Distinct Biological Roles for the Akt Family in Mammary Tumor Progression

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Abstract

The phosphatidylinositol 3′ kinase/Akt pathway is frequently dysregulated in cancer, which can have unfavorable consequences in terms of cell proliferation, survival, metabolism, and migration. Increasing evidence suggests that Akt1, Akt2, and Akt3 play unique roles in breast cancer initiation and progression. We have recently shown that in contrast to Akt1, which accelerates mammary tumor induction in transgenic mice, Akt2 promotes metastasis of tumor cells without affecting the latency of tumor development. Despite the distinct phenotypic outputs resulting from Akt1 or Akt2 activation, very little is known about the mode by which such unique functions originate from these highly related kinases. Here we discuss potential mechanisms contributing to the differing functional specificity of Akt1 and Akt2 with respect to migration, invasion, and metastasis. Cancer Res; 70(11): 0F1–5. ©2010 AACR.

Introduction

The primary breast tumor on its own is rarely fatal, and mortality is due mainly to the growth of tumor cells at distant sites following metastatic spread. For this reason, elucidating the signaling pathways involved in the metastatic cascade is an important goal for developing therapeutics in efforts to reduce cancer mortality rates. The formation of secondary metastases involves multiple steps including migration out of the primary tumor and intravasation into the vascular system, survival in the blood stream, extravasation out of the blood stream, and survival and proliferation at the secondary site.

The Akt family of serine-threonine kinases consists of three members: Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ (reviewed in ref. 1). Akt has been shown to be the primary downstream mediator of the effects of the phosphatidylinositol 3′ kinase (PI3K), and through the phosphorylation of target substrates Akt impinges on numerous cellular processes. All Akt family members share a similar domain structure with an N-terminal plekstrin homology domain, a linker domain, a central kinase domain, and a C-terminal hydrophobic domain. In order to be fully activated, Akt requires phosphorylation at two specific amino acid residues, threonine 308 and serine 473, located in the kinase domain and C-terminal hydrophobic domain, respectively. Phosphorylation is achieved by the action of two independent protein kinases: phosphatidylinositol-dependent kinase 1 (PDK1), which phosphorylates threonine 308, and the mammalian target of rapamycin complex-2 (mTORC2), which phosphorylates serine 473. Upon activation, Akt moves to the cytoplasm and nucleus where it phosphorylates downstream target proteins. Several modes of Akt pathway dysregulation have been identified in human breast cancer and ultimately affect a number of processes including cell growth, survival, proliferation, metabolism, and motility and/or invasion.

In addition to amplification and mutational activation of Akt itself, alterations in upstream signaling components [amplification and/or overexpression of growth factor receptors and PDK1, mutational activation of PI3K, and loss of phosphatase and tensin homologue deleted on chromosome 10 (PTEN)] have also been noted (reviewed in ref. 2). Thus, as a central converging node for multiple activation events, Akt inhibition represents an attractive therapeutic target.

Using two different transgenic mouse models of oncogene-induced mammary tumorogenesis, we have recently shown that, in contrast to Akt1, which plays an important role in breast cancer induction, Akt2 is primarily involved in metastatic dissemination (3–5). Interestingly, Akt1-expressing mammary tumors not only displayed a highly differentiated pathology, but in the case of an ErbB2-driven tumor model, Akt1 expression actually decreased the proportion of tumor-bearing mice that develop lung metastases (5). Thus, the two Akt family members achieve opposing functions in terms of breast tumor cell metastasis. In light of these observations and previous cell-based studies revealing that Akt1 relays antimigratory and anti-invasive signals in breast cancer cells, the exact mechanism by which these highly related kinases induce such distinct phenotypic outputs is an appealing avenue of investigation.

In terms of cell motility and invasion, the specific role of the individual Akt family members seems to depend, in large
part, on the cell type being studied. In breast epithelial cells, several in vitro studies have suggested that Akt1 and Akt2 play opposing roles in migration and invasion, with Akt2 exerting promigratory effects. Early studies showed that, of the Akt family members, overexpression of only Akt2 could duplicate the invasive phenotype of PI3K transfected breast cancer cells (6). Furthermore, expression of a kinase dead Akt2, but not Akt1 or Akt3, prevented invasion induced by PI3K activation or ErbB2 overexpression (6). Akt2 overexpression in breast and ovarian cancer cells was shown to upregulate β1-integrin and increase in vitro invasion as well as metastasis in vivo (6). Together, these results indicate that among the members of the Akt family, Akt2 may have particular importance in mediating PI3K-dependent effects on cellular adhesion, motility, invasion, and metastasis in breast cancer cells.

Subsequently, a number of separate studies have implicated Akt1 as an inhibitor of breast epithelial cell motility and invasion. The Brugge laboratory used an MCF10A breast epithelial cell line expressing the insulin like growth factor 1 receptor in transwell assays to assess the contribution of Akt1 and Akt2 to cell migration (7). In this model they showed that downregulation of Akt1 using short hairpin RNA (shRNA) dramatically increased cell migration. In contrast, Akt2 downregulation did not affect migration, and the concomitant downregulation of Akt1 and Akt2 abrogated the migratory effect of Akt1 knockdown, suggesting that expression of Akt2 is required for this phenotype. They further showed that shRNA-mediated downregulation of Akt1 increased activation of the extracellular signal-regulated kinase (ERK) and concluded that Akt1-mediated suppression of ERK signaling is responsible for the antimigratory effect of Akt1. More recently, Iliopoulos and colleagues showed that epithelial-mesenchymal transition in MCF10A cells induced by Akt1 knockdown correlated with a downregulation of the miR-200 family of microRNAs (8). In agreement with these knockdown studies in MCF10A cells, Akt1 overexpression in breast cancer cell lines was shown to result in decreased migration and invasion (9). This study further showed that the inhibitory effect was mediated through the proteasomal degradation of nuclear factor of activated T cells, via Akt-mediated activation of the mouse double minute 2 ubiquitin ligase. It therefore seems that Akt1-dependent inhibition of migration can be achieved by several distinct mechanisms.

Consistent with these tissue culture experiments, we have provided in vivo evidence supporting the opposing functions of Akt1 and Akt2, with Akt1 inhibiting and Akt2 promoting metastasis in transgenic mouse models of breast cancer (3, 5). Furthermore, two groups have reported interbreeding of an MMTV-ErbB2 mouse strain with a germline knockout of Akt1. Consistent with the importance of Akt1 in tumor induction, germline ablation of Akt1 resulted in severely impaired tumor induction in both of these studies (10, 11). In agreement with our original observation that ectopic expression of Akt1 results in suppression of ErbB2-induced metastatic spread (5), Maroulakou and colleagues noted an enhanced invasive appearance in ErbB2/Akt1-deficient tumors (11). In contrast, the Ju and colleagues study did not note any metastases in five tumor-bearing ErbB2/Akt1-deficient animals and the authors concluded that Akt1 also plays an important role in promoting tumor metastasis (10). However, whether the metastatic defect is a secondary consequence of Akt1 ablation at the level of cell survival or a defect in tumor microenvironment remains to be elucidated. Also consistent with our observations that overexpression of Akt2 enhances metastasis (3), germline deletion of Akt2 in MMTV-ErbB2 mice was shown to decrease lung metastases (11). Germline ablation of Akt3 had little effect on tumor latency in MMTV-ErbB2 mice but decreased metastasis (11). In this regard it would be interesting to examine the effect of mammary specific expression of activated Akt3 in transgenic mouse models of breast cancer. Collectively, these in vitro and in vivo studies highlight the distinct functions of Akt1 and Akt2 in breast cancer induction and invasiveness; however, the molecular mechanisms downstream of Akt1 and Akt2 that are responsible for these differing phenotypic outputs are unclear.

The Akt Kinases Induce Distinct Biological Functions

Studies using genetically engineered animals have formally shown that the Akt family members are not redundant and that each kinase fulfills unique functions. Indeed, homozygous knockout of the individual Akt genes in mice results in distinct phenotypes (reviewed in ref. 12). However, very little is known about differences in substrate specificity or pathway activation downstream of Akt1 and Akt2. There are a number of hypotheses that may account for Akt1- or Akt2-mediated effects on biological outcomes including specific target substrate phosphorylation, differential activation, protein-protein interactions, or subcellular localization, and each of these potential mechanisms will be discussed in detail below (Fig. 1).

Activation Levels

The Akt1 and Akt2 transgenes that we used in our mouse models were constitutively active owing to the mutation of the threonine and serine residues to phosphomimetic aspartic acids. Thus, in these mouse models, differences in Akt activation do not supply an explanation for the phenotypic differences. However, in human breast cancer similar mutations do not exist, and therefore, regulation of Akt kinase activity is an important consideration. Akt1 and Akt2 are recruited to membrane phospholipids at similar affinities, and both are activated by the same upstream kinases. Therefore, it is unlikely that any differences in activation are achieved through these mechanisms. Once activated, Akt signaling is attenuated by two groups of phosphatases, protein phosphatase 2A (PP2A) and the plekstrin homology domain and leucine-rich repeat protein phosphatases (PHLPP), which target the kinase domain threonine and hydrophobic domain serine residues, respectively. Although PP2A targets all three
Akt family members, specificity is exhibited by the PHLPP family of phosphatases. PHLPP1 specifically dephosphorylates Akt2 and Akt3, whereas PHLPP2 dephosphorylates Akt1 and Akt3 (13). In this regard, altered expression of either of the two PHLPP proteins will differentially affect the activation status of Akt1 and Akt2. Given that the amino acids surrounding the Akt serine residue are highly conserved between Akt1 and Akt2, immunostaining for activation of a specific Akt family member is difficult. Thus in human breast cancer, examination of the levels of the individual PHLPP proteins may serve as a useful surrogate marker to infer activation of either Akt1 or Akt2.

**Substrate Specificity**

Activated Akt influences a number of proteins that have been implicated in cell migration and/or metastasis either through direct phosphorylation or through the modulation of upstream regulators. A critical process for directional cell migration is integrin recycling from the trailing end of a moving cell to lamellipodia at the leading edge. Recycling of αvβ3 and α5β1 integrins is promoted indirectly by Akt through glycogen synthase kinase 3 (GSK3; ref. 14). Akt may further regulate β1 integrin recycling through the phosphorylation of ACAP1 (ArfGAP with coiled-coil, ankyrin repeat, and pleckstrin homology domains), leading to increased migration (15). Thus through the inactivation of GSK3 and the activation of ACAP1, Akt plays a positive role in regulating cell migration. A recently identified Akt target substrate known as Girdin localizes to lamellipodia at the leading edge of migrating cells (16). Downregulation of Girdin in breast cancer cells was shown to decrease migration and invasion in vitro and inhibit metastasis in nude mice (16). Although GSK3, ACAP1, and Girdin have been implicated in cell migration, to date no targeting by a specific Akt family member has been shown for these substrates.

Pak1, another substrate involved in cell migration, is differentially activated by Akt1 and Akt2. Interestingly, both Akt1 and Akt2 associate with Pak1; however in fibroblasts Akt1 has been shown to activate Pak1, whereas Akt2 inhibits Pak1 activation (17). Although in fibroblasts Akt1 promotes migration, Akt2 seems to be the promigratory family member in mammary epithelial cells. Thus, because both Akt1 and Akt2 can interact with Pak1, it is conceivable that in the context of the mammary epithelial cell, Akt2 may activate Pak1, leading to the subsequent induction of cell migration. With this knowledge in mind, however, inherent Akt substrate specificity is unlikely to be imparted solely by Akt kinase activity itself. Indeed, most substrates are phosphorylated equally well by Akt1 and Akt2 when...
tested in in vitro kinase assays. Furthermore, the kinase domain is highly conserved between the Akt proteins, and experiments conducted using chimeric Akt molecules in which the kinase domains were interchanged did not show any noticeable effects in terms of phenotypic readouts (17, 18). Thus, it is likely that specific functions of the Akt family members are regulated at the level of interacting partners or through distinct subcellular localization.

Protein-Protein Interactions

Very few unique interacting proteins have been identified for the individual Akt family members. Interestingly, whereas the plenty of SH3 domains (POSH) adaptor protein has been shown to be phosphorylated by both Akt1 and Akt2, only Akt2 interacts directly with POSH (19, 20). However, POSH has thus far only been implicated in the induction of apoptotic cell death, making it unlikely that Akt2-specific association with POSH would account for differences in metastasis between our Akt1- and Akt2-expressing mouse models of mammary tumorigenesis. In addition to POSH, another protein that interacts with a particular Akt family member is Tcl1 (T-cell leukemia-lymphoma), a protein implicated in T-cell malignancies. Tcl1 interacts specifically with Akt1, and this interaction leads to increased Akt1 activation and translocation to the nucleus (21). The exact effect this has on Akt1-mediated substrate phosphorylation remains to be determined. Aside from a number of nuclear substrates that become available for targeting by Akt1, this relocalization also reduces Akt1 from a number of nuclear substrates that become available for targeting by Akt1, this relocalization also reduces Akt1 phosphorylation in these cellular compartments.

Localization

One possible explanation for differential behavior of members of the Akt family derives from the observation that in contrast to Akt1, Akt2 localizes predominantly adjacent to the collagen IV matrix during cellular attachment (6). This localization difference may bring the Akt kinases in proximity to different downstream substrates and may provide a mechanism for contrast in the processes regulated by each family member. The overall conservation between Akt1 and Akt2 at the amino acid level is quite high, especially within the kinase domain. Examination of the conservation between the two proteins within domains reveals the greatest divergence to be between the linker and C-terminal extension regions of the proteins. Interestingly, defects in cell migration in Akt2 null mouse embryonic fibroblasts can be rescued by expressing a chimeric Akt1 protein containing the Akt2 plekstrin homology and linker domain, but not one in which only the kinase domain is swapped (17). In support of these observations, insulin stimulation of adipocytes leads to greater plasma membrane recruitment of Akt2 and GLUT4 translocation to the plasma membrane downstream of Akt2 was shown to be dependent on the plekstrin homology and linker domains of Akt2 (18). It will be interesting to examine the localization of Akt1 and Akt2 in mammary tumor-derived cells and evaluate whether targeting particular family members to distinct subcellular compartments affects the invasive ability.

Concluding Remarks

The Akt kinases regulate a spectrum of biological processes through the phosphorylation of diverse target substrates. In terms of breast cancer invasion and metastasis, the exact mechanism by which the highly related Akt kinases exert such opposing effects is currently an enigma. Candidate hypotheses include differences in activation levels, interacting partners, downstream substrates, or subcellular localization, or quite possibly a combination of several of these mechanisms. Akt plays an important role in promoting a variety of cellular processes that are involved in tumorigenesis and is, thus, an attractive target for cancer therapy. Determining the factors that govern this individuality will open avenues of drug targeting for specific aspects of the cancer phenotype. Furthermore, enhanced Akt activation is observed not only in breast cancer; a number of human malignancies display hyperactivation of the PI3K/Akt pathway. Thus, Akt inhibitors may have therapeutic applications in a broad spectrum of human cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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