A New Mutational *akt*ivation in the PI3K Pathway

Joan Brugge,[1] Mien-Chie Hung,[2] and Gordon B. Mills[3,*]
[1]Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA
[2]Department of Cellular and Molecular Oncology, M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA
[3]Department of Systems Biology, M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA
*Correspondence: gmills@mdanderson.org
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Although multiple members of the phosphatidylinositol-3-kinase pathway (PI3K) are targeted by germline or somatic mutations, functional mutations in the three *akt* isoforms have proven elusive. This is somewhat surprising, as AKT represents a key node in the PI3K pathway, exhibiting transforming activity when incorporated into the AKT8 retrovirus. A recent report in *Nature* identifies a transforming E17K PH domain mutation in *akt1* in breast (8%), colorectal (6%), and ovarian (2%) cancers. E17K-*akt1* transforming activity appears due to PtdIns(3,4)P2- and PtdIns(3,4,5)P3-independent recruitment of AKT1 to the membrane. This novel observation raises important theoretical and clinical questions.

Introduction

The PI3K pathway is more frequently activated by genomic aberrations than any other signaling pathway across many cancer lineages (Table 1). Multiple pathway components are targeted by germline or somatic mutation, amplification, rearrangement, methylation, overexpression, and aberrant splicing (Hennessy et al., 2005; Karni et al., 2007; Kumar and Hung, 2005; Manning and Cantley, 2007). A major challenge in determining the frequency and spectrum of mutations resides in the difficulty of differentiating the core PI3K pathway from the wider PI3K signaling network that includes interactions with the p53, RAS/MAPK, TGFβ, NfκB, WNT, and other pathways (Hennessy et al., 2005; Kumar and Hung, 2005; Manning and Cantley, 2007). The PI3K pathway is also activated by many cell surface receptors as well as intracellular linkers and signaling molecules (Table 1). Thus, most human cancers exhibit activation of the PI3K pathway, leading to the expectation that PI3K pathway targeting will have broad antitumor activity.

Germline mutations associated with cancer-predisposing "hamartoma" syndromes provide key insights into PI3K network wiring. The TSC1/2 tuberous sclerosis complex integrates information from the growth factor-sensing arm of the network, encompassing PI3K, PTEN, and AKT, and the energy-sensing LKB1/AMPK arm, with the latter being dominant, passing the message to Rheb, mTOR, and other downstream mediators. This represents a simplistic model, as each member of the pathway has multiple additional inputs and outputs (Hennessy et al., 2005; Kumar and Hung, 2005; Manning and Cantley, 2007). In *Drosophila*, mutations upstream of TSC1/2 alter cell number and size, while those downstream of TSC1/2 affect only cell size, supporting the concept that the pathway bifurcates and integrates at multiple levels. Somatic mutations in patient tumors also help to define the pathway. With few exceptions, *pik3ca* and *pten* mutations are rarely seen together, suggesting that they mediate similar cellular functions. In contrast, *pdk1*amplification or *b-raf* mutation and *pten* mutation or loss are frequently concordant, indicating that they mediate different cellular events and outcomes or act in concert to stimulate the pathway.

A New First *akt* in the Mutation Play

Recent observations added an E17K mutation in the PH domain of *akt1* to the panoply of known PI3K pathway aberrations (Carpten et al., 2007). This mutation results in PI3K-independent membrane recruitment of AKT1, recapitulating the effects of the AKT8 murine leukemia retrovirus GAG-AKT fusion protein. E17K-*akt1* exhibits transforming activity in vitro and in vivo, albeit at lower levels than myristoylated AKT. Membrane targeting is critical to this transforming activity.

Arising Questions

As with all major new observations, this report raises important questions for the future. The mechanism by which the E17K mutation in the PH domain increases membrane association and AKT activation in the absence of PtdIns(3,4)P2 or PtdIns(3,4,5)P3 while still facilitating activation in the presence of growth factors requires further exploration. In addition, the finding that PDK1 and PDK2 sites of E17K-AKT1 are phosphorylated raises the question of whether PDK1 and TORC2 are constitutively associated with the membrane in cells with mutant *akt1*. Is recruitment of the mutant PH domain to the membrane due to altered binding specificity for other membrane phospholipids (e.g., PI4,5P2, phosphatidylcholine, phosphatidylserine) or, as PH domains can also bind

Table 1. Spectrum of PI3K Pathway Aberrations in Cancer

Molecule	Alteration	Frequency	Approximate Cases per Year (U.S.)	Tumor Lineage
PI3K Pathway				
akt1	PH domain mutation	8%	17,100	breast
		6%	9000	colorectal
		2%	400	ovary
akt1, *2*	amplification	low (≤5%)		ovary, pancreas, breast (rare)
folliculin Birt Hogg Dube syndrome	mutation	<5%		renal cancer, gastric cancer, endometrial cancer
Forkhead family	translocations	>50%		alveolar rhabdomyosarcoma
		low		acute leukemia
pdk1	amplification overexpression	20%	21,000	breast
lkb1 Peutz Jeghers	mutation	30%	52,300	lung
pik3ca	activating mutations[a]	40%	16,480	endometrial[f]
		20%–40%	30,000–60,000	bowel
		30%	64,000	breast
		5%–15%		head and neck and esophageal[g]
		<5%	1000	serous epithelial ovarian cancer
pik3cb	amplification	5%	1000	serous epithelial ovarian cancer
		5%	13,000	breast cancer
pten Cowdens	mutations[b]	≥50%		glioma, melanoma, prostate, endometrial cancer,[h] endometrioid ovarian cancer[i]
PTEN	decreased expression (e.g., LOH, methylation)	>50%	85,000	breast, leukemia
p85	activating mutations	rare		ovary, bowel, glioma
p70s6k	amplification	≤30%	64,000	breast
p70s6k	aberrant splicing	unknown		unknown
tcl1	rearrangement	unclear		T cell leukemia, chronic, lymphocytic leukemia
tsc1/2 Tuberous sclerosis	mutation	<5%		renal cell cancer
Interacting Pathways				
*egfr*vIII	alternate splicing deletion aa 6–273	>80%		glioma
		>50%	107,320	breast
		40%	69,800	non-small-cell lung cancer
egfr	mutation[c]	10%	17,400	lung cancer[j]
egfr	amplification	variable		breast, lung, bowel, head, and neck
her2	amplification	10%–20%	21,500–43,000	breast cancer
		8%	1600	high-grade ovarian cancer
		<10%	<17,500	lung cancer
her2	mutation[d]	<5%	8700	lung cancer
bcr/abl	translocation	>90%	>4050	CML

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Table 1. *Continued*

Molecule	Alteration	Frequency	Approximate Cases per Year (U.S.)	Tumor Lineage
kit	mutation	>80%		GIST
		12%		ovarian germ cell tumors
		variable		sarcomas
ras	mutation[e]	70%–90%	23,650–30,400	pancreatic cancer
		~35%	56,000	lung[k]
		≤5%	10,700	breast
		30%		AML
		55%		thyroid
		15%		melanoma
pdgfr	mutation	rare		GIST
		>80%		dermatofibrosarcoma protuberans
met	mutation	10%	17,400	lung
	amplification	10%	14,900	colorectal
	mutation	27%		head and neck
flt3	mutation	20%–25%		AML
Estrogen receptor	expression	60%–70%	128,000–150,000	breast cancer

[a]*pik3ca* mutations are late events in endometrial, esophagus, and likely breast cancers.
[b]*pten* mutations are frequently mutually exclusive with *pik3ca* mutations except in endometrial cancer, where they coexist in ~25% of cases.
[c]*egfr* mutations are mutually exclusive with *kras* mutations.
[d]*her2* mutations are not mutually exclusive with *kras* mutations.
[e]Mainly *kras* mutations.
[f]*pik3ca* mutations occur in <10% of cases of complex atypical endometrial hyperplasias.
[g]*pik3ca* mutations have not been documented in Barrett's esophagus.
[h]*pten* mutations also occur at ~50% frequency in the precursor lesion of endometrial cancer, complex atypical hyperplasia.
[i]*pten* mutations are also common it the potential precursor lesion of endometrioid ovarian cancers, endometriosis.
[j]*egfr* mutations mostly occur in lung cancers arising in nonsmokers, women, adenocarcinomas, and people of Asian origin.
[k]*kras* mutations occur more commonly in lung adenocarcinomas than squamous cancers.

proteins, does it interact with a protein complex? AKT1 functions both in the membrane and nucleus. What regulates the dissociation of activated E17K-*akt1* from the membrane and translocation to the nucleus? Alternatively, does E17K-*akt1* mediate transformation as a membrane protein? What is the frequency of E17K-*akt1* mutations in other tumor lineages and tumor subtypes? Similarly, is E17K-*akt1* mutation an early or late event during tumor development?

Clinical Implications

Genetic aberrations can provide molecular markers of prognosis and therapy responsiveness as well as therapeutic targets (Hennessy et al., 2005). Cell line and animal model studies suggest that, while AKT2 increases motility, invasion, and metastases, AKT1 is not competent in these processes (Arboleda et al., 2003; Hutchinson et al., 2004). The mechanisms underlying these functional differences are as yet unknown. Thus it will be important to determine whether the E17K-*akt1* mutation contributes to patient prognosis or predicts responsiveness to current therapeutic approaches. Tumors with the E17K-*akt1* mutation may be less aggressive, resulting in improved outcomes. A network approach wherein the combinatorial effects of genomic aberrations in the PI3K network including E17K-*akt1* are considered systematically defining "pathway activity" may provide superior prognostic and predictive power.

The PI3K pathway regulates multiple cellular outcomes, including survival, growth, proliferation, angiogenesis, migration, metabolism, and glucose homeostasis, and therefore must be under tight homeostatic control ensuring that cellular inputs are integrated into appropriate outcomes. Indeed, potent feedback and feedforward pathway networks are of theoretical and therapeutic importance. For example, TORC1 complex inhibition with rapamycin analogs increases AKT phosphorylation and activity through feedback loops (O'Reilly et al., 2006), potentially explaining the disappointing activity of TORC1 complex inhibitors in cancer patients. A systems biology approach to development of robust PI3K network computational models in normal cells integrating the effect of aberrations present in cancer patients will be required for efficient implementation of targeted therapeutics and, in particular, for combinato-

rial therapy. As E17K-AKT1 is constitutively recruited to the membrane, it may not be under the normal homeostatic feedback loop from p70S6K to IRS1, potentially contributing to its transforming activity.

With the PI3K pathway representing a high-quality target, many different companies and academic efforts are developing drugs targeting pathway components (Hennessy et al., 2005). Clinical trials are underway with inhibitors targeting PI3K, AKT, and the TORC1 complex. PI3K pathway aberrations may render tumor cells more sensitive to pathway inhibition due to oncogene "addiction" due to genomic instability or "adaptation" due to homeostatic feedback loops. Thus, despite the importance of the PI3K pathway to normal cellular function, there may be a therapeutic index that allows implementation of PI3K pathway-targeted therapeutics.

The specific mutational aberration in tumors may determine the efficacy of drugs targeting particular PI3K pathway components. Targeting an upstream molecule in the pathway may have limited efficacy in patients with downstream activating mutations. As full activation of E17K-AKT1 appears to be dependent on phosphorylation by PDK1 and PDK2 (Carpten et al., 2007), cells carrying this mutation may be sensitive to both upstream and downstream pathway inhibitors. Alternatively, as E17K-AKT1 is associated with the membrane in the absence of PI3K activity, tumors with E17K-*akt1* mutations may be insensitive to upstream inhibitors.

The E17K-*akt1* mutation questions whether pan-AKT or isoform-specific inhibitors will be optimally effective. Will AKT1 inhibitors be selectively effective in patients with the E17K-*akt1* mutation with decreased toxicity due to preservation of AKT2 activity? In contrast, a pan-AKT inhibitor may be required for efficacy in individuals with *pik3ca* or *pten* mutations, while an *akt2*-selective inhibitor may be required in patients with AKT2 amplification.

Implications for the Cancer Genome Atlas Pilot Project

The Cancer Genome Atlas (TCGA) will perform high-content analysis of DNA copy number, transcriptional profiles for mRNA and regulatory RNAs, RNA splicing, methylation, and candidate gene sequencing. The TCGA pilot will provide an unprecedented quality, quantity, and density of clinically relevant information with the potential to increase the pace of progress in improving patient outcome. This analysis of 500 gliomas, ovarian cancers, and lung cancers is designed to detect events that occur with at least 5% frequency. Thus, mutations with similar frequency to E17K-*akt1* mutations should be detected. However, previous large-scale sequencing efforts assessing all three AKT isoforms in multiple cancer lineages failed to identify the E17K-*akt1* mutation (Greenman et al., 2007; Sjoblom et al., 2006). Other aberrations in AKT family members were identified, including germline single-nucleotide polymorphisms and rare (<1%) *akt2* and *akt3* somatic mutations of unknown significance. This suggests that current high-throughput sequencing technologies have the potential to "miss" functionally relevant mutations and identifies a need for additional technology development and evaluation.

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