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Review

Akt: Versatile Mediator of Cell Survival and Beyond

Dohoon Kim and Jongkyeong Chung*

National Creative Research Initiatives Center for Cell Growth Regulation and Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon 305-701, Korea

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The serine/threonine kinase Akt has been intensely studied for its role in growth factor-mediated cell survival for the past 5 years. On the other hand, the ongoing research effort has recently uncovered novel regulatory mechanisms and downstream effectors of Akt that demonstrate the involvement of Akt in other cellular functions such as cell cycle progression, angiogenesis, and cancer cell invasion/metastasis. Furthermore, recent studies using whole model organisms suggest additional roles for Akt in important diseases such as aging and diabetes. The following review addresses these recent advances in the understanding of Akt function.

Keywords: PI3 kinase, Apoptosis, Cell cycle, Metastasis, Animal models

PI3 kinase, now well-known as an important upstream regulator of cell survival, growth, malignant transformation, vesicle trafficking, and cytoskeletal regulation, was first suggested to play a role in cell survival as it was found to be activated during the colony stimulating factor 1 (CSF-1) mediated cell proliferation and survival (Varticovski *et al.*, 1989). It was also later discovered that it is required for the prevention of apoptosis in various cell types by growth factors (Scheid *et al.*, 1995; Yao and Cooper, 1995; Takashima *et al.*, 1996). Akt was finally identified as the crucial link between the PI3 kinase and the prevention of apoptosis (Franke *et al.*, 1997; Dudek *et al.*, 1997). This opened the floodgates for Akt research. Subsequent searches for the downstream targets of Akt led to the discovery of Bad, caspase-9, and Forkhead transcription factors. Each is a component of apoptotic machinery that is inhibited by Akt to prevent apoptosis. In addition to utilizing such a multi-faceted approach to preventing apoptosis, Akt blocks apoptosis that is induced by a wide range of apoptotic stimuli, and in a wide range of cell types. Furthermore, studies using constitutively active and dominant negative forms of Akt have demonstrated that Akt is both necessary and sufficient for survival. Collectively, these findings support the role of Akt as perhaps the most important mediator of survival in the cell.

In addition to its important role in promoting cell survival, the versatile Akt plays numerous other roles in the cell. One of the first roles that was attributed to Akt was its regulatory role in glycogen synthesis via the phosphorylation of glycogen synthase kinase 3 (GSK-3). Later it was discovered that Akt is also highly involved in cell-cycle progression, angiogenesis, and cancer cell invasion/metastasis. In this article, the numerous roles of Akt in the cell, in cell survival and beyond, will be discussed in detail with special emphasis on the most recently discovered roles that are attributed to Akt.

The PI3 kinase and upstream regulation of Akt activity

There is actually a family of the PI3 kinases extant in the cell, each with a distinct mode of regulation and substrate specificity (reviewed in Fruman *et al.*, 1998). The predominant form of the PI3 kinase exists as a heterodimer of a catalytic subunit (molecular weight 110 kD) and a regulatory subunit (molecular weight 85 kD). The PI3 kinase is activated by a variety of transmembrane receptors on the plasma membrane. These receptors are activated, usually by tyrosine autophosphorylation, upon ligand binding, and recruit the PI3 kinase to their cytoplasmic side of the plasma membrane. Once localized in the plasma membrane, the PI3 kinase phosphorylates the D-3 position of the inositol ring of phosphoinositides in the membrane. PI(3,4)P_2 and PI(3,4,5)P_3 that are generated by the PI3 kinase mediate the activation of Akt (Franke *et al.*, 1997; Stokoe *et al.*, 1997). The phosphoinositide products of the PI3 kinase are thought to activate Akt by (1) recruiting Akt to the plasma membrane, and (2) recruiting PDK1, an activator of Akt, to the membrane also. The recruitment occurs because both Akt and PDK1

*To whom correspondence should be addressed.
Tel: 82-42-869-2620; Fax: 82-42-869-8160
E-mail: jchung@mail.kaist.ac.kr

contain PH domains, which have a high affinity for PI(3,4)P_2 and PI(3,4,5)P_3. At the membrane, PDK1 and a yet unidentified PDK2 phosphorylate two residues on Akt (Thr-308 and Ser-473), fully activating it (Alessi *et al.*, 1997). Besides allowing Akt and its activators to come into close proximity, the binding of the PI3 kinase-generated phosphoinositides to the PH domain of Akt appears to cause a conformational change in Akt that allows it to be phosphorylated by PDK1 and the putative PDK2 (Alessi *et al.*, 1997; Stokoe *et al.*, 1997).

Until now nearly all modes of Akt activation seem to occur via PI3 kinase. A wide variety of upstream signalings have been demonstrated to activate Akt via the PI3 kinase. The most well documented activators of the PI3 kinase/Akt-signaling pathway are the growth factors. A wide variety of growth factors such as EGF, PDGF, NGF, IGF, etc., have been demonstrated to activate the PI3 kinase/Akt signaling, and this mechanism accounts for the growth factor requirement of most cell lines for survival and proliferation.

There are a number of other important signaling molecules besides growth factors that modulate the PI3 kinase/Akt signaling. Integrins bind to integrin receptors and activate the PI3 kinase, leading to Akt activation (Khwaja *et al.*, 1997; Shaw *et al.*, 1997); this may be an important mechanism in PI3 kinase/Akt mediated cell invasion/metastasis, and anchorage-dependent cell survival. G-protein coupled receptors (Murga *et al.*, 1998), angiotensin II (Ushio-Fukai *et al.*, 1999), and oncogenic Ras (Franke *et al.*, 1995) also activate the PI3 kinase, which leads to Akt activation. Recently, extracellular zinc was shown to activate the PI3 kinase/Akt signaling (Kim S. *et al.*, 2000), the first demonstration of the involvement of zinc signaling. Recently, cAMP was shown to either activate (Shong, personal communication) or inactivate (Kim S. *et al.*, 2001; Wang *et al.*, 2001) the PI3 kinase/Akt signaling, depending on the cell type and cellular context. This suggests a means of cross-talk between the cAMP and the PI3 kinase signaling pathways.

On the other hand, there have been a few observations of the PI3 kinase-independent regulation of Akt. The Ca^{2+}/calmodulin-dependent protein kinase (CaMKK) that is activated by an increase in Ca^{2+} levels was found to directly phosphorylate Akt, activating it in a PI3 kinase-independent manner (Yano *et al.*, 1998). This mechanism may play an important role in the selective survival of active neurons, which increased the intracellular Ca^{2+} activity, during neural development. Protein kinase C-related kinase 2 (PRK2) inhibits Akt in a PI3 kinase-independent manner (Koh *et al.*, 2000). The C-terminal fragment of PRK2, generated by caspase cleavage during apoptosis, binds Akt and inhibits its activity. This finding demonstrates a mechanism for inhibiting the antiapoptotic function of Akt during the progression of apoptosis.

The upstream signaling mechanisms regulating Akt activity are outlined in Fig. 1.

Akt and apoptosis

Activated Akt has the capacity to phosphorylate a wide variety of substrate proteins in order to perform its various functions in the cell. A most important discovery in the Akt function was made using synthetic peptides with sequences that are related to the phosphorylation site of GSK-3 as substrates for Akt kinase activity. As a result, the consensus site for Akt phosphorylation, RXRXXS/T, was identified (Alessi *et al.*, 1996). The subsequent searches for apoptosis-related proteins that contain this sequence led to the identification of numerous proteins that are involved in apoptosis as targets of Akt. It has also given us an extensive and detailed picture of how Akt inhibits apoptosis. The various mechanisms by which Akt inhibits apoptosis are shown in Fig. 2.

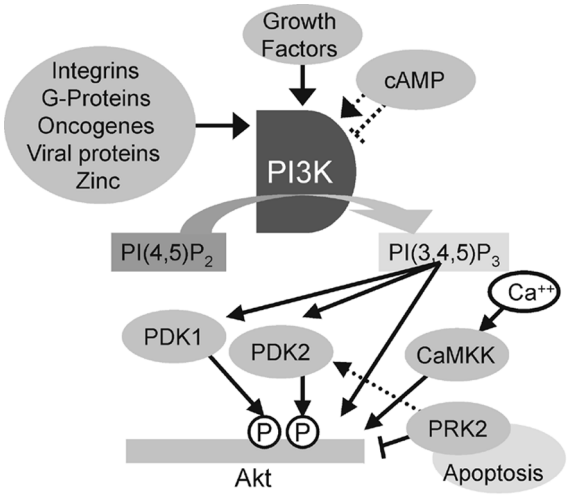

Fig. 1. The upstream signaling mechanisms regulating Akt activity.

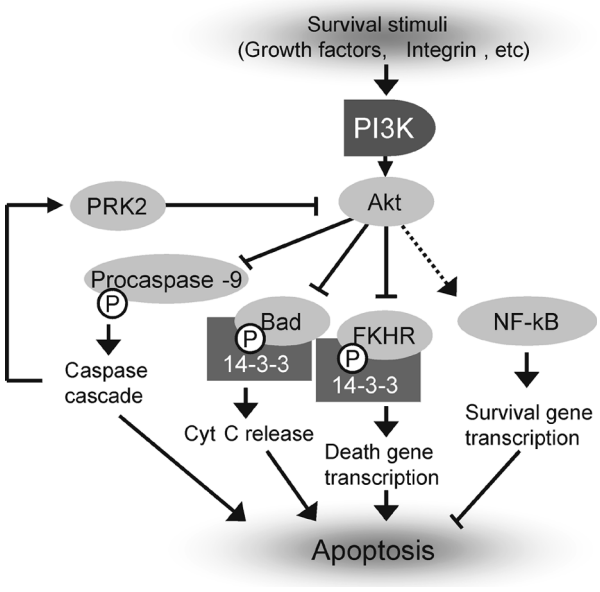

Fig. 2. Inhibition of Apoptosis by Akt.

Bad Bad was the first protein that is directly involved in apoptosis to be identified as a target of Akt. Bad is a member of the Bcl-2 family, which converges on the mitochondrial outer membrane to regulate cell survival (reviewed in Gottlieb, 2000). In the absence of Akt activity, Bad binds with another pro-survival member of the Bcl-2 family, Bcl-XL, and induces cell death, most likely by inhibiting the function of Bcl-XL to block the release of cytochrome *c* from mitochondria to the cytoplasm (Kharbanda *et al.*, 1997; Kennedy *et al.*, 1999). However, activated Akt phosphorylates Bad at Ser-136 (Datta *et al.*, 1997), causing it to dissociate from Bcl-XL in the mitochondrial membrane and associate with the adaptor protein 14-3-3 instead. This results in the sequestration of Bad to the cytosol (Zha *et al.*, 1996). Thus, Bad that is phosphorylated by Akt cannot induce cell death.

Caspase 9 During apoptosis, cytochrome c that is released into the cytoplasm binds the CED-4 homologue, Apaf-1. This causes it to bind, cleave, and activate the cysteine protease procaspase-9, which propagates the apoptotic caspase cascade that results in the activation of the 'executioner' caspases, caspase 3 and caspase 7 (reviewed in Cohen, 1997). Interestingly, Akt phosphorylates procaspase-9 at Ser-196 (Cardone *et al.*, 1998), rendering it resistant to processing and activation. Although it may appear redundant for Akt to act both upstream and downstream of cytochrome c in preventing apoptosis, the phosphorylation of procaspase-9 by Akt must have a physiological significance, as the cells that express caspase-9 with the Ser-196 mutated to alanine and underwent apoptosis that was resistant to Akt activity (Cardone *et al.*, 1998)

FKHR1 Akt phosphorylates and inactivates the Forkhead transcriptional factors. In the absence of survival signalings (i.e. phosphorylation by Akt), the Forkhead proteins enter the nucleus and are thought to induce the transcription of various cell-death related genes, such as FasL (Fas ligand) (Brunet *et al.*, 1999). However, active Akt induces the phosphorylation of a specific site on the FKHR1 molecule that causes it to be excluded from the nucleus (Biggs *et al.*, 1999; Brunet *et al.*, 1999), therefore losing its transcriptional activity. As with Bad, FKHRL1 binds 14-3-3 (Brunet *et al.*, 1999); 14-3-3 may generally function to sequester Akt targets away from their sites of action.

NF-κB NF-κB is another factor that is involved in cell survival. It has been identified as a functional target of Akt (Ozes *et al.*, 1999; Romashkova *et al.*, 1999). NF-κB is a family of transcription factors, which induce the expression of a wide variety of genes, especially those involved in survival, such as the Bcl-2 family member Bfl-1, and the caspase inhibitors c-IAP1 and c-IAP2 (Wang *et al.*, 1998; Zong *et al.*, 1999). Binding with IκB sequesters it to the cytoplasm. Upon phosphorylation of IκB by IKKalpha and IKKbeta, IkB is degraded and NF-κB can enter the nucleus to induce transcription (reviewed in May and Ghosh, 1997). It must be noted that NF-κB does not appear to be directly phosphorylated by Akt, but indirectly activated. The exact mechanism of NF-κB activation by Akt is still in question. There are conflicting reports that Akt activates NF-κB through IκB (Ozes *et al.*, 1999; Kane *et al.*, 1999), or through indirect phosphorylation of the catalytic p65 subunit of NF-κB (Sizemore *et al.*, 1999). Recently, NF-κB was found to mediate the induction of the MMP-9 production by Akt, leading to increased cell invasive potential (Kim D. *et al.*, 2001). This will be described in detail later.

Akt and glucose metabolism

The first function that was attributed to Akt was glycogen metabolism. The first discovered substrate for Akt was GSK-3. GSK-3 is an important regulatory kinase with various targets, such as glycogen synthase, the translation initiation factor eIF2B, and the transcription factor C/EBP. It is involved in various functions, such as metabolic regulation, development, and oncogenesis. Akt that is activated by insulin phosphorylates and inactivates GSK-3 (Cross *et al.*, 1995), revealing the link between insulin signaling and the synthesis of glycogen from glucose. In addition, Akt that is activated by insulin enhances glucose uptake by increasing the expression levels of the glucose transporters, GLUT1 and GLUT3 (Barthel *et al.*, 1999), and by inducing the translocation of GLUT4 (Kohn *et al.*, 1996). Also, Akt may induce glycolysis, the metabolic breakdown of glucose. Akt phosphorylates and activates 6-phosphofructo-2-kinase, one of the enzymes that are involved in the glycolysis pathway (Deprez *et al.*, 1997). Recently, an Akt knockout mouse was studied for the first time, demonstrating a novel role of Akt in glucose homeostasis (Cho H. *et al.*, 2001). This strongly suggests an important role for Akt in glucose metabolism-related diseases, such as diabetes mellitus.

Akt and cell-cycle progression

While the role of Akt in preventing apoptosis has been well-established through research over the last 5 or so years, knowledge on the role of Akt in cell-cycle regulation has just recently begun to burgeon. The involvement of Akt in cell-cycle progression was first observed in IL-2 dependent T-cell lymphomas, which undergo G1 arrest in IL-2-deficient conditions (Ahmed *et al.*, 1997). When constitutively active myristylated Akt was overexpressed in this cell line, the cells were not subjected to G1 arrest. More direct evidence on the promotion of the cell-cycle progression by Akt was obtained when the co-expression of Akt rescued cells from PTEN-induced cell-cycle arrest (Paramio *et al.*, 1999).

As in apoptosis, Akt appears to regulate cell-cycle progression through a large number of targets (Fig. 3). The first identified indirect target of Akt in cell-cycle regulation was the oncogene c-Myc. Akt activity increased the

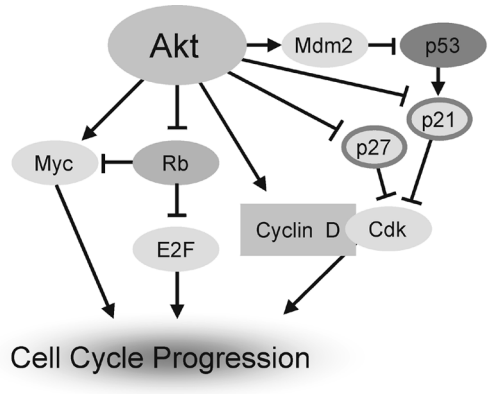

Fig. 3. Promotion of cell-cycle progression by Akt.

transcription of c-Myc (Ahmed *et al.*, 1997), which (when hyperactivated or overexpressed) is a strong promoter of cell-cycle progression, causing the cells to exit G0 and proliferate (reviewed in Evan and Littlewood, 1993).

The tumor suppressor retinoblastoma (Rb) has also been identified as a target of Akt. In the dephosphorylated state, Rb binds to and thus inactivates the regulatory proteins, such as E2F and c-Myc, which are required for cell proliferation. Akt phosphorylated and deactivated Rb, leading to the activation of E2F (Brennan *et al.*, 1997).

Another interesting target of Akt that is involved in cell-cycle progression is cyclin D1. During cell-cycle progression, when sufficient quantities exist, cyclin D1 associates various cyclin dependent kinases (Cdks) to allow the cell to exit the G0 stage and progress past the G1 stage. This is done as the Cdk/cyclin complex phosphorylates and inactivates the Rb protein (Matakeyama *et al.*, 1994; Resnitzky and Reed, 1995). Akt is an important link between growth factor stimulation and cyclin D1 levels. Cyclin D1 has a very short half-life, which is due in part to the degradation that is induced by the Akt target GSK-3, and is present in sufficient numbers only in the presence of stimulation by growth factors (reviewed in Terada *et al.*, 1999). Akt activation by serum stimulation also leads to the enhanced translation of cyclin D1 (Muise-Helmericks *et al.*, 1998).

In addition, Akt also indirectly regulates the Cdk inhibitor, p27. p27 is related to the p53 downstream effector, p21. Both p27 and p21 function to block the activation of Cdk-cyclin dimers (reviewed in Pruitt and Der, 2001). Studies using the PTEN tumor suppressor first implied a relationship between Akt and p27, as the downregulation of the Akt activity by PTEN was correlated with an increase in levels of p27, which lead to G1 arrest (Li and Sun, 1998; Sun *et al.*, 1999). This was later confirmed when Akt was shown to diminish p27 expression levels during its promotion of prostate cancer progression (Graff *et al.*, 2000), and during IGF-1-stimulated cell-cycle progression of skeletal muscle cells (Chakravarthy *et al.*, 2000). Interestingly, Akt was recently found to phosphorylate and inactivate p21 as well (Zhou *et al.*, 2001). p21, whose activity is strongly correlated with a nuclear localization, was found to be phosphorylated at Thr-145 by activated Akt. Upon phosphorylation, p21 is translocated to the cytosol, and loses its ability to block cell proliferation.

Finally, an exciting recent discovery is that the oncogene Mdm2 is a direct target of Akt in cell-cycle regulation (Mayo and Donner, 2001). Mdm2 is an important regulator of p53, a crucial tumor suppressor in the cell that halts the cell-cycle in G1 in response to DNA damage or other cellular stresses, or triggers cell apoptosis if G1 arrest is not possible, through the transcriptional activation of genes that are involved in cell-cycle control or apoptosis (reviewed in Levine, 1997). Mdm2 and p53 co-exist in a negative feedback loop, where p53 induces the transcription of Mdm2, and Mdm2 binds to and inhibits the tumor suppressor function of p53 by promoting its ubiquitin-dependent degradation. Phosphorylation of residues Ser-166 and Ser-186 by Akt promoted the nuclear localization of Mdm2, where it bound p53, resulting in reduced transcriptional activity and expression levels of p53 (Mayo and Donner, 2001). This finding may help explain the ability of Akt to suppress apoptosis that is initiated by such a wide variety of stresses, such as anoikis (Kwaja *et al.*, 1997), growth factor deprivation (Dudek *et al.*, 1997), and UV radiation (Kulik *et al.*, 1997).

Although many functional targets of Akt in cell-cycle progression have been implicated, in contrast to Akt and apoptosis, the overall picture of cell-cycle regulation by Akt is still vague. The targets that have been mentioned, with the exception of Mdm2 and p21, are not direct targets of phosphorylation by Akt. The link between Akt and its targets still need to be identified. In addition, it is uncertain whether Akt regulates each target through separate mechanisms, or regulates some of the targets in a single mechanism. For example, the cyclin D1 upregulation by Akt may lead to the inhibition of Rb and the upregulation of c-Myc that was observed in previous studies.

Akt and angiogenesis

Many recent studies on endothelial cell lines also demonstrated a role for Akt in angiogenesis, the formation of blood vessels. Akt was first implicated in angiogenesis in a study that demonstrated that the vascular endothelial growth factor promotes endothelial cell survival through the activation of Akt (Gerber *et al.*, 1998). It was also reported that Tie2, which has angiopoietin-1 as a ligand, activates Akt via the PI3 kinase (Kontos *et al.*, 1998). Akt was found to mediate the survival of epithelial cells by proangiogenic stimuli, such as shear stress (Dimmeler *et al.*, 1998), angiopoietin-1 (Kim I. *et al.*, 2000a; Papapetropoulos et al, 2000), and angiopoietin-2 (Kim I. *et al.*, 2000b). This further demonstrates its important role in angiogenesis.

The first and only identified direct target of Akt in the promotion of angiogenesis is endothelial nitric oxide synthase (eNOS) (Dimmeler *et al.*, 1999; Fulton *et al.*, 1999). Through

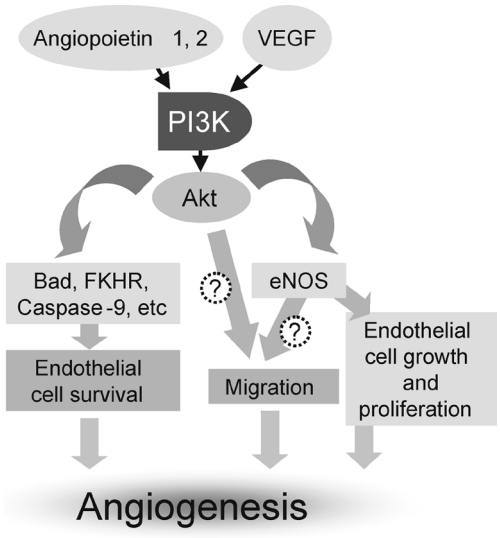

Fig. 4. Regulation of angiogenesis by Akt.

its product, the highly reactive nitric oxide (NO) free radical, eNOS stimulates the growth and proliferation of endothelial cells (reviewed in Goligorsky *et al.*, 1999), and induces vascular permeability and vasodilatation (reviewed in Fukumura and Jain, 1998), functions which enhance angiogenesis and vascular blood flow. Although there is some debate pertaining to the exact site of phosphorylation, Akt was shown to phosphorylate eNOS, leading to an increase in the NO production in endothelial cells (Dimmeler *et al.*, 1999; Fulton *et al.*, 1999).

The role of Akt in regulating eNOS activity and angiogenesis holds vast clinical implications, especially in the aspect of tumor progression. The formation of new blood vessels (neovascularization) and sufficient blood flow to the tumor is vital for its growth beyond a certain size. Akt, NOS, and angiogenesis have each been implicated, through numerous studies, in tumor progression. This strongly supports an Akt-to-eNOS-to-angiogenesis link to tumor progression. However, a direct link between Akt and angiogenesis has not yet been demonstrated in vivo. Future studies that utilize transgenic organisms are required to resolve this matter. Figure 4 outlines the known role of Akt in regulating angiogenesis.

Akt, cell migration, and invasion/metastasis

The ultimate stage of tumor progression, and the most deadly, is the gain of an invasive/metastatic phenotype. Cell invasion and metastasis, which is the migration of individual tumor cells away from the primary tumor to form secondary tumors on different sites in the body, signals the worst-case scenario for cancer patients. The most recently discovered and potentially important role of Akt, one that is just beginning to be unraveled, is the role of Akt in cell migration and invasion/metastasis.

An early clue that Akt may be involved in cancer invasion and metastasis is the high correlation of angiogenesis with metastasis. Countless clinical documentations have linked neovascularization within a tumor to metastasis. Tumor neovascularization seems to be a prerequisite to an invasive and metastatic phenotype. Indeed, inhibitors of angiogenesis, such as the cyclooxygenase-2 (Cox-2) inhibitor, also inhibit metastasis (Masferrer *et al.*, 2000). The recently discovered role of Akt in angiogenesis, described previously, hints at a possible role for Akt in tumor cell invasion/metastasis.

Another hint of Akt involvement in cancer cell invasion/metastasis is the increasing evidence for a role of Akt in cell motility/migration. In addition to the changes in cell adhesion properties (so that the tumor cell can detach from surrounding cells) and increased expression and activation of extracellular proteases to degrade the extracellular matrix (ECM), the increased cell migration (motility) that is mediated by changes in the cytoskeleton is an important prerequisite to cancer cell invasion. This leads to metastasis (reviewed in Stetler-Stevenson *et al.*, 1993; Takeichi, 1993; Bohle and Kalthoff, 1999). VEGF stimulation activates Akt that leads to endothelial cell migration (Radisavljevic *et al.*, 1999; Morales-Ruiz *et al.*, 2000), a function that is necessary for angiogenesis. In addition, Akt is required for efficient chemotaxis to cAMP in the slime mold *Dictyostelium* (Meili *et al.*, 1999).

The first direct evidence of the regulation of cancer invasion/metastasis was just recently demonstrated (Kim D. *et al.*, 2001). Using the highly metastatic HT1080 fibrosarcoma cell line, Akt, localized at the leading edge of migrating cells, was demonstrated to regulate cell migration and invasion in a manner that is highly dependent on its kinase activity and membrane-translocating ability. The modulation of cell migration by Akt contributed to its effect on cell invasion.

In the same study (Kim D. *et al.*, 2001), Akt was shown for the first time to modulate the expression of matrix metalloproteinase-9 (MMP-9) in the regulation of cell invasion. The matrix metalloproteinases are a group of zinc-dependent, ECM degrading proteases that are required in cancer cell invasion/metastasis (reviewed in Stetler-Stevenson *et al.*, 1993). The increased expression of the MMPs (especially MMP-2, MMP-7, and MMP-9) is correlated with the progression and metastatic potential of cancer cells. Especially, MMP-9 is expressed in a large variety of malignant cells and degrades collagen, the major component of the ECM and basement membrane. Akt modulated the expression levels of MMP-9 by inducing the transcriptional activity of NF-kB, a target that was mentioned previously in the regulation of apoptosis (Kim D. *et al.*, 2001). In support of this finding, Akt activity and MMP-9 expression levels were both shown to be selectively upregulated in the cells in the perivascular tumor areas, i.e. those with the highest metastatic potential (Kubiatowski *et al.*, 2001).

This newly discovered function of Akt, along with Rac, explains the well-documented involvement of the PI3 kinase

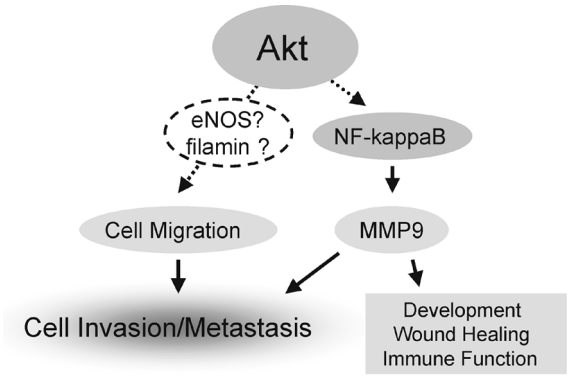

Fig. 5. A preliminary outline of Akt functions in cell invasion/metastasis and other biological phenomena.

pathway in cancer cell invasion (Keely *et al.*, 1997; Shaw *et al.*, 1997). As in apoptosis and cell-cycle regulation, the emerging discoveries point to the Akt regulating cancer cell invasion/metastasis through multiple targets and mechanisms. Whereas a link between Akt and MMP-9 production has been demonstrated, the mechanisms by which Akt modulates cell migration remains a mystery. The previously mentioned eNOS appears to be a viable candidate as an Akt effector in the VEGF-stimulated endothelial migration (Dimmeler *et al.*, 2000). However, whether or not a similar mechanism is present in tumor cell migration still needs to be addressed. Also, the leading edge localization of Akt during cancer cell migration (Kim D. *et al.*, 2000), and the requirement of cytoskeletal restructuring during migration, implies that Akt may directly phosphorylate some component that is involved in cytoskeletal regulation, such as filamin. Finally, we cannot rule out the possibility that Akt may regulate cell invasion and metastasis through more aspects than just metalloproteinase production and cell migration. Much research is still needed on this newly discovered function of Akt. Figure 5 shows a preliminary sketch of Akt functions in cancer cell invasion/metastasis, as well as other important biological phenomena.

Conclusions and future prospects

With so many important substrates and functions, Akt can be thought of as a major hub in cellular signaling, a central component that connects diverse upstream signalings to even more diverse physiological outputs. Such a versatile and important role in the cell implies that Akt is involved in the regulation of many important biological phenomena, and that errors in Akt signalings may lead to various diseases. Indeed, the described role of Akt in cell survival, proliferation, angiogenesis, and invasion/metastasis have firmly established it as a major promoter of tumor progression, in accordance with clinical observations (Cheng *et al.*, 1992; Bellacosa *et al.*, 1995; Cheng *et al.*, 1996).

However, there may also be other crucial biological functions of Akt. According to recent studies, Akt is likely involved in the phenomena of aging. Homologues of Akt in *Caenorhabditis elegans* and in yeast regulate the stress resistance and aging in these organisms (Wolkow *et al.*, 2000; Fabrizio *et al.*, 2001). In *Caenorhabditis elegans*, the disruption of the PI3 kinase/Akt signaling led to a metabolically suppressed state where antioxidant levels were elevated and lifespan increased severalfold. In non-dividing yeast, mutations in the Akt homologue Sch9 lead to oxidative stress resistance and increased the yeast lifespan threefold. While *Caenorhabditis elegans* and yeast are evolutionarily distant from mammals, the described phenomena are highly analogous to the effects of dietary restriction in mammals, There a lowered metabolism, lower levels of free radicals, and increased lifespan are observed (reviewed Finch *et al.*, 1997). where, the role of Akt in the mammalian lifespan may well be conserved. Indeed, the mentioned role of Akt in glucose metabolism may be related to this mechanism. In addition, Akt is reported to phosphorylate and enhance telomerase activity (Kang *et al.*, 1999), a function that may also have implications in aging and carcinogenesis.

The regulation of MMP-9 by Akt may also have other important functions. Besides being required for cancer invasion and metastasis, matrix metalloproteinases play important roles in normal physiological functions, such as development, immune activity, and wound healing. Through the regulation of MMP-9, and possibly other MMPs, Akt may also be involved in these functions.

The most promising field in Akt research appears to be genetics research that uses multicellular organisms. As was mentioned, Akt has already been extensively studied in *Caenorhabditis elegans.* The homologues of Akt and upstream/downstream signaling components, such as the PI3 kinase (age-1), PDK1 (pdk-1), and FKHR1 (daf-16), have been identified and characterized (Morris *et al.*, 1996; Paradis and Ruvkun, 1998; Paradis *et al.*, 1999). In fact, the discovery of daf-16 as a target of Akt in *Caenorhabditis elegans* led to the identification of its mammalian homologue, FKHR1, as an Akt effector. Studies using transgenic fruit flies demonstrated the role of Akt in suppressing apoptosis and regulating cell size, in the context of the whole organism (Staveley *et al.*, 1998; Verdu *et al.*, 1999; Cho K. *et al.*, 2001). In addition, a recent study using an Akt knockout mouse (Cho H. *et al.*, 2001), mentioned previously, strongly confirmed the role of Akt in glucose metabolism, which was extensively studied using cell lines.

As evidenced by these results, studies using whole organisms allow the function of Akt to be studied in a physiologically relevant manner. Accordingly, future research on the various Akt targets and functions, using transgenic organisms, will be required to confirm the functions that are attributed to Akt through studies using cell lines. For example, while the phosphorylation and inactivation of Bad by Akt in specific cell lines is well established (as Bad is expressed at low levels and not ubiquitously), this mechanism may play only a minor role in the organism. Likewise, recent findings

suggest that serum and glucocorticoid-activated kinase (SGK) may have some functions that overlap with those of Akt (reviewed in Scheid and Woodgett, 2001).

In addition to confirming the already discovered roles of Akt, research using transgenic animals is necessary for addressing the possible biological/pathological roles of Akt, such as aging and diabetes. Finally, genetic screening systems using transgenic animals should allow the discovery of novel targets and functions of Akt, which could not be discovered using conventional methods.

Since the discovery of GSK-3 as its first target in 1995, research groups around the world have made hundreds to thousands of discoveries on Akt. They have identified a menagerie of substrates that implicate Akt in cellular functions as diverse as glucose uptake and metastasis. There are so many research groups that study the various aspects of Akt signaling, as well as so many novel and exciting discoveries that are being made each month that pertain to Akt, that whole new aspects on Akt may need to be addressed by the time this review is published. The combined research effort on Akt in recent years is prodigious. But, the continued exploration into Akt only seems to further emphasize its importance and open up even more avenues for Akt research. Akt will remain in the spotlight in cellular signaling and molecular pathology for years to come.

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