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# AKT/PKB SIGNALING MECHANISMS IN CANCER AND CHEMORESISTANCE

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## 1. ABSTRACT

During the past decade, Akt (also known as protein kinase B, PKB) has been extensively studied. It regulates a variety of cellular processes by mediating extracellular (mitogenic growth factor, insulin and stress) and intracellular (altered tyrosine receptor kinases, Ras and Src) signals. Activation of Akt by these signals is via its pleckstrin homology (PH) domain binding to products of phosphatidylinositol 3-kinase (PI3K). This process is negatively regulated by a dual phosphatase PTEN tumor suppressor. Today, more than 30 Akt substrates have been identified. These phosphorylation events mediate the effects of Akt on cell survival, growth, differentiation, angiogenesis, migration and metabolism. Further, PI3K/PTEN/Akt pathway is frequently altered in many human malignancies and overexpression of Akt induces malignant transformation and chemoresistance. Thus, the Akt pathway is a major target for anti-cancer drug development. This review focuses on Akt signaling mechanism in oncogenesis and chemoresistance, and ongoing translational efforts to therapeutically target Akt.

## 2. BACKGROUND

Akt was originally identified as the oncogene transduced by AKT8 acute transforming retrovirus. The AKT8 retrovirus was isolated from an AKR mouse thymoma in 1977. This virus induces malignant transformation in the mink lung epithelial cell line CCL-64 and tumor formation, specifically thymic lymphoma, in nude mice (1). A decade later, its defective retrovirus was

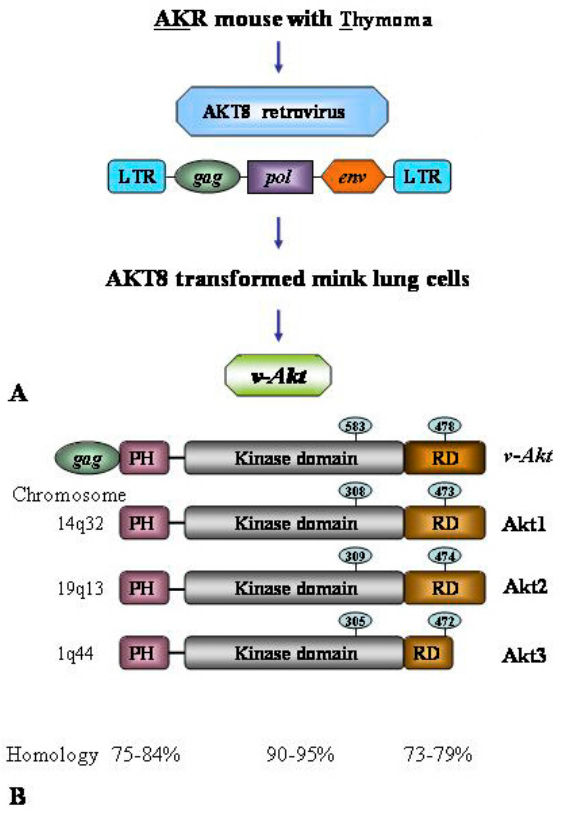


**Figure 1.** Identification and comparison of Akt. (A) v-Akt was cloned from mink lung cells transformed with AKT8 retrovirus. The AKT8 virus was isolated from a thymoma in AKR mouse. (B) Domain structure of Akt. The chromosomal localization of Akt isoforms is listed on left and the percentage of homology is shown on the bottom.

identified from mink lung epithelial cells infected with AKT8 virus, and was shown to contain a cell-derived oncogenic sequence, which was termed Akt (2, Figure 1).

In early 1990, sequence analysis of the Akt viral oncogene and its cellular homolog revealed that it encodes a serine-threonine protein kinase, composed of a carboxy-terminal kinase domain very similar to that of PKC and PKA and an amino terminal PH domain (3). Akt was also cloned based on its homology with PKC or PKA by two additional groups, who named it RAC or PKB (4, 5). To date, the protein is most commonly referred to as Akt/PKB.

## 3. COMPARISON OF AKT ISOFORMS

### 3.1. Sequence and expression pattern

Three major isoforms of Akt encoded by three separate genes have been identified in mammalian cells. Akt1/PKBα and Akt2/PKBβ were the first isolated isoforms (3-7). Akt3/PKBγ was subsequently cloned through homology screening (8, 9). While Akt1 is the true human homologue of v-akt (98% identity at the amino acid level), Akt2 and Akt3 are v-akt closely related kinases (6, 9). The three isoforms of Akt/PKB are highly homologous to v-akt. The overall homology between these three isoforms is >85%. They share a very similar structure, which contains an N-terminal PH domain, a central kinase domain, and a serine/threonine-rich C-terminal region. All three Akt/PKB isoforms possess conserved threonine and serine residues (T308/S473 in Akt1, T309/S474 in Akt2 and T305/S472 in Akt3) that together with the PH domain are critical for Akt/PKB activation. The C-terminal regions between these three isoforms are more diverse (homology 73%~84%) as compared to the kinase domain (homology 90%~95%), suggesting that C-terminal regions may represent functional difference between Akt1, Akt2 and Akt3 (Figure 1).

Although Akt1, Akt2, and Akt3 display high sequence homology, there are clear differences between them in terms of biological and physiological function: 1) overexpression of wild type (WT)-Akt2, but not Akt1 and Akt3, transforms NIH 3T3 cells and induces invasion and metastasis in human breast and ovarian cancer cells (10, 11); 2) *Akt2*, but not *Akt1* and *Akt3*, is frequently amplified in certain types of human cancer even though alterations of three isoforms of Akt have been detected at kinase and protein levels in human malignancies (6, 12-19); 3) *Akt1* expression is relatively uniform in various normal organs whereas high levels of *Akt2* and *Akt3* mRNA are detected in skeletal muscle, heart, placenta and brain (6, 9, 20, 21); 4) Akt2 but not Akt1 plays un unique role in muscle differentiation (22, 23) and 5) *Akt1*-, *Akt2*- and *Akt3*-deficient mice displayed different phenotypes (24-27).

### 3.2. Phenotype of the Akt knock-out mouse

A knockout study demonstrated that mice deficient in *Akt2* are impaired in the ability of insulin to lower blood glucose because of defects in the action of the hormone on skeletal muscle and liver. *Akt2*$^{-/-}$ mice are born without apparent defects, but develop peripheral insulin resistance and nonsuppressible hepatic glucose production, resulting in hyperglycemia accompanied by inadequate compensatory hyperinsulinemia (24), similar in some important features to type 2 diabetes in human. These phenotypic characteristics are not compensated by the presence of *Akt1* and *Akt3*, reflecting differences of substrate specificity in insulin-responsive tissues. In contrast, *Akt1*-deficient mice do not display a diabetic phenotype. The mice are viable but display impairment in organismal growth with smaller organs than wild type littermates (25, 26). Such relatively subtle phenotypic changes in *Akt1*$^{-/-}$ mice suggest that Akt2 and Akt3 may substitute to some extent for Akt1 (26). In contrast, a recent report shows that *Akt3* knock out mice exhibit a uniformly reduced brain size, affecting all major brain regions, suggesting a central role of Akt3 in postnatal development of the brain (27). Nevertheless, these data indicate that there are non-redundant functions between 3 isoforms of Akt in certain tissues and/or cell types.

## 4. AKT ONCOGENIC ACTIVITY AND ITS ROLE IN CHEMORESISTANCE

### 4.1. Mechanism of Akt activation

Akt is activated by a variety of stimuli, including growth factors, protein phosphatase inhibitors, and cellular

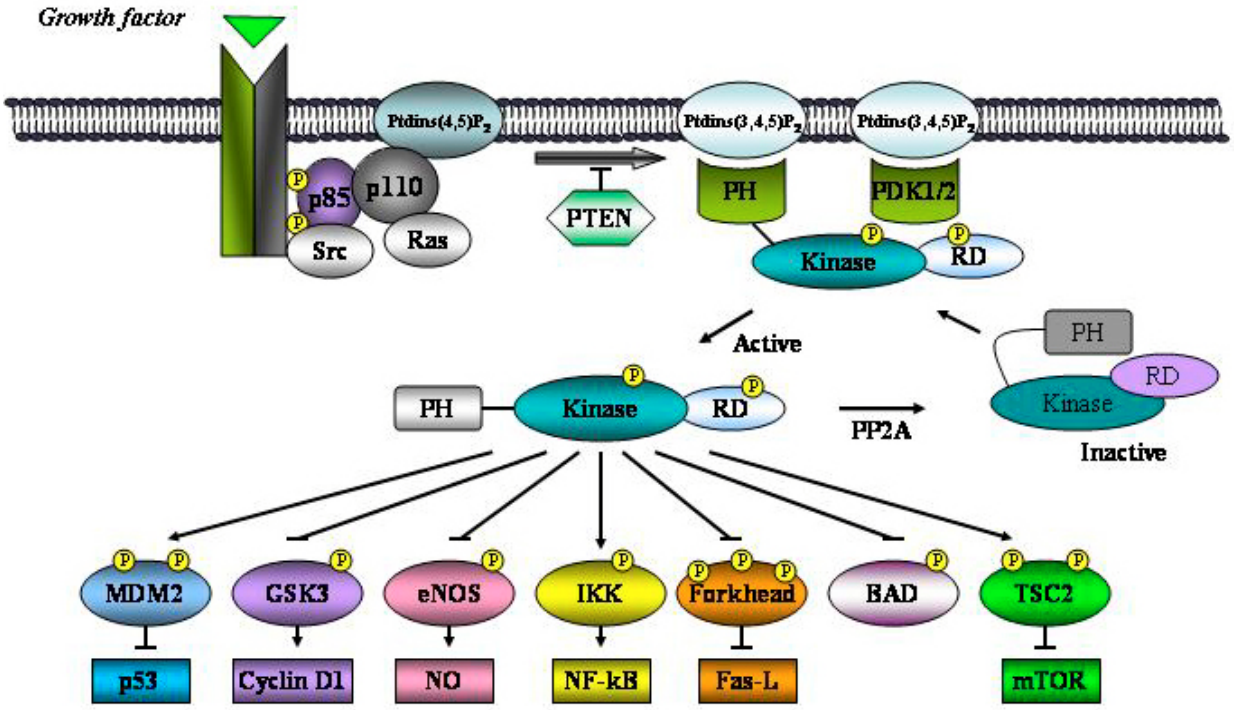


**Figure 2.** Schematic representation of Akt activation. Extracellular (growth factors) and intracellular (active Ras and Src) stimuli activate phosphatidylinositol 3-kinase, which results in production of phosphatidylinositol-3,4,5-trisphosphate (PIP3). PH domains of Akt and PDK1 subsequently bind to PIP3 leading to phosphorylation of Thr-308 and Ser-473 and activation of Akt.

stress in a PI3K-dependent manner (28-31). Activation of Akt depends on the integrity of the PH domain, which binds to PI3K products PtdIns-3,4-P2 and PtdIns-3,4,5-P3, and on the phosphorylation of $Thr^{308}$ ($Thr^{309}$ in Akt2 and $Thr^{305}$ in Akt3) in the activation loop and $Ser^{473}$ ($Ser^{474}$ in Akt2 and $Ser^{472}$ in Akt3) in the C-terminal activation domain by PDK1 and ILK or DNA-PK (Figure 2, ref. 32-34). The activity of Akt is negatively regulated by *PTEN*, a tumor suppressor gene that is mutated in a number of human malignancies. *PTEN* encodes a dual-specificity protein and lipid phosphatase that reduces intracellular levels of PtdIns-3,4-P2 and PtdIns-3,4,5-P3 in cells by converting them to PtdIns-4-P1 and PtdIns-4,5-P2, respectively, thereby inhibiting the PI3K/Akt pathway (35, 36).

### 4.2. Transforming activity of Akt

Previous studies demonstrated that overexpression of WT-Akt2, but not WT-Akt1, in NIH 3T3 cells resulted in malignant transformation (10). Ahmed *et al.* also showed that Akt1 is not tumorigenic when overexpressed in the nontumorigenic rat T cell lymphoma cell line 5675. In contrast, *v-akt*-expressing 5675 cells and active forms of Akt-expressing chicken embryo fibroblasts were highly tumorigenic (37, 38). Since v-akt arose by way of an in-frame fusion of the viral *Gag* and *Akt*, the oncogenic difference between v-akt and Akt1 may be due to myristoylation of the amino-terminus of v-akt (3, 20, 38). Several lines of evidence show that the PH domain of Akt is required for its membrane translocation and activation, and that attachment of a membrane-targeting sequence (myristoylation/palmitoylation) to the amino-terminus of Akt is sufficient to induce its maximal activation (39). Recent data also show that overexpression of constitutively active *Akt1* and *Akt3*, but not kinase-dead *Akt1* (Myr-Akt1-K179M) and *Akt3*, in NIH 3T3 cells leads to oncogenic transformation (14 and unpublished data). These results suggest that the kinase activity of Akt contributes to the control of cell transformation.

### 4.3. Alterations of Akt pathway in human cancer

*Akt2* locates at chromosome 19q13, which it is frequently overpresented in human cancers. Amplification of the *Akt2* has been observed in 15% of human ovarian carcinomas and 20% of human pancreatic cancers (6, 12, 17, 19). In contrast to *Akt2*, *Akt1* has been reported to be amplified in only a single human gastric carcinoma (2). Because of its location at chromosome band 14q32, proximal to the *IGH* locus, *Akt1* had been proposed as a candidate gene targeted by 14q32 chromosome rearrangements in human T-cell malignancies, prolymphocytic leukemias, and mixed lineage childhood leukemia. However, no such alteration of *Akt1* was detected in more than 30 hematologic specimens examined (unpublished data). Accumulated studies have shown frequent overexpression and/or activation of Akt in different human malignancies (12-19, 40-42). Alterations of Akt were predominantly observed in late stage and high-grade tumors, suggesting that Akt plays an important role in tumor progression rather than initiation.

### 4.4. Akt and chemoresistance

Recent studies indicate that overexpression of HER-2/neu or Xiap renders tumor cells resistant to TNFα

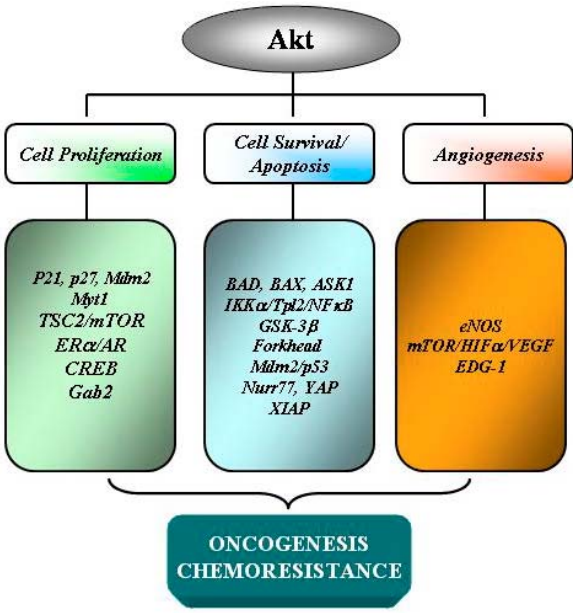


**Figure 3.** Mmechanism of Akt involvement in human oncogenesis and chemoresistance.

or to chemotherapeutic agents through activation of the PI3K/Akt pathway (43-45). Cancer cells either expressing constitutively active Akt or containing *Akt* gene amplification are also far more resistant to paclitaxel than cancer cells expressing low levels of Akt (46). We have recently observed that cisplatin-sensitive ovarian cancer cells (A2780s and OV2008) transfected with constitutively active Akt2 become resistant to cisplatin, whereas overexpression of dominant-negative (DN) Akt2 renders cisplatin-resistant ovarian cancer cells (A2780cp and C13) susceptible to cisplatin-induced apoptosis (47, 48). In addition, we previously reported inhibition of tumorigenecity and invasiveness of pancreatic cancer cell lines by antisense Akt2 (12) and recently demonstrated that PI3K/Akt is a critical target for farnesyltransferase inhibitor (FTI) and geranylgeranyltransferase I inhibitor (GGTI) -induced apoptosis. Constitutively active Akt overcame FTI-277 and GGTI298-induced programmed cell death (49, 50). Taken together, these data indicate that the Akt pathway is a critical target for cancer intervention and that activation of this pathway is associated with chemoresistance in human cancer.

The molecular mechanism by which Akt induces transformation and drug resistance is still not fully understood. It is believed that Akt anti-apoptotic activity and induction of cell cycle progression largely contribute to these processes (Figure 3).

## 5. NORMAL CELLULAR FUNCTION OF AKT

### 5.1. Anti-apoptosis

#### 5.1.1 . Apoptotic proteins

In numerous cell types, it has been shown that Akt induces cell survival and suppresses apoptotic death induced by a variety of stimuli. A major identified target of Akt is BAD, which is a BH3 domain-containing proapoptotic protein that binds Bcl-2 and Bcl-XL and inhibits their anti-apoptotic potential (51). When BAD is phosphorylated on Ser1-36 by Akt, it does not exhibit proapoptotic activity in cells. It has also been shown that Akt activates PAK1, which in turn phosphorylates BAD at Ser-112 resulting in its release from Bcl-$_{xL}$ complex (52). Once phosphorylated, BAD is released from a complex with Bcl-2/Bcl-$x_L$ that is localized on the mitochondrial membrane, and forms a complex with 14-3-3 proteins (51-53).

BAX is a 21-kDa protein that is important in controlling cell death, particularly in hematopoietic cells. Cells that overexpress BAX show enhanced apoptosis (54), whereas BAX-null cells display resistance to both spontaneous and induced apoptosis. The BAX protein is normally found in the cytoplasm heterodimerized to anti-apoptotic Bcl-2 family members such as Mcl-1 and Bcl-$x_L$; however, once the cell is exposed to an apoptotic stimulus, BAX translocates to the mitochondria (55–57), where it is thought to form oligomers. These promote apoptosis by forming large transmembranous pores, resulting in the loss of mitochondrial membrane potential and the release of cytochrome *c* (58, 59). We and others have shown that ectopic expression of Akt inhibits BAX conformational change and mitochondrial translocation induced by chemotherapeutic reagents. A recent study suggests that Akt might phosphorylate BAX at Ser-184 (60).

A previous study shows Akt inhibition of apoptosis at the postmitochondrial level (61). An X-linked inhibitor of apoptosis protein (XIAP) has been recognized as an important antiapoptotic protein by direct interaction and inhibition of activated caspases 9, 3 and 7 at postmitochondrial level (62-68). It is known that a number of chemotherapeutic reagents induce XIAP degradation leading to programmed cell death (69-71). Elevated level of XIAP rendered cells resistant to cisplatin whereas knockdown XIAP sensitized cells to apoptosis induced by cisplatin and trail (70, 72, 73). We have recently demonstrated that XIAP is a direct substrate of Akt. Akt phosphorylates XIAP and inhibits XIAP ubiquitination/degradation induced by cisplatin (74). Knockdown XIAP by RNA interference or antisense XIAP largely abrogates Akt-induced cisplatin resistance (74). Therefore, XIAP is a major target of Akt at postmitochondrial level.

Human caspase-9 has been reported to be phosphorylated by Akt, resulting in attenuation of its activity (75). However, the phosphorylation site is not conserved in other mammalian species, suggesting that this regulation of Akt is not likely to be a major physiological regulatory pathway.

#### 5.1.2. Other cell survival- related molecules

Accumulated evidence has shown that JNK is activated by a number of chemotherapeutic drugs and plays an essential role in anti-tumor reagents-induced programmed cell death (76, 77). Knockout JNK renders cells resistant to DNA damage-stimulated apoptosis (78). It has been shown that JNK mediates chemotherapeutic

drug-induced apoptosis by phosphorylation of Bim, Bmf and Bid (79). We and others have previously demonstrated that Akt inhibited JNK activation induced by cisplatin through phosphorylation of apoptosis signal regulating kinase ASK1. The phosphorylation of ASK1 by Akt inhibited its kinase activity and failed to stimulate JNK activation (59, 80, 81).

Forkhead transcription factor (FoxO1, FoxO3, FoxO4, previously known as FKHR, FKHRL1 and AFX) is important in the induction of apoptosis (82, 83). Their target genes include FasL and Bim, which plays a pivotal role in death receptor and mitochondrial pathways. Akt phosphorylates FoxO at three serine/threonine sites (84-86). Upon phosphorylation of FoxO proteins by Akt, FoxO binds to 14-3-3 proteins which results in translocation of FoxO to the cytosol from the nucleus and consequently inactivation of its function as a transcription factor (84, 85). Akt has been demonstrated to phosphorylate Yes-associated protein (YAP) and induce its association with 14-3-3 proteins. As is the case for FoxO, this results in the localization of YAP into the cytosol (87). YAP is a transcriptional co-activator which binds to p73 and promotes the transcription of its target genes. As p73 is a member of p53 family that plays an important role in the induction of apoptosis, Akt phosphorylation of YAP impairs the transcriptional activity of p73 and attenuates the induction of pro-apoptotic gene expression in response to DNA damaging agents.

There have been some indications that Akt can induce the expression of pro-survival genes, including IAPs and Bcl2. This may be due to positive cross-talk between the Akt and NFκB pathways. Activation of NFκB is dependent on the phosphorylation and degradation of IκB, an inhibitor of NFκB, by the IκB kinase (IKK) complex. Akt has been shown to regulate IKK activity in both direct and indirect manner. It has been shown that Akt interacts with and phosphorylates IKKα on Thr-23 (88, 89). Several studies have also provided evidence that Akt phosphorylates Ser/Thr kinase Tpl-2 (or Cot) on Ser-400, resulting in IKK complex activation (90, 91).

### 5.2. Cell cycle progression

#### 5.2.1. Cell cycle regulators

Akt targets several key cell cycle regulators including $p21^{cip1/waf1}$, $p27^{kip1}$, and MDM2. Akt phosphorylates $p21^{cip1/waf1}$ on residues Thr-145 and Ser-146 (92-94). The phosphorylation of Thr-145 inhibits p21 nuclear localization and affinity to Cdk2, Cdk4 and PCNA leading to activation of cyclin/CDK and DNA replication. However, phosphorylation of Ser-146 enhances protein stability of p21 that may result in cell survival (93, 94). Human $p27^{kip1}$, another major cyclin/CDK inhibitor, is also phsophorylated by Akt on Thr-157, even though this site is not conserved in other species (95-97). As Thr-157 resides within a nuclear localization signal (NLS) region, Akt phosphorylation of Thr-157 leads to p27 exclusion from the nucleus. Our laboratory has shown that Akt decreases TSC1/TSC2 tumor complex by phosphorylation of TSC2 resulting in the destabilization of p27 (98). In addition, Akt phosphrylation of forkhead protein inhibits p27 at transcriptional level (99). Taken collectively, Akt downregulates p27 at different levels leading to activation of cyclin/Cdk and cell cycle progression (95-99)

MDM2 is a major negative regulator of p53 tumor suppressor. Loss of p53 function has been thought to be a major mechanism of chemoresistance (100). MDM2 has ubiquitin E3 ligase activity, directly binds to p53 and targets it for ubiquitination and proteasome degradation (101, 102). Akt has been shown to phosphorylate MDM2 on Ser-166 and Ser-186 and induce MDM2-mediated p53 ubiquitination, even though there is still controversial regarding subcellular localization of Akt phosphorylated MDM2 (103-105).

#### 5.2.2. mTOR and TSC2

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase and is best known as a regulator of cell cycle progression and cell proliferation by integrating signals from nutrients (amino acids and energy) and growth factors (106-108). The best known biochemical function of mTOR is to regulate protein translation by initiation of mRNA translation and ribosome synthesis leading to an increased rate of cell growth (an increase in cell mass and size), which is required for supporting the rapid proliferation (an increase in cell number). A model for mTOR function suggests that mTOR regulates primarily the rate of cell growth and secondarily cell cycle progression. The mTOR-dependent downstream effectors, S6K1, 4E-BP1, and eIF4E, also regulate the rate of cell cycle progression. When quiescent U2OS cells are stimulated with serum to enter $G_1$ phase from $G_0$, overexpression of S6K1 and eIF4E accelerates S phase entry, while reduced expression of S6K1 with RNAi or overexpression of a dominant mutant of 4E-BP1 inhibits the rate of S phase entry (109, 110). Moreover, overexpression of rapamycin-resistant mutants of S6K1 or overexpression of eIF4E partially rescues the rapamycin-induced delay in $G_1$ progression to S phase, indicating that S6K1 and eIF4E are downstream mediators of mTOR-dependent cell division (109, 110).

It has been shown that Akt mediates insulin and nutrient activation of mTOR pathway through both direct and indirect mechanism. Akt phosphorylates mTOR at serine-2448 and possible serine-2446, which fit the Akt phosphorylation consensus motif (RXRXXS/T) in 3T3-L1 adipocytes, HEK293 cells and intact skeletal muscle (111-115). Although one might expect that Akt phosophorylation of serine-2448 would activate mTOR, there is no direct evidence to support such an effect. In fact, when expressed in HEK293 cells, nonphosphorylatable mTOR-S2448A (converting serine to alanine) or mTOR-S2446/2448A exhibit the same effect as overexpressed wild type mTOR in mediating insulin-stimulated phosphorylation of S6K1 and 4E-BP1. These data suggest that mTOR is not a direct target of Akt. We and others have demonstrated that Akt interacts with and phosphorylates tuberin, a product of tumor suppressor tuberous sclerosis complex (TSC) 2 gene. A possible mechanism for Akt activation of mTOR has been proposed by finding of tumor suppressor TSC2 linking Akt to mTOR.

Tuberous sclerosis is an autosomal dominant disorder developing hamartomas in multiple organs and is caused by mutation of either the *TSC1* or the *TSC2* tumor suppressor gene. TSC1 and TSC2 function as a complex to inhibit cell growth. Overexpression of TSC2 and TSC1 inhibited mTOR activity and blocked the increase in phosphorylation of S6K1 and 4E-BP1 in response to nutrients or growth factor stimulation (116-118). We and others have shown that Akt phosphorylates TSC2 at multiple serine/threonine sites and causes to degradation of TSC2 and TSC1 and disruption of TSC1/TSC2 complex (98, 11-123), resulting in release of its inhibition of mTOR. Further, expression of nonphosphorylatable TSC2 mutants with alanine substitutions at Akt phosphorylation sites blocks growth factor-induced S6K1 activation. TSC2 has been shown to interact with overexpressed TOR in *Drosophila* but this interaction does not occur in mammalian cells (124).

Several studies have shown TSC2/TSC1 inhibition of mTOR through TSC2 GAP activity to hydrolyze Rheb-GTP to inactive Rheb-GDP form. Rheb is a GTP-binding protein and overexpresing Rheb increases S6K1 and 4E-BP1 phosphorylation but does not induce the activity of a rapamycin-resistant form of S6K1, suggesting that Rheb signaling to S6K1 is through mTOR and not through a parallel pathway. However, no evidence shows that Rheb directly activates mTOR. Disruption of Rheb in *S. cerevisiae* leads to an increase in the uptake of arginine and lysine by the amino acid permease Can 1p (125-128). This implies that Rheb may control mTOR indirectly by changing amino acid level. Recent studies have demonstrated that mTOR functions as part of a larger signaling complex. Two mTOR-associated proteins, Raptor and GβL, have been identified by sequencing proteins that coimmunoprecipitated with mTOR. The GβL binds to kinase domain of mTOR whereas Raptor links mTOR to S6K1 and 4E-BP1 by binding to their TOR signaling (TOS) motifs, leading to mTOR-dependent phosphorylation of S6K and 4E-BP1 in response to nutrients or growth factors (129-134). However, there is no evidence that these two proteins are involved in Akt regulation of mTOR.

In addition, a number of transcriptional factors that associate with cell cycle control are regulated by Akt. Cyclic AMP (cAMP)-response element binding protein (CREB), is phosphorylated by Akt on Ser-133. This process results in increased affinity of CREB to its co-activator CRB, leading to transcriptional upregulation of cell cycle associated genes such as cyclin D1 (135). Estrogen receptor (ER)α is also phosphorylated by Akt (136). The phosphorylated ERα will induce its target gene expression which is thought to involve anti-estrogen resistance (136, 137).

Finally, angiogenesis induced by Akt may also associate with its transforming activity and chemoresistance. Accumulated evidence shows that Akt plays a central role in the sprouting of new blood vessels by mediating many angiogenic growth factors and regulating downstream target molecules that are potentially involved in blood vessel growth. It is known that VEGF has various functions on endothelial cells, the most prominent of which is the induction of proliferation and differentiation by selectively binding to the Flk-1/KDR receptor and subsequent activation of Akt pathway (138). Constitutively active Akt also induces VEGF mRNA expression by stabilization (139) and enhanced translation (140) of HIF1α through regulation of mTOR pathway. Moreover, Akt phosphorylates eNOS on Ser-1177, resulting in enzymatic activation of eNOS (141), which leads to production of NO and angiogenesis.

## 6. AKT PATHWAY AS A POTENTIAL THERAPEUTIC TARGET FOR CANCER INTERVENTION

Although cytotoxic chemotherapeutic drugs are first-line agents for cancer, chemoresistance remains a major therapeutic hurdle. The prospect of gene targeted anti-tumor agents as a therapeutic approach for cancer, particularly for the chemoresistant disease, has generated considerable excitement.

As described above, Akt pathway is essential for cell survival, cell cycle progression and angiogenesis. Amplification/overexpression/activation of PIK3CA (p110α) enzymatic subunit of PI3K and Akt as well as somatic mutation of gene encoding p110α are frequently detected in human malignancy (142-146). Inhibition of PI3K and/or Akt induces programmed cell death in cancer cells (144). Expression of constitutively active Akt results in cancer cells resistance to cisplatin and taxol-induced apoptosis, whereas dominant negative Akt sensitizes the cells to chemotherapeutic drugs (147, 148). Thus, PI3K/Akt pathway is a critical target for cancer intervention and inhibition of PI3K and/or Akt could overcome a subset of chemoresistant cancers.

### 6.1. Biological approaches

Biological approaches include antisense, dominant-negative, antibody of PI3K and Akt as well as peptides to mimic and compete pleckstrin-homology (PH) domain of Akt binding to PI3K products, PtdIns-3,4-P2 and PtdIns-3,4,5-P3. We and other have previously demonstrated that the introduction of antisense Akt2 or DN-Akt into several Akt-overexpressing cancer cell lines abrogates endogenous Akt expression and diminishes their invasiveness and tumor formation in nude mice (12, 149). Antisense oligonucleotides of Akt can inhibit Akt pathway and induce apoptosis in different cell lines[141] and cell growth and survival can also be inhibited by the expression of dominant negative (DN) forms of PI3K and Akt (150, 151). Our recent data show that expression of DN-Akt in NIH3T3 cells remarkably reduces v-H-ras-induced colony formation and tumor formation (unpublished data). Moreover, consistent with the tumor-inhibitory effects of DN-PI3K and DN-Akt is the demonstration of the inhibition of Ras and BCR/ABL malignant transformation with p85ΔiSH2 and DN-Akt, respectively (152, 153). Microinjection of AKT2 antibody into myoblasts can also specifically block their function, i.e., induction of myotube (154). Further studies are required to investigate the effects of antibodies of PI3K and Akt on human cancer cell growth.

### 6.2. Pharmacological approaches for upstream inhibition of Akt

Although wortmanin and LY294002 efficiently abrogate PI3K activity and have been widely used in the cell culture system (155, 156), they have not been applied for clinical trails due to either toxicity (LY294002) or a short of half-live (wortmanin). We have demonstrated that farnesyltransferase inhibitor (FTI)-277, originally designed to block Ras oncoprotein, inhibits PI3K/Akt pathway and induces apoptosis in a number of human cancer cell lines (49, 157). FTIs are highly effective at inhibiting tumor growth without toxicity to normal cells. However, the mechanism by which they inhibit tumor growth is not well understood (158-160). FTIs are unable to induce apoptosis in Raf transformed NIH 3T3 cells even though MAPK pathway is inhibited by FTIs (158, 159), indicating that FTIs may target other cell survival pathway(s) regulated by Ras or other farnesylated proteins. Interestingly, our data showed that FTI-277 induces apoptosis only in Akt2-overexpressing human cancer cell lines. Furthermore, overexpression of Akt2, but not oncogenic H-Ras, sensitizes NIH 3T3 cells to FTI-277; and a high serum level prevents FTI-277-induced apoptosis in H-*Ras*- but not *Akt2*-transformed NIH 3T3 cells (49, 157). These data suggest that FTIs specifically target the PI3K/Akt pathway to inhibit tumor cell growth and may be candidate agents for reversing resistance of human cancer to cytotoxic chemotherapeutic drugs.

### 6.3. Akt inhibitors

The importance of Akt in cell survival, growth, cell transformation and human malignancy has prompted the search for specific and safe pharmacological inhibitors for Akt. Five recent reports including ours have identified the compounds as potential Akt inhibitors that reduce Akt kinase activity in a number of cancer cell lines (161-165).

Hu *et al.* synthesized a phosphatidylinositol analogue (1L-6-hydroxy-methyl-chiro-inositol 2*(R)*-2-*O*-methyl-3-*O*-octadecylcarbonate) and showed that it inhibited Akt by compete with phosphatidylinositol (161). This compound reduces the resistance of human leukemia cells to chemotherapeutic drugs and ionizing radiation (161). Chaudhary *et al.* demonstrated that the plant-derived pigment curcumin reduces Akt activity resulting in cell growth arrest in several prostate cancer cell lines (162). A compound synthesized from the natural plant compound rotenone (degeulin) has also been identified as a potential Akt and PI3K inhibitor in malignant human bronchial epithelial cells (163). Meuillet *et al.* have used a novel strategy to identify a group of D-3-deoxy-phosphatidyl-myo-inositols that bind to the PH domain of Akt, trapping it in the cytosol and preventing its activation in response to growth factors (164). We have recently identified a small molecule inhibitor of Akt, API (Akt/PKB signaling inhibitor)-2/TCN, by screening the National Cancer Institute Diversity Set (165). API-2 inhibited the kinase activity of Akt resulting in suppression of cell growth and induction of apoptosis in human cancer cells harboring constitutively activated Akt. API-2 is highly selective for Akt and does not inhibit the activation of PI3K, PDK1, PKC, SGK , PKA, STAT3, Erk-1/2, or JNK. Furthermore, API-2 potently inhibited tumor growth in nude mice of human cancer cells where Akt is aberrantly expressed/activated but not of those cancer cells where it is not. These findings suggest that API-2 exerts anti-tumor activity largely by inhibition of Akt (165).

## 7. PERSPECTIVE

In the past decade, the mechanism for Akt activation, lipid second messenger-mediated phosphorylation of Akt, has been well characterized. However, the physiological and pathological difference of three isoforms of Akt remains elusive. While Akt is a key molecule in cell survival, downstream targets that mediate this action are still obscure. As Akt plays a pivotal role in human cancer development and chemoresistance, it is essential that future work is aimed at developing pharmacological reagents as well as genetic and biochemical approaches that not only identify novel roles for Akt but also verify the physiological functions previously ascribed. The generation of a potent and specific Akt inhibitors, especially isoform-specific Akt inhibitors, would certainly revolutionize the study of the processes mediated by Akt in the same way inhibitors of MAP kinase kinase 1 activation (e.g., PD98059, PD184352, U0126) have on our understanding of processes regulated by the classical MAP kinase pathway. More importantly, such drugs or in combination with conventional chemotherapeutic agents would reasonably improve the outcome of human cancer.

## 8. ACKNOWLEDGEMENT

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