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Physiological roles of PKB/Akt isoforms in development and disease

B. Dummler* and B.A. Hemmings*[1]
*Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland

Abstract

PKB (protein kinase B, also known as Akt) is a serine/threonine kinase that is important in various signalling cascades and acts as a major signal transducer downstream of activated phosphoinositide 3-kinase. There are three closely related isoforms of PKB in mammalian cells, PKBα (Akt1), PKBβ (Akt2) and PKBγ (Akt3), and this review discusses recent advances in our understanding of the functions of these isoforms in the regulation of adipocyte differentiation, glucose homeostasis and tumour development.

Introduction

The serine/threonine kinase PKB (protein kinase B, also called Akt) constitutes an important node in diverse signalling cascades and acts as a major signal transducer downstream of activated PI3K (phosphoinositide 3-kinase). Mammalian cells contain three genes that encode three closely related and highly conserved isoforms of PKB, termed PKBα/Akt1, PKBβ/Akt2, and PKBγ/Akt3. Since our last review in *Biochemical Society Transactions* [1], several significant advances have been made in dissecting the isoform-specific physiological functions of these kinases. This review covers recent advances in the understanding of PKB isoform functions that have emanated from the analysis of new PKB mutant mouse models. In particular, involvement of PKB isoforms in the regulation of adipocyte differentiation, glucose homoeostasis and tumour development is discussed.

The PI3K/PKB pathway regulates adipocyte differentiation

Several recent studies have shown that the PI3K/PKB pathway is required for adipocyte differentiation. Genetic evidence for an implication of PKB in adipocyte differentiation has been inferred from the analysis of PKBα/PKBβ double knockout mice. Histological analysis of the brown adipose tissue of PKB$\alpha^{-/-}$PKB$\beta^{-/-}$ neonates revealed very thin dorsal pads which contained no visible lipid droplets in the cells [2]. In addition, MEFs (mouse embryonic fibroblasts) derived from these PKB$\alpha^{-/-}$PKB$\beta^{-/-}$ mice or from PKB$\alpha^{-/-}$ mice were not able to differentiate into adipocytes in a standard *in vitro* adipogenesis-induction assay [2,3]. Similarly, adipocyte differentiation is blocked in 3T3-L1 preadipocytes when PKBα expression is down-regulated by RNAi (RNA interference) [4]. Baudry et al. [3] showed that ectopic expression of wild-type PKBα rescues adipocyte differentiation in PKB$\alpha^{-/-}$ MEFs and expression of a constitutively active form of PKBα (m/p-HA-PKBα) induces differentiation of PKB$\alpha^{-/-}$ MEFs even in the absence of adipogenic treatment. These results, and the fact that PKB$\beta^{-/-}$ MEFs are not impaired in their ability to differentiate into adipocytes [2], suggest that regulation of adipocyte differentiation is a specific function for PKBα in these cellular systems.

Adipocyte differentiation is mediated by temporally regulated expression of numerous genes. PPARγ (peroxisome-proliferator-activated receptor γ) is a key regulator of this transcriptional program, and is induced before the transcriptional activation of most adipocyte-specific genes. PKB$\alpha^{-/-}$ MEFs are unable to induce PPARγ expression, which may in part account for their failure to differentiate into adipocytes [2,3]. Some evidence suggests that the induction of PPARγ transcription is linked with PKB-mediated phosphorylation and inactivation of FKHR (forkhead in rhabdosarcoma; Foxo1) transcription factor. In PKB$\alpha^{-/-}$PKB$\beta^{-/-}$ MEFs, as well as in 3T3-L1 cells with RNAi-mediated PKBα-knockdown, phosphorylation of FKHR is severely reduced and coincides with a failure to induce PPARγ expression [2,4]. Furthermore, a constitutively active form of FKHR, in which all three putative PKB phosphorylation sites are mutated, inhibits the induction of PPARγ expression and adipocyte differentiation [5].

In an effort to identify other downstream effectors of PKB via gene expression profiling, 80 genes were found whose expressions were up-regulated in wild-type MEFs during adipogenesis but were significantly reduced in PKBα-deficient MEFs under the same conditions [3]. Among the identified genes are KLF15 (Krüppel-like transcription factor 15), a known regulator of adipogenesis, and also the protease Ren1 (Renin 1). The mechanism of transcriptional regulation of these candidates by PKBα and their contributions to the adipocyte differentiation programme remains to be determined.

Key words: adipocyte differentiation, cancer, glucose homoeostasis, mouse model, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), protein kinase B (PKB).

Abbreviations used: AS160, Akt substrate of 160 kDa; FKHR, forkhead in rhabdosarcoma; GAP, GTPase-activating protein; MEF, mouse embryonic fibroblast; PI3K, phosphoinositide 3-kinase; PIKfyve, phosphoinositide 5-kinase; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PKB, protein kinase B; PPARγ, peroxisome-proliferator-activated receptor γ; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RNAi, RNA interference.

[1]To whom correspondence should be addressed (email brian.hemmings@fmi.ch).

Role of PKB isoforms in glucose homoeostasis

The contributions of the different PKB isoforms to insulin signalling and maintenance of glucose homoeostasis have been studied extensively using knockout mouse models and RNAi knockdown approaches. Of the three mammalian PKB isoforms, PKBβ is strongly correlated with the regulation of glucose homoeostasis and is the predominant PKB isoform expressed in insulin-responsive tissues. Targeted disruption of the PKBβ locus in mice leads to defective insulin signalling as evident by impaired insulin-stimulated glucose uptake in muscle and adipocytes, and failure to suppress hepatic glucose output [6,7]. Consequently, PKBβ-null mice display glucose intolerance and insulin resistance, and a substantial portion of these mice develops a severe form of diabetes that is accompanied by β-cell failure. In contrast, normal glucose homoeostasis is maintained in PKB$\alpha^{-/-}$ as well as in PKB$\gamma^{-/-}$ mice, and analysis of PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ mice showed that absence of PKBγ does not intensify the diabetes-like phenotype of PKB$\beta^{-/-}$ mice [8–11]. Interestingly, Bae et al. [12] showed that glucose uptake could be completely restored in adipocytes derived from PKB$\beta^{-/-}$ MEFs by ectopic expression of PKBβ, whereas ectopic expression of PKBα at comparable levels was ineffective at rescuing insulin action. RNAi knockdown of PKBα and/or PKBβ in 3T3-L1 adipocytes similarly showed a primary role of PKBβ in this process [13]. At the cellular level, insulin increases glucose uptake into muscle and fat cells mainly by initiating the translocation of a special glucose transporter, GLUT4, from intracellular storage vesicles to the cell surface. Although it is still not clear how PKB impinges on GLUT4 cycling, there is evidence for a number of potential PKB substrates linking insulin signalling via PI3K/PKB to GLUT4 translocation. The first one, AS160 (Akt substrate of 160 kDa), contains at least five phosphorylation sites that conform to the PKB substrate consensus sequence. Phosphorylation of these sites is increased upon insulin stimulation in 3T3-L1 adipocytes, and mutation of the sites blocks the ability of insulin to stimulate exocytosis of GLUT4 [14]. AS160 contains a GAP (GTPase-activating protein) domain for Rab proteins, and phosphorylation of AS160 inhibits its GAP activity. The Rab protein which is regulated by AS160 GAP activity has yet to be identified. Intriguingly, Rab GTPases are involved in the regulation of membrane trafficking and could thus potentially link GLUT4 translocation with AS160 phosphorylation [15]. Of note, reduced insulin-stimulated AS160 phosphorylation was found in skeletal muscle of Type 2 diabetic patients and was associated with a decrease in PKB activation loop phosphorylation [16]. A second PKB substrate that may play a role in insulin-regulated GLUT4 trafficking is PIKfyve (phosphoinositide 5-kinase) [17]. PIKfyve appears to have a role in the sorting of GLUT4 from internalized endosomes into GLUT4 storage vesicles. A third PKB substrate that seems to be involved in the regulation of insulin-stimulated GLUT4 translocation is Synip [18]. However, the physiological relevance of Synip phosphorylation is controversial because substitution of alanine for the PKB-phosphorylated residue does not prevent insulin-dependent GLUT4 accumulation at the cell surface [19]. It would be interesting to assess the phosphorylation levels of these substrates in insulin-responsive tissues of PKB$\beta^{-/-}$ mice. Reduced phosphorylation would validate these targets as PKBβ substrates and provide a potential molecular mechanism that accounts for the glucose intolerance and insulin resistance in these mice.

Lessons from mouse cancer models of the PI3K/PKB pathway

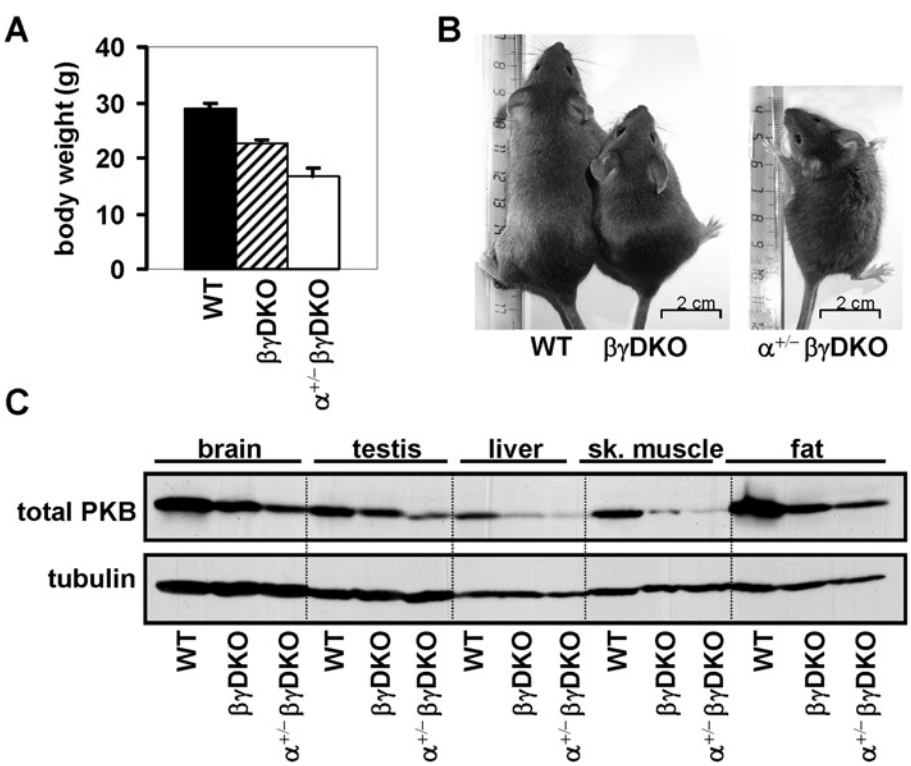
The PI3K/PKB pathway is an important driver of cell proliferation, cell growth and cell survival, all events that favour tumorigenesis. Accordingly, constitutive PKB signalling presents a major means whereby tumour cells achieve uncontrolled proliferation, and PKB is one of the most frequently hyperactivated protein kinases in human cancers [20]. Hyperactive PKB is, because of its anti-apoptotic activity, also linked to the resistance of many cancers to treatment with cytotoxic agents. Aberrant activation of PKB in human cancer can occur by diverse mechanisms. In a large number of cancers the tumour-suppressor gene PTEN (phosphatase and tensin homologue deleted on chromosome 10) is inactivated. PTEN is a lipid phosphatase whose main function is to convert the membrane PIP_3 (phosphatidylinositol 3,4,5-trisphosphate), the product of PI3K activity, into phosphatidylinositol 4,5-bisphosphate. PTEN inactivation leads to the accumulation of high PIP_3 levels at the membrane and consequently to constitutive activation of PKB. To assess the importance of PKB activation in tumorigenesis directly, various transgenic mice have been generated that express a constitutively active form of PKB under the control of different tissue-specific promoters. Analysis of these mice has shown hypertrophy (heart, pancreatic β-cells), intraepithelial neoplasia (prostate) and lymphomas (T-cells) as a consequence of PKB hyperactivity in the respective tissues. Although constitutively active PKB obviously promotes cell proliferation, it is, on its own, in many tissues not sufficient for tumour induction. For instance, expression of constitutively active PKBα in mammary glands markedly accelerates tumour induction when co-expressed with an ErbB2 transgene, but is not sufficient to induce tumours when expressed alone [21–24]. Nevertheless, a recent study has now provided genetic evidence that tumours induced by PTEN inactivation are to a large extent dependent on PKB signalling. Whereas a homozygous null mutation of PTEN results in early embryonic lethality in mice, PTEN heterozygous (PTEN$^{+/-}$) mice are viable and develop a wide range of tumours at an early age, with a high tumour incidence in endometrium, prostate, thyroid, adrenal medulla, intestine and mammary gland [25,26]. Chen et al. [27] crossed PTEN$^{+/-}$ mice with PKBα-deficient mice and could show that PKBα-deficiency protects the PTEN mutant mice from developing tumours. PTEN$^{+/-}$PKB$\alpha^{-/-}$mice had a marked decrease in tumour incidence and development compared

Table 1 | **Phenotypes of mice with combined ablations of PKB isoforms**

Genotype	Phenotype
PKB$\alpha^{-/-}\beta^{-/-}$	Develop to term but die shortly after birth; severe growth deficiency, impaired skin and bone development, impeded adipogenesis, skeletal muscle atrophy
PKB$\alpha^{-/-}\gamma^{-/-}$	Lethal at around embryonic day 11 (E11); multiple developmental defects, such as increased apoptosis in developing nervous system, abnormalities in the cardiovascular system, decreased vasculature
PKB$\beta^{-/-}\gamma^{-/-}$	Normal embryonic development and postnatal survival; growth deficiency, reduced brain and testis size, impaired glucose homoeostasis
PKB$\alpha^{+/-}\beta^{-/-}\gamma^{-/-}$	Normal embryonic development and postnatal survival; severe growth deficiency; the phenotype of these mice was not extensively studied

Figure 1 | **???????** Q1

(**A**) Growth deficiency in PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ and PKB$\alpha^{+/-}$PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ mice. Body weights of 12-week-old male PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ ($\beta\gamma$DKO), PKB$\alpha^{+/-}$PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ ($\alpha^{+/-}\beta\gamma$DKO) and wild-type (WT) mice are shown. (**B**) Top view of 12-week-old male wild-type, PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ and PKB$\alpha^{+/-}$PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ mice. (**C**) Stepwise reduction of functional PKB alleles is reflected by concomitant decrease in total PKB protein levels in tissues. Adapted from [8] with permission. © 2006, the American Society for Microbiology.



with PTEN$^{+/-}$ mice in many tissues, with the most effective tumour inhibition observed in prostate, endometrium and small intestine.

This result has important implications for the development of PKB-targeted cancer therapy, as inhibition of individual PKB isoforms may be sufficient for effective treatment. General PKB inhibitors, which inhibit all three isoforms, induce some metabolic toxicities [28], whereas, for example, an isoform-specific inhibitor for PKBα would not compromise PKBβ-mediated functions in glucose metabolism. Of note, a group from Merck Research Laboratories recently described novel allosteric PKB inhibitors that are isoform-selective [29,30]. Although promising results were achieved in cell culture experiments, the poor solubility and pharmacokinetic properties of these inhibitors have so far precluded a thorough evaluation in animal tumour models.

In several recent studies, differential roles for PKBα and PKBβ were proposed in aggressive malignant cell behaviour. While all three isoforms possess *in vitro* transformation ability [31], opposing functions for PKBα and PKBβ in cell migration, cell proliferation and invasiveness of cancer cells have been reported. For instance, while activation of PKBα during ErbB2-induced mammary tumorigenesis promotes tumour growth and incidence in mice, it appears to reduce metastatic progression of these tumours [22]. Similarly, Yoeli-Lerner et al. [32] reported that PKBα-mediated signalling inhibits migration and invasion of breast cancer cells. In contrast with PKBα, overexpression of PKBβ appears to increase the invasiveness and metastatatic potential of breast cancer cells in animal models [33]. In addition, Heron-Milhavet et al. [34] showed that PKBα promotes cell proliferation, whereas PKBβ promotes cell cycle exit in myoblasts and fibroblast cell lines, due to differential interaction of these PKB isoforms with the cell cycle inhibitor $p21^{Cip1}$. For future studies, it will be interesting to assess how deletions of the two other PKB isoforms affect tumour incidence and development in PTEN mutant mice.

Life with a single isoform of PKB

To understand the *in vivo* functions of the three PKB isoforms, mouse mutant models lacking individual PKB isoforms (PKB$\alpha^{-/-}$ [35,36], PKB$\beta^{-/-}$ [6,7] and PKB$\gamma^{-/-}$ [10,11] mice) or various possible combinations of PKB isoforms (PKB$\alpha^{-/-}$PKB$\beta^{-/-}$ [2], PKB$\alpha^{-/-}$PKB$\gamma^{-/-}$ [37] and PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ [8] mice) have been generated and analysed. Mice lacking individual PKB isoforms are viable and display relatively subtle phenotypes, but combined deficiency of PKBα/PKBγ or PKBα/PKBβ causes lethality at the embryonic and the neonatal stage respectively (for phenotypes of PKB double knockout mice, see Table 1). The lethality of these double knockout mice suggests extensive functional overlap in between isoforms *in vivo*. Interestingly, PKBα appears to have a dominant role in embryonic development and postnatal survival. Mice in which only the PKBα isoform remains, such as PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ mice and even PKB$\alpha^{+/-}$PKB$\beta^{-/-}$PKB$\gamma^{-/-}$ mice, survive with minimal dysfunctions, despite a dramatic reduction of total PKB levels in many tissues (Figure 1) [8]. Overall, the analyses of PKB loss-of-function mouse models established a role for PKB isoforms in cell proliferation, cell growth, differentiation and glucose metabolism *in vivo*. The distinct phenotypes of these mice suggest an order of apparent importance of the three PKB genes for specific functions. There are non-redundant functions for PKBβ and PKBγ in glucose homoeostasis and brain respectively, and a major role for the PKBα isoform in embryonic development, growth and survival.

We apologize to our colleagues whose work could not be included owing to space limitations. We thank Elisabeth Fayard and David Restuccia for critical reading of the manuscript. B.D. is supported by Krebsliga Schweiz (KFS 1167-09-2001 and KFS 01002-02-2000). The Friedrich Miescher Institute is part of the Novartis Research Foundation.

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Received 16 December 2006