Akt Signaling in Oncology

Authors H. Unger, K.L. van Golen

Key words AKT/PKB, cancer motility, metastasis, inflammatory breast cancer, prostate cancer

Summary

Akt, also known as protein kinase B (PKB), is a major signal transducer of the phosphoinositide 3-kinase (PI3K) pathway and since its discovery has emerged as a central player in cell growth, proliferation, metabolism, and survival. The mechanisms of activation of the Akt kinases have been more clearly defined, though the specific functional roles of the Akt isoforms, Akt1, Akt2, and Akt3, continue to be under investigation, particularly their potential roles in cancer invasion and metastasis. The frequent aberrant activation of Akt/PKB in human cancer has made it an attractive target for pharmacological and therapeutic treatment, making the potential specific roles of each of the three Akt isoforms highly relevant.

(BJMO 2009;Vol 3;4:139-144)

Introduction

The serine/threonine kinase Akt, or protein kinase B (PKB), has recently been a focus of intense research as a reflection of the recognized central role of the Akt kinases in both normal physiology and disease development. Initial work on Akt was conducted in 1977, when a transforming murine leukemia virus from a T-cell lymphoma localized in the thymus of the spontaneously lymphomatous AKR/J mouse was isolated and called AKT-8.[1] AKT-8 was able to produce foci of malignant transformation in mink lung epithelial cells and thymic tumors, while other cell lines were negative for transformation, suggesting the virus contained a previously undescribed oncogene.[2] The retrovirus isolated from mink lung epithelial cells contained oncogenic sequences of cellular origin and the oncogene was named *v-akt*.[2] DNA fragments containing two human homologues of the *akt* gene, *AKT1* and *AKT2*, were identified and permitted the discovery of an amplification of an *AKT1* allele in primary gastric adenocarcinoma.[2,3] This was the first evidence for a role of Akt in tumorigenesis and the pathogenesis of some human malignancies. Shortly thereafter, the *AKT1* gene was mapped to chromosome 14, confirming its human origin.

In 1991 independent lines of research converged on the identification of genes relating to Akt/PKB. Two groups used homology-based approaches to identify genes that encoded a serine/threonine protein kinase similar to protein kinase A (PKA) and protein kinase C (PKC), which was called RAC-PK (related to PKA and PKC) and subsequently named *PKBα/Akt1*.[4] These studies suggested an important role for Akt/PKB in signal transduction pathways. Later that year v-*Akt* was cloned as the oncogene transduced by AKT-8, which highlighted the important role for *AKT* in transformation and cancer.[5] A second isoform of the protein, PKBβ/Akt2, was identified in human cell lines. Currently, three mammalian isoforms of PKB/Akt have been identified, PKBα/Akt1, PKBβ/Akt2, and PKBγ/Akt3, all of which are expressed differentially on mRNA and protein level and play crucial roles in cellular homeostasis.

The Akt kinases belong to the general class of AGC kinases and are composed of three domains: an N-terminal pleckstrin homology (PH) domain, a kinase domain, and a short regulatory hydrophobic C-terminal tail. The isoforms share the same structural organization, though they differ slightly in the localization of their regulatory phosphorylation sites, and sequence homologies of the kinase domains exceed 87%.[6] Activation of the Akt kinases is a complex cascade involving engagement of receptor tyrosine kinases, membrane binding, translocation, and phosphorylation (*Figure 1*). Akt is a key downstream effector of phosphoinositide 3-kinase (PI3K). Upon stimulation with a growth factor such as insulin-like growth factor-1 (IGF-1), PI3K is activated and synthesizes the phosphoinositide second messenger PtdIns-3,4,5-P_3 (PIP3) from membrane bound PtdIns-4,5-P_2 (PIP2).

PIP3 binds to the PH domain of Akt and targets it to the plasma membrane. The binding of PIP3 to the PH domain anchors Akt to the membrane and allows its phosphorylation and activation. Full activation of Akt/PKB involves a conformational change and phosphorylation of two highly conserved residues.[7] The major activating phosphorylation event involves a threonine residue, Thr308, within the kinase domain activation loop which is phosphorylated by phosphoinositide-dependent kinase-1 (PDK1). The phosphorylation of Ser473 in the hydrophobic motif is required for the full activity of Akt and is presumably phosphorylated by the mTORC2 (mammalian target of rapamycin complex 2) complex.[8] Other implicated mechanisms of Ser473 phosphorylation include PDK1, integrin-linked kinase, and autophosphorylation.[9] Once fully activated, Akt loses PIP3 binding and translocates within the cell to the cytoplasm, nucleus, mitochondria, and other organelles. Akt/PKB is then able to transduce signals through phosphorylation of a number of substrates including, glycogen synthase kinase 3β, BAD, MDM2, human caspase 9, FOXO, NF-κB transcription factors, mTOR, Raf, p21, BRCA-1, and Rac1, many of these being implicated in cancer-associated phenotypes.

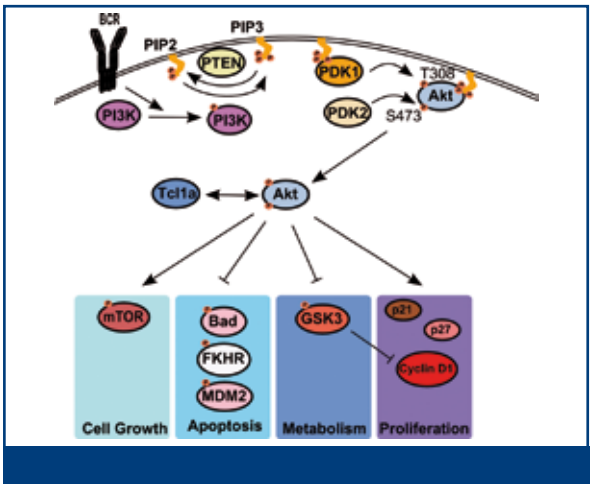

Figure 1. Overview of the Akt/PKB signaling pathway. Akt, activated by phosphorylation on its threonine 308 and serine 473 residues, phosphorylates and mediates many known downstream effectors and signals implicated in cancer phenotypes.

Akt Isoforms

The term Akt encompasses the three highly conserved cellular homologues, Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ, found in mammals. Because Akt isoforms are ubiquitously expressed in all tissues, including the cellular compartments of the stroma, investigation into the roles the Akt isoforms play in cancer phenotypes has provided insights into the pathophysiological mechanisms of tumor development and metastasis. Although each Akt kinase is activated through similar mechanisms and responds similarly to stimuli, their different tissue-specific expression patterns suggest distinct roles. Akt1 is the most abundant and ubiquitously expressed isoform. Akt2 is also ubiquitously expressed, though Akt2 transcripts are especially abundant in insulin-responsive tissues such as skeletal muscle and brown fat.[10] Akt3 has a more restricted tissue expression and can be found in neuronal tissues.[11]

Mouse knockouts were the first experiments uncovering isoform-specific functions of Akt1, Akt2, and Akt3. Akt1-null mice displayed developmental defects and growth retardation, Akt2-null mice had defects in glucose homeostasis and developed insulin-resistant diabetes, Akt3-null mice on the other hand displayed defects in brain development and had a decreased brain size.[11] Although studies have shown that functional redundancy between the isoforms does exist, data suggest that there are functional differences at the cellular level.

Akt/PKB is involved in a number of cellular processes including survival, proliferation, growth, metabolism, and tumorigenesis, and it is crucial to delineate the roles each of the isoforms play in these processes. Akt1 has traditionally been shown to be involved in cell survival by negatively regulating and blocking the function of pro-apoptotic proteins and processes. In addition, Akt1 plays a role in promoting cell growth, primarily through the activation of the mTOR complex 1 (mTORC1) and stimulating cell proliferation by interrupting cell-cycle regulation.[11] Akt1 also plays pivotal roles in both physiological and pathological angiogenesis, being required for endothelial cell migration.[12]

Akt1 was first implicated in human cancer shortly after its discovery in 1977.[2] Increased kinase activity of Akt1 is associated with poor prognosis in prostate and breast carcinomas. Given the high frequency of PTEN (phosphatase and tensin homolog deleted on chromosome 10) mutations in cancer, it is thought that in a majority of tumors, Akt activation results from loss of PTEN.[13] PTEN is a tumor suppressor that negatively regulates Akt activation by directly inactivating the PI3K/Akt pathway, acting as a lipid phosphatase of phosphorylated phosphoinositides. PTEN is frequently inactivated in a number of human tumors, resulting in hyperactivation of Akt signaling, phosphorylation of downstream substrates, and resulting in cellular transformation and tumorigenesis.

Independent studies demonstrate that active Akt1 de-

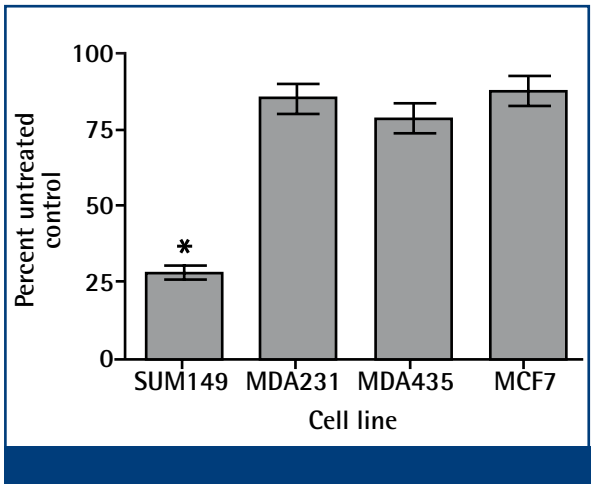

Figure 2. Treatment of SUM149 inflammatory breast cancer (IBC) cells and MDA231, MDA435, and MCF7 non-IBC cells with siRNA specifically directed against Akt1 causes significantly reduced invasion of SUM149 IBC cells in a Matrigel invasion assay.

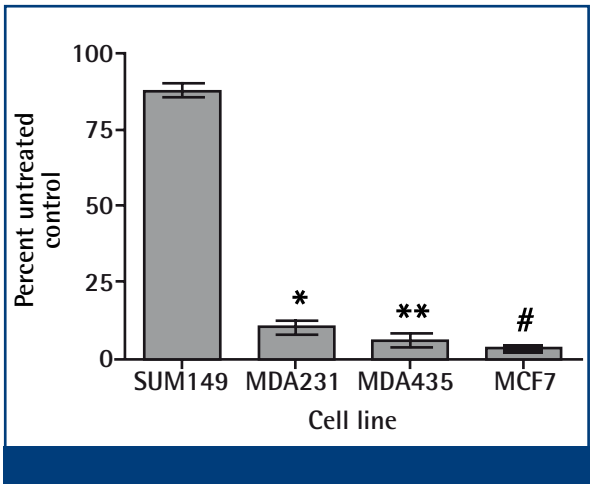

Figure 3. Treatment of SUM149 IBC cells and MDA231, MDA435, and MCF7 non-IBC cells with siRNA specifically directed against Akt2 causes significantly decreased invasion of non-IBC cells in a Matrigel invasion assay.

creases mammary epithelial cell migration. The inhibitory effect of Akt1 on migration is mediated through proteasomal degradation of the NFAT (Nuclear Factor of Activated T cells) transcription factor.[14] Using siRNA, knockdown of Akt1, but not Akt2, led to an increase in the migratory capabilities of mammary epithelial cells. Stimulation of the cells with IGF-IR and down-regulation of Akt1 caused increased activation of the ERK/MAPK pathway, which contributed to enhanced migration and demonstrated the importance of Akt1 in potentially cross-regulating the ERK signaling pathway.[15] Additionally, overexpression of constitutively active Akt1 phosphorylates and targets the tumor suppressor Tuberous Sclerosis Complex 2 (TSC2) for degradation, leading to reduced Rho GTPase activity and decreased motility and invasion of breast cancer cells.[16]

Akt2 was first reported to be involved in tumorigenesis when found to be amplified and overexpressed in ovarian cell lines and tumors, causing an aggressive phenotype.[17] Akt2 activity is elevated in approximately 40% of primary ovarian cancers and overexpression of Akt2 is seen in 10% of pancreatic and 5% of breast cancers.[7] As Akt1 has been shown to have an inhibitory effect on breast cancer invasion, Akt2 plays a pro-invasive role in the disease. Increased expression of Akt2 by the transcription factor Twist enhances breast cancer migration.[11] Akt2 up-regulates β1 integrins, interacts with PKC and promotes adhesion and migration of breast cancer cells.[15]

In direct contrast, our group has found opposite roles for Akt1 and Akt2 in inflammatory breast cancer (IBC) invasion. Using siRNA to specifically knockdown Akt1 levels, it was found that *in vitro* invasive capabilities of IBC cells were significantly inhibited, while the invasiveness of the non-inflammatory breast cancer cells was not affected (*Figure 2*). In contrast, siRNA knockdown of Akt2 did not effect the IBC cells but significantly decreased the invasiveness of the non-inflammatory breast cancer cells (*Figure 3*), implicating Akt1 in the highly invasive phenotype of IBC.

Akt3 remains understudied. It is expressed abundantly in neuronal tissues and is involved in the protection of motoneuronal death.[18] Akt3 mRNA is upregulated in estrogen receptor (ER)-negative breast tumors and has increased activity in ER-negative breast cancers and androgen-insensitive prostate cancer cell lines (PCa).[19] Though Akt3 mRNA expression is not limited to ER-negative breast cancer cells, data suggest that Akt3 contributes to the aggressiveness of steroid hormone-insensitive cancers.

Clinical Applications

The importance of the Akt/PKB pathway in human cancers is firmly established and presents an exciting target for molecular therapeutics, serving as a convergence point for growth stimuli and a central role in transducing oncogenic signals. Thus, putting the issues of using Akt as a diagnostic marker and developing targeted drugs for the treatment of cancers with Akt involvement at the forefront of translational cancer research.

There is a definite need to identify biomarkers that are able to distinguish clinically aggressive forms of a tu-

mor. In prostate cancer for example, a disease where four times as many men are treated for the disease than would have died from it if left untreated, high levels of active phosphorylated Akt (pAkt) are associated with a high Gleason-grade. High pAkt levels are an excellent indicator of PSA failure and predictor of poor clinical outcome.[20] Recent identification of Akt1 mutations in *in situ* breast carcinoma demonstrates that it is a potential early indicator for chemoprevention strategies.[21]
Therapeutic targeting of the Akt/PKB pathway includes Akt, its upstream regulators and downstream effectors. Various Akt inhibitors are better classified *in vitro*, including commercially available Wortmannin and LY294002. Thus far, translation of these inhibitors to everyday cancer therapy has failed. However, in PC-3 PCa cells adhered to collagen-I, inhibition of Akt with LY294002 induced caspase activation, suggesting that decreased Akt activation increases sensitivity of PCa cells to Docetaxel. Although combinations of inhibitors targeting PI3K, Akt, and mTOR, with various chemotherapies and radiation have been explored in preclinical studies, only a few clinical trials with Akt inhibitors are reported to date. The most developed Akt inhibitor is Perifosine; a lipid-based inhibitor. *In vitro* Perifosine is able to inhibit translocation of Akt to the cell membrane and sensitize cancer cells to apoptosis induced by radiation.[22] phase I and II clinical trials investigating Perifosine concluded that its use as monotherapy is associated with limited efficacy and high toxicity. However, phase I trials of the combinational use of Perifosine with chemotherapeutic agents such as taxanes and gemcitabine show that these drugs can be safely administered. Currently, other phase II clinical trials are using Perifosine in combination with conventional therapies targeting multiple myeloma.[24] Another Akt inhibitor, the nucleoside analogue Triciribine (API-2), is currently being investigated in phase I clinical trials targeting metastatic solid tumors with high pAkt levels.[24] This agent showed to have minimal efficacy with serious toxicities, presumably due to Akt2 inhibition.
Currently, there is a lack of Akt-specific inhibitors as well as isoform-selective Akt inhibitors. However, a few novel selective inhibitors have been developed: Akti-1, Akti-2, and Akti-1/2. These inhibitors are able to inhibit kinase activity, phosphorylation and activation of the corresponding isoforms reversibly. In LnCaP PCa cells, both Akt1 and Akt2 must be inhibited for maximal caspase-3 induction and apoptosis. Apoptosis induced by any three of these inhibitors could not be reversed by overexpression of active Akt3, suggesting that Akt3 cannot compensate for Akt1 and Akt2.[25]
Though these compounds show great promise *in vitro*, poor solubility and pharmacokinetics have inhibited *in vivo* studies. Further research on these selective Akt compounds is required as the potential redundant, distinct, or opposite functions of the three Akt/PKB isoforms are explored.

Novel Approaches and concepts

As questions concerning upstream regulators, downstream substrates, and potential isoforms-specific pathways in cancer remain unanswered, Akt/PKB continues to be a focus of intense research. One unique concept regarding Akt involves its role as both a kinase and a substrate of GTPases. A putative Akt/PKB phosphorylation site (ydRIRpISYp) can be found in nearly all Rho proteins. Rho proteins are important in nearly every cellular process, particularly reorganization of the actin cytoskeleton. One of the first suggestions that Rho proteins can be Akt/PKB substrates came from experiments showing that recombinant Rac1 GTPase could be phosphorylated on serine 71 by active Akt/PKB.[26] *In vitro* phosphorylation of Rac1 attenuated its activation without affecting its GTPase activity suggesting that Akt acts upstream of Rac1 and modulates its activity. Phosphorylation of Rac1 by Akt results in decreased Rac1 activity and decreased osteoclast motility, which can be reversed by PI3K inhibitors.[27] Likewise, downregulation of RhoC GTPase activity in PC-3 PCa cells leads to a marked increase of Rac and Cdc42 GTPase activity, corresponding with decreased serine 71 phosphorylation of these two GTPases.[28] The mechanism(s) by which serine 71 phosphorylation of the Rho proteins prevent activation is currently unknown but may be due to changes within the activating switch region of the GTPases.
In contrast, several studies have suggested that Akt/PKB may act as a downstream substrate of the Rac and Rho GTPases. Activation of Cdc42 and/or Rac1 disrupts the normal polarization of mammary epithelial cells in the collagen matrix, leading to increased invasiveness through the activation of PI3K by Cdc42/Rac1.[29] In addition, the regulatory subunit of PI3K (p85) binds to GTP-bound Rac/Cdc42, causing activation of PI3K/Akt.[30]
Although the relationship between Akt and the Rho proteins still remains unclear, it was found that Akt/PKB is active in SUM149 IBC cells due to loss of PTEN. Increased Akt activity results in high levels of serine-phosphorylated RhoC GTPase. Inhibition of Akt/PKB activity, either by Akt1 pharmacologic inhibitors, introduction of siRNA to Akt1, or re-in-

Key messages for clinical practice

1. Although often spoken of as a single entity, Akt/PKB actually consists of three isoforms, each of which with distinct cellular functions. Targeting of these individual isoforms will need to be specific.

2. The different isoforms of Akt/PKB have different roles in different cancer types. The effect of targeting Akt/PKB isoforms will need to be cancer-type specific.

3. Current pharmacological inhibitors of Akt/PKB tend to have problems such as lack of specificity, low efficacy, high toxicities and poor pharmacokinetics. However, some evidence suggests that these compounds hold promise as adjuvant therapies as opposed to monotherapies. More combinatorial studies will need to be performed to determine optimal combinations of Akt/PKB inhibitors and conventional chemotherapeutics.

4. Interactions with other Akt/PKB substrates, such as the Rho GTPases, may provide attractive surrogate targets for the PI3K/AKT pathway, with less deleterious effects.

troduction of PTEN, leads to a significant decrease in RhoC-mediated invasion. Likewise, introduction of a RhoCS73A mutant into the IBC cells significantly decreases their invasive capabilities. The mechanism through which Akt/PKB phosphorylation of RhoC affects the cells invasive capabilities is currently under investigation. RhoC activity does not appear to be affected by serine phosphorylation, however, serine 73 lies within the switch II region of the guanine nucleotide binding pocket. Each switch region is critical for effector binding and X-ray crystallography suggests that GTP binding causes RhoC to undergo two conformational changes that may be affected by phosphorylation, thus altering its effector binding capabilities.

Conclusion

As research continues, the Akt kinases have been assigned an increasing number of cellular functions in both physiological and disease processes. Akt/PKB has been shown to be at a crossroads of oncogenic and tumor suppressor signaling networks. Studies indicate that individual functions may be revealed for Akt1, Akt2, and Akt3. As the molecular mechanisms of Akt isoform-specific roles continue to be explored, the knowledge of these precise functions is crucial for the design of highly specific and effective Akt-targeted therapeutics for cancer treatment.

Glossary

- Akt/Protein Kinase B (Akt/PKB): Serine/threonine kinases activated by the PI3K pathway and involved in several cellular processes such as survival, growth and motility.
- Inflammatory breast cancer (IBC): A highly aggressive and rare form of locally advanced breast cancer. This form of breast cancer manifests rapidly within 6 months and is by definition, classified as T4d due to invasion of the dermal lymphatics overlying the breast. Blockage of the dermal lymphatics by tumor emboli results in primary skin changes resembling inflammation.
- Ras Homology GTPase (Rho GTPase): Monomeric GTP-binding proteins which act as binary switches, transiently moving between an inactive-GDP-bound state to an active-GTP-bound state back to an inactive state. The activation/inactivation of the proteins comprises the tightly regulated GTPase cycle. The main role of these proteins is to reorganize the actin cytoskeleton leading to cellular motility and invasion.

References

1. Staal SP, Hartley JW, Rowe WP. Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. Proc Natl Acad Sci USA 1977;74(7):3065-67.

2. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: Amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci USA 1987;84:5034-37.

3. Staal SP, Hartley JW. Thymic lymphoma induction by the AKT8 murine retrovirus. J Exp Med 1988;167(3):1259-61.

4. Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA.

Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. Proc Natl Acad Sci USA 1991;88:4171-75.

5. Bellacosa A, Testa JR, Staal SP, Tsichlis PN. A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region.Science 1991;254(5029):274-7.

6. Bellacosa A, Kumar CC, Cristofano AD, Testa JR. Activation of Akt kinases in cancer. Adv in Cancer Res. Elsevier Academic Press pp.30-71;vol.94.

7. Paez JG, Sellers WR. PI3K/PTEN/AKT Pathway: A critical mediator of oncogenic signaling. Cancer Treat Res 2003;115:145-67.

8. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rector-mTOR complex. Science 2005;307(5712):1098-1101.

9. Persad S. Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase: critical roles for kinase activity and amino acids arginine 211 and serine 343. J Biol Chem 2001;276(29):27462-67.

10. Testa JR, Bellacosa A. Akt plays a central role in tumorigenesis. Proc Natl Acad Sci USA 2001;98(20):10983-85.

11. Manning BD, Cantley LC. Akt/PKB signaling: navigating downstream. Cell 2007;129(7):1261-74.

12. Ackah E. Akt1/protein kinase Balpha is critical for ischemic and VEGF-mediated angiogenesis. J Clin Invest 2005;115(8):2119-22.

13. Sun M. Akt1/PKBalpha kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. Am J Pathol 2001;159(2):431-34.

14. Yoeli-Lerner M, Yiu GK, Rabinovitz I, Erhardt P, Jauliac S, Toker A. Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT. Mol Cell 2005;20(4):539-45.

15. Irie HY. Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial-mesenchymal transition. J Cell Biol 2005;171(6):1023-33.

16. Liu H. Mechanism of Akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for TSC2. Proc Natl Acad Sci USA 2006;103(11):4134-38.

17. Cheng JQ. Akt2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci USA 1992;89(19):9267-70.

18. Nawa M, Kanekura K, Hashimoto Y, Aiso S, Matsuoka M. A novel Akt/PKB-interacting protein promotes cell adhesion and inhibits familial amyotrophic lateral sclerosis-linked mutant SOD-1 induced neuronal death via inhibition of PP2A-mediated dephosphorylation of Akt/PKB. Cell Signal 2008;20(3):493-500.

19. Nakatani K. Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. J Biol Chem 1999;274(31):21528-32.

20. Kreisberg JI. Phosphorylation of Akt is an excellent predictor of poor clinical outcome in prostate cancer. Cancer Res 2004;64:5232-36.

21. Dunlap J. Phosphotidylinositol-3-kinase and Akt1 mutations occur early in breast carcinoma. Breast Cancer Res Treat 2009(in press).

22. Vink SR. Phase I and pharmacokinetic study of combined treatment with perifosine and radiation in patients with advanced solid tumours. Radiother Oncol 2006;80(2):207-11.

23. Marsh W. A phase II trial of perifosine in locally advanced, unresectable, or metastatic pancreatic adenocarcinoma. Am J Clin Oncol 2007;30(1):26-31.

24. LoPiccolo J, Blumenthal GM, Bernstein WB, Dennis PA. Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. Drug Resist Updat 2008;11(1-2):32-47.

25. Lindsley CW. Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors. Bioorganic and Medicinal Chem Letters 2005;15:761-764.

26. Kwon T, Kwon DY, Chun J, Kim JH, Kang SS. Akt protein kinase inhibits Rac1-GTP binding through phosphorylation at serine 71 of rac1. J Biol Chem 2000;275(1):423-38.

27. Fukuda A. Regulation of osteoclast apoptosis and motility by small GTPase binding protein rac1. J Bone Miner Res 2005;20(12):2245-53.

28. Yao H, Dashner EJ, van Golen CM, van Golen KL. RhoC GTPase is required for PC-3 prostate cancer cell invasion but not motility. Oncogene 2006;25(16):2285-96.

29. Keely PJ, Westwick JK, Whitehead IP, Der CJ, Parise LV. Cdc42 and rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. Nature 1997; 390(6660):632-34.

30. Zheng Y, Bagrodia S, Cerione RA. Activation of phosphoinositide-3 kinase activity by Cdc42Hs binding to p85. J Biol Chem 1994;269(29):18727-29.

Correspondence address

Authors: H. Unger, K.L. van Golen
The University of Delaware, Newark, Delaware

Please send all correspondence to:
Kenneth L. van Golen, PhD
320 Wolf Hall
The University of Delaware
Newark, Delaware, 19716 USA
Tel: (302)-831-2669
Fax: (302)-831-2281
klvg@udel.edu

Conflict of interest: the authors have nothing to disclose and indicate no potential conflicts of interest.