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Structure, activation and biological effects of AKT or protein kinase B

Lecturer Daniela MIRICESCU[1], MD, PhD, Assoc. Prof. Alexandra TOTAN[1], MD, PhD, Assist. Prof. Constantin STEFANI[2], MD, PhD, Assist. Prof. Iulia-Ioana STĂNESCU[1], MD, PhD, Assist. Prof. Ana Maria Alexandra STANESCU[3], MD, PhD, Assist. Prof. Ioana Ruxandra RUSU[4], MD, PhD, Assist. Prof. Radu RADULESCU[1], MD, PhD, Assist. Prof. George Costin RUSU[5], MD, PhD, Prof. Maria GREABU[1], MD, PhD

[1] Department of Biochemistry, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Department of Family Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania,
[3] "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[4] Department of Anatomy and Embryology, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[5] Department of Modern Languages, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[6] "Carol Davila" University Central Emergency Military Hospital, Bucharest, Romania

ABSTRACT

AKT or protein kinase B is a serine / threonine kinase that plays a crucial role in cell proliferation, survival, growth, and glucose metabolism. So far, there have been discovered 3 isoforms of AKT, the most widespread in the tissues is AKT1. All isoforms present similar structure being activated by the phosphorylation process at the level of 2 hydroxyl amino acids serine and threonine. After activation, AKT will phosphorylate a number of protein substrates which it will activate or inhibit, finally leading to lipids, proteins, glycogen or nucleotides synthesis. In this review, we will discuss the structure of these protein kinases, the molecular mechanism of activation and the phosphorylation effects on other cellular structures.

Keywords: protein kinase, phosphorylation, survival, proliferation

AKT IDENTIFICATION AND STRUCTURE

AKT or protein kinase B belongs to the super protein kinase family named AGC after the kinases members, c-AMP-dependent protein kinase A (PKA), protein kinase G (PKG) and protein kinase C (PKC). AGC kinases exhibited similar activation mechanisms and structural homology within the catalytic domain (1-3).

AKT is a serine / threonine kinase that was initially discovered in 1987 by Stefan Staal as the likely transforming gene component, v-AKT of AKT8 provirus. In the same study, Staal identified the human homologue of v-Akt, AKT 1 which was increased at patients with gastric adenocarcinomas (4).

In 1995, Richard Roth and co-workers discovered that AKT is activated by insulin (5). In mammals have been identified three AKT genes, termed AKT1/PKBα, AKT2/PKBβ and the last one AKT3 / PKBŸ. Of all 3 isoforms, AKT 1 is the most widely distributed at the tissue level, being involved in

Corresponding author:
Assoc. Prof. Alexandra Totan, MD, PhD, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
E-mail: alexandratotan99@gmail.com

cell growth and survival (6-8). AKT 2 is found at the muscular and adipocyte levels contributing to insulin mediated glucose homeostasis (8,9). The last isoform, AKT 3, has been identified especially at brain and endocrine tissues (testes) levels (8-11).

All AKT izoforms have a highly conserved structure: an N-terminal pleckstrin domain or PH domain, a kinase domain and a C-terminal regulatory tail which contains the hydrophobic motif (11).

The N-terminal domain contains about 100 amino acids, which is similar to other protein kinases that binds 3-phosphoinositides and interacts with membrane lipid products such as phosphotidylinosiol 3,4,5 trisphosphate (PIP3) produced by phosphatidylinositol 3-kinase (PI3K) (11-13).

At catalytic domain level phosphorylation of the Thr residue occurs both in the case of PKB but also in PKA and PKC, thus leading to the partial activation of these protein kinases (11,15,16).

The C-terminal domain contains about 40 amino acids having the following sequence in hydrophobic motif F-X-X-F / Y-Ser / Thr-Y / F, where X can be any amino acid. For the whole AGC protein kinases family phosphorylation of Ser and Thr residues is required for full activation in this hydrophobic motif (11). Akt 3 isoforms phosphorylate various substrates that contain in the C-terminal region the following amino acid sequence: RXRXX-Ser /Thr, for exemple PRAS40 (proline-rich Akt substrate of 40 kDa) can be phosphorylated by all 3 isoforms but Akt 1 phosphorylates actin associated with palladin protein(11,17).

AKT ACTIVATION

AKT signaling pathway is activated by various stimuli that are capable of inducing PIP3 formation by PI3K such as tyrosine kinase receptors, integrins, T and B cell receptors, cytokine receptors or receptors coupled with G proteins. In the extracellular domain of tyrosine kinase receptors (RTK), growth factors binds and will cause autophosphorylation of the receptor. Class 1 of PI3K binds to the phosphorylated receptor directly or *via* an adapter protein such as insulin receptor substrate 1/2 (IRS 1/IRS2).The PI3Ks will further catalyze phosphorylation of phosphatidyl inositol 4, 5 bisphostat (PIP2) to PIP3(11,17).

PTEN (phosphate and tensin homology) performs dephosphorylation of PIP3 at PIP2. The interaction between AKT PH domain and 3-phosphoinositol induces a conformational change in AKT and PDK1 (3-phosphoinositide-dependent protein kinase 1) will phosphorylate Thr 308. For maximum activity, mTOR (mammalian target of rapamycin complex) will phosphorylate AKT Ser 473 from hydrofobic motif (11,17,18).

Dephosphorylation of the 2 hydroxyl amino acids is carried out by PP2A (protein phosphatase 2A) specific Thr 308 and PHLPP (PH-domain leucine-rich-repeat-containing protein phosphatases) for Ser 473. Once activated Akt will dissociate from the membrane and further phosphorylate a wide variety of substrates, which are contained in the structure Ser or Thr such as protein or lipid kinases, transcriptional factors, metabolic enzymes (11,17).

AKT AND BIOLOGICAL EFFECTS

Active AKT is involved in cell survival, growth and proliferation and glucose uptake as can be seen from Table 1.

The AKT / PKB signaling pathway plays a crucial role in regulating cell survival, helping the cells in the fight against apoptosis. Apoptosis is characterized in mammalian cells as an early process that is associated with the loss of mitochondrial integrity followed by the release of cytochrome c.

The cytochrome c released then binds to the apoptotic protease-activating factor (Apaf -1) which it activates. Apaf-1 binds and activates capase-9 (proteases with cysteine residues), and initiates a caspase cascade, which are regulated by anti-apoptotic effectors (Bcl-2 and Bcl-xL) or pro-apoptotic proteins (Bad, Bid, Bik, Bax and Bak) (19,20).

Bad is a member of the Bcl-2 protein family that is phosphorylated by AKT on Ser 136, so it no longer exhibits pro-apoptotic activity at the cell level and promotes cell survival (20,21,22).

SAK (stress-activated protein kinase) is a family of protein kinases that regulates cellular response to stress or cytokines, consisting of 2 groups of kinases: JNK and p38 MAP kinases (23,24).

ASK1 (apoptosis signal-regulating kinase) is a MAP kinase that usually induces apoptosis, will interact with AKT and is phosphorylated at Ser 83, thus inhibiting the apoptotic process and promoting cell survival. AKT will phosphorylate both MLK3 (mixed lineage kinase 3) on Ser 674 and SEK 1 on Ser 78, the activity of these kinases will also be inhibited as in the case of ASK1, promoting cell survival and not apoptosis (25,26).

AKT promotes the regulation of cell survival through transcriptional factors that are responsible for pro and anti-apoptotic genes. The family of Fox or FH (forkhead) transcriptional factors has four Fox protein isoforms:Fox01, Fox02, Fox03,

TABLE 1. *The effects of AKT phosphorylation on different substrates (adapted from) (11).*

Cellular function	Substrate	Amino acid	AKT phosphorylation effect
Cell survival	BAD	Ser 136	Release of Bcl-2 proteins
	MLK3	Ser 674	Apoptosis inhibition
	ASK1	Ser 83	Apoptosis inhibition
	SEK1	Ser 78	Apoptosis inhibition
	FOX01	Thr 24, Ser 256, 319	Apoptosis inhibition
	FOX03	Thr 32, Ser 253, 315	Apoptosis inhibition
	FOX04	Thr 28, Ser 139, 258	Apoptosis inhibition
	MDM2	Ser 133	Inactivation of p53
	Ik-B kinase	Thr 23	Transcriptional activity of NF-kB
	CREB	Ser133	Activation of antiapoptotic genes
	YAP	Ser 127	Suppresor of apoptosis
Cell growth	TSC complex (TSC1/TSC2)	Ser 939,981, 1130, 1132,Thr 11462	Synthesis of proteins, lipids and nucleodites
Glucose homeostasis	GSK3α	Ser 21	Glycogen synthesis
	GSK3β	Ser9	Glycogen synthesis
Cell proliferation	Cyclin D,	Thr 58, 286	G1/S progression
	Cyclin B	Ser 354	G2/M transition

Fox04 which can be directly phosphorylated by AKT. Phosphorylated Fox proteins promote cell survival through their action on specific target genes that normally inhibit cell survival (27,28).

Family of nuclear transcription factor kB (NF-kB) is a key regulator of immune response, and a deregulation of its activity leads to the development of pathologies such as autoimmune diseases and cancer (29,30).

NF-kB is activated by phosphorylation of the kinase complex IkB (inhibitor of kappa B kinases), which leads to its nuclear translocation and transcription of specific survival genes for Bcl-xL and caspase inhibitors (30-32).

Mdm2 (murine double minute) is an oncogene product induced by p53, the major regulator of cell death in response to stress, especially when DNA damage occurs. AKT phosphorylates Mdm2 at 2 Ser residues, resulting in promoting inactivation or degradation of p53 and undermine the p53 to mediate pro-apoptotic transcriptional responses (33-35). CREB (Cyclic AMP (Camp)-response element binding protein) is a transcription factor, which can be phosphorylated by AKT on Ser 133, inducing expression of some antiapoptotic genes such as Bcl-2 (36).

YAP (Yes-associated protein) is phosphorylated by AKT on Ser 127 and in the phosphorylated form is a suppressor for apoptosis mediated by p73 transcriptional activity (37,38).

AKT is involved in regulating cell growth through its effects on the tuberous sclerosis complex 1 and 2 (TSC1 / TSC2) and the mTORC signaling pathway. The primary mechanism by which AKT activates mTORC is the phosphorylation on Ser 2448 and TSC complex inhibition . TSC complex acts as a GAP specific for Ras-related GTPase Rheb, which will promote conversion of Rheb-GDP to Rheb-GTP and mTORC1 activation, which will further determine synthesis of proteins, lipids and nucleotides and autophagy (39). mTOR phosphorylates S6K1(kinases p70S6K1) and 4E-BP1(eIF4E-binding protein 1), leading to increased translation and synthesis of cell-cycle-regulating and ribosomal proteins(17,39).

AKT is involved in phosphorylation of glycogen synthase kinase 3 at the N-terminus Ser residue, GSK-3α, Ser 21 and for GSK3β, Ser 39. Phosphorylated GSK3, inhibits its kinase activity and also inhibits glycogen synthase. AKT-mediated inhibition of GSK3 activity, but dephosphorylates and activates glycogen synthase via PP1 (phosphoprotein phosphatase) which is activated by insulin or glucose leading to glycogen synthesis (17).

There is a close relationship between AKT and GSK3 in terms of metabolism and cell survival: phosphorylation and inbibation of GSK-3 mediates some of the effects of AKT. Phosphorylation of GSK-3 by AKT was considered to be a mechanism by which cell proliferation is also achieved (11,17).

AKT can phosphorylates protein tyrosine phosphatase 1B (PTB1B), which prevents insulin receptor (IR) dephosphorylation and translocation of glucose transporter 4 (GLUT 4) from vesicular intracellular compartments to the plasma membrane and intracellular glucose uptake (11,17).

AKT is involved in the control of the cell cycle beeing essential for meiosis, and dispensable for mitosis, by phosphorylating some target proteins that will lead to their activation or inactivation. AKT activetes cyclin B/CDK1 by phosphorylation, and coordinates the activation of cyclin B/cdk1

(cyclin dependent kinase 1) at the centrosome and in the nucleus. Cyclins B, D,E are activated which will finally activate cdk2 and cdk1 which will determine G2 / M transition, cdk4 / 6 and chk2 will determine G1 / S transition (40).

CONCLUSIONS

AKT is a serine-threonine kinase that is activated by phosphorylation, which further phosphorylates a number of proteins that contain Ser or Thr residues. These phosphorylations are essential for cell proliferation, growth and survival. In conclusion, the study of molecular mechanisms of AKT activation and further phosphorylation are crucial for a healthy human body.

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