3CA* mutant cancer cells exhibited unexpectedly low AKT signaling.

We considered the possibility that the reduced AKT phosphorylation could reflect down-modulation through known feedback regulatory mechanisms (Haruta et al., 2000; O'Reilly et al., 2006). In this case, downstream effectors might be active even though p-AKT levels are suppressed. To test this, we examined RPPA data corresponding to the AKT substrates GSK3β and TSC2 in the NCI60 panel. Phosphorylation of both substrates was reduced in *PIK3CA* mutant cells compared to *PTEN* null cells (Figures 1E and 1F). Immunoblot studies confirmed a tight

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