Minireview

Forkhead transcription factors are targets of signalling by the proto-oncogene PKB (C-AKT)

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ABSTRACT

Following the discovery that the proto-oncogene protein kinase B (PKB) functions as a downstream element in signalling from phosphoinositide 3′-kinase (PI3-kinase) (Burgering & Coffer, 1995), PKB has been shown to mediate a diverse array of PI3-kinase dependent cellular responses. Most recently PKB-dependent phosphorylation of 3 members of the family of Forkhead transcription factors has been demonstrated to play a role in PI3-kinase dependent effects on transcription. This review focuses on this newly discovered function of PKB in conveying the diversity of PI3-kinase dependent cellular responses.

Key words: Protein kinase B; c-akt.

Protein kinase B (PKB, also known as c-akt) is a PH domain-containing serine/threonine kinase that has transforming capabilities. In mammalian cells, PKB is activated on treatment with most peptide growth factors such as insulin and PDGF. Activation of the receptor for these factors results in the recruitment to the plasma membrane and activation of phosphoinositide 3′-kinase (PI3-kinase). Active PI3-kinase produces 3′ phosphorylated inositol lipids that act as second messengers to recruit PH domain-containing proteins to the plasma membrane. By this mechanism, PKB is recruited, activated, and released into the cytosol (reviewed in Coffer et al. 1998). Once activated, PKB can phosphorylate a range of proteins and thereby control several cellular processes. First, apoptotic signals can be overcome by PKB activity. This survival signal of PKB is transduced by direct phosphorylation and inactivation of the pro-apoptotic factors caspase-9 and BAD (Dudek et al. 1997; Cardone et al. 1998). Secondly, a role for PKB in protein translation has been shown. PKB mediates the phosphorylation of the translational repressor 4E-BP-1 and PKB can activate the p70S6-kinase that phosphorylates the S6 ribosomal subunit and stimulates protein synthesis (Burgering & Coffer, 1995; Gingras et al. 1998). Thirdly, nitric oxide production is under the direct control of PKB. By phosphorylation of eNOS, PKB is able to enhance NO production and thereby influence processes such as vascular remodelling and maintenance of blood glucose levels (Dimmler et al. 1999; Fulton et al. 1999). Fourth, PKB is involved in regulating metabolic processes by directly controlling the activity of GSK3 and PFK2, enzymes that are involved in glycogen metabolism (Deprez et al. 1997; van Weeren et al. 1998). Furthermore, PKB activity affects the recruitment of glucose transporters to the plasma membrane, and PKB controls the expression of certain genes such as PEPCK, fatty acid synthase and IGFBP-1 (Kohn et al. 1996; Cichy et al. 1998; Liao et al. 1998; Wang & Sul. 1998). Direct targets of PKB that can mediate effects on transcription, however, had yet to be identified. By means of genetic complementation studies, a pathway was elucidated in *Caenorhabditis elegans* that showed control of a Forkhead-type transcription factor (DAF-16) by a cascade that consists of insulin receptor or IGFI receptor-like and PI3 kinase-like proteins (Ogg et al. 1997). Mammalian orthologues of this Forkhead transcription factor are AFX, FKHR and FKHRL1. Interestingly, these proteins each contain 3 putative PKB phosphorylation sites, and therefore we and

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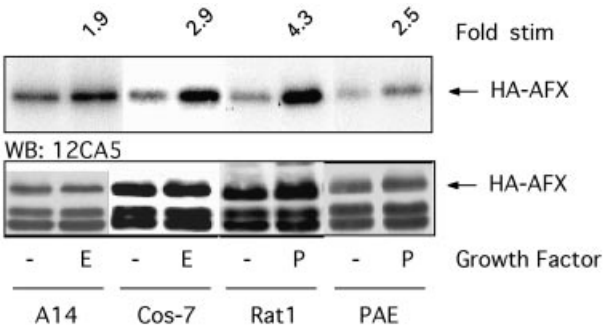

Fig. 1. Growth factor-induced phosphorylation of AFX. A14, Cos-7, Rat1 and PAE cells transiently expressing HA-AFX were labelled for 3 h with ^{32}P-orthophosphate and treated with EGF (E) or PDGF (P) for 30 min. Immunoprecipitated HA-AFX was subsequently analysed for the amount of incorporated radiolabelled phosphate. Equal expression was verified by western blotting with the 12CA5 antibody (WB: 12CA5).

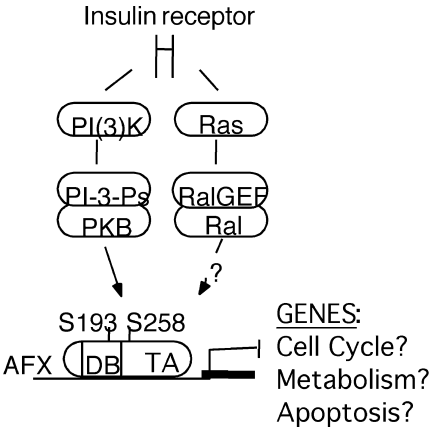

Fig. 3. A model for insulin-mediated inhibition of AFX-dependent transcription. The insulin-induced pathways leading to phosphorylation and inactivation of AFX are described in the text. DB, DNA-binding domain; TA, transactivation domain.

others hypothesised that the Forkhead transcription factors are under direct control of PKB activity.

All 3 Forkheads have now been shown to be phosphorylated and regulated following addition to cells of insulin or IGFI (Brunet et al. 1999; Guo et al. 1999; Kops et al. 1999; Rena et al. 1999), but it is likely that other growth factors can also achieve this. Figure 1 shows growth factor-induced phosphorylation of epitope-tagged AFX. PDGF treatment of Rat1 cells and PAE cells expressing wildtype PDGFR results in increased phosphorylation of AFX and treatment of COS-7 cells with EGF and of A14 cells with EGF or insulin showed a similar effect. This indicates that phosphorylation of AFX can be accomplished by a variety of receptor tyrosine kinases, including those that have been shown to activate PKB.

Cotransfection of AFX with active forms of PKB or its upstream activator PI3-kinase results in growth factor-independent phosphorylation of AFX (Fig. 2*a*) and furthermore PKB is able to phosphorylate AFX in vitro (Fig. 2*b*). This was also shown for FKHRL1 and FKHR (Brunet et al. 1999; Rena et al. 1999). The PI3-kinase/PKB pathway is not the only pathway that leads to phosphorylation of the transcription factors. For AFX another route has been identified and was shown to be Ras signalling to the Ral GTPase (Fig. 3, and Kops et al. 1999). Although not formally shown, there are data to suggest that pathways other than PI3-kinase/PKB operate on FKHR, FKHRL1, and DAF-16.

As in *C. elegans*, activation of the PI3-kinase/PKB pathway in mammalian cells antagonises Forkhead activity. Using a range of reporter constructs that carry an insulin response element (IRE) it was shown for all 3 transcription factors that activation of these reporters by the Forkheads can be inhibited by insulin signalling to PKB, and for AFX it was shown that insulin signalling to Ral can do the same thing (Brunet et al. 1999; Guo et al. 1999; Kops et al. 1999).

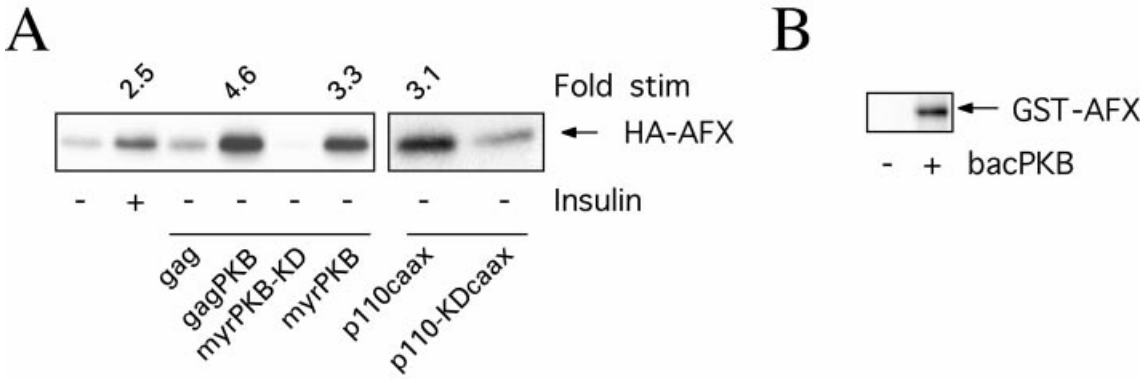

Fig. 2. PKB phosphorylates AFX. A, Growth factor-independent phosphorylation of AFX by active forms of PKB and PI3-kinase. A14 cells were transfected with HA-AFX together with active PKB (gagPKB, myrPKB) or the respective control, or active PI3-kinase (p110caax) and its control. Phosphorylation of AFX was analysed as in Fig. 1. KD; kinase-dead. B; PKB phosphorylates GST-AFX in vitro. Bacterially purified GST-AFX was incubated with (+) or without (−) active PKB (bacPKB) in the presence of radioactive phosphate.

How this inhibition is accomplished, however, is not clear for all 3 proteins. FKHRL1 is relocalised to the cytoplasm on phosphorylation by PKB, and we have observed a similar translocation for AFX, but it is doubtful whether this is the only way of inhibiting AFX. Activation of Ral by an active form of its exchange factor RLF, for instance, inactivates AFX, but does not cause a change in subcellular distribution.

FKHRL1 has been shown to upregulate the gene for FasL, a ligand for the death receptor Fas (Brunet et al. 1999). By inhibiting FKHRL1, PKB can inhibit FasL expression and thus ensure cellular survival. Similarly, FKHR overexpression results in apoptosis, which can be overcome by expression of active PKB, whereas apoptosis induced by expression of an unphosphorylatable form of FKHR cannot be inhibited by PKB (Tang et al. 1999). Other functions of the Forkheads may emerge shortly, however. Overexpression of AFX, for instance, inhibits cell growth but this does not seem to be via apoptosis. Furthermore, FKHR has been shown to regulate the promoter of the mouse and human gene for glucose-6-phosphatase (Fig. 3).

Of the 3 forkhead proteins, 2 have previously been shown to be involved in tumorigenesis. Chromosomal translocations that cause leukaemia or rhabdomyosarcoma involve the genes for AFX and FKHR, respectively, and create highly active chimeric transcription factors, namely MLL-AFX and PAX3-FKHR (Sublett et al. 1995; Borkhardt et al. 1997). Another implication of the translocations, however, is that one allele of the Forkheads is no longer functional. Since AFX is an X-linked gene, this means that such a translocation in males results in the functional loss of AFX activity. Since AFX-like proteins are involved in the inhibition of cell growth, loss of AFX function might contribute to oncogenesis. The translocation therefore might have a dual effect. On the one hand, it creates a constitutively active fusion protein and on the other the loss of activity of a potential tumour suppressor. A similar argument can be made for FKHR. Cells have 2 FKHR alleles, so the translocation of 1 allele still leaves 1 functional gene. However, the created PAX3-FKHR fusion protein can upregulate the genes encoding MET receptor (HGF receptor) and PDGF receptor (Epstein et al. 1998; Ginsberg et al. 1998) which are strong activators of PKB and therefore inactivators of FKHR. By upregulating these genes, the translocation might contribute to the functional inactivation of the second intact gene. In conclusion, the discovery that certain Forkhead family members are targets of PKB signalling provides new insights into the mechanism not only of PKB-induced oncogenesis, but possibly also into oncogenesis induced by translocations involving the Forkhead genes.

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