Akt Signaling and Cancer: Surviving but not Moving On

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Abstract

The frequent deregulation of the phosphoinositide 3-kinase/Akt survival signaling pathway in cancer has prompted significant interest in blocking this pathway to treat cancer. Recently, however, two studies have shown that the Akt isoform Akt1 limits the invasive migration of breast cancer cells. These studies suggest that Akt1 may have a dual role in tumorigenesis, acting not only pro-oncogenically by suppressing apoptosis but also anti-oncogenically by suppressing invasion and metastasis. We discuss the possible implications of these findings for therapeutic development of Akt inhibitors to treat cancer. (Cancer Res 2006; 66(8): 3963-6)

One decade ago, the Tsichlis laboratory discovered that the Akt/protein kinase B (PKB) serine/threonine protein kinase is a target of phosphoinositide 3-kinase (ref. 1). With the subsequent discovery that Akt increases cell survival in a PI3K-dependent manner (2), it became rapidly evident that this pathway could be an effective target for antineoplastic therapies. We now know that Akt controls a plethora of cellular responses, and that the three Akt isoforms, Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ), are ubiquitously expressed in all cell types and tissues, although Akt3 seems to have a more restricted expression pattern. In normal and cancer cells, Akt regulates both growth and survival mechanisms and does so by phosphorylating a large number of substrates. Most stimuli that induce these responses activate PI3K, a lipid kinase that catalyzes the synthesis of the membrane phospholipid PtdIns-3,4,5-P_3 from PtdIns-4,5-P_2, effectively recruiting Akt to the plasma membrane by direct interaction of PtdIns-3,4,5-P_3 with the Akt pleckstrin homology domain. Full activation of Akt also requires phosphorylation of two conserved residues, Thr^{308} and Ser^{473}. Once phosphorylated and active, Akt relocalizes to several subcellular locations where it phosphorylates proteins, such as Forkhead transcription factors (FOXO), GSK-3, BAD, and MDM2, some of which contribute to antiapoptotic signaling [recently reviewed by Woodgett (3)].

Importantly, many of the enzymes that either mediate the Akt signal, such as MDM2 (4), or regulate Akt activity, such as the tumor suppressors PTEN (5) and PHLPP (6), are frequently mutated in human tumors, thereby up-regulating Akt activity and increasing tumor cell growth and survival. Similarly, oncogenic mutations in PI3K itself have been detected in human tumors (7). Several laboratories have also reported increased Akt activity, phosphorylation, and even protein expression in tumors of the breast, prostate, ovary, and pancreas (8). These and numerous other studies have thus provided overwhelming evidence that efficient signaling through the PI3K/Akt signaling axis promotes growth and survival, and that any genetic perturbation of this pathway will increase the survival of cancer cells that would normally undergo apoptosis.

With this as the backdrop, our laboratory embarked on a study to evaluate the importance of Akt in mediating cancer cell motility and invasion. Growth and survival are, of course, not the only phenotypes that prevail in carcinomas, which are cancers of epithelial origin. Additionally, cell motility and invasion through basement membrane are important phenotypes that are ultimately responsible for the progression of primary tumors into metastases (9). Our interest in this area of research was stimulated by a key discovery made by the Mercurio laboratory several years ago, showing that the $\alpha_6\beta_4$ integrin, a tumor-associated antigen, promotes breast and colon cancer cell migration and invasion by activating PI3K (10). Other groups have since reported that in certain cells, ectopic expression of various Akt isoforms increases cell migration and invasion, at least *in vitro*. For example, Akt can stimulate secretion of matrix metalloproteases, known to be required for degradation of the extracellular matrix (11). In fibroblasts, Akt signaling enhances activation of various small GTPases, including Rac, and thus lead to remodeling of the actin cytoskeleton and enhanced cell motility (12). Similarly, expression of activated Akt in fibrosarcoma or pancreatic cancer cells increases their invasion through Matrigel (11, 13, 14), an effect recapitulated by overexpression of Akt2 in breast and ovarian cancer cells (15). Expression of Akt can also promote epithelial-mesenchymal transition (EMT), a process intimately associated with tumor progression to invasive and metastatic carcinoma (16).

Our studies revealed the unexpected finding that expression of activated Akt1, activated either as a myristoylated membrane-bound form or a phosphorylation site mutant, potently blocked the *in vitro* migration and invasion of three distinct breast cancer cell lines through Matrigel (17). This effect required the kinase activity of Akt1 because an inactive catalytic site mutant did not block invasion. Invasion could be blocked by expression of activated Akt1 or by stimulating cells with the physiologic ligand insulin-like growth factor-I (IGF-I). To rigorously establish that endogenous Akt1 blocks invasion, we designed two distinct Akt1 small interfering RNA (siRNA) sequences specific to Akt1, which did not affect expression of Akt2. As predicted, Akt1 siRNA enhanced breast cancer cell migration and Matrigel invasion. The obvious implication of this finding is that the two Akt isoforms must have specific and nonredundant roles in regulating invasion. If Akt2 also blocks invasion, then silencing Akt1 expression alone with siRNA would have no effect on migration because Akt2 would take over by redundancy.

Our results only suggested that the three Akt isoforms may function in a distinct manner in regulating cell migration and invasion (17). A separate study from the Brugge laboratory has validated and extended these observations (18). This laboratory had been studying the role of IGF-I and its receptor (IGF-IR) in mediating hyperproliferation and antiapoptotic signaling in

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doi:10.1158/0008-5472.CAN-06-0743

three-dimensional organ cultures of the MCF-10A breast epithelial cell line. In previous studies, they had shown that MCF-10A cells expressing IGF-IR stimulated with IGF-I develop filled lumens because of the failure of the centrally located cells to undergo apoptosis (19), which is a key event in the morphogenesis of the parental MCF-10A cells. Because IGF-I is a potent activator of the PI3K and Akt pathway, and because Akt is a crucial survival kinase, they speculated that interfering with Akt expression would reverse the survival signaling induced by IGF-I stimulation. They developed specific short hairpin RNAs (shRNA) targeting Akt1 and Akt2 and obtained similar counterintuitive results when the Akt1 shRNA was introduced into organ cultures. Silencing Akt2 expression reversed the hyperproliferative and antiapoptotic effect of IGF-I, as one would predict. However, silencing Akt1 expression had a drastically different result, not only enhancing cell migration induced by IGF-I or epidermal growth factor (EGF) but also facilitating an EMT in the cells (18). In combination with EGF stimulation, expression of wild-type Akt1 also blocked migration in a Transwell assay. Interestingly, the increased EMT-like conversion induced by Akt1 shRNA was reversed by down-regulation of Akt2. Therefore, the first conclusion to be drawn from both studies is that Akt1 and Akt2 in opposition to each other in breast cell motility, invasion, and EMT morphogenesis.

Both studies also reported on two distinct signaling mechanisms, which Akt1 uses to suppress cell motility and invasion. In our own work, we focused on the transcription factor nuclear factor of activated T cells (NFAT) as a target of Akt in the invasion response (Fig. 1). We reasoned that NFAT was a good candidate for several reasons. First, we knew that NFAT transcriptional activity is elevated in highly invasive breast cancer cell lines, and that blocking NFAT activity results in decreased Matrigel invasion (20). Work from other laboratories had also shown that expression of activated Akt in T cells results in accumulated cytoplasmic, inactive NFAT (21). Lastly, it is known that many targets of Akt are actually inactivated by the Akt signal, a good example being the FOXO3a transcription factor that is phosphorylated in the nucleus by Akt and then exported to the cytoplasm, thereby terminating its transcriptional activity (22). Indeed, we found that activated Akt1 potently blocks NFAT transcriptional activity, whereas Akt1 siRNA increased this activity. We also showed that the ability of Akt1 siRNA to increase invasion could be reversed by coexpressing a dominantly interfering NFAT mutant, showing that NFAT is at least one effector of the Akt signaling pathway leading to suppression of invasion. Furthermore, we showed that the mechanism by which Akt1 blocks NFAT is to accelerate its proteasomal degradation. This is mediated by the Akt substrate and E3 ubiquitin ligase MDM2.

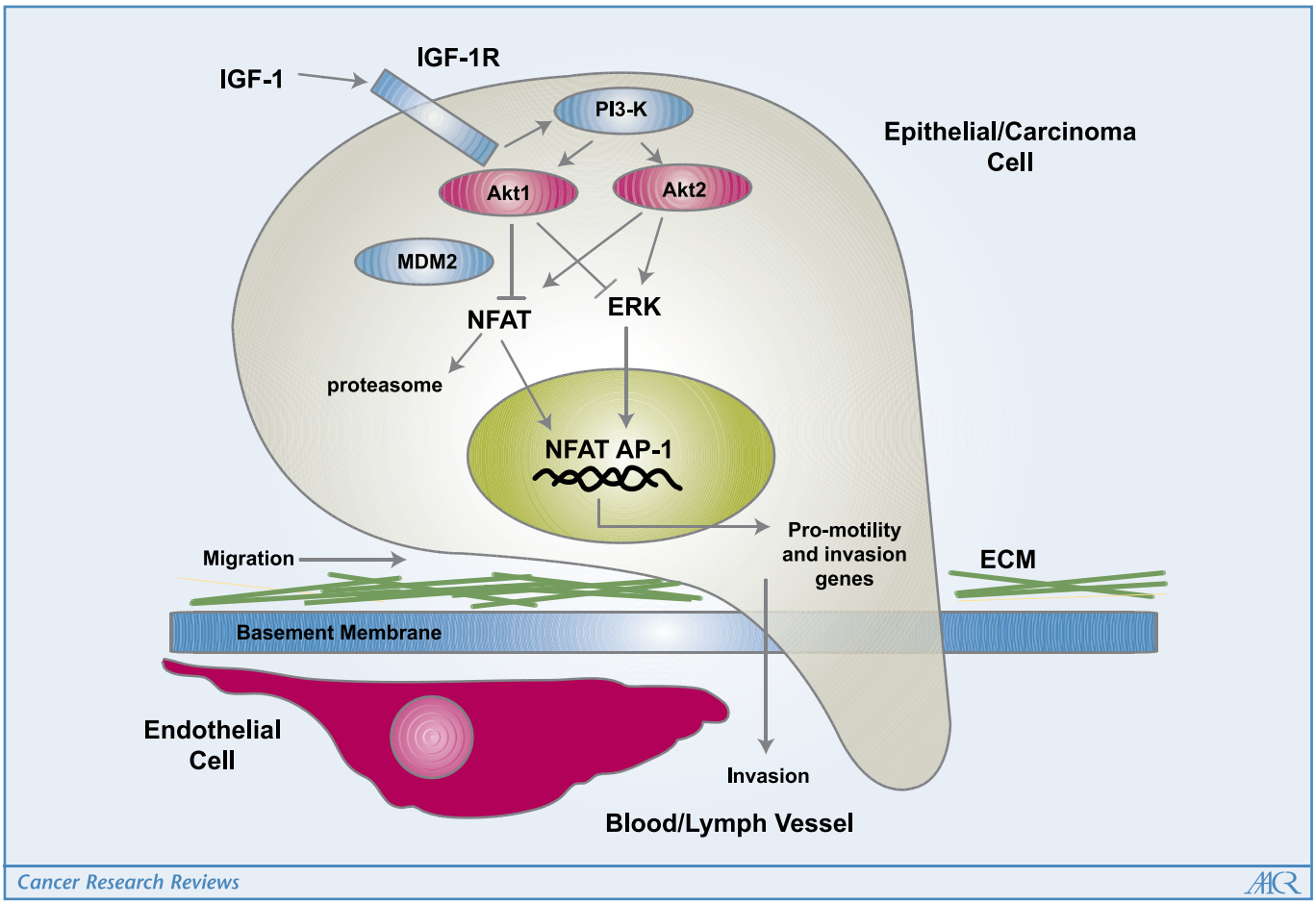

Figure 1. Contrasting functions of Akt1 and Akt2 on epithelial cell motility and invasion. Stimulation of breast epithelial or carcinoma cells with IGF-I initiates the IGF-IR signaling pathway, leading to activation of PI3K, which in turn activates Akt1 and Akt2. Akt1 suppresses both NFAT and ERK activity (*red arrows*) and in turn blocks cell migration and invasion. In contrast, Akt2 may positively regulate both NFAT and/or ERK (*green arrows*) and facilitate invasion. In the absence of Akt1, active NFAT and ERK (through nuclear AP-1) contribute to a transcriptional program, which leads to the induction of promotility and invasion genes, leading to breakdown of the extracellular matrix (*ECM*) and intravasation into the vasculature.

In the presence of active Akt, MDM2 is stabilized and able to ubiquitinate NFAT, leading to its degradation and therefore inhibition of migration and invasion. We speculate that NFAT likely induces a transcriptional program in cancer cells, leading to the induction of genes that promote invasion, including matrix metalloproteinases, cytokines, and others. Although we know very little about the identity of these genes, a recent study showed that autotaxin/ENPP2, a promotility factor, is an NFAT target gene (23). Future studies will shed more light on the nature of other genes that mediate migration and invasion in an NFAT-dependent manner.

The Brugge article reported on a different mechanism by which suppression of Akt1 expression increases MCF-10A cell migration and EMT. For two reasons, they focused on the extracellular signal-regulated kinase (ERK) pathway. First, hyperactivation of ERK through Ras mutations or mitogenic stimulation typically results in induction of EMT. Second, overexpression of Akt1 is known to suppress ERK activity (24). Consistent with both observations, Akt1 shRNA dramatically induced ERK activity, whereas Akt2 shRNA had no effect (18). They further showed that the induction of migration in cells transduced with Akt1 shRNA was due to ERK activation, because the phenotype could be reversed with specific ERK inhibitors. The reverse was also true: when Akt1 was overexpressed, it suppressed EGF-stimulated migration and ERK activation.

It is not entirely surprising to find two distinct mechanisms which contribute to the inhibitory activity of Akt1 on motility and invasion. Indeed, as multiple mechanisms have been shown to operate downstream of Akt leading to cellular survival, it is not surprising to find that more than one pathway downstream of Akt1 is essential to block cellular motility and invasion. For example, in immune cells the efficient induction of several cytokines requires two coordinately regulated signals emanating from the T cell receptor, one being calcium flux, which leads to the nuclear translocation and activation of NFAT, and the second being the ERK pathway, which is required for efficient activation of activator protein-1 (API-1; ref. 25). Thus, in the same way that many immune response genes are induced by the coordinate activation of both NFAT and AP-1, the same may hold true for Akt1 signaling in epithelial cells where NFAT and ERK could cooperate to mediate suppression of motility and invasion. Whether other Akt-dependent pathways must also function in parallel to ERK and NFAT to mediate motility and invasion is as yet unknown.

Although clearly more work is required to fully comprehend the implication of these studies, some key issues are worth evaluating at this early stage. First, although Matrigel invasion assays and three-dimensional organ cultures provide much insight into molecular mechanisms which govern motility responses, these are *in vitro* assays, and as such, validation of these results *in vivo* is required. However, one report has already addressed this issue. The Muller laboratory reported that whereas expression of activated Akt1 in ErbB2 transgenic mouse mammary tumors enhanced their proliferation, it also resulted in fewer metastatic lesions compared with control animals (26). This would be the expected result if Akt1 functions as a suppressor of invasion or metastasis. Future studies using inducible deletion of individual Akt isoforms in various tumor models may provide further validation of the Akt-specific effects on proliferation versus invasion and metastasis.

One obvious extrapolation of these studies is that small-molecule inhibitors that target Akt1 may actually enhance invasion and metastasis, rather than suppress it. Akt inhibitors have been developed recently, which seem to be isoform specific towards Akt1 and Akt2 at relatively low doses (27). As expected, in cell culture models, these inhibitors potentiate tumor cell apoptosis in the presence of apoptosis-inducing ligands, such as tumor necrosis factor–related apoptosis-inducing ligand. It will clearly be interesting to see if these inhibitors recapitulate the effects of the Akt siRNA on migration and invasion, both *in vitro* and *in vivo*.

There are many other unanswered questions arising from these studies. What are the differences between Akt1 and Akt2, which affords them such contrasting effects on epithelial cell motility? Although the Akt1 and Akt2 knockout mice show distinct phenotypes, with Akt1 null mice exhibiting growth defects, and Akt2 null mice exhibiting mainly defects in glucose homeostasis, these differences have yet to be correlated to differences at the level of substrate selectivity or signaling functions of the two Akt isoforms. One answer may lie in the relative abundance of each Akt isoform in a given cell type or tissue, or perhaps in their unique and specific intracellular localization. If this proves to be the case, then it is likely that the function of Akt as an inhibitor of invasive migration may hold true only on a subset of tissues. Given that Akt2 is the predominant isoform that is amplified in breast and ovarian tumors, that activating mutations of Akt2 have been detected in colon cancer, and that expression of Akt2 increases invasion of cancer cells *in vitro*, it is tempting to speculate that Akt2-specific inhibitors would be the most appealing avenue for clinical therapy. Additional weight is added to this proposition by the independent finding that only Akt2 emerged from an unbiased siRNA screen of kinases, which increase apoptosis when down-regulated (28). Why should Akt1 expression suppress migration and invasion in epithelial cells yet enhance it in other cells, such as fibroblasts? This question presumably reflects some feature of signaling or expression background in different cell types, but it has yet to be resolved. In closing, evidence continues to accumulate that different isoforms of Akt have different functions in cells, including in settings of human neoplasia. To develop effective new cancer therapeutics that limit relevant Akt signals, it will be important to gain a more complete picture of the precise roles of these kinases and of specific PI3K/Akt pathways in cancer, in more specific rather than general signaling terms.

Acknowledgments

Received 2/24/2006; accepted 2/24/2006.

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Cancer Res 2006;66:3963-3966.