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PI3K: Downstream AKTion Blocks Apoptosis

Minireview

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Extracellular stimuli are transmitted intracellularly by signaling cascades that involve the interaction of macromolecules and/or the generation of second messenger molecules that transduce signaling events over a distance between the origin and target of a signal. One class of such second messenger molecules is generated via phosphorylation of phosphoinositides on the D-3 position by phosphoinositide 3-kinase (PI3K). These products of PI3K can act on multiple downstream effectors that include Src homology-2 (SH2) and Pleckstrin homology (PH) domains of serine/threonine and tyrosine kinases and various cytoskeletal proteins (reviewed by Carpenter and Cantley, 1996). The role of PI3K in intracellular signaling has been underscored by its implication in a plethora of biological responses. Although it is unlikely that these multiple responses will be explained by the action of a single downstream target, recent research from several laboratories indicates that a signaling pathway from PI3K to the serine/threonine protein kinase Akt/PKB may mediate some cellular responses of PI3K (Burgering and Coffer, 1995; Cross et al., 1995; Franke et al., 1995; Kohn et al., 1995), including protection from apoptosis (Dudek et al., 1997; Kauffmann-Zeh et al., 1997; Khwaja et al., 1997; Kulik et al., 1997).

PI3K and Phosphoinositides

Multiple forms of PI3K with distinct mechanisms of regulation and different substrate specificities exist in mammalian cells (reviewed by Carpenter and Cantley, 1996; Zvelebil et al., 1996). The various forms of PI3K are able to phosphorylate the D-3 position of phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate (PtdIns-4-P), and phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P_2) to produce phosphatidylinositol-3-phosphate (PtdIns-3-P), phosphatidylinositol-3,4-bisphosphate (PtdIns-3,4-P_2), and phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P_3), respectively. PtdIns-3-P is constitutively produced in the absence of growth factor stimulation and is implicated in vesicle trafficking. PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 are nominally absent in quiescent cells, but they appear acutely within seconds to minutes of addition of extracellular stimuli and are ultimately degraded by phosphatases (Figure 1). In most cells that have been investigated, PtdIns-3,4-P_2 appears with a lag after the peak in PtdIns-3,4,5-P_3, suggesting that it results from hydrolysis of the D-5 phosphate from PtdIns-3,4,5-P_3. There is evidence that PtdIns-3,4-P_2 can also be synthesized by pathways that do not involve production of PtdIns-3,4,5-P_3. In vitro, this lipid can be produced by the p170/mCpk-type PI3K that phosphorylates PtdIns-4-P at the D-3 position (Figure 1) or by a kinase that phosphorylates PtdIns-3-P at the D-4 position (not shown). Thus, the relative levels of PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 are independently controlled by a complex set of kinases and phosphatases that have not yet been fully characterized. The importance of this regulation is emphasized by recent evidence that certain SH2 and PH domains interact with PtdIns-3,4,5-P_3, but not with PtdIns-3,4-P_2 (reviewed by Carpenter and Cantley, 1996), while the serine/threonine protein kinase Akt/PKB is activated by PtdIns-3,4-P_2 but not by PtdIns-3,4,5-P_3 (Franke et al., 1997; Klippel et al., 1997).

Regulation of the Akt/PKB Kinase by PI3K

Akt/PKB is homologous to the PKA and PKC families of protein kinases (hence named PKB or Related to A and C protein kinase: RAC-PK). It is also the cellular homolog of the retroviral oncogene v-*akt* (see references in Burgering and Coffer, 1995; Cross et al., 1995; Franke et al., 1995; Kohn et al., 1995). In vivo, the activity of Akt/PKB is regulated by serum and growth factors that activate PI3K (Burgering and Coffer, 1995; Franke et al., 1995, 1997; Kohn et al., 1995; Alessi et al., 1996; Andjelkovic et al., 1996). Three types of experiments have suggested that PI3K is necessary and sufficient for growth factor-dependent activation of Akt. First, PDGF receptor mutants that are deficient in activating PI3K fail to mediate activation of Akt/PKB (Burgering and Coffer, 1995; Franke et al., 1995). Second, dominant-inhibitory alleles of PI3K prevent activation of Akt (Burgering and Coffer, 1995), and constitutively activated

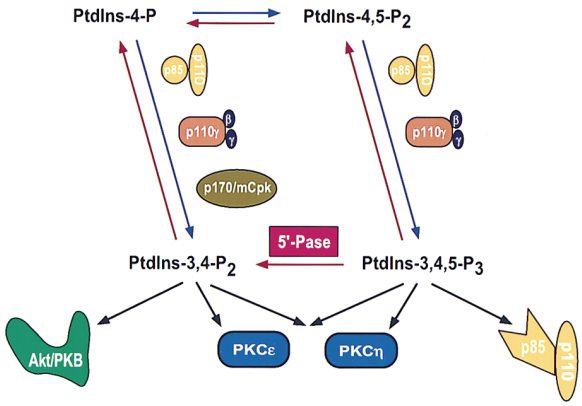

Figure 1. Downstream Targets of Phosphoinositide 3-Kinases

Various lipid kinases and phosphatases are involved in determining the ratio of phosphoinositides in vivo. Several proteins specifically bind PtdIns-3,4-P_2 and/or PtdIns-3,4,5-P_3 in vitro. Among them are certain SH2 and PH domain–containing proteins that specifically bind PtdIns-3,4,5-P_3, including the p85 regulatory subunit of PI3K (reviewed by Carpenter and Cantley, 1996) and the Akt/PKB PH domain that binds PtdIns-3,4-P_2 (Franke et al., 1997). Several Ca^{2+}-independent atypical PKC isoforms are activated by PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 (reviewed by Carpenter and Cantley, 1996).

PI3K increases Akt/PKB activity independent of growth factor stimulation (Klippel et al., 1996; Marte et al., 1996; Franke et al., 1997). Third, the PI3K inhibitor wortmannin blocks activation of Akt/PKB by growth factors (Burgering and Coffer, 1995; Franke et al., 1995, 1997; Kohn et al., 1995; Andjelkovic et al., 1996). Akt/PKB activation is partially dependent upon the activity of Ras (Franke et al., 1995), and activated mutants of R-Ras and Ras may stimulate Akt/PKB by activating PI3K (Klippel et al., 1996; Marte et al., 1996). Although these studies indicate that PI3K is necessary to activate this enzyme in response to certain extracellular stimuli, an additional pathway for activation has been suggested that is independent of PI3K and could involve p38/HOG (Alessi et al., 1996; Konishi et al., 1996). The diversity of factors leading to the activation of Akt/PKB may explain the existence of conflicting models of Akt/PKB regulation.

Akt/PKB molecules are able to dimerize and to interact with other proteins through an NH_2-terminal region that includes a PH domain (Konishi et al., 1996; Franke et al., 1997, and references therein). Homo-oligomerization of Akt/PKB is induced by interaction with PtdIns-3,4-P_2 and increases Akt/PKB activity (Franke et al., 1997); homo-oligomerization is also involved in the regulation of the serine/threonine kinase Raf and could possibly be a general mechanism of regulation of serine/threonine kinases that will have to be examined further (reviewed by Marshall, 1996). The integrity of the NH_2-terminal PH domain is required for in vivo activation of Akt/PKB by several growth factors, by constitutively active PI3K, and also by certain pathways independent of PI3K (Franke et al., 1995, 1997; Andjelkovic et al., 1996; Klippel et al., 1996; Konishi et al., 1996). In contrast, the Akt/PKB PH domain is not required for Akt/PKB activation by okadaic acid or insulin in cells overexpressing the insulin receptor (Kohn et al., 1995; Andjelkovic et al., 1996). These results indicate that the relative importance of the Akt/PKB PH domain for activation depends on the cell type or the stimulus used.

Treatment of cells with inhibitors of serine/threonine phosphatases and direct treatment of purified Akt/PKB with phosphatases has shown that Akt/PKB phosphorylation is critical for its activity. The major phosphorylation sites required for activation have been identified as threonine 308 and serine 473, and MAPKAP kinase 2 is able to phosphorylate one of these sites (serine 473) to partially activate Akt/PKB in vitro (Alessi et al., 1996). Since MAPKAP kinase 2 is activated by p38/HOG under conditions of cellular stress that do not activate PI3K, it may contribute to PI3K-independent activation of Akt/PKB in vivo (Alessi et al., 1996; Konishi et al., 1996). The kinase that phosphorylates Akt/PKB in its catalytic loop on threonine 308 and activates Akt/PKB in the growth factor- and PI3K-dependent pathway is not known.

Akt/PKB as a Direct Effector of PI3K

A direct mechanism of Akt/PKB activation by PI3K that involves the binding of PtdIns-3,4-P_2 to the Akt/PKB PH domain has been described recently (Franke et al., 1997; Klippel et al., 1997). PtdIns-3,4,5-P_3 does not activate Akt/PKB and may cause some inhibition of the basal activity. The failure of the structural isomer PtdIns-4,5-P_2 to activate Akt/PKB supports the view that the activation of Akt/PKB by PtdIns-3,4-P_2 is physiologically relevant. PtdIns-3,4-P_2 causes dimerization of Akt/PKB, which could be the mechanism of activation (Franke et al., 1997). These results suggest the model presented in Figure 2. Activation of PI3K by growth factors results in the production of PtdIns-3,4-P_2 at the membrane. Akt/PKB binds to this lipid, dimerizes, and is stabilized in a partially active state. The location at the membrane and/or the dimerization then enhances the ability of Akt/PKB to be phosphorylated.

Although Ras is involved in activation of Akt/PKB (Franke et al., 1995; Klippel et al., 1996; Marte et al., 1996), Akt/PKB does not appear to be in the pathway leading to the activation of MAPK (Franke et al., 1995). This is consistent with the fact that R-Ras activates Akt/PKB but does not activate MAPK (Marte et al., 1996). These results indicate the existence of a Ras signaling cascade parallel to the Raf/MEK/MAPK pathway: Ras binds and activates PI3K, causing activation of Akt/PKB. The only known protein target of Akt/PKB is glycogen synthase kinase-3 (GSK3): Akt/PKB phosphorylates N-terminal sequences in GSK3 in vitro that are important for GSK3 inhibition by insulin stimulation in vivo, thereby linking PI3K and Akt/PKB to insulin-dependent glycogen synthesis (Cross et al., 1995). There is also evidence that $p70^{S6kinase}$ is downstream of constitutively activated Akt/PKB (Burgering and Coffer, 1995).

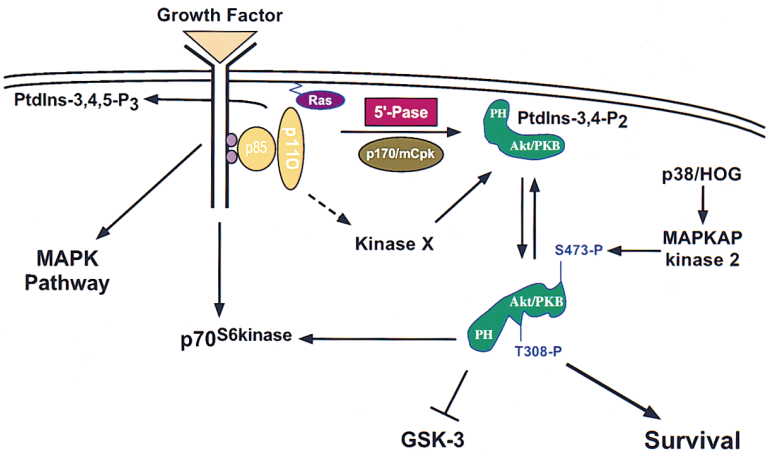

Figure 2. Akt/PKB Is an Effector of PI3K In Vivo

Akt/PKB is activated by growth factors that stimulate PI3K. Akt/PKB activity is regulated by PtdIns-3,4-P_2 (Franke et al., 1997; Klippel et al., 1997) as well as by phosphorylation on serine and threonine residues (Alessi et al., 1996) that may stabilize a transiently induced conformational change. Akt/PKB participates in the regulation of $p70^{S6kinase}$ (Burgering and Coffer, 1995), but is not sufficient for MAPK activation (Franke et al., 1995). GSK3 is the first identified direct target of Akt/PKB (Cross et al., 1995). One prominent biological role of Akt/PKB is the PI3K-dependent regulation of survival (Dudek et al., 1997; Kauffmann-Zeh et al., 1997; Khwaja et al., 1997; Kulik et al., 1997).

PI3K, Akt/PKB, and Cell Survival

Exciting insights into the function of Akt/PKB have been revealed by studies investigating its function in PI3K-dependent pathways that are involved in the regulation of cell survival. Previous studies have demonstrated that PI3K is involved in serum-dependent survival of PC12 cells (reviewed by Carpenter and Cantley, 1996). Recent results indicate that PI3K also mediates type 1 insulin-like growth factor (IGF-1)-dependent survival of Rat-1 and COS-7 cells (Kulik et al., 1997) and granule neurons (D'Mello et al., 1997).

A role for Akt/PKB in IGF-1-mediated cell survival has been indicated (Dudek et al., 1997): overexpression of Akt/PKB prevents apoptosis in primary cultures of cerebellar neurons that are induced by survival factor withdrawal or inhibition of PI3K. The expression of dominant-negative forms of Akt/PKB interferes with growth factor-mediated survival in these cells, indicating that Akt/PKB is necessary and sufficient for neuronal survival. Studies from Weber and colleagues have shown that overexpression of constitutively activated Akt/PKB also blocks UV-induced apoptosis in Rat-1 and COS-7 cells (Kulik et al., 1997). A different approach taken by Downward and colleagues indicated that activated Akt/PKB prevents apoptosis that is induced by detachment of MDCK cells from their extracellular matrix (anoikis; Khwaja et al., 1997).

Finally, Evan and colleagues demonstrated that mutants of V12 Ras that selectively stimulate PI3K and Akt/PKB but not the Raf/MEK/MAPK pathway are able to prevent c-*myc*-induced cell death in Rat-1 cells (Kauffmann-Zeh et al., 1997). In this system, activated forms of PI3K and Akt/PKB are sufficient to prevent apoptosis that is induced by c-*myc*. $p70^{S6kinase}$ activity was not necessary for the prevention of apoptosis caused by deregulated c-*myc* (Kauffmann-Zeh et al., 1997), nor was it necessary for Akt/PKB-dependent neuronal survival in primary cerebellar neurons (Dudek et al., 1997). Therefore, IGF-1 and certain other growth factors stimulate a cell survival pathway that involves Ras-dependent stimulation of PI3K, leading to activation of Akt/PKB. This pathway appears to be independent of MAPK and $p70^{S6kinase}$ and to prevent apoptosis induced by a variety of cellular challenges. Its importance in tissue culture systems as well as in primary neuronal cells suggests that this pathway may be of general significance. Further studies are needed to determine if Akt/PKB can be a suitable target for drug therapy directed at neurodegenerative and other degenerative human diseases.

Many groups are currently extending these findings and contributing to the further understanding of Akt/PKB in survival in other cell systems. GSK3 has not been implicated in the regulation of survival (see references in Kauffmann-Zeh et al., 1997), suggesting that Akt/PKB has more widespread roles in cell regulation and employs distinct subsets of substrates in different signaling systems. Additional direct downstream targets of Akt/PKB other than GSK3 must exist that have not been identified. Recent studies have provided some insight into the mechanism by which cell survival factors cause modifications to members of the Bcl-2 family of proteins (reviewed by Gajewski and Thompson, 1996). The exact mechanisms of prevention of apoptosis by Akt/PKB are undetermined; future studies will address whether Akt/PKB mediates survival by phosphorylation and inhibition of proteins that are involved in programmed cell death. The observation that PI3K and Akt/PKB are involved in cell survival certainly could explain why so many oncoproteins, growth factors, and survival factors have evolved mechanisms for activation of PI3K.

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Note Added in Proof

While this minireview was in the proof stages, we learned that S. G. Kennedy, A. J. Wagner, S. D. Conzen, J. Jordán, A. Bellacosa, P. N. Tsichlis, and N. Hay had obtained results ([1997] Genes Dev., in press), similar to those of Kaufmann-Zeh et al. (1997), demonstrating that activated Akt will suppress apoptosis that is induced by serum withdrawal and ectopic expression of *myc* in Rat-1 fibroblasts.