

## **PROTEIN KINASE B/AKT : Structure, Functions and Regulation.**

PROTEİN KİNAZ B/AKT : Yapısı, Fonksiyonları ve Regülasyonu.

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### **ABSTRACT:**

*Protein kinase B (PKB)/Akt is a serine/threonine kinase activated by growth hormones and implicated in prevention of apoptosis, glycogen metabolism and glucose uptake. PKB/Akt regulates a variety of cellular functions including cell survival, cell growth, cell differentiation, cell cycle progression, transcription, translation and cellular metabolism. This review will summarize the structure and functions of PKB/Akt and indicates the evidence that PKB/Akt is an important mediator of physiological effects of insulin and several growth factors and plays a key role in various cellular functions.*

**Key Words:** *Protein kinase B, Insulin*

### **ÖZET:**

*Protein kinaz B/Akt, büyüme hormonları ile aktive olan serin/treonin kinazdır ve apoptozisin önlenmesi, glikojen metabolizması ve glukoz emiliminde rolü vardır. PKB/Akt hücre hayatıyetinin devamı, hücre büyümesi ve farklılaşması , hücre döngüsü, transkripsiyon, translasyon ve hücre metabolizması gibi çeşitli hücrel işlevlerini regüle etmektedir. Bu derleme PKB/Akt nin yapısını , fonksiyonlarını özetlemekte ve PKB/Akt nin insulinin ve bazı büyüme faktörlerinin fizyolojik etkilerine aracılık etmesinin yanısıra çeşitli hücrel fonksiyonlardaki anahtar rolünün önemini de vurgulamaktadır.*

**Anahtar Kelimeler:** *Protein kinaz B, İnsulin*

### **INTRODUCTION:**

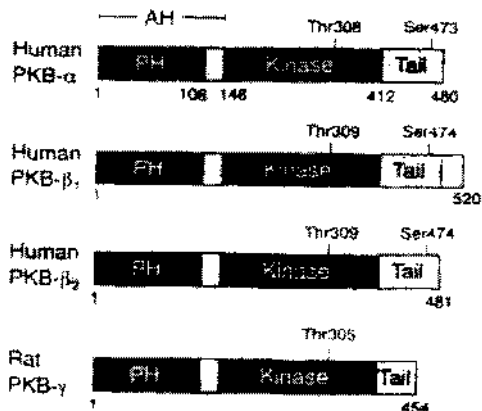
Akt (protein kinase B) identified in 1991. Akt was also termed PKB or Rac. Akt is a 60 kDa serine/threonine kinase. As a result of its homology with both protein kinase C (73% similarity with the kinase domain of PKC) and protein kinase A ( 68% similarity with the kinase

domain of PKA) it was named as protein kinase B (PKB) or RAC-PK (Related to the A and C kinases) (1, 2). Akt was also identified as the product of the oncogene v-akt of the acutely transforming retrovirus AKT8 found in a rodent T-cell lymphoma (3). All three names have been used widely to describe this kinase. PKB/Akt is a target for phosphoinositide 3-OH kinase and plays a key role in various cellular functions.

### Structure of Protein Kinase B/Akt

PKB/Akt is a serine/threonine kinase composed of an NH<sub>2</sub>-terminal pleckstrin homology (PH) domain and a COOH-terminal catalytic domain (2). The PH domain (aminoacids 1-106) makes up the major part of the amino-terminal regulatory domain of PKB/Akt, which spans residues 1-147 and has been referred to as the Akt-homology (AH) domain. The kinase domain stretches from residues 148-411 with the carboxy-terminal tail region (amino acids 412-480) accounting for the remainder of the protein (Fig. 1) (4).

There are 4 known PKB/Akt isoforms. PKB $\alpha$ , PKB $\beta_1$ , PKB $\beta_2$  and PKB $\gamma$ . PKB $\alpha$  also termed Akt-1 or RAC-PK $\alpha$  and PKB $\beta$  termed as Akt-2 or RAC-PKB $\beta$ . (Fig. 1) (5, 6, 7).



**Figure 1:** Schematic representation of the four known PKB/Akt isoforms.(4)

PKB/Akt molecules are able to dimerize and to interact with other proteins through an NH<sub>2</sub>-terminal PH domain (7) Homo-oligomerization of Akt is induced by interaction with Phosphatidylinositol-3,4-diphosphate and increases Akt activity (8). The integrity of NH<sub>2</sub>-terminal PH domain is required for in vivo activation of Akt by several growth factors, by constitutively active PI-3 kinase (PI3K) and also by certain pathways independent of PI3K (8,9). In contrast, the Akt PH domain is not required for PKB/Akt activation by okadaic acid or insulin in cells overexpressing the insulin receptor (10) These results indicate that the relative

importance of the Akt PH domain for activation depends on the cell type or the stimulus used. For example, it is activated upon T-cell antigen receptor engagement or upon an active form of phosphoinositide 3-kinase (PI3K) in T lymphocytes. Genot et al investigated the small GTPase Rac-1 is implicated in this pathway. This is the first report of a membrane receptor employing Rac-1 as a downstream transducer of Akt activation (11).

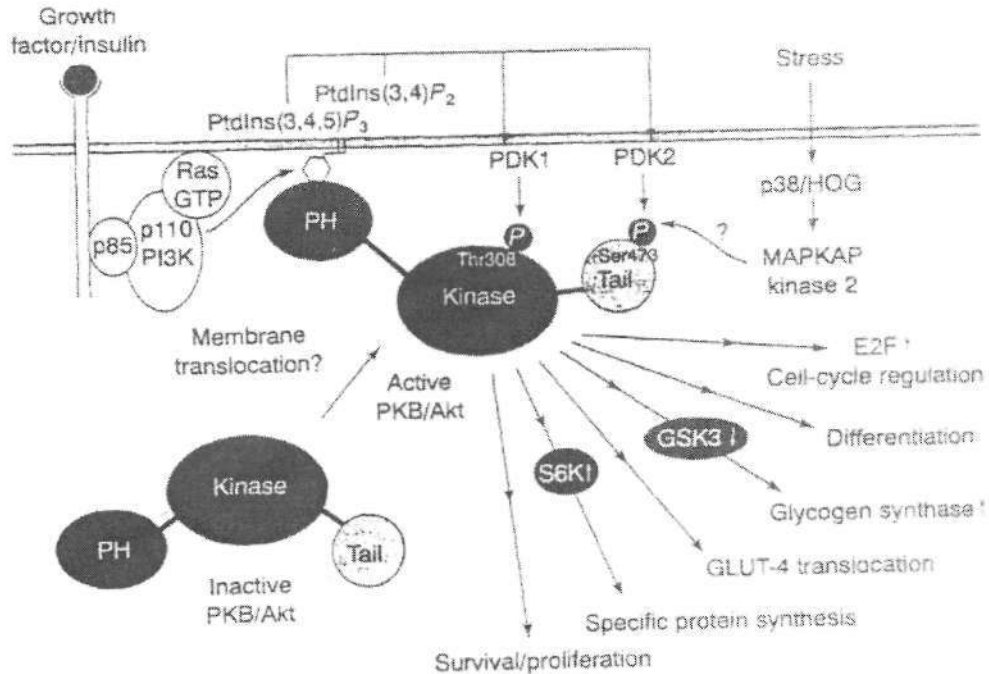
### **Regulation of PKB/Akt**

Full activation of PKB/Akt requires phosphorylation of Thr308 and Ser473. PKB/Akt is activated in response to treatment of cells with a wide variety of growth stimuli, including platelet derived growth factor (PDGF), epidermal growth factor (EGF), insulin, thrombin and nerve growth factor (NGF). Lipid kinase PI3K is involved in regulation of PKB/Akt (Fig.2) (12,13).

Synthesis of activated forms of PI3K results in stimulation of PKB/Akt. After the activation of PI3K, the membrane-bound lipid phosphatidylinositol 3,4,5,-triphosphate (PIP3) is synthesized and recruits 3-phosphoinositide-dependent kinase (PDK1) by binding to its PH domain (14,15). IP3 and phosphatidylinositol 3,4-bisphosphate recruit PKB/Akt to the plasma membrane by interacting with the PH domain of PKB/Akt (8). PDK1 phosphorylates Thr308 of PKB/Akt and PDK2 phosphorylates Ser473 of Akt (14). PKB/Akt phosphorylated at both sites becomes active and phosphorylates target proteins. On the other hand, mitogen-activated protein kinase (MAPK)- activated protein kinase 2 (MAPKAP-kinase 2) can phosphorylate Ser473 in vitro (16) and might contribute the activation of PKB/Akt by certain cellular stresses. However, MAPKAPkinase -2 is responsible for the phosphorylation of Ser473 .

PKB/Akt following growth factor stimulation of cells. Other stimuli such as heat shock, hyperosmolarity and cAMP activate PKB/Akt in a PI3K-independent manner (7,17).

Kroner et al. reported the major PKB subtype in platelets is PKB $\alpha$  which is activated by phosphorylation of Thr308 and Ser473 and has a constitutively phosphorylated Thr450 that does not contribute to PKB activation . Their data reveal a PI3K-independent PKB activation in which PKC $\alpha$ /beta regulates the phosphorylation of Ser473 in PKB $\alpha$ . The independent control of the phosphorylation sites may contribute to fine regulation of PKB $\alpha$  activity (18).



**Figure 2:** A model for the activation of PKB/Akt by PI3K-dependent mechanisms (12,13).

### Functions of PKB/Akt

PKB/ Akt regulates a variety of cellular functions including cell survival, cell growth, cell differentiation, cell cycle progression, transcription, translation and cellular metabolism .

PKB/Akt mediates the effect of PI3kinase on some cellular events, such as apoptosis and protein synthesis (13,19). It is also thought to mediate the phosphorylation and inactivation of glycogen synthase kinase-3 (GSK-3) by insulin (20). GSK-3 is a negative regulator of glycogen synthesis, it inhibits GS (Glycogensyntase) activity (21). Phosphorylation of GSK-3 by PKB/Akt results in its inactivation and the consequent activation of glycogen synthesis.

Another target for PKB/Akt is the ribosomal protein S6 kinase(14). This kinase is responsible for altering the pattern of protein synthesis.

In addition, PKB/Akt has been found to stimulate glucose uptake and glucose transporter- 4 (GLUT-4) translocation and induce adipocyte differentiation (22,23). Overexpression of PKB/Akt prevents apoptosis in primary cultures of cerebral neurons that are induced by survival factor withdrawal or inhibition of PI3K. Activated forms of PI3K and PKB/Akt are sufficient to prevent apoptosis (24). Previous data (25) indicated that survival of

several cell line is PI3K-dependent and the new results show that IGF-1 protects cerebellar neurons from apoptosis by activating PKB/Akt.

In summary, extracellular stimuli that induce PKB/Akt activation include growth factors, cytokines and antigen receptors . Most, if not all, of these stimuli promote cell survival and proliferation. Although mechanisms by which PKB/Akt activation contributes to cell proliferation are not fully understood, some of the PKB/Akt targets are involved in cell survival (26).

In addition to the effects of PKB/Akt, endothelial nitric oxide syntase (eNOS) also phosphorylated by PKB/Akt at serinell79 and this phosphorylation enhances its ability to generate nitric oxide (NO). Because NO is an important regulatory of vasomotor tone. ,PKB/Akt functions as key regulator of vasomotor tone in vivo (27).

### **PKB/Akt and Insulin**

PKB/Akt is particularly important in mediating several metabolic actions of insulin. PKB/Akt is activated PI3K- dependent manner to regulate glucose transport, glycogen synthesis, cell survival and gene expression in response to insulin. PKB/Akt is also mediating protection against apoptosis by insulin -like-growth factor-1 (28).

The importance of PKB/Akt activation for the metabolic action of insulin is unclear. In vivo insulin administration in rats and humans rapidly activates PKB/Akt in skeletal muscle (29,30). Furthermore, insulin-stimulated PKB/Akt kinase activity in skeletal muscle from Non-Insulin-Dependent-Diabetes Mellitus (NIDDM) subjects was significantly reduced compared with control subjects and this reduction was not linked to a reduced protein expression of PKB/Akt (30). The ability of PKB/Akt to inhibit glycogen synthase kinase is a critical step in the activation of GS by insulin (29,31) . PKB/Akt may regulate glycolysis via activation of phosphofructo-2-kinase (32).

Insulin has differential effect on PKB/Akt isoforms in a tissue and species specific manner. Insulin administration in rats rapidly activates Akt-1 in skeletal muscle with minimal effect on Akt-2 and no effect on Akt-3 (33) In humans, insulin activates all isoforms in muscle although the effect on Akt-3 is minimum (31). In vitro administration of insulin on rat adipocytes results in an activation of Akt-1 and Akt-2 but not Akt-3. Whereas in rat hepatocytes in vitro insulin activates Akt-1, with very small effects on Akt-2 and no effect on Akt-3 (33). In human adipocytes, insulin activates Akt-2 (34) effects on other isoforms have not been studied.

In lean rats insulin stimulated Akt-1 and Akt-2 activity in muscle, liver and adipose tissue is increased. In obese rats, insulin stimulated Akt-1 activity decreases in muscle and in adipose tissue but increases in liver. Insulin stimulated Akt-2 activity decreases in muscle and liver but increases in adipose tissue. PI3K activity associated with insulin reseptor substrate-1 (IRS-1) or phosphotyrosine was reduced in tissues of obese rats because of lower IRS-1 protein levels and reduced insulin reseptor and IRS-1 phosphorylation. In adipose tissue of obese rats PI3K activity and activation of Akt-2 was increased, glucose transport was reduced. These findings suggest that PDK-dependent effect on glucose transport in adipocytes are not mediated primarily by Akt-2. In insulin resistant state Akt-1 and Akt-2 is less impaired than activation of PI3K. The mechanisms in insulin action on Akt-1 and Akt-2 activities in tissues of insulin resistant obese rats involve tissue - and isoform-specific changes in both expression and activation (35).

The activation mechanism of PKB/Akt mediated by IRS-1 is unclear. Full activation of PI3K is not required for activation of PKB/Akt at least in cells expressing IRS-1, whereas PI3K mostly contributes to activation of PKB/Akt in cells expressing IRS-1. However, very small amount of PI3K activity associated with IRS proteins is sufficient for the full activation of PKB/Akt because of the particular subcellular localization of IRS/PI3K complex. Nevertheless, IRS-1 mediates the signals to activation of PKB/Akt by the PI3K-dependent pathway and the PI3K-independent pathway, such as Ras-dependent. Ueki et al demonstrated that PKB/Akt in liver plays a pivotal role in systemic glucose homeostasis and that PKB/Akt activation might be sufficient for reducing insulin resistance even without full activation of PI3K (36).

## CONCLUSION

Protein kinase B/Akt is activated in response to treatment of cells with growth stimuli, such as insulin. Full activation of PKB/Akt needs phosphorylation of Thr 308 and Ser 473. A key enzyme in PKB/Akt activation is PI3K which triggers the dual phosphorylation. The activation of PKB/Akt is also mediated by a PI-3 independent manner. Activated PKB/Akt provides a survival signal to cells that protects them from apoptosis, functions as a key regulator of vasomotor tone, mediates the effects of PI3K such as protein synthesis, glycogen synthesis, glucose transport, gene expression in response to insulin. Insulin stimulation on PKB/Akt is tissue-specific and also isoform-specific. Importantly, whereas a defect on stimulation of PKB/Akt may play a role in pathogenesis of insulin resistance and diabetes.

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