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Focus on Molecules: Akt (PKB)

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1. Structure/activation

The serine (Ser)/threonine (Thr) kinase Akt, also known as protein kinase B (PKB), belongs to the AGC (cAMP-dependent, cGMP-dependent and protein kinase C) kinase family. In humans, there are three Akt genes: *Akt1* (NM_005163, NM_001014432, NM_001014431; the 3 splice variants encode the same protein), *Akt2* (NM_001626), and *Akt3* (NM_005465, NM_181690; the 2 splice variants encode a longer (Akt3.1) and a shorter (Akt3.2) isoform).

Akt proteins contain an N-terminal pleckstrin homology (PH) domain, a short helical region, a Ser/Thr specific kinase domain, and a C-terminal hydrophobic regulatory domain (Fig. 1A). A crystal structure of activated kinase domain of Akt2 (106L_A) is shown in Fig. 1B. Activation of Akt begins with the binding of a ligand (e.g., growth factor) to its cell surface receptor, which activates phosphatidylinositol (PtdIns) 3-kinase (PI3K). PI3K phosphorylates PtdIns(4,5)P_2 (PIP_2) to generate PtdIns(3,4,5)P_3 (PIP_3). The level of PIP_3 is also regulated by PTEN (phosphatase and tensin homologue deleted on chromosome 10) that dephosphorylates PIP_3 to generate PIP_2 (Fig. 1C). Accumulation of PIP_3 is a key step in activation of Akt because it recruits Akt and PDK1 (3-phosphoinositide-dependent protein kinase 1) to the plasma membrane by interacting with their PH domains. PDK1 phosphorylates Akt at a conserved Thr residue in the kinase domain, which stabilizes the activation loop of the kinase (Fig. 1C). Full activation of Akt requires phosphorylation at a conserved Ser residue in the regulatory domain, which is mediated (at least under certain conditions) by mTOR Complex 2 (mTORC2) (Sarbassov et al., 2005) (Fig. 1C). Once activated, Akt can dissociate from the plasma membrane.

2. Function

Although the three Akt isoforms have highly homologous sequences and are activated in a similar manner, mouse knockouts have revealed distinct physiological functions for different Akt isoforms (Yang et al., 2004). Akt1-deficient mice display developmental defects and exhibit increased neonatal mortality (approx. 40%), and the surviving adults show approx. 20% reduction in body weight. Akt2-deficient mice are born without apparent defects, but adult mice display a profound diabetic phenotype. In Akt3-deficient mice, the size and weight of the brain are reduced by approx. 25%.

One of the physiological processes that are regulated by Akt is angiogenesis. Angiogenesis is a process in which new blood vessels grow from pre-existing ones. Hypoxia induces angiogenesis, and the mechanism involves stabilization of hypoxia-inducible factor 1α (HIF1α), a key transcriptional activator of multiple pro-angiogenic factors including vascular endothelial growth factor (VEGF). Instead of interfering with degradation of HIF1α, Akt induces transcription and translation of HIF1α.

Akt impacts physiological processes by controlling cellular functions such as cell survival, growth, proliferation, and migration (Manning and Cantley, 2007). While over 100 Akt substrates have been identified, this information is not sufficient to understand how Akt governs a given cellular response. This is because some of the same substrates are phosphorylated when cells undergo different responses. For example, cell survival and proliferation are associated with Akt-dependent phosphorylation of some of the same proteins, e.g., GSK3, Forkhead transcription factors (FOXO) and murine double minute 2 (MDM2). Additional information related to the timing of the phosphorylation event and/or its subcellular location is needed to determine the way in which Akt-driven phosphorylation of a given substrate governs cellular responses. Thus while identifying Akt substrates is an important first step in developing new therapeutic options, a complete understanding of signaling events that govern physiology and pathology is a prerequisite for the success of such development.

In certain cellular contexts, Akt can also crosstalk with other kinase pathways (Manning and Cantley, 2007). For example, Akt blocks Ras-Raf-MEK-ERK signaling by directly phosphorylating and inhibiting Raf-1. Akt signaling also crosstalks with the NF-κB pathway and the c-Jun N-terminal kinase (JNK)/p38 apoptotic pathway.

3. Disease involvement

Deregulation of Akt is implicated in the pathogenesis of cancer, diabetes and multiple ocular diseases. Akt plays a key role in cancer progression and is constitutively activated in many types of cancer. Akt was originally identified as a retroviral oncogene product, v-Akt, which can transform rodent cells. Other oncogenic events associated with abnormal activation of Akt include overexpression of the Akt positive regulator PI3K and mutations of the Akt negative regulator PTEN. Akt fosters tumorigenesis via two distinct mechanisms: it promotes tumor cell survival by inhibiting apoptosis under conditions in which cells should normally die; and it stimulates tumor cell proliferation under conditions in which cells should normally be growth arrested. Besides its role in tumor cells, Akt is a key signaling molecule in endothelial cells (ECs) exposed to pro-angiogenic factors, and therefore also affects tumor angiogenesis. The central role of Akt in cancer progression makes it an attractive target of molecular-targeted cancer therapy, and at least some of the advances made in the tumor field are likely to be applicable to patients afflicted with angiogenesis-based ocular diseases.

Given that Akt constitutes a signaling node for growth factors, cytokines and hormones, it is not surprising that deregulation of Akt is involved in a range of ocular diseases, including age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), diabetic macular edema (DME), retinal vascular occlusion, and retinopathy of prematurity (ROP). Akt is a key contributor to intracellular signaling cascades by which pro-angiogenic factors drive cellular events intrinsic to angiogenesis in these ocular diseases. Besides its role in angiogenesis, Akt is also an endogenous retinal survival factor. Modulation of Akt activity can critically affect cell survival during hyperglycemic stress in the context of diabetes. Glaucoma is an optic neuropathy characterized by a progressive degeneration of retinal ganglion cells (RGCs) and their axons. Akt is activated as a self-defense mechanism of RGCs against injury.

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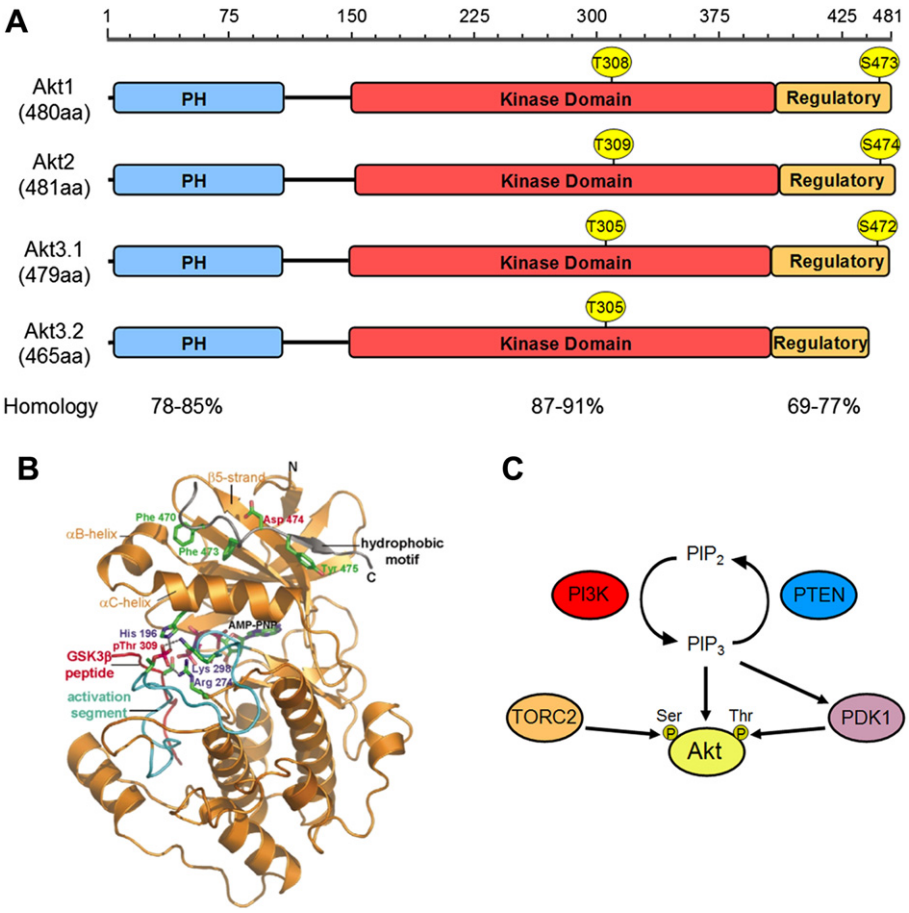

Fig. 1. The structure and regulation of the Akt family of protein kinases. (A) Diagram of the domains of human Akt proteins drawn to scale. The percentage homology between each domain of the Akt isoforms is 78–85% in the PH domain, 87–91% in the kinase domain, and 69–77% in the regulatory domain (for Akt3, Akt3.1 was used for the homology comparisons in the regulatory domain). Also indicated are the two phosphorylation sites shown to be essential for Akt activation. (B) Diagram of the crystal structure of activated kinase domain of Akt2-PIF in complex with a glycogen synthase kinase 3 (GSK3)-peptide substrate and AMP-PNP (an ATP analog). The hydrophobic motif of Akt2 is replaced with that of PIFtide, a potent mimic of a phosphorylated hydrophobic motif, to achieve an activated state of the kinase. Reprinted by permission from Macmillan Publishers Ltd: *Nature Structural Biology* 9(12): 940–944. © 2002. (C) Schematic representation of Akt activation and regulation; see text for additional information.

4. Future studies

As Akt is activated by a wide variety of pro-angiogenic factors, Akt is an attractive target for therapeutic intervention in cancer, AMD, PDR, and other ischemic retinopathies. However, caution needs to be taken when inhibiting Akt. The functions of Akt vary in a cell- and stimulus-context-dependent manner. For instance, in cultured adipocyte 3T3-L1 cells, although insulin and platelet-derived growth factor (PDGF) both activate PI3K/Akt, only insulin promotes GLUT-4 translocation and glucose uptake. This example highlights the fact that signaling cascades that lead to distinct outcomes can include some of the same participants. A better understanding of how Akt is activated may be instrumental to effective and selective Akt-based therapies.

VEGF is a potent pro-angiogenic factor and mediator of both physiological and pathological ocular angiogenesis. Although the PI3K/Akt pathway is essential for many VEGF-induced angiogenic alterations in ECs, the mechanism by which VEGF receptor VEGFR-2 activates PI3K/Akt has been only partially resolved. Grb2-adapter binder 1 (Gab1) is required for acute (within minutes) activation of PI3K/Akt in VEGF-stimulated ECs, but not at later times (Laramee et al., 2007). In light of the fact that *in vivo* angiogenesis involves prolonged (hours/days) exposure to elevated VEGF, the as yet undiscovered, Gab1-independent mechanism to activate PI3K is of great importance and interest.

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