Histol Histopathol (2011) 26: 651-662

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Akt1 and Akt2: Differentiating the aktion

Lisa Heron-Milhavet, Nabil Khouya, Anne Fernandez and Ned J. Lamb

Cell Cycle and Myogenesis, Institut de Génétique Humaine, CNRS UPR1142, Montpellier, France

Summary. Kinases of the Akt family are integral and essential components in growth factor signaling pathways activated downstream of the membrane bound phospho-inositol-3 kinase. In light of strong homologies in the primary amino acid sequence, the three Akt kinases were long surmised to play redundant and overlapping roles in insulin signaling across the spectra of cell and tissue types. Over the last 10 years, work using mouse knockout models, cell specific inactivation, and more recently targeted gene inactivation, has brought into question the redundancy within Akt kinase isoforms and instead pointed to isoform specific functions in different cellular events and diseases. Here we concentrate on the differential roles played by Akt1 and Akt2 in a variety of cellular processes and in particular during cancer biogenesis. In this overview, we illustrate that while Akt1 and 2 are often implicated in many aspects of cellular transformation, the two isoforms frequently act in a complementary opposing manner. Furthermore, Akt1 and Akt2 kinases interact differentially with modulating proteins and are necessary in relaying roles during the evolution of cancers from deregulated growth into malignant metastatic killers. These different actions of the two isoforms point to the importance of treatments targeting isoform specific events in the development of effective approaches involving Akt kinases in human disease.

Key words: Akt isoforms, Proliferation, Cancer, Differentiation, Migration, Metastasis

Introduction

Reversible protein phosphorylation plays a widespread and essential role in the regulation of most cellular functions. Among the kinases responsible for the phosphorylation of a variety of cellular substrates, Akt kinases have been the subject of intensive research over the past 20 years. Akt is a serine threonine protein kinase activated by different stimuli via phosphatidylinositol 3 kinase (PI3K)-dependent mechanisms. The *Akt* gene is the cellular homolog of the *v-akt* oncogene transduced by AKT8, an acute transforming retrovirus in mice that was originally described in 1977 and isolated from an AKR thymoma cell line (Staal et al., 1977). For full activation, Akt is phosphorylated at two sites, one within the T-loop of the catalytic domain (Thr308 on Akt1) by the phosphoinositide-dependent kinase 1 (PDK1) (Alessi et al., 1997) and the second in the carboxyl terminal hydrophobic domain (Ser473 on Akt1) by the Rictor kinase part of the mammalian target of rapamycin complex 2 (mTORC2) (Sarbassov et al., 2005). These activating phosphorylations of Akt need membrane targeting followed by translocation of the activated kinase to the nucleus (Andjelkoviç et al., 1997). Once Akt has been phosphorylated and activated, it phosphorylates many other proteins – for example glycogen synthase kinase 3 (GSK3) and forkhead box family of transcription factors (FOXOs) – thereby regulating a wide range of cellular processes involved in protein synthesis, cell growth and survival. proliferation, metabolism and migration (reviewed by Manning and Cantley, 2007; Restuccia and Hemmings, 2010).

The Akt family is expressed as three isoforms; Akt1, Akt2 and Akt3 (Vivanco and Sawyers, 2002; Toker and Yoeli-Lerner, 2006). The primary amino acid sequences of mouse, rat and human Akt1 and Akt2 have been aligned in Figure 1A using ClustalW showing 81% homology between Akt1 and Akt2 from these different species. This alignment also reveals 98% inter mammalian species homology for Akt1 or Akt2. In Fig. 1B, C, the sequence alignment between the last 130 amino acids from human Akt1 and Akt2 is shown to illustrate some major differences between these isoforms in the putative secondary structure prediction. Indeed, in Fig. 1C, the alpha helix and beta sheet structures are partially inverted between the two isoforms from amino

Offprint requests to: Dr. Lisa Heron-Milhavet, Cell Cycle and Myogenesis, IGH, CNRS UPR1142, 141 rue de la cardonille, 34396 Montpellier, France. e-mail: lisa.heron-milhavet@igh.cnrs.fr

acids 410 to 460. Even though the Akt isoforms share a similar primary structure with a conserved amino-terminal pleckstrin homology domain, a central serine-threonine catalytic kinase domain and a small carboxyl-terminal regulatory domain, they differ in their biochemical properties. Here, using high resolution 2-dimensional gel electrophoresis, we show Akt2 having a slightly higher molecular weight and resolving as a more basic form than Akt1 in mouse myoblast extracts (Fig. 2A-C). Whereas Akt1 is ubiquitously expressed, Akt2 is highly expressed in insulin-sensitive tissues, including liver, skeletal muscle, pancreas and adipose tissue, and its protein expression is drastically increased during differentiation of both adipose tissue (Hill et al., 1999) and skeletal muscle (Vandromme et al., 2001). In contrast, Akt3 displays more restricted tissue distribution with high levels in brain and testis and little or no expression in visceral organs and muscle tissue. The PH domain anchors all 3 kinase isoforms at the plasma membrane. Indeed, overexpressed myristylated forms of Akt1 and Akt2 are constitutively membrane bound, as shown in Figure 3. Gene ablation experiments in mouse

Fig. 1. Primary amino acid sequence alignment of Akt1 and Akt2 isoforms in mouse, rat and human and secondary structure prediction of human Akt1 and human Akt2. **A.** Protein sequence alignment of both Akt1 and Akt2 isoforms in mouse, rat and human. All sequence alignments have been performed using the ClustalW2 shared software from EBI, UK (http://www.ebi.ac.uk/Tools/clustalw2/index.html). **B.** Sequence alignment for the last 130 amino acids of human Akt1 and Akt2. In both sequence alignments (**A and B**), different residues are shown in red and similar residues are shown in blue. **C.** Secondary structure prediction for the last 130 amino acids of human Akt1 and Akt2 using GOR4 shared software from PBIL, France (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html).

models show that each Akt isoform plays a different role in metabolism and growth. For example, Akt1 knock-out mice are smaller than their wild-type counterparts and Akt1-null cells display higher rates of apoptosis, indicating a critical role for Akt1 in cell growth and survival (Chen et al., 2001; Cho et al., 2001a). In contrast, Akt2 knock-out mice develop type 2 diabetes-like phenotypes with impaired glucose metabolism, suggesting a role for Akt2 in the maintenance of glucose homeostasis (Garofalo et al., 2003 ; Cho et al., 2001b). Concerning Akt3, it has been implicated in brain development because Akt3 knock-out mice display impaired brain growth (Tschopp et al., 2005). Even though the role of each isoform has been depicted with single isoform deletion, problems of functional redundancy and overlap and/or compensation of gene expression have been revealed in double knock-out mice. For instance double deletions of both Akt1 and Akt2 caused lethality shortly after birth (Peng et al., 2003) while the double deletion of Akt1 and Akt3 was lethal in early embryonic stages (Yang et al., 2005). In this review, we will focus on the distinct roles and substrate specificity of Akt1 and Akt2 isoforms in cell proliferation and differentiation of normal or transformed cell lines as well as during cell migration, invasiveness and thus tumorigenesis.

Akt isoform specificity in cell proliferation and the development of cancer

Over the past two decades, a multitude of studies have revealed the unequivocal importance of signalling through the PI3K, PTEN and Akt pathways leading to tumorigenesis. Indeed the Akt kinases were found to be hyper-activated in a majority of tumors (Cicenas, 2008; see section below). Because Akt1 and Akt2 isoforms are ubiquitously expressed in all cells and tissues, although at different levels, investigating the role played by Akt isoforms in the control of cell proliferation has provided insights into the pathophysiological mechanisms of cancer development. In the study of Akt isoform-specificity, we showed that Akt1, but not Akt2, played an essential role in G1/S transition and proliferation (Heron-Milhavet et al., 2006). Using microinjection of antibodies, we first suggested true isoform-specific roles of Akt1 and Akt2 in cell proliferation (Vandromme et al., 2001) later confirmed by siRNA depletion in mouse C2 myogenic cells (Heron-Milhavet et al., 2006). In order to perform conclusive studies on the role of Akt isoforms in normal and in transformed cells, isoform-specific tools have to be validated. Santi and Lee recently examined the distinct subcellular localizations of the three Akt isoforms using antibodies available to distinguish Akt1, Akt2 and Akt3. According to this study, Akt1 was localized in the cytoplasm, Akt2 was colocalized within mitochondria while Akt3 was localized in both the nucleus and nuclear membrane (Santi and Lee, 2010). These results diverge from our own observations with endogenous or ectopically expressed HA- or GFP-tagged Akt1 and Akt2 showing Akt2 to be predominantly nuclear, while Akt1 was cytoplasmic and membrane bound in unstimulated cells. As shown in rat embryonic fibroblasts (REF52 cells) upon activation using growth factors, both endogenous and overexpressed tagged Akt1 undergo a brief nuclear translocation (Andjelkovic et al., 1997). In addition, cell fractionation experiments on normal fibroblasts and the use of isoform-specific antibodies have shown a predominant nuclear localization of Akt2, even before growth factor stimulation (Heron-Milhavet et al., 2006).

Data showing mitochondrial localization for Akt2 are also controversial in light of a recent finding concerning the C-terminal modulator protein CTMP. This protein, described as binding Akt C-terminal region (Maira et al., 2001), appears to preferentially associate with Akt1 (our unpublished data). It has also been recently shown to be localized in the mitochondrial compartment (Parcellier et al., 2009) and further work is clearly required to clarify these observations (see below).

As far as the implication of the different Akt isoforms in human cancer are concerned, Liu and colleagues (2009) recently reviewed the incidence of genetic alterations in Akt1 and Akt2 isoforms. An activating mutation in the PH domain of Akt1 (E17K) –

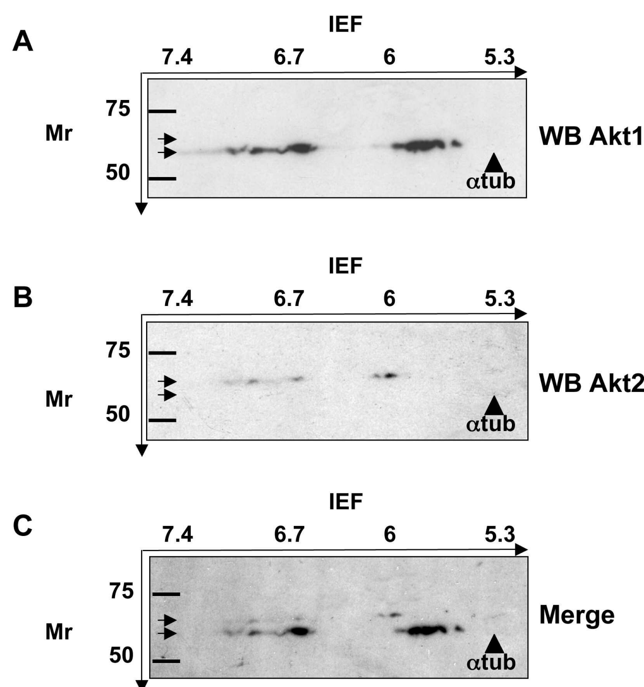

Fig. 2. Akt1 and Akt2 distinction by high resolution 2-dimensional PAGE. Protein extracts from C2.7 mouse proliferative myoblasts were separated by high resolution 2-dimensional PAGE. Western blot using isoform specific anti-Akt1 **(A)** and anti-Akt2 **(B)** antibodies revealed migration of both isoforms as compared to tubulin (shown as a black triangle). The merge image of both results is shown in panel **C**.

which results in growth factor-independent membrane translocation of Akt and increased Akt phosphorylation levels – was identified in melanomas, breast, colorectal and ovarian cancers (Carpten et al., 2007; Davies et al., 2008). Another study showed the expression of Akt1 protein and mRNA was similar in gliomas and normal control tissues, whereas the protein and mRNA levels of Akt2 increased with the pathological grade of malignancy, while that of Akt3 decreased with increasing grade malignancy (Mure et al., 2010). Taken together, there is clear evidence that deregulation, particularly amplification of each isoform, accompanies many tumor types, thus confirming the important role of this family of kinases in human tumorigenesis.

The protein kinase Akt has frequently been linked to induction and progression of cell growth particularly of breast-derived adenocarcinomas during breast cancer. Using fluorescence in situ hybridization (FISH) analysis, Kirkegaard and colleagues have shown that, in estrogen receptor-positive breast carcinomas, Akt1 and Akt2 genes were mutated (essentially deletion mutations) in 5% and 22% of the cases respectively (Kirkegaard et al., 2010). In the context of the complex in vivo environment, Akt1 was shown to promote, whereas Akt2 inhibits, mammary tumor induction and growth (Maroulakou et al., 2007). This outcome could not have been predicted from the results of earlier in vitro studies, but is in agreement with our "in cellulo" study of Akt1 and Akt2 (Heron-Milhavet et al., 2006). In 2008, it was also shown that Akt1 and Akt2 have distinct roles in lactogenic differentiation and involution in mammary gland development. The ablation of Akt1 delays the differentiation of mammary epithelia during pregnancy while lactation and extinction of Akt2 have the opposite effect (Maroulakou et al., 2008). It has also been shown that coexpression of Akt1 and ErbB2 can accelerate early stages of ErbB2-mediated tumorigenesis through increased cellular proliferation but interferes with subsequent metastatic progression by inducing mammary epithelial differentiation (Hutchinson et al., 2004).

Another recent report described distinct biological roles of the Akt family in mammary tumor progression (Dillon et al., 2009) where Akt1 plays an essential role in breast cancer induction and Akt2 is primarily involved in metastatic dissemination. This conclusion was reinforced in a recent review recapitulating the role of components of PI3 kinase pathway in mammary gland development and breast cancer (reviewed by Wickenden and Watson, 2010). Thus the two Akt members frequently perform distinct but complementary functions illustrated in breast tumor progression (See below and

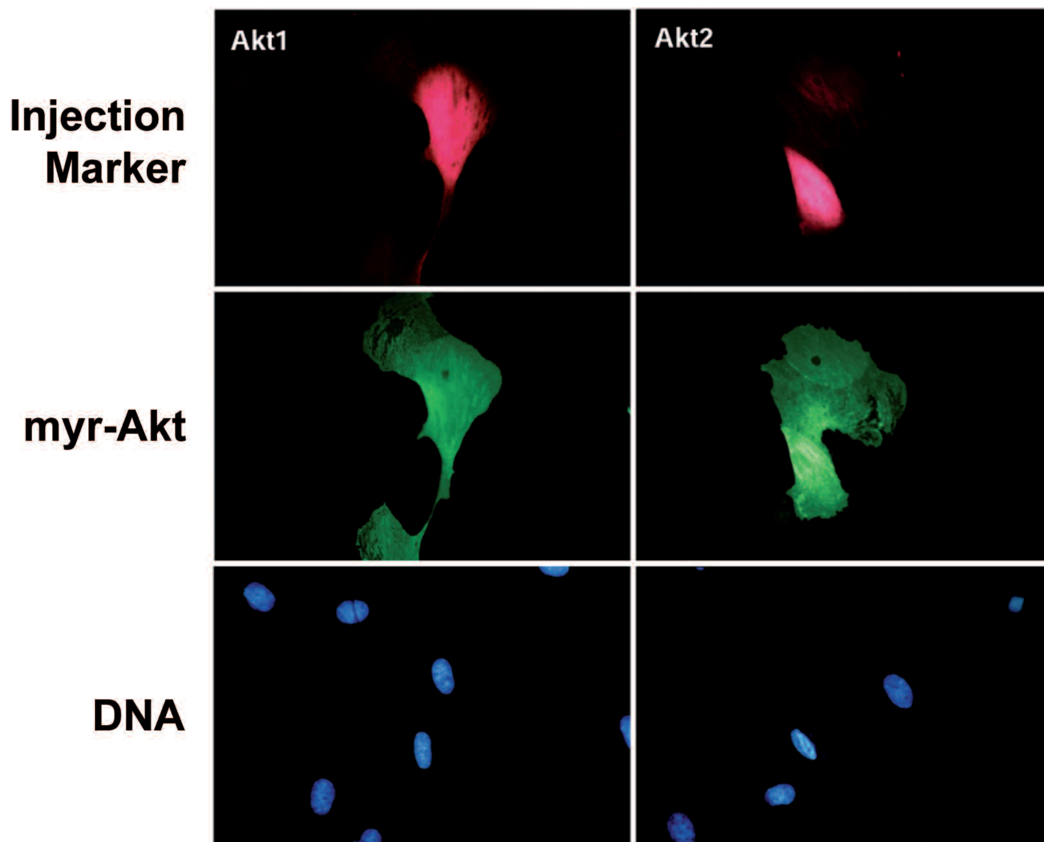

Fig. 3. Microinjection of using CMV driven CDNA encoding for myr-Akt1 and myr-Akt2 into human myoblasts. Injections were performed using myristylated GFP-tagged isoforms (in green) along with Texas-red Dextran as the injection marker (in red). DNA was stained with Hoechst (in blue).

scheme in Fig. 4).

Akt-isoform specificity and cell migration and invasion

Cancer lethality significantly increases when tumor cells acquire phenotypes that eventually lead to metastasis, which is responsible for more than 90% of all cancer patient deaths. Metastasis involves the spreading of cancer cells from their initial site of neogenesis to other locations in the body and subsequent anchorage and growth into tumors. Metastasis develops through a variable cellular sequence that includes relaxed proliferation control and resistance to apoptosis, Epithelial to Mesenchymal Transition (EMT), detachment from the primary tumor site and escape into the blood or lymph networks followed by reattachment, *de novo* growth and inception of an autonomous blood supply (angiogenesis) at the new location.

The level of phosphorylated Akt was found to be much higher in metastatic breast cells compared to non-metastatic tumors cells (Qiao et al., 2007). It is now accepted that activated Akt phosphorylates GSK3ß on serine 9, causing inactivation and enhanced proteolytic degradation of GSK3ß, which consequently increases the stability of transcription regulators such as SNAIL. This in turn decreases transcription of the trans-membrane proteins, including E-cadherin, a protein primarily implicated in forming adhesions between adjacent cells, thereby facilitating their detachment and subsequent entry into the blood or lymph (Julien et al., 2007). The decrease in E-cadherin is also a hallmark of the Epithelial to Mesenchymal Transition (Grille et al., 2003). EMT is a process that plays an important role both during development and in oncogenesis. The developmental switch characteristic of EMT renders tumor cells undergoing this process less adherent, therefore more invasive and metastatic. Akt is known to be an enhancer of cell motility in distinct cell types (reviewed by Chin and Toker, 2010), but it is still unclear what role each isoform plays. While Akt1 and Akt2 have been studied, the role of Akt3 in cell motility is still poorly examined or unknown. In fibroblasts, ectopic overexpression of Ak1 increased cell motility (Higuchi et al., 2001) and a similar conclusion that Akt1 was required for cell migration was reached using fibroblasts from Akt1 knock-out mice (Zhou et al., 2006). Mouse embryonic fibroblasts (MEF) lacking Akt1 exhibit impaired migration and peripheral ruffling in response to PDGF stimulation, whereas MEF lacking Akt2 are normal (Kim et al., 2008). Moreover, overexpression of Akt1, but not Akt2, is sufficient to restore PDGF-induced cell migration in an Akt1/Akt2 deficient background (Kim et al., 2008). While the

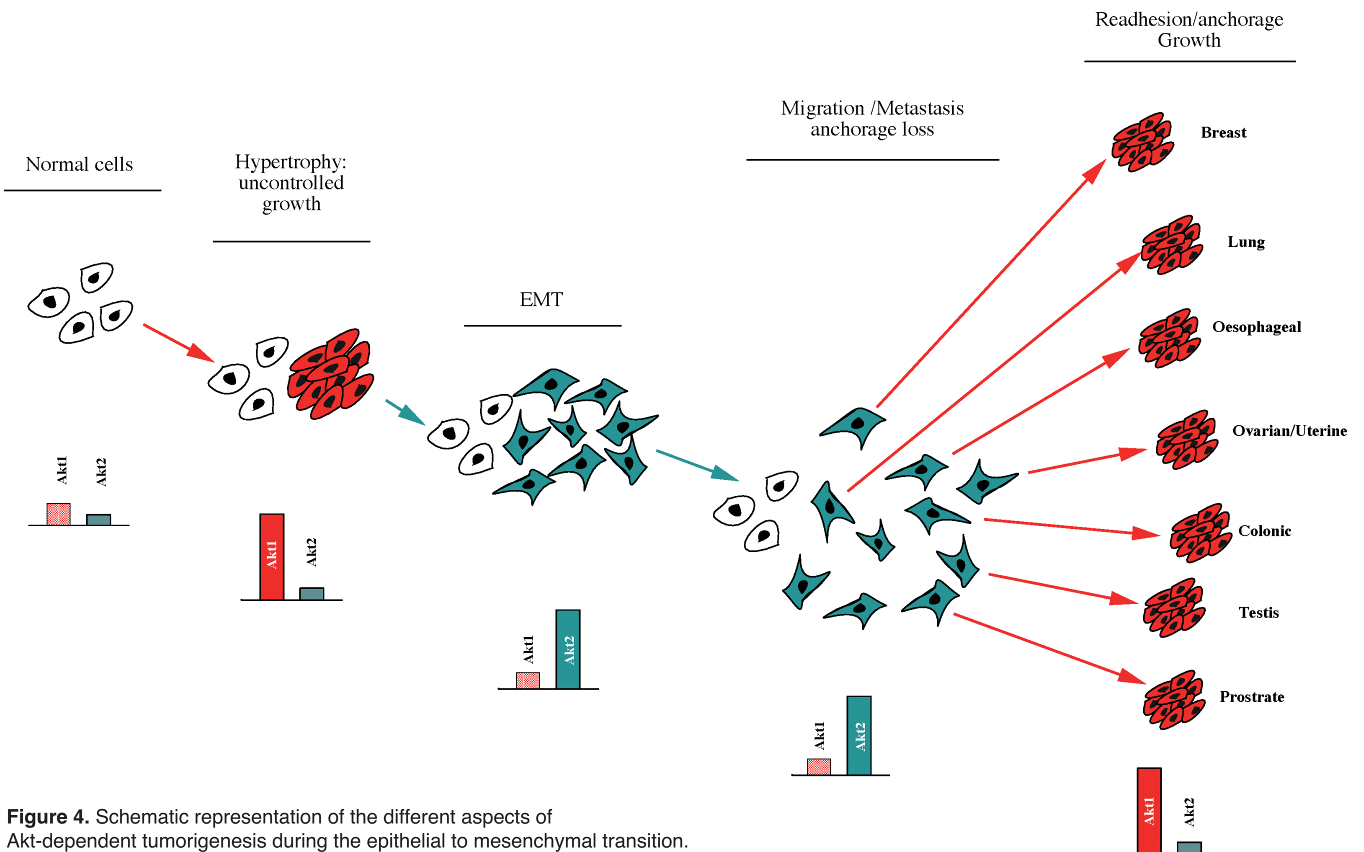

Figure 4. Schematic representation of the different aspects of Akt-dependent tumorigenesis during the epithelial to mesenchymal transition.

mechanisms behind this are unknown, it has been suggested that Akt1 controls cell migration by selectively translocating to the leading edge and activating Rac through phosphorylation (Kim et al., 2008). Thus, Akt1 seems to be the predominant isoform promoting cell motility in fibroblasts. In contrast, in cells of endodermal origin, such as breast epithelial cells, overexpression of Akt1, but not Akt2, blocked cell migration, invasion and thus metastasis through proteosomal degradation of the transcription factor NFAT (Yoeli-Lerner et al., 2005). Akt2 has also been shown to enhance the migratory and invasive phenotypes in other endodermal/epithelial cells, such as breast carcinoma cells, by up-regulating ß1 integrins and promoting adhesion and invasion of breast cancer cells in vitro, and also metastasis in vivo (Arboleda et al., 2003). Moreover, it has been shown that Akt2 is specifically involved in the epithelial to mesenchymal transition (Irie et al., 2005; Aktas et al., 2009; Iliopoulos et al., 2009; Ptaszynska et al., 2010).

Ablation of Akt1 inhibits, whereas ablation of Akt2 accelerates, tumor induction and growth by polyoma middle T and ErbB2 (Maroulakou et al., 2007). However, mammary adenocarcinomas developing in Akt1 knock-out mice are more invasive than those seen in Akt2 knock-out or wild-type mice, supporting that knockdown of Akt1 may enhance tumor invasiveness, a process independent of tumor induction and growth in epithelial cells. These findings were consistent with observations demonstrating that Akt1 down-regulation or ablation promotes migration and invasiveness of human epithelial cells in culture (Irie et al., 2005), perhaps by initiating EMT. Thus, inhibition of individual Akt isoforms may have both desirable and undesirable effects during oncogenesis. Iliopoulos et al. have begun to identify and characterize some downstream targets of different Akt isoforms (Iliopoulos et al., 2009). They described a set of microRNAs that are differentially regulated by the three Akt isoforms in non transformed mammary epithelial cells (MCF10A) stimulated with insulin-like growth factor I (IGF-I). They were able to demonstrate that an imbalance between Akt1 and Akt2 deregulates the abundance of the miR-200 family of microRNA that controls EMT and stem cell renewal programs (Iliopoulos et al., 2009). The same was true both in mouse and human tumors. Down-regulation of the miR-200 microRNA family members in these cell types appears to depend not on Akt activity *per se*, but on the balance between Akt1 and Akt2 isoforms. Knocking down exclusively Akt1, but not Akt2 or the double knockdown (Akt1 and Akt2) would promote EMT by decreasing the abundance of the miR-200 microRNA family members. While there is clear evidence that Akt1 is involved in migration of some cell types, mostly of ectodermal origin, the inverse is true in epithelial cell types, which make up the bulk of human tumor types, and where Akt2 stimulates motility and Akt1 reduces it. Again, these observations support the hypothesis that these two isoforms play different and opposing roles, even though such roles may inverse in a cell type-specific manner.

Re-adhesion of cells is also a critical step in cancer metastasis. Re-inforced adhesion of cancer cells can be activated by extracellular forces such as pressure, and the implication of Akt isoforms in this process was investigated in 2008 (Wang and Basson, 2008). Reducing Akt1, but not Akt2, reduced cancer cell adhesion associated with increased (15 mm Hg) extracellular pressure. To identify the determinants of this Akt isoform specificity, the PH domain and/or hinge regions of Akt1 and Akt2 were exchanged. The Akt1 PH domain and hinge regions were identified as functional domains which jointly permit Akt1 translocation and phosphorylation in response to extracellular pressure (Wang and Basson, 2008).

Taken together these studies point to complementary roles of Akt1 and Akt2 in epithelial tumor metastasis, with Akt2 involved in the first step of invasiveness, the EMT, leading to reduced adhesion and increased migration, and Akt1 involved in the latter stage of tumor anchoring through re-adhesion (Fig. 4). Nevertheless, the question remains of how these two closely related kinases may perform such distinct functions in the same cellular environment. The simplest possibility would be through their specific phosphorylation of distinct substrates as well as their specific intracellular localization.

Akt isoform-specific substrates

By systematically permutating the amino-acid sequence surrounding the Akt phosphorylation site in GSK3, Alessi et al. (1996) derived an optimal peptide sequence for Akt phosphorylation (R-X-R-X-X-S/T; where X is any amino acid). This Akt consensus motif is a common feature of known substrates of Akt, and its presence predicts reasonably well whether a given protein may be phosphorylable by Akt kinases in vitro. Without any isoform specificity, targets of Akt with implications in apoptosis have been discovered; BAD regulation by Akt has exemplified the molecular pathways linking survival factor signalling to apoptosis suppression (reviewed by Franke and Cantley, 1997). Another important class of Akt targets are proteins involved in the stress-activated/mitogen activated protein kinase (SAPK/MAPK) cascades. A close functional relationship between the Akt survival pathway and SAPK/MAPK cascades activated by numerous cellular stresses and linked to apoptosis was reported in 2008 (reviewed by Franke, 2008). Finally, some Akt substrates with specific metabolic functions have also been identified. These include the insulin-mediated increase in glycogen synthesis and suppression of glycogenolysis through the Akt-dependent phosphorylation and inactivation of GSK3α and ß, and other metabolic effects of Akt activation include the phosphorylation and activation of 6-phosphofructo-2-kinase (reviewed by Franke, 2008).

Are there possible isoform-specific Akt1 and Akt2 signaling pathways that would lead to substrate selectivity between the two isoforms? As examples in this exciting area of research, it has been reported that Akt2, rather than Akt1, was the principal isoform responsible for the stimulation of Glut4 translocation in adipocytes (Katome et al., 2003). Another example was described above relating that the knock-down of Akt1 led to an increase in the migration of mammary epithelial cells whereas the knock-down of Akt2 did not (Irie et al., 2005). In these different examples, the possible occurrence of Akt isoform-specific substrates with differential accessibility, or ability to be recognized, must exist. Other recent studies have examined specificity in Akt isoform substrates and an actin-associated protein palladin was identified as an Akt1-specific substrate that modulates invasive migration of breast cancer cells. Akt1, but not Akt2, phosphorylated palladin at Ser507 in a domain critical for F-actin bundling (Chin and Toker, 2010). As detailed above, increased expression of Akt1 was shown to reduce cell migration in epithelial cells, in particular migration induced through ERK-MAPK pathways; on the contrary, Akt2 upregulated ß1 integrin expression, promoted cell invasiveness and metastasis (Arboleda et al., 2003). Palladin is thought to have anti-migratory and anti-invasive activity at least in breast cancer cells. These findings identified palladin as a specific substrate for Akt1 that regulates cell motility and provides a putative molecular mechanism that accounts at least in part for the functional distinction between Akt isoforms in breast cancer cell signalling with respect to cell migration (Chin and Toker, 2010). In the switch of C2C12 cells from proliferating to differentiating phenotypes, Gherzi and colleagues also identified Pitx2 as a substrate phosphorylated by Akt2, which is effectively implicated in modulating the interchange between proliferation and differentiation in these mouse myoblasts. Indeed, when the differentiation program is initiated, phosphorylation of Pitx2 by Akt2 impairs its association with the Cyclin D1 mRNA-stabilizing complex thus shortening the half-life of Cyclin D1 (Gherzi et al., 2010). Prohibitin-1 is another protein likely playing an important function in cell metabolism and proliferation and has been characterized as an Akt1 substrate in MiaPaCa-2 cells (Han et al., 2008), where Akt1 may regulate the cellular functions of prohibitin-1 via its phosphorylation. Within the last ten years, the quest for other isoform specific Akt substrates has increased in an attempt to understand the specific roles of Akt isoforms even though the conclusions drawn from some of these studies remain controversial. Among the groups working to elucidate the isoform-specific substrates, we identified p21 as a specific phospho-substrate of Akt1 and we showed that Akt2 did not phosphorylate p21 but instead bound to p21 (Heron-Milhavet et al., 2006). It had been previously shown that Akt could phosphorylate p21 without defining which isoform. This conclusion was reinforced by our data showing that only the Akt1 isoform phosphorylated p21, inducing its release from cdk2 and cytoplasmic relocalization. In contrast, Akt2 binds p21 competing with phosphorylation by Akt1 and inducing accumulation of p21 in the nucleus (Heron-Milhavet et al., 2006). We further demonstrated that Akt1, but not Akt2, plays another major role in early cell cycle exit and myogenic differentiation through, at least in part, its specific interaction with a second member of the prohibitin family, Prohibitin-2/REA (Heron-Milhavet et al., 2008). This member of the prohibitin family has also been implicated in various cellular processes including cancer cell proliferation and adhesion (Sievers et al., 2010).

Because we found that, in myoblasts and fibroblasts, the interaction of Ak2 with p21 is an important event in stabilizing p21 in the nucleus and subsequent regulation of cell cycle, we have recently been focusing on determining the region of Akt2 involved in this interaction. We have determined that the region of p21 involved in binding Akt2 spans the T145 phosphorylation site (Heron-Milhavet et al., 2006). To study the region involved in p21 binding, we are exploiting different C-terminal and N-terminal mutant forms of Akt2 and human full length p21. Our ongoing experiments indicate a 30 amino acid region within the last 70 amino acids of Akt2 (LHM, AF and NJL; manuscript in preparation).

Activation of Akt and isoform-specific protein kinase inhibition

PDK1 activates at least 23 AGC kinases by phosphorylating a specific Thr or Ser residue located within the T-loop of the kinase domain (Alessi et al., 1997). For Akt1 and Akt2, maximal activation also requires phosphorylation of a second serine located in the C-terminal part of the catalytic domain within a region known as the hydrophobic motif (Yang et al., 2002). It has been established that insulin-induced activation of Akt1 and Akt2, although markedly diminished, was not abolished in mouse models expressing a form of PDK1 mutated in the PH domain and in ES cells derived from it (Bayascas et al., 2008). Inhibition of Akt resulted from reduced phosphorylation of Thr308, which is catalyzed by PDK1, rather than altered phosphorylation of Ser473, which is mediated by mammalian target of rapamycin complex-2 (mTORC2) (Bayascas et al., 2008). Although isoform-specific substrates of Akt1 and Akt2 have now been described, it is still unclear if differential isoform-specific Akt activation mechanisms exist, principally because the tools available to date do not distinguish between Akt1 and Akt2 phosphorylation and/or activation. In this respect, overexpression of either isoform constitutively bound to the membrane (Fig. 3) is also associated with uncontrolled Akt activation even though neither isoform can translocate to the nucleus.

Several studies have found Akt2 to be amplified or overexpressed at the mRNA level in various tumor cell

lines and in a number of human malignancies, such as colon, pancreatic and breast cancers. One recent study measuring the levels of Akt isoform phosphorylation and thus activation state, described that assessing activation of Akt isoforms by monitoring Akt phosphorylation state would be a more clinically significant marker than measuring only Akt2 amplification or overexpression (Cicenas, 2008). The implication of the PI3K pathway has also been described in chronic myelogenous leukemia (CML) after activation by Bcr-Abl chimera protein which promotes leukemogenesis in these cells (Hirano et al., 2009). Indeed Bcr-Abl repression continuously activates Akt1, Akt2 and Akt3 via phosphorylation on Ser473, resulting in CML cell proliferation. Akt activation is also a common finding in pediatric malignant gliomas where overexpression of activated Akt was observed in 42 of 53 tumors (Pollack et al., 2010). With respect to apoptosis, a novel mechanism was recently described involving recruitment of PDK1 to the SHPS-1 signaling complex which is required for IGF-I-stimulated Akt-T308 phosphorylation and resulting inhibition of apoptosis (Shen et al., 2010). Finally, a further mechanism leading to cisplatin resistance in lung cancer cells was identified describing Akt, without specifying the isoform, as the downstream target of GRP78 in mediating drug resistance in these cells (Lin et al., 2011).

Recently, the intriguing possibility of activation of Akt through tyrosine phosphorylation in transformed cells has been raised. Although not expressed in normal mammary gland tissue, protein tyrosine kinase 6 (PTK6) is expressed in the majority of human breast tumors examined to date and it has been reported that Akt, again without specifying the isoform, is a potential direct substrate of PTK6 through phosphorylation of Tyr315 and 326, events which would promote Akt activation in response to epidermal growth factor (Zheng et al., 2010). More data will be required before a direct connection between Akt activation, and in particular which isoform, is established.

Since hyperactivation of the Akt pathway has been detected in up to 50% of all human tumors and is closely associated with chemoresistance (Sun et al., 2001; Altomare et al., 2005), Akt has become an attractive target for anti-cancer drug discovery. To study the increase in radiosensitivity of human carcinoma cell lines, selective inhibition of Ras, PI3K and Akt isoforms has been carried out, revealing that inhibition of Akt1 reduced tumor cell radiation survival whereas inhibition of Akt2 or Akt3 was less effective; selective inhibition of Akt1 has thus been proposed as a viable approach to sensitizing many tumor cells to cytotoxic therapies (Kim et al., 2005). Alternatively, in squamous cell carcinomas, Akt1 downregulation is common, while Akt2 up-regulation is widespread. Here activation of up-regulated Akt2 involving serine phosphorylation (i.e. 473) correlates with high grade tumors, the most dangerous forms. These findings suggest that Akt2 would be a possible therapeutic tumor target in squamous cell carcinomas (O'Shaughnessy et al., 2007). These data confirm that Akt1 and Akt2 isoforms appear to have distinct cell-dependent effects, the nature of which may interchange in a manner that depends on the cellular background, such that in some cell types Akt1 is associated with poor prognosis while in others, this role will be prevalent for Akt2.

In 2005, Merck Research Laboratories described potent Akt1 and Akt2 dual inhibitors. Compounds from this series, which contain a 5-tetrazolyl moiety, generally exhibit more potent inhibition of Akt2 than Akt1 (Zhao et al., 2005). At the same time another group at Merck identified two Akt inhibitors that exhibited enzyme isoform specificity. The first inhibited only Akt1, while the second inhibited both Akt1 and Akt2 isoforms (both compounds being reversible) and blocked phosphorylation and activation of the corresponding Akt by PDK1 (Barnett et al., 2005). In 2008, the same group described potent, allosteric dual Akt1 and Akt2 inhibitors with improved aqueous solubility that translated into enhanced cellular activity and resulting caspase-3 induction (Zhao et al., 2008). Importantly, the discovery and development of these small Akt inhibitor molecules enabled clearer insight into the implication of each isoform in tumorigenesis. For example, it has now been shown that inhibition of both Akt1 and Akt2 selectively sensitized tumor cells, but not normal cells, to apoptotic stimuli (DeFeo-Jones et al., 2005).

Confirming the efficacy of targeting Akt in the resistance of cancer cells to apoptosis, a recent study reported the identification of another small chemical inhibitor of Akt, API-1, which binds to the PH domain of Akt (thus inhibiting all three members of the Akt family by blocking their membrane translocation). Treating cancer cells with API-1 inhibited Akt phosphorylation on threonine 308 and inactivated all three kinase members. Inhibition of Akt by API-1 also selectively resulted in cell growth arrest and apoptosis in human cancer cells that harbour constitutively activated forms of Akt (Kim et al., 2010).

Another small-molecule inhibitor of Akt phosphorylation was recently presented in phase I clinical trials and administered to patients with tumors displaying increased Akt phosphorylation. Modest decreases in tumor-associated Akt phosphorylation were detected following treatment with triciribine phosphate, although the significance of these conclusions must be tempered by the variable dose levels used and the small sample size, characteristic of phase I trials (Garett et al., 2010).

Akt protein inhibitors and modulators

A number of naturally occurring protein inhibitors and modulators of Akt have also been described. It has been known for a number of years that PDK1 is the kinase that phosphorylates Thr308 on Akt1 or Thr309 on Akt2. Furthermore, a mutation in the PH domain of PDK1 inhibits Akt activation, leading to smaller size

animals and insulin resistance in mouse models (Bayascas et al., 2008). Another protein which interacts with Akt through the PH domain is casein kinase 2-interacting protein-1 (CKIP-1) which also has an Akt inhibitory function. Once bound through a site in the N-terminal of CKIP-1, CKIP-1 forms complexes with all three Akt isoforms through their PH domains, an interaction that specifically decreases Akt kinase activity. Stable expression of CKIP-1 in cells results in Akt inactivation and inhibition of cell growth in vitro, suggesting that CKIP-1 would be a candidate tumor suppressor through inhibition of Akt's functions (Tokuda et al., 2007). A positive role of CKIP-1 during the proliferation and differentiation of skeletal muscle C2 myoblasts has also been shown previously downstream of the insulin stimulation of the PI3K pathway (Safi et al., 2004).

The carboxyl-terminal modulator protein CTMP is another protein which interacts with Akt and has been of interest for our laboratory. First discovered in 2001, CTMP was identified as a protein partner for Akt that specifically binds the COOH terminus of Akt at the plasma membrane (Maira et al., 2001). In humans, mice and rats, CTMP is one member of the thioesterase family (thioesterase superfamily member 4), a family of proteins which have remained unchanged since bacteria. CTMP binding to the C-terminal region of Akt inhibits its phosphorylation on Ser 473 and Thr 308 leading to the suggestion that CTMP was a negative regulatory component in the pathway controlling Akt activation (Maira et al., 2001). Three years later, additional insights into the implication of CTMP-Akt in cancer arose, where loss of CTMP function and/or expression reduced its inhibitory effects on Akt and promoted tumorigenesis. Indeed, when primary glioblastomas and glioblastoma cell lines were studied for the consequences of CTMP deletion, mutation, promoter hypermethylation and mRNA expression, Knobbe and colleagues (Knobbe et al., 2004) found that both hypermethylation and transcriptional down-regulation of CTMP genes could be related to tumor state. In 2009, the role of CTMP in multistage lung tumorigenesis revealed that lentiviral overexpression of CTMP altered the Akt signalling pathway and inhibited DNA synthesis and cell cycle progression in lungs of 9-week-old K-ras(LA1) mice. Increased apoptosis was also observed in lungs of 13-week-old K-ras(LA1) mice (Hwang et al., 2009). When several cell permeable peptides from the N-terminal region of CTMP (termed TAT-CTMP1-4) were screened in vitro, at least one was found to induce significant apoptosis in pancreatic adenocarcinoma cell lines. These data indicate that inhibiting Akt with CTMP may be of therapeutic benefit in the treatment of pancreatic adenocarcinoma and, when combined with established therapies, may result in an increase in tumor cell death (Simon et al., 2009). Apart from all these studies describing the possible implication of CTMP in tumorigenesis, CTMP was recently shown to associate with mitochondrial proteins, localize to mitochondria and become phosphorylated following treatment of cells with insulin-mimetic compounds (Parcellier et al., 2009; Piao et al., 2009). Moreover, overexpression of CTMP increased the sensitivity of cells to apoptosis, most likely due to the inhibition of Hsp70 function (Piao et al., 2009). These data suggested that in addition to its role in Akt inhibition, CTMP may play a key role in mitochondria-mediated apoptosis by localizing to mitochondria.

Other important protein interactions with Akt isoforms have also been identified. Some of these, such as CKIP-1, negatively regulate all three Akt members and the consequences of this interaction will by nature be cell type specific. CTMP, another Akt interacting protein, also appears to have negative effects on Akt, but in addition it may also play a role in insulin signalling. Finally, p21, a cdk modulator protein implicated in cdk-cyclin kinase complex formation, shows Akt isoform-specific effects by binding essentially to Akt2. It will be interesting to determine the nature of these interactions in different cell types or tumors and examine the relationship of Akt binding to either CTMP or CKIP-1.

Conclusion

From these data, a better focused picture of Akt signaling is beginning to emerge. The importance of this family of kinases is now clearly established in very varied group of cellular functions. Furthermore, it is now obvious that despite close sequence homology at the primary amino acid level, the different Akt isoforms have different and frequently opposing effects in cells. In normal cells, and during muscle differentiation, Akt1 plays an important role in modulating and promoting cell growth and proliferation. It is also essential to prevent apoptosis during normal cell proliferation and the early events of myogenic differentiation. Akt2 plays an essential and opposing role in the arrest and exit from the proliferation cell cycle and progression through muscle cell differentiation (Vandromme et al., 2001; Heron-Milhavet et al., 2006). It is also primarily involved in the epithelial to mesenchymal transition (reviewed by Wickenden and Watson, 2010). At least part of the molecular basis for a differential action in cell proliferation is now clear and involves differential interaction with p21 (Heron-Milhavet et al., 2006) and potentially CTMP (our unpublished observations). Deregulation of either Akt1 or Akt2 is therefore implicit in the development of most tumor types. Here a picture emerges where up-regulation of one kinase isoform is implicated in the transformed phenotype, whereas the normal counterbalancing role of the other isoform is down-regulated or otherwise compromised.

References

Aktas B., Tewes M., Fehm T., Hauch S., Kimmig R. and Kasimir-Bauer S. (2009). Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic

breast cancer patients. Breast Cancer Res. 11, R46.

Alessi D.R., Caudwell F.B., Andjelkovic M., Hemmings B.A. and Cohen P. (1996). Molecular basis for the substrate specificity of protein kinase B; comparison with MAPKAP kinase-1 and p70 S6 kinase. FEBS Lett. 399, 333-338.

Alessi D.R., James S.R., Downes C.P., Holmes A.B., Gaffney P.R., Reese C.B. and Cohen P. (1997). Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr. Biol. 7, 261-269.

Altomare D.A., You H., Xiao G.H., Ramos-Nino M.E., Skele K.L., De Rienzo A., Jhanwar S.C., Mossman B.T., Kane A.B. and Testa J.R. (2005). Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. Oncogene 24, 6080-6089.

Andjelkoviç M., Alessi D.R., Meier R., Fernandez A., Lamb N.J., Frech M., Cron P., Cohen P., Lucocq J.M. and Hemmings B.A. (1997). Role of translocation in the activation and function of protein kinase B. J. Biol. Chem. 272, 31515-31524.

Arboleda M.J., Lyons J.F., Kabbinavar F.F., Bray M.R., Snow B.E., Ayala R., Danino M., Karlan B.Y. and Slamon D.J. (2003). Overexpression of AKT2/protein kinase Bbeta leads to up-regulation of beta1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. Cancer Res. 63, 196-206.

Barnett S.F., Defeo-Jones D., Fu S., Hancock P.J., Haskell K.M., Jones R.E., Kahana J.A., Kral A.M., Leander K., Lee L.L., Malinowski J., McAvoy E.M., Nahas D.D., Robinson R.G. and Huber H.E. (2005). Identification and characterization of pleckstrin-homology-domain-dependent and isoenzyme-specific Akt inhibitors. Biochem. J. 385, 399-408.

Bayascas J.R., Wullschleger S., Sakamoto K., García-Martínez J.M., Clacher C., Komander D., van Aalten D.M., Boini K.M., Lang F., Lipina C., Logie L., Sutherland C., Chudek J.A., van Diepen J.A., Voshol P.J., Lucocq J.M. and Alessi D.R. (2008). Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance. Mol. Cell. Biol. 28, 3258-3272.

Carpten J.D., Faber A.L., Horn C., Donoho G.P., Briggs S.L., Robbins C.M., Hostetter G., Boguslawski S., Moses T.Y., Savage S., Uhlik M., Lin A., Du J., Qian Y.W., Zeckner D.J., Tucker-Kellogg G., Touchman J., Patel K., Mousses S., Bittner M., Schevitz R., Lai M.H., Blanchard K.L. and Thomas J.E. (2007). A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448, 439-444.

Chen W.S., Xu P.Z., Gottlob K., Chen M.L., Sokol K., Shiyanova T., Roninson I., Weng W., Suzuki R., Tobe K., Kadowaki T. and Hay N. (2001). Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. Genes Dev. 15, 2203-2208.

Chin Y.R. and Toker A. (2010). The actin-bundling protein palladin is an Akt1-specific substrate that regulates breast cancer cell migration. Mol. Cell. 38, 333-344.

Cho H., Thorvaldsen J.L., Chu Q., Feng F. and Birnbaum M.J. (2001a). Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J. Biol. Chem. 276, 38349-38352.

Cho H., Mu J., Kim J.K., Thorvaldsen J.L., Chu Q., Crenshaw E.B. 3rd, Kaestner K.H., Bartolomei M.S., Shulman G.I. and Birnbaum M.J. (2001). Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292, 1728-1731.

Cicenas J. (2008). The potential role of Akt phosphorylation in human cancers. Int. J. Biol. Markers 23, 1-9.

Davies M.A., Stemke-Hale K., Tellez C., Calderone T.L., Deng W., Prieto V.G., Lazar A.J., Gershenwald J.E. and Mills G.B. (2008). A novel AKT3 mutation in melanoma tumours and cell lines. Br. J. Cancer 99, 1265-1268.

DeFeo-Jones D., Barnett S.F., Fu S., Hancock P.J., Haskell K.M., Leander K.R., McAvoy E., Robinson R.G., Duggan M.E., Lindsley C.W., Zhao Z., Huber H.E. and Jones R.E. (2005). Tumor cell sensitization to apoptotic stimuli by selective inhibition of specific Akt/PKB family members. Mol. Cancer Ther. 4, 271-279.

Dillon R.L., Marcotte R., Hennessy B.T., Woodgett J.R., Mills G.B. and Muller W.J. (2009). Akt1 and akt2 play distinct roles in the initiation and metastatic phases of mammary tumor progression. Cancer Res. 69, 5057-5064.

Franke T.F. (2008). PI3K/Akt: getting it right matters. Oncogene 27, 6473-6488.

Franke T.F. and Cantley L.C. (1997). Apoptosis. A Bad kinase makes good. Nature 390, 116-117.

Garrett C.R., Coppola D., Wenham R.M., Cubitt C.L., Neuger A.M., Frost T.J., Lush R.M., Sullivan D.M., Cheng J.Q. and Sebti S.M. (2010). Phase I pharmacokinetic and pharmacodynamic study of triciribine phosphate monohydrate, a small-molecule inhibitor of AKT phosphorylation, in adult subjects with solid tumors containing activated AKT. Invest. New Drugs (in press).

Garofalo R.S., Orena S.J., Rafidi K., Torchia A.J., Stock J.L., Hildebrandt A.L. Coskran T., Black S.C., Brees D.J., Wicks J.R., McNeish J.D. and Coleman K.G. (2003). Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. J. Clin. Invest. 112, 197-208.

Gherzi R., Trabucchi M., Ponassi M., Gallouzi I.E., Rosenfeld M.G. and Briata P. (2010). Akt2-mediated phosphorylation of Pitx2 controls Ccnd1 mRNA decay during muscle cell differentiation. Cell Death Differ. 17, 975-983.

Grille S.J., Bellacosa A., Upson J., Klein-Szanto AJ.., van Roy F., Lee-Kwon W., Donowitz M., Tsichlis P.N. and Larue L. (2003). The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res. 63, 2172-2178.

Han E.K., Mcgonigal T., Butler C., Giranda V.L. and Luo Y. (2008). Characterization of Akt overexpression in MiaPaCa-2 cells: prohibitin is an Akt substrate both in vitro and in cells. Anticancer Res. 28, 957-963.

Héron-Milhavet L., Franckhauser C., Rana V., Berthenet C., Fisher D., Hemmings B.A., Fernandez A. and Lamb N.J. (2006). Only Akt1 is required for proliferation, while Akt2 promotes cell cycle exit through p21 binding. Mol. Cell. Biol. 26, 8267-8280.

Heron-Milhavet L., Mamaeva D., LeRoith D., Lamb N.J. and Fernandez A. (2010). Impaired muscle regeneration and myoblast differentiation in mice with a muscle-specific KO of IGF-IR. J. Cell. Physiol. 225, 1-6.

Higuchi M., Masuyama N., Fukui Y., Suzuki A. and Gotoh Y. (2001). Akt mediates Rac/Cdc42-regulated cell motility in growth factor-stimulated cells and in invasive PTEN knockout cells. Curr. Biol. 11, 1958-1962.

Hill M.M., Clark S.F., Tucker D.F., Birnbaum M.J., James D.E. and Macaulay S.L. (1999). A role for protein kinase Bbeta/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. Mol. Cell. Biol. 19, 7771-7781.

Hirano I., Nakamura S., Yokota D., Ono T., Shigeno K., Fujisawa S., Shinjo K. and Ohnishi K. (2009). Depletion of Pleckstrin homology domain leucine-rich repeat protein phosphatases 1 and 2 by Bcr-Abl promotes chronic myelogenous leukemia cell proliferation through continuous phosphorylation of Akt isoforms. J. Biol. Chem. 284, 22155-22165.

Hutchinson J.N., Jin J., Cardiff R.D., Woodgett J.R. and Muller W.J. (2004). Activation of Akt-1 (PKB-alpha) can accelerate ErbB-2-mediated mammary tumorigenesis but suppresses tumor invasion. Cancer Res. 64, 3171-3178.

Hwang S.K., Lim H.T., Minai-Tehrani A., Lee E.S., Park J., Park S.B., Beck G.R. Jr and Cho M.H. (2009). Repeated aerosol delivery of carboxyl-terminal modulator protein suppresses tumor in the lungs of K-rasLA1 mice. Am. J. Respir. Crit. Care Med. 179, 1131-1140.

Iliopoulos D., Polytarchou C., Hatziapostolou M., Kottakis F., Maroulakou I.G., Struhl K. and Tsichlis P.N. (2009). MicroRNAs differentially regulated by Akt isoforms control EMT and stem cell renewal in cancer cells. Sci. Signal. 2, ra62.

Irie H.Y., Pearline R.V., Grueneberg D., Hsia M., Ravichandran P., Kothari N., Natesan S. and Brugge J.S. (2005). Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial-mesenchymal transition. J. Cell. Biol. 171, 1023-1034.

Julien S., Puig I., Caretti E., Bonaventure J., Nelles L., van Roy F., Dargemont C., de Herreros A.G., Bellacosa A. and Larue L. (2007). Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. Oncogene 26, 7445-7456.

Katome T., Obata T., Matsushima R., Masuyama N., Cantley L.C., Gotoh Y., Kishi K., Shiota H. and Ebina Y. (2003). Use of RNA interference-mediated gene silencing and adenoviral overexpression to elucidate the roles of AKT/protein kinase B isoforms in insulin actions. J. Biol. Chem. 278, 28312-28323.

Kim I.A., Bae S.S., Fernandes A., Wu J., Muschel R.J., McKenna W.G., Birnbaum M.J. and Bernhard E.J. (2005). Selective inhibition of Ras, phosphoinositide 3 kinase, and Akt isoforms increases the radiosensitivity of human carcinoma cell lines. Cancer Res. 65, 7902-7910.

Kim E.K., Tucker D.F., Yun S.J., Do K.H., Kim M.S., Kim J.H., Kim C.D., Birnbaum M.J. and Bae S.S. (2008). Linker region of Akt1/protein kinase Balpha mediates platelet-derived growth factor-induced translocation and cell migration. Cell. Signal. 20, 2030-2037.

Kim D., Sun M., He L., Zhou Q.H., Chen J., Sun X.M., Bepler G., Sebti S.M. and Cheng J.Q. (2010). A small molecule inhibits Akt through direct binding to Akt and preventing Akt membrane translocation. J. Biol. Chem. 285, 8383-8394.

Kirkegaard T., Witton C.J., Edwards J., Nielsen K.V., Jensen L.B., Campbell F.M., Cooke T.G. and Bartlett J.M. (2010). Molecular alterations in AKT1, AKT2 and AKT3 detected in breast and prostatic cancer by FISH. Histopathology 56, 203-211.

Knobbe C.B., Reifenberger J., Blaschke B. and Reifenberger G. (2004). Hypermethylation and transcriptional downregulation of the carboxyl-terminal modulator protein gene in glioblastomas. J. Natl. Cancer Inst. 96, 483-486.

Lin Y., Wang Z., Liu L. and Chen L. (2011). Akt is the downstream target of GRP78 in mediating cisplatin resistance in ER stress-tolerant human lung cancer cells. Lung Cancer 71, 291-297.

Liu P., Cheng H., Roberts T.M. and Zhao J.J. (2009). Targeting the phosphoinositide 3-kinase pathway in cancer. Nat. Rev. Drug Discov. 8, 627-644.

Maira S.M., Galetic I., Brazil D.P., Kaech S., Ingley E., Thelen M. and Hemmings B.A. (2001). Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and *v-akt* at the plasma membrane. Science 294, 374-380.

Manning B.D. and Cantley L.C. (2007). AKT/PKB signaling: navigating downstream. Cell 129, 1261-1274.

Maroulakou I.G., Oemler W., Naber S.P. and Tsichlis P.N. (2007). Akt1 ablation inhibits, whereas Akt2 ablation accelerates, the development of mammary adenocarcinomas in mouse mammary tumor virus (MMTV)-ErbB2/neu and MMTV-polyoma middle T transgenic mice. Cancer Res. 67, 167-177.

Maroulakou I.G., Oemler W., Naber S.P., Klebba I., Kuperwasser C. and Tsichlis P.N. (2008). Distinct roles of the three Akt isoforms in lactogenic differentiation and involution. J. Cell. Physiol. 217, 468-477.

Mure H., Matsuzaki K., Kitazato K.T., Mizobuchi Y., Kuwayama K., Kageji T. and Nagahiro S. (2010). Akt2 and Akt3 play a pivotal role in malignant gliomas. Neuro. Oncol. 12, 221-232.

O'Shaughnessy R.F., Akgül B., Storey A., Pfister H., Harwood C.A. and Byrne C. (2007). Cutaneous human papillomaviruses down-regulate AKT1, whereas AKT2 up-regulation and activation associates with tumors. Cancer Res. 67, 8207-8215.

Parcellier A., Tintignac L.A., Zhuravleva E., Cron P., Schenk S., Bozulic L. and Hemmings B.A. (2009). Carboxy-Terminal Modulator Protein (CTMP) is a mitochondrial protein that sensitizes cells to apoptosis. Cell. Signal. 21, 639-650.

Peng X.D., Xu P.Z., Chen M.L., Hahn-Windgassen A., Skeen J., Jacobs J., Sundararajan D., Chen W.S., Crawford S.E., Coleman K.G. and Hay N. (2003). Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev. 17, 1352-1365.

Piao L., Li Y., Yang K.J., Park K.A., Byun H.S., Won M., Hong J., Kim J.L., Kweon G.R., Hur G.M., Seok J.H., Cho J.Y., Chun T., Hess D., Sack R., Maira S.M., Brazil D.P., Hemmings B.A. and Park J. (2009). Heat shock protein 70-mediated sensitization of cells to apoptosis by Carboxyl-Terminal Modulator Protein. BMC Cell. Biol. 10, 53.

Pollack I.F., Hamilton R.L., Burger P.C., Brat D.J., Rosenblum M.K., Murdoch G.H., Nikiforova M.N., Holmes E.J., Zhou T., Cohen K.J. and Jakacki R.I. (2010). Akt activation is a common event in pediatric malignant gliomas and a potential adverse prognostic marker: a report from the Children's Oncology Group. J. Neurooncol. 99, 155-163.

Ptaszynska M.M., Pendrak M.L., Stracke M.L. and Roberts D.D. (2010). Autotaxin signaling via lysophosphatidic acid receptors contributes to vascular endothelial growth factor-induced endothelial cell migration. Mol. Cancer Res. 8, 309-321.

Qiao M., Iglehart J.D. and Pardee A.B. (2007). Metastatic potential of 21T human breast cancer cells depends on Akt/protein kinase B activation. Cancer Res. 67, 5293-5299.

Restuccia D.F. and Hemmings B.A. (2010). From man to mouse and back again: advances in defining tumor AKTivities in vivo. Dis. Model. Mech. 3, 705-720.

Safi A., Vandromme M., Caussanel S., Valdacci L., Baas D., Vidal M., Brun G., Schaeffer L. and Goillot E. (2004) Role for the pleckstrin homology domain-containing protein CKIP-1 in phosphatidylinositol 3-kinase-regulated muscle differentiation. Mol. Cell. Biol. 24, 1245-1255.

Santi S.A. and Lee H. (2010). The Akt isoforms are present at distinct subcellular locations. Am. J. Physiol. Cell. Physiol. 298, C580-591.

Sarbassov D.D., Guertin D.A., Ali S.M. and Sabatini D.M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-1101.

Shen X., Xi G., Radhakrishnan Y. and Clemmons D.R. (2010). PDK1 Recruitment to the SHPS-1 Signaling Complex Enhances Insulin-like Growth Factor-I-stimulated AKT Activation and Vascular Smooth Muscle Cell Survival. J. Biol. Chem. 285, 29416-29424.

Sievers C., Billig G., Gottschalk K. and Rudel T. (2010). Prohibitins are required for cancer cell proliferation and adhesion. PLoS One 5, e12735.

Simon P.O. Jr, McDunn J.E., Kashiwagi H., Chang K., Goedegebuure P.S., Hotchkiss R.S. and Hawkins W.G. (2009). Targeting AKT with the proapoptotic peptide, TAT-CTMP: a novel strategy for the treatment of human pancreatic adenocarcinoma. Int. J. Cancer 125, 942-951.

Staal S.P., Hartley J.W. and Rowe W.P. (1977). Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. Proc. Natl. Acad. Sci. USA 74, 3065-3067.

Sun M., Wang G., Paciga J.E., Feldman R.I., Yuan Z.Q., Ma X.L., Shelley S.A., Jove R., Tsichlis P.N., Nicosia S.V. and Cheng J.Q. (2001). AKT1/PKBalpha kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. Am. J. Pathol. 159, 431-437.

Toker A. and Yoeli-Lerner M. (2006). Akt signaling and cancer: surviving but not moving on. Cancer Res. 66, 3963-3966.

Tokuda E., Fujita N., Oh-hara T., Sato S., Kurata A., Katayama R., Itoh T., Takenawa T., Miyazono K. and Tsuruo T. (2007). Casein kinase 2-interacting protein-1, a novel Akt pleckstrin homology domain-interacting protein, down-regulates PI3K/Akt signaling and suppresses tumor growth *in vivo*. Cancer Res. 67, 9666-9676.

Tschopp O., Yang Z.Z., Brodbeck D., Dummler B.A., Hemmings-Mieszczak M., Watanabe T., Michaelis T., Frahm J. and Hemmings B.A. (2005). Essential role of protein kinase B gamma (PKB gamma/Akt3) in postnatal brain development but not in glucose homeostasis. Development 132, 2943-2954.

Vandromme M., Rochat A., Meier R., Carnac G., Besser D., Hemmings B.A., Fernandez A. and Lamb N.J. (2001). Protein kinase B beta/Akt2 plays a specific role in muscle differentiation. J. Biol. Chem. 276, 8173-8179.

Vivanco I. and Sawyers C.L. (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat. Rev. Cancer 2, 489-501.

Wang S. and Basson M.D. (2008). Identification of functional domains in AKT responsible for distinct roles of AKT isoforms in pressure-stimulated cancer cell adhesion. Exp. Cell Res. 314, 286-296.

Wickenden J.A. and Watson C.J. (2010). Key signalling nodes in mammary gland development and cancer. Signalling downstream of PI3 kinase in mammary epithelium: a play in 3 Akts. Breast Cancer Res. 12, 202.

Yang J., Cron P., Thompson V., Good V.M., Hess D., Hemmings B.A. and Barford D. (2002). Molecular mechanism for the regulation of protein kinase B/Akt by hydrophobic motif phosphorylation. Mol. Cell 9, 1227-1240.

Yang Z.Z., Tschopp O., Di-Poï N., Bruder E., Baudry A., Dümmler B., Wahli W. and Hemmings B.A. (2005). Dosage-dependent effects of Akt1/protein kinase Balpha (PKBalpha) and Akt3/PKBgamma on thymus, skin, and cardiovascular and nervous system development in mice. Mol. Cell. Biol. 25, 10407-10418.

Yoeli-Lerner M., Yiu G.K., Rabinovitz I., Erhardt P., Jauliac S. and Toker A. (2005). Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT. Mol. Cell 20, 539-550.

Zhao Z., Leister W.H., Robinson R.G., Barnett S.F., Defeo-Jones D., Jones R.E., Hartman G.D., Huff J.R., Huber H.E., Duggan M.E. and Lindsley C.W. (2005). Discovery of 2,3,5-trisubstituted pyridine derivatives as potent Akt1 and Akt2 dual inhibitors. Bioorg. Med. Chem. Lett. 15, 905-909.

Zhao Z., Robinson R.G., Barnett S.F., Defeo-Jones D., Jones R.E., Hartman G.D., Huber H.E., Duggan M.E. and Lindsley C.W. (2008). Development of potent, allosteric dual Akt1 and Akt2 inhibitors with improved physical properties and cell activity. Bioorg. Med. Chem. Lett. 18, 49-53.

Zheng Y., Peng M., Wang Z., Asara J.M. and Tyner A.L. (2010). Protein tyrosine kinase 6 directly phosphorylates AKT and promotes AKT activation in response to epidermal growth factor. Mol. Cell. Biol. 30, 4280-4292.

Zhou G.L., Tucker D.F., Bae S.S., Bhatheja K., Birnbaum M.J. and Field J. (2006). Opposing roles for Akt1 and Akt2 in Rac/Pak signaling and cell migration. J. Biol. Chem. 281, 36443-36453.

Accepted February 25, 2011