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UNDERSTANDING THE PATHWAY

Regulation, Role, and Targeting of Akt in Cancer

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The serine-threonine kinase *AKT*, also known as protein kinase B (PKB), was identified in 1977 as the proto-oncogene of the v-Akt oncogenic murine thymoma virus.[1] Subsequent research has demonstrated that genetic events activating Akt occur in most types of cancer. Activation of Akt promotes many of the processes critical to the malignant phenotype. Thus, Akt is an attractive therapeutic target for cancer. However, its critical role in many physiologic processes suggests that achieving an acceptable therapeutic index with Akt inhibitors may be a challenge. In *Journal of Clinical Oncology*, Yap et al[2] report the results of, to our knowledge, the first-in-man phase I clinical trial of Akt inhibitor MK-2206. In addition to determining the maximum-tolerated dose of MK-2206, the study included a pharmacodynamic analysis of hair follicles in the majority of patients, and of paired tumor biopsies in a maximum-tolerated dose expansion cohort. The results provide strong evidence that significant inhibition of Akt is feasible in patients. However, limited single-agent antitumor activity was observed. This Understanding the Pathway report will highlight the current understanding of the role and regulation of Akt signaling in cancer and the implications for further development of therapeutic strategies against this critical signaling node.

Akt is a member of the AGC family of protein kinases. Akt has three isoforms: Akt1 (also known as PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). Akt1 and Akt2 are expressed in most tissue types; Akt3 expression is generally restricted to neuronal tissue and the testes.[3] The three isoforms share over 80% homology and are characterized by three conserved functional domains: an amino-terminal pleckstrin homology (PH) domain that regulates intracellular trafficking of the protein, a central catalytic domain, and a carboxy-terminal regulatory domain. Activation of all three Akt isoforms is dependent on phosphatidylinositol 3-kinase (PI3K).[4] PI3K is stimulated by a variety of signals, including growth factor and G protein–coupled receptors on the cell surface. Activation of PI3K results in the generation of 3′-phosphorylated phosphatidylinositols in the cell membrane, which recruit Akt and other PH domain–containing proteins to the cell membrane. At the cell membrane, Akt comes into proximity with PDK1, another PH domain–containing serine-threonine kinase, which phosphorylates Akt at the Thr308 residue of its catalytic domain. The activated conformation of Akt is further stabilized by phosphorylation at the Ser473 residue, either by the mammalian target of rapamycin complex 2 in response to growth factor stimulation or by

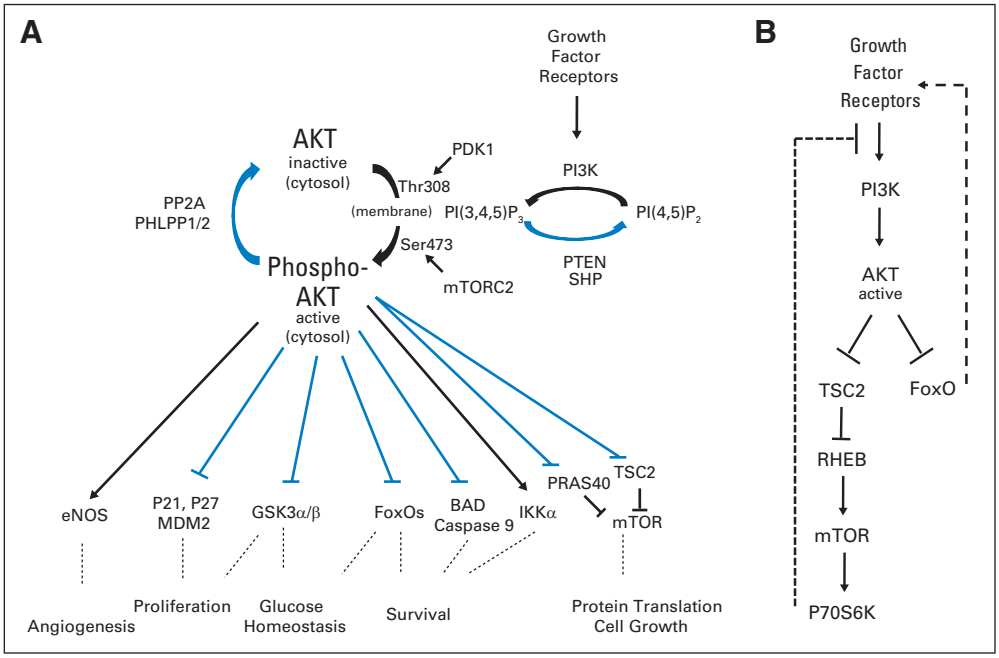

Fig 1. (A) Akt signaling pathway. Activation of Akt by growth factor receptors instigated by stimulation of phosphatidylinositol 3-kinase (PI3K) after ligand binding. Activated PI3K generates phosphatidylinositol (3,4,5)P_3 (PIP_3), which recruits inactive Akt to cell membrane. Akt is phosphorylated at Thr308 (by pyruvate dehydrogenase [lipoamide] kinase isozyme 1 [PDK1]) and Ser473 (by mammalian target of rapamycin complex 2 [mTORC2]), which activates catalytic activity. Akt phosphorylates multiple substrates, including endothelial nitric oxide synthase (eNOS), p21, p27, glycogen synthase kinase 3 alpha (GSK3α)/(GSK3β), forkhead box O (FoxO) transcription factors, Bcl-2–associated death promoter (BAD), caspase 9, inhibitor of nuclear factor kappa-B kinase alpha (IKKα), proline-rich Akt substrate 40 (PRAS40), and tuberous sclerosis protein 2 (TSC2). Effects of these and other substrate phosphorylation events affect indicated cellular processes, often promoting malignant phenotype of cancer cells. (B) Known feedback signaling loops within PI3K-Akt pathway, which may affect consequences of Akt inhibition. PTEN, phosphatase and tensin homolog.

DNA-dependent protein kinase after DNA damage.[5,6] Akt activity is negatively regulated primarily by phosphatases that dephosphorylate phosphatidylinositols at the cell membrane (phosphatase and tensin homolog [PTEN], SHP2) or phosphorylation sites on Akt itself (PP2A, PHLPP1, PHLPP2).

More than 50 substrates of Akt have been identified, some of which are illustrated in Figure 1.[4,7-9] Through these and other effectors, Akt regulates a variety of cellular processes, including proliferation, survival, motility, angiogenesis, and metabolism/glucose homeostasis. There is strong evidence that at least some of these activities are specific to different Akt isoforms. For example, targeted deletion of the *AKT2* gene in mice has resulted in impaired glucose uptake and hyperglycemia, which were not observed with knockout of either *AKT1* or *AKT3*.[10] Knockout of *AKT3* has produced specific developmental defects in brain development.[11] Interestingly, although most cancer cell types demonstrate dependence on Akt1 and Akt2, there is evidence that melanomas, which developmentally arise from the neural crest, depend on Akt3.[12,13] Studies in breast cancer cells have also identified contradictory effects of Akt1 and Akt2 on growth factor–induced motility and epithelial-mesenchymal transition.[14] Taken together, the findings suggest that the relative activity of inhibitors against different Akt isoforms may affect both their efficacy and toxicity. The development of isoform-specific inhibitors may allow for further interrogation of the therapeutic potential of the different Akts.

The successful clinical development of several targeted therapies (ie, trastuzumab for human epidermal growth factor receptor 2 (*HER2*)/*neu*–amplified breast cancer[15-17] and vemurafenib for *BRAF*-mutant melanoma[18]) has depended critically on the identification of somatic genetic alterations that result in dependence on those targets.[19] There are a variety of genetic events occurring in cancer that activate Akt, including activating point mutations and/or amplifications of *AKT, PIK3CA,* Ras family members, and growth factor receptors.[20] In addition, loss of expression or catalytic function of PTEN results in constitutive activation of AKT.[21-24] Although all of these events may activate Akt, the frequent finding of more than one of these genetic changes in individual tumors suggests that they have nonoverlapping functions.[20] Measurement of phosphorylated (activated) Akt levels in both tumors and cancer cell lines has confirmed quantitative differences in Akt activation with different mutations and sensitivity to Akt inhibition correlated with phospho-Akt levels.[25-28] These findings suggest that tumors with elevated levels of phospho-Akt, particularly resulting from loss of PTEN, may be most likely to respond to Akt inhibitors.[28] In the present study of MK-2206, pretreatment status of the PI3K-Akt pathway was only determined in nine patients and revealed that only two patients harbored alterations predicted to activate the Akt pathway (one patient, PTEN loss; one patient, PTEN loss and *KRAS* mutation).[2] One of those two patients seemed to have one of the best clinical responses in the study, with 23% reduction in target lesion size and approximately 60% decrease in a serum tumor marker. Although this correlation of activity with genetic aberration in the pathway is intriguing, clearly evaluation in additional patients with such alterations, and with activating *PIK3CA* or *AKT* mutations, is necessary and warranted to test this hypothesis. However, as alterations in the PI3K-Akt pathway are often detected in tumors with concurrent genetic aberrations in other pathways (ie, activating *BRAF* mutation in melanomas with PTEN loss[29,30]), it is unclear if even these markers will correlate with single-agent efficacy with Akt inhibitors.

In addition to the presence of concurrent mutations, compensatory signaling after Akt inhibition may necessitate combinatorial approaches to achieve significant antitumor activity. Investigators have recently demonstrated that Akt inhibition results in increased expression and activation of multiple growth factor receptors, particularly HER3, in a variety of cancer cell lines.[31] This induction reduced the antiproliferative effects of Akt inhibitors in vitro and in vivo, whereas combined treatment with an Akt inhibitor and HER-family inhibitor lapatinib produced marked synergy. This finding is similar to those in previous reports of compensatory activation of PI3K-Akt signaling after inhibition of the mitogen-activated protein kinase pathway and of Akt after mammalian target of rapamycin complex 1 inhibition.[32-37] In the current phase I study of MK-2206, the reported analysis of paired tumor biopsies was restricted to measurement of phospho-Akt levels.[2] The preclinical findings with Akt inhibitors, and the clinical experience with other targeted agents, support the critical need in future studies for evaluation beyond on-target/pharmacodynamic markers to understand resistance to such treatments and expedite the development of effective combinatorial approaches.

In summary, the results presented by Yap et al[2] give reason for hope that Akt inhibitors may be administered safely at doses that inhibit Akt activity. Clear hypotheses regarding patient selection for single-agent treatment and combinatorial approaches are now ready for testing, but they will depend critically on vigorous concurrent translational research using both pre- and on-treatment tumor specimens. Although such studies will require the investment of significant time and resources in the associated clinical trials, the potential benefit is the rapid, rational, and—it is hoped—more effective development of therapeutic strategies incorporating Akt inhibitors.

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