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PKB/Akt–Dependent Regulation of Cell Motility

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The prosurvival activity of phosphoinositide 3 kinase (PI3K)/Akt (also known as protein kinase B, PKB) pathway has been investigated in great detail in human physiology and disease. Accumulating evidence is emerging that this signaling axis also actively engages with the migratory process in motile cells, including metastatic cancer cells. Interference with the role of PI3K/Akt–mediated cell motility impairs cellular development and attenuates malignant progression of cancer metastasis. Because metastasis is responsible for 90% of mortality in cancer patients, the acceleration of cancer cell spreading observed in association with hyperactivation of the PI3K pathway, triggered for example by chemotherapy/radiotherapy in the clinic, has heightened awareness of the conflict between "good drugs" and unfavorable effects. Here, we discuss recent studies on PI3K/Akt–regulated cell motility in both physiological and pathological settings, with the aim of a better understanding of how activities of the PI3K/Akt axis initiate and transmit "migratory signals" that stimulate cell movement. We focus in particular on its direct influence on cell migration and invasion, epithelial-mesenchymal transition, and cancer metastasis.

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Membrane targeting is an initial step in the activation of a broad variety of signaling proteins during the development of multicellular organisms. Acidic phospholipids that constitute about 11% of total lipid in the plasma membrane (1) act as the major signaling messengers of basic cellular processes such as migration, differentiation, mitosis, and polarity. Phosphatidylinositol (4,5)-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3), two phosphoinositides that make up only 1% of membrane phospholipid molecules, have been shown to have a critical influence collaboratively on the activity of distinct signaling cascades. PI3K, one of an evolutionarily conserved intracellular lipid kinase family that converts PIP2 to PIP3, and the phosphatase and tensin homolog that reverses this process, constitute a functional switch that results in the precise temporal and spatial regulation of signaling. Lipid-mediated membrane recruitment arises by the specific interaction of lipid-binding domains and target proteins with a remarkable affinity. The pleckstrin homology domain, one of approximately 11 investigated lipid-binding motifs, is composed of approximately 100 amino acids and occurs in a broad range of proteins that associate functionally with the intracellular membrane. Upon stimulation, pleckstrin homology domain–containing proteins such as Akt, phosphoinositide-dependent kinase 1 (PDK1), and phospholipase C (PLC) recognize and bind to newly generated and enriched PIP3, resulting in its transient membrane relocalization and consequent activation. For example, when Akt associates with the membrane, it is rapidly phosphorylated by PDK1 on threonine 308 (T308) and by the mammalian target of rapamycin complex 2 (mTORC2) on serine 473 (S473), which

results in maximal Akt activation in the mediation of downstream signaling. Indeed, although still not fully understood, the PI3K/Akt/mTOR signaling axis has emerged as a pivotal node of many signaling cascades in human physiology and pathology. Genetic depletion of Akt isoforms or epigenetic inhibition of its activity results in functional abnormalities in mice. In the clinic, diabetes and cancer are tightly associated with aberrant Akt activity, which has attracted great attention in attempts to develop specific inhibitors as therapy for these diseases. Given that the contribution of this signaling axis to cell proliferation and survival has already been widely discussed (2–4), in this review we discuss recent discoveries that highlight the influence of PI3K/Akt/mTOR signaling (Figure 1) on cell migration and invasion, focusing particularly on its direct involvement in the modulation of cytoskeleton.

PI3K/Akt–Regulated Cell Migration in the Developing Embryo

Defined by distinctions in morphology, migratory behavior, and molecular signatures, epithelial and mesenchymal cells constitute the two major cell types in metazoa. Their interconversion (epithelial-mesenchymal transformation, EMT) is a frequent event during embryo development and is a fundamental mechanism in the formation of adult organs and tissues. Unlike epithelial cells, which are organized into adherent sheets restricted by intercellular junctions, mesenchymal cells are characterized by their irregular shape, disrupted cell–cell contact, and high motility. During gastrulation of the early embryo, the first EMT results in the organization of the mesoderm (5). Several studies in different model organisms have provided evidence that activated PI3K/Akt signaling contributes to EMT-driven mesoderm formation. At the onset of zebrafish gastrulation, activated Akt colocalizes with actin bundles at the leading edge of mesendodermal cells that undergo an EMT during internalization (6). In response to signaling attractants, such as platelet-derived growth factor (PDGF), the mesoderm cells rapidly orientate and migrate to the signal source, where they exhibit elevated membrane protrusive potential. Treating migrating mesendodermal cells with a PI3K/Akt inhibitor suppresses the formation of membrane protrusive structures, including filopods and pseudopods, and reduces the asymmetrical distribution of Akt at the leading edge. This is accompanied by strongly reduced cell migration, which indicates an influence of PI3K/Akt signaling on cell polarity and motility in the gastrula embryo. Interestingly, although migration speed is markedly reduced, inhibition of the PI3K/Akt pathway does not influence the direction of cell migration. Analysis of PDGF signaling in

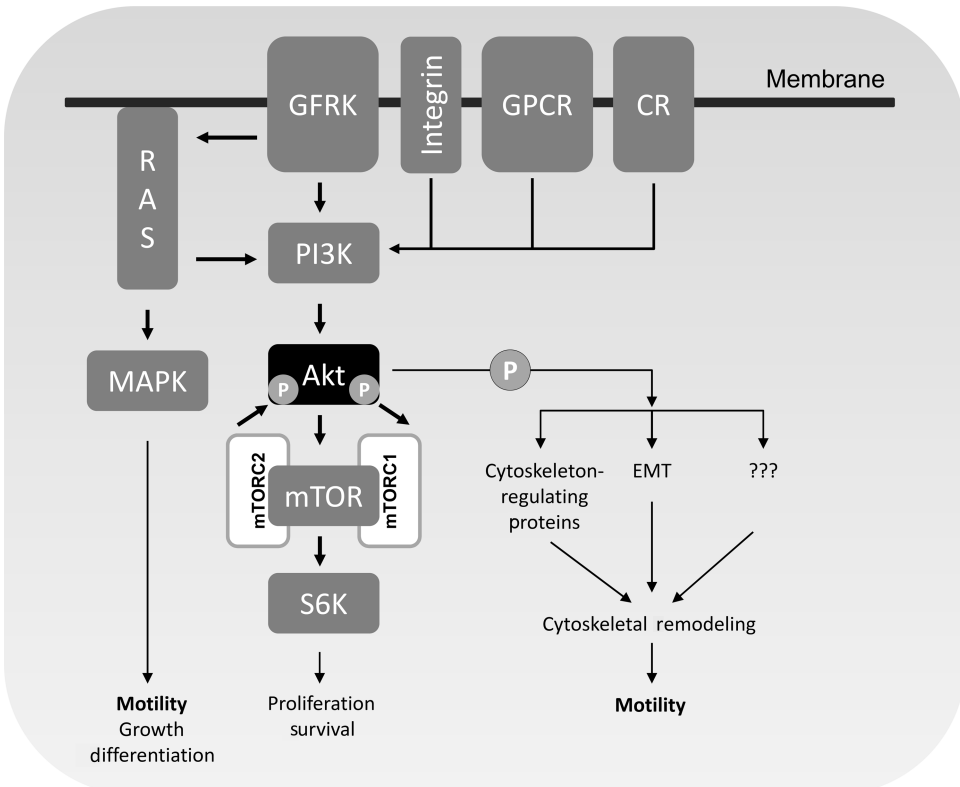

Figure 1. Classical phosphoinositide 3 kinase (PI3K)/Akt signaling pathway. Upon activation by growth factor receptor kinases (GFRKs), as well as integrin, G-protein-coupled receptor (GPCR), and cytokine receptor (CR) signaling, PI3K is phospho-activated to convert phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which triggers Akt membrane targeting. Maximal activity of Akt requires threonine 308 (T308) phosphorylation mediated by phosphoinositide-dependent kinase 1 (PDK1) and serine 473 (S473) phosphorylation by mTOR complex 2 (mTORC2) or DNA-dependent protein kinase (DNAPK). Activated Akt phosphorylates a number of substrates that perform different functions in a variety of cellular conditions, including several cytoskeleton-regulating proteins and epithelial-mesenchymal transformation (EMT)–activating proteins that specifically regulate cell motility. To be noted, other pathways that crosstalk with PI3K/Akt are capable of regulating cell migration and invasion in a PI3K/Akt independent manner. These aspects, however, are neither illustrated in this figure, nor discussed in the text. MAPK = mitogen-activated protein kinase.

the early chick embryo, especially during gastrulation, indicated that induced expression of *N*-cadherin on the plasma membrane of mesoderm cells, particularly enriched in cellular protrusions, is probably a key determinant of cell migration. Interference with PDGF signaling suppressed *N*-cadherin expression, inhibited Akt phosphorylation, and associated with decreased cell migration, thus highlighting an active signaling route from PDGF to PI3K/Akt and *N*-cadherin during cell migration. Further studies confirmed that inhibition of PI3K/Akt, when PDGF signaling is maintained intact, also inhibits *N*-cadherin expression, suggesting that PDGF-mediated signaling drives cell migration, at least in part, by upregulation of *N*-cadherin and activation of the PI3K/Akt pathway, thus defining this signaling axis as a central node during mesoderm cell migration (7). Consistent with these data, cyclo-oxygenase-1–mediated activation of prostaglandin E2/G-protein-coupled EP4 receptor signaling axis elevated the velocity of mesodermal cell migration through the activation of the PI3K/Akt signaling node (8). Taken together, these data demonstrate that PI3K/Akt activity is crucial to the promotion of mesodermal cell migration during vertebrate gastrulation.

Given the fact that the intracellular PI3K/Akt signalosome is at the crossroads of many membrane-bound receptor kinase signaling pathways, disturbance of its activity causes severe defects during embryogenesis. Indeed, dysregulation of PI3K/Akt activity either by upregulation/downregulation of the phosphatase and tensin homolog or by the overexpression of constitutively active Akt results in abnormal gastrulation (9–11). However, dependence of active Akt proteins on cell migration during the whole course of embryonic development is not yet understood, particularly at gastrulation, when EMT-associated cell migration is critical for developing embryos (12,13). Because Akt knockout mice display severely impaired embryo development, future studies should investigate in detail whether mesodermal formation is affected in Akt knockout mice.

Activation of PI3K/Akt at the Leading Edge of the Plasma Membrane of Postnatal Motile Cells

Increased cell motility is often reflected in enhanced dynamic remodeling of the cytoskeleton, characterized by distinct changes in cell morphology and polarity and is associated with differentially activated biochemical signaling on the membrane. Cell migration has been suggested to follow two major patterns: collective and individual (14). In collective migration, intercellular coupling forces remain intact during cell movement, and thus migrating cells exhibit cohesive migration as an entity. In contrast, in single-cell (amoeboid, mesenchymal) migration, cells lose cell–cell contact and generate moving force individually (15). Both migration modes of animal cells display high diversity. There is emerging evidence that border cells determine directed group migration in response to the surrounding environment, in particular to chemotactic cues and invading pathogens. Interestingly, in a recent study using live-imaging microscopy, single cells were seen to promote a directional collective movement by transducing extrinsic signaling cues to the group (16), indicating that mutual interdependence of distinct migration behavior is the basis of an overall migratory mechanism.

Akt family members are broadly expressed in most cell types, and a basal activity is maintained in cultured cells. Less-motile epithelial cells that display apical–basal polarity often display an even distribution of basal level of phosphorylated Akt on the plasma membrane, overlapping with cortical actin filaments (Figure 2A). This epithelial integrity is disrupted by the introduction of migration-promoting factors such as Twist, a master regulator of EMT (17), which results in solitary cells by the cleavage of intercellular junctions and a transition from apical–basal to front–rear polarity and elevated cell motility. In these conditions, phosphorylated Akt is substantially enriched on unique membrane structures, colocalizing with strengthened actin bundles (Figure 2B). Indeed, Twist-expressing cells undergo EMT and trigger the paracrine signaling loop. For example, when normal NMuMG epithelial cells were incubated with the medium that was used to culture MDCK/Twist1 cells, NMuMG cells started to undergo EMT within 6 hours (Figure 2C, ii). In particular, only those cells at the edge show remarkably activated Akt and vimentin, which are colocalized on membrane protrusions. This invasive behavior is also frequently observed in many types of highly migratory cancer cells that often cause metastases (Figure 2D). Activated Akt at the leading edge participates in the regulation of cell polarity and reorganization of the cytoskeleton and mediates contraction of the cellular body, thus facilitating directed migration of the cell.

Transition of cell polarity from apical–basal to front–rear axis, usually termed EMT, is a predominant characteristic of cell migration induced by extracellular stimuli such as growth factors. This leads to local asymmetric redistribution of signaling molecules over the plasma membrane, often associated with specific reprogramming of gene expression and/or repression that impacts on intercellular and cell–substratum interactions during migration. The first evidence of Akt involvement in the regulation of polarity plasticity came from studies on Dictyostelium, in which the Akt protein is an evolutionarily conserved homolog of mammalian Akt1. In response to cAMP stimulation, Akt was recruited to the membrane and activated, which led to the direct phosphorylation on threonine 579 (T579) and activation of PAKa, a key regulator of cell migration belonging to the p21-activated serine/threonine kinase family (18,19).Inhibition of T579 phosphorylation dramatically decreased cell migration velocity, probably because of disrupted myosin 2 assembly and impaired polarity (20). Although this phosphorylation site is not present in mammalian PAK1, it has been shown that another site (serine 21) is phosphorylated by Akt, which is important for its binding to the noncatalytic region of tyrosine kinase adaptor protein (Nck) and the promotion of directed cell migration induced by growth factors (21). Nevertheless, a recent report pointed out that PAK1 could also mediate feed-forward signals, facilitating membrane targeting and the activation of Akt proteins through direct interaction but in a catalytic activity–independent manner (22). Interestingly, selective interference with Akt1 by expression of its kinase-dead form or by knockdown considerably inhibited PAK1-promoted cell migratory and invasive capacity, suggesting that Akt is an essential downstream effector of PAK1 in stimulating cell motility. Consistently, another earlier report also showed that a dominant negative mutation of Akt efficiently attenuated PDGF/Rac/Cdc42–promoted cell migration in mammalian fibroblasts (23). Although these data did not suggest a mechanism

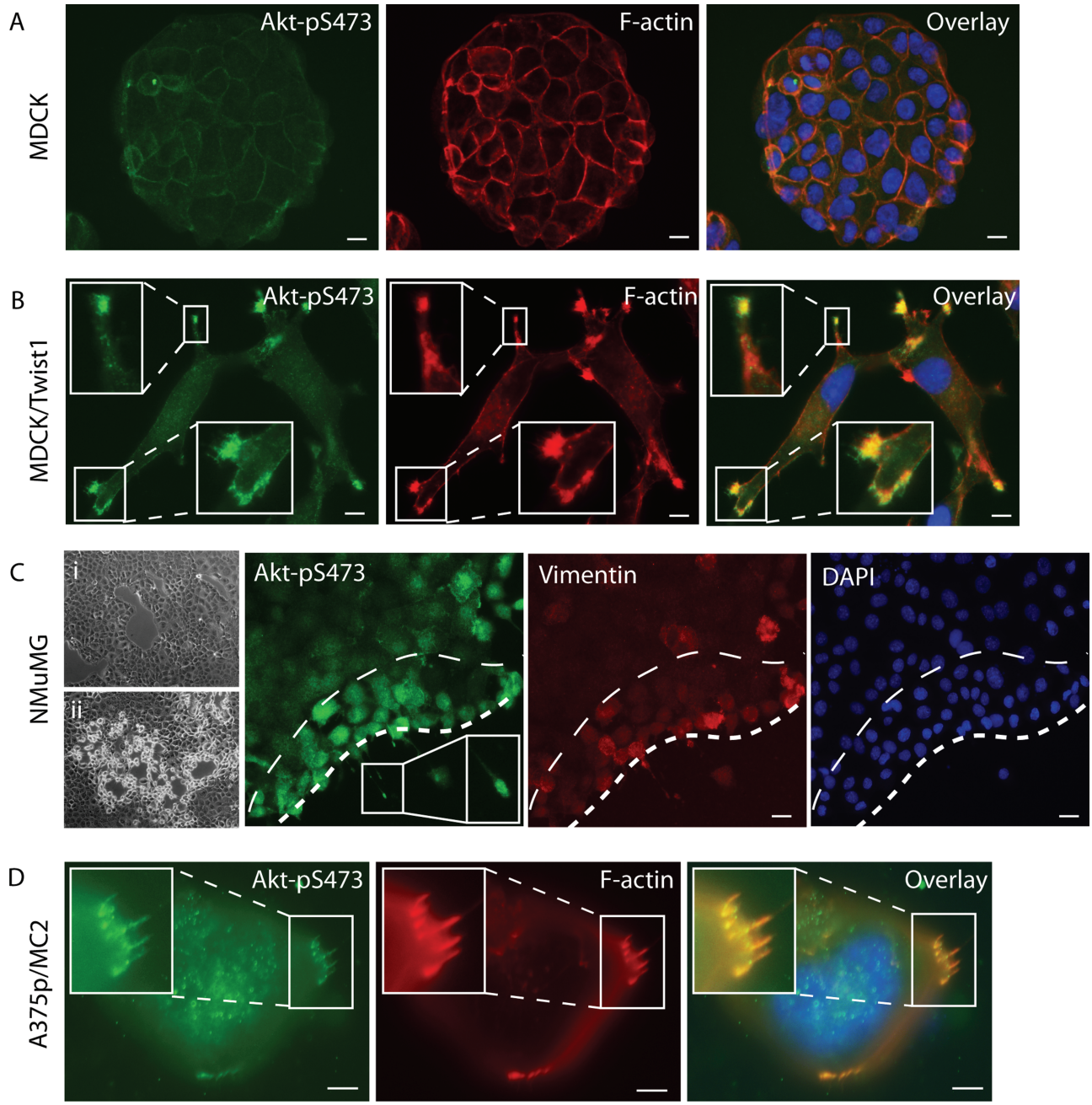

Figure 2. Phosphorylated Akt colocalizes with highly polymerized actin at membrane protrusions in motile cells. **A**) MDCK exhibits classical epithelial phenotype. **B**) Upon ectopically introducing Twist, the intercellular contact is disrupted, multiple membrane protrusion forms and F-actin is specifically accumulated and colocalizes with increased Akt phosphorylation. **C**) Epithelial cells NMuMG start to undergo epithelial-mesenchymal transformation when incubated with conditional medium from MDCK/Twist1 cell culture. **D**) This morphological change is remarkably observed in many types of invasive cancer cell such as metastatic melanoma cell A375p/MC2.

by which PAK1/Akt complex formation is regulated, the concept of Akt proteins and direct regulation of cell motility along the PAK signaling pathway was established.

Akt-Regulated Cytoskeleton-Related Proteins

Whatever signaling pathways are activated, cell motility is driven ultimately by adapted cytoskeletal remodeling. Extensive studies have focused on how dynamic polymerization and stabilization of intracellular filaments regulates cell migration (24,25). The concept of a migration cycle (26–28) simplified this complex and heterogeneous process into four fundamental steps: 1) initiation and polarization, 2) formation of membrane protrusions at the leading edge and membrane retraction at the trailing edge, 3) contraction of the cell body, and 4) re-establishment of adhesion between cell and substratum. Several key components closely associated with the cellular filaments that are important for cytoskeleton dynamics have been shown to be activated by Akt-mediated phosphorylation.

Akt and Actin

Vascular endothelial cell migration is a critical process in angiogenesis. Microvascular endothelial cells show enhanced actin polymerization upon stimulation by vascular endothelial growth factor (VEGF), associated with elevated motility and Akt activation. Inhibition of Akt activity by expression of a kinase-dead mutant abrogated actin bundle formation and blocked cell locomotion. This effect was enhanced when myristylated Akt was expressed (29), indicating that Akt is a critical mediator of VEGF-induced endothelial cell migration through actin reorganization. Similarly, interfering with Akt activity in embryonic fibroblasts effectively blocked PI3K-transduced migratory signals (30). In neutrophils, activation of G-protein-coupled chemokine receptors leads to F-actin polymerization and cytoskeleton contraction as a result of PIP3 signaling. This rearrangement pattern of actin ensures pseudopod extension in human neutrophils during chemoattractant stimulation, which is dependent on Akt activity (31). In breast cancer cells, enhanced cell migration and invasion is often associated with increased filopodia production, which occurs in an active Akt-dependent manner. Treating the human breast cancer cell line BT549 with the small-molecule allosteric inhibitor API-2, which specifically targets only Akt proteins, blocked filopodia formation (32). This was also seen in integrin-like kinase–induced cellular actin rearrangement and motility, for which Akt activity was indispensable (33).

Taken together, these observations support the model that Akt activation potentially influences cell motility through direct modulation of actin. Interestingly, further studies have provided evidence that actin preferentially binds to phosphorylated Akt at pseudopodia with enhanced bundles (34,35). As to the question of whether actin is a substrate of Akt, one study showed that actin is markedly concentrated in an immunocomplex containing phosphorylated Akt, 14-fold higher than in a corresponding complex containing inactive Akt (36). It was demonstrated further that actin can be phosphorylated by Akt (37) and that cortical remodeling of actin associated with cell migration is strongly dependent on Akt proteins.

Akt and Actin-Tethering Molecules

Actin polymerization is a highly dynamic process. Actin-rich membrane structures frequently observed in highly motile cells, such as filopodia, pseudopodia and invadopodia, need to be strategically stabilized to function properly. This stabilization usually results from the activity of actin-associated proteins that prevent the degradation of newly formed actin filaments. The Akt phosphorylation enhancer (APE), also termed girders of actin filaments (girdin), is an actin-binding protein that maintains the integrity of actin filaments, particularly the actin meshwork at the leading edge of migrating cells. Depletion of APE/girdin dramatically destabilizes actin bundles, resulting in ablation of stress fibers and cortical actin structure. This leads to loss of directional migratory capacity and illustrates the crucial activity of APE in the coordinated regulation of cell migration. Notably, Enomoto and colleagues demonstrated that APE/girdin is phosphorylated by Akt on serine 1416 (S1416) (38). In response to an extracellular stimulus such as EGF, S1416 phosphorylation triggers the rapid translocation of APE/girdin from the junctions between actin filaments to the leading edge, colocalized with phosphorylated Akt. This phosphorylation pattern seems to be crucial for cell migration, as expression of a nonphosphorylatable mutant S1416A or knocking down Akt-attenuated cell movement. Indeed, recent observations in the clinic that APE/girdin is overexpressed in malignant human cancers and that this is associated with metastatic potential (39–41) further demonstrated that APE is a stringent mediator able to signal Akt-controlled cell motility in both physiological and pathological settings. Curiously, it was reported that Akt phosphorylates kank, a kidney ankyrin repeat-containing protein, which subsequently led to negative regulation of the assembly of stress fibers and RhoA activation, thus blocking cell migration (42). It is not yet clear whether this reflects a negative feedback signaling loop related to the cessation of cell movement. A further actin-associated cross-linker protein, filamin A (43,44), was shown to be phosphorylated by Akt on serine 2152 (45,46), mediating caveolin-1–driven cancer cell migration along the IGF pathway. Moreover, the sodium–hydrogen exchanger isoform 1 (NHE1) was also shown to be a key mediator of stress fiber disassembly induced by insulin and PDGF, and this activity required Akt-directed phosphorylation on serine 648 (S648) (47). S648 phosphorylation of NHE1 was proposed to be critical for the growth factor–initiated cytoskeletal reorganization that favors cell migration and invasion, at least in part through the disassembly of cellular filamentous structures. Although it is not clear mechanistically how S648 phosphorylation initiates cell motility, the involvement of NHE1 in the promotion of cell migration by modulation of the cytoskeleton has been demonstrated in different cell types (48–51). Recently, NHE1 was discovered to interact with cortactin, an actin regulatory protein that promotes invadopodium formation and maturation in many cancer cells. NHE1 is selectively recruited to invadopodia in invasive breast cancer cells (52), where it locally regulates pH-dependent interactions of promigratory proteins, stabilizing newly formed actin-rich invadopodia. This action favors invadopodial elongation of cancer cells and, thus, their dissemination from the primary site to distant organs. Whether NHE1 recruitment is dependent on S648 phosphorylation is a crucial question for further studies.

Akt and F-Actin Assembly

Toker and colleagues have shown another aspect of Akt activation that affects cell motility negatively (53). The actin-binding protein palladin crosslinks and scaffolds other actin-associated proteins, maintaining the integrity of the actin meshwork in cells. Interestingly, palladin is specifically phosphorylated by Akt1 on serine 507 (S507), which leads to disruption of F-actin bundles. This Akt-dependent inhibition phenotype mediated by its isoform-specific substrate was observed in breast cancer cells in which expression of Akt1 suppressed cell movement. This is consistent to some extent with further observations that the ratio of Akt isoforms determines cell fate. Indeed, unlike the antimigratory activity of palladin mediated by Akt1 phosphorylation, Akt2 contributes to palladin stability independent of S507 phosphorylation (54). Similarly, Akt1 also phosphorylates and thus destabilizes the tuberous sclerosis complex 2 (TSC2), a Rho GTPase regulator that influences cell migration, which leads to an attenuated migratory phenotype due to impaired F-actin assembly (55). The opposing

roles of Akt isoforms in the regulation of cell motility will be discussed later.

Akt and Intermediate Filaments

The role of intermediate filaments in cell motility has been studied extensively (56,57). The type 3 filamentous protein vimentin is the most abundant cellular intermediate protein that maintains normal cell and tissue integrity. Based on broad investigations, vimentin appears to be one of the most important of the factors that are substantially upregulated when cells become highly motile, particularly during EMT, and therefore promote migration and invasion of different cell types, both in physiological and pathological conditions such as cancer. Although it possesses a nonclassical motif, vimentin is phosphorylated by Akt1 on serine 39 (S39) (58). This phosphorylation pattern protects vimentin from degradation and thereby regulates cancer cell invasion in aggressive mesenchymal-originated soft-tissue sarcoma, as was demonstrated using nonphosphorylatable or phospho-mimicking mutants. Clearly, it is important to know whether vimentin phosphorylation is also part of a general mechanism in other cancer types, such as breast cancer, in which vimentin is strongly expressed in lung metastases and is considered to be a promising target in cancer therapy (59,60).

However, the precise mechanisms how some of the Akt substrates regulate cell motility are still unclear. For example, two independent studies reported in 2009 that a component of E3 ligase, S-phase kinase-associated protein 2 (Skp2), was phosphorylated by Akt (61,62). Akt-mediated phosphorylation on serine 72 activated Skp2-dependent E3 ligase activity and promoted cell migration through maintaining Skp2 in cytoplasm. Depletion of Skp2 in MEF cells strongly inhibited cell migration, a phenotype that was rescued by re-expression of a potential phospho-mimicking mutant Skp2_S72D but not Skp2_S72A mutant (61), indicating a role of S72 phosphorylation in regulation of cell motility. Although the mechanism needs further investigation, aberrant overexpression of Skp2 and its cytoplasmic localization in highly invasive human cancer cells indirectly supports the experimental observations. Nevertheless, uncertainties have been reported (63–65). Regardless of the variety of models applied in the studies, the global effects of S72 phosphorylation need to be revisited.

As discussed above, remodeling of the cell skeleton is the direct precursor of cell migration. Migration potential arises because of a synergistic effect of all the three basic elements—filamentous actin, microtubule, and intermediate filament vimentin. In addition, Akt regulates microtubule dynamics at its plus end through Akt/GSK3β axis-dependent activation of microtubule binding protein adenomatous polyposis coli, thus promoting cell migration (66–69). Cooperative interactions between intracellular filament-associated proteins regulate cell motility through the dynamic assembly of cellular protrusions, focal adhesions, and retractions in response to extracellular stimuli. The specific impact of Akt on the activities of these proteins provides unambiguous evidence that Akt may directly influence cell movement (Figure 3), in addition to its well-known prosurvival activity. This aspect of metastatic tumor progression is particularly important because understanding the mechanism of Akt-promoted metastasis should help in the search for more specific drug targets for halting metastasis.

Akt-Regulated Cell Motility Through Signaling Crosstalk

Akt and VEGFR/eNOS Signaling

In addition to targeting cytoskeletal proteins by phosphorylation, Akt also interacts with other promigratory proteins, thus

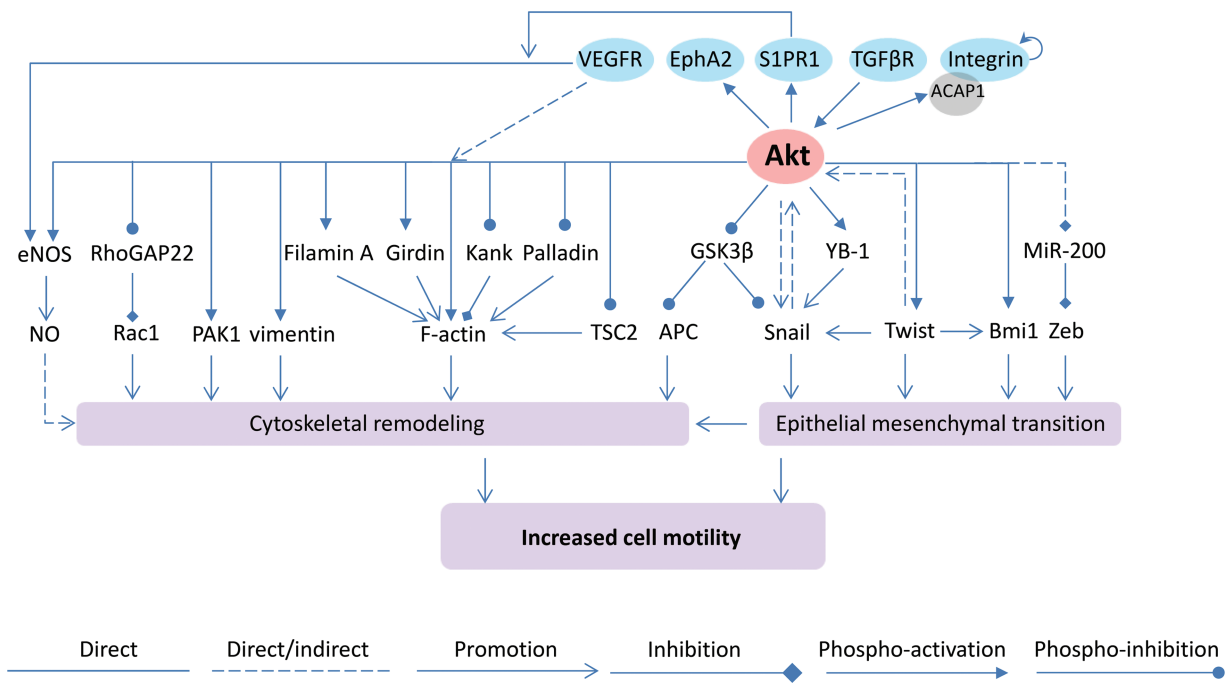

Figure 3. Akt is activated through upstream signalosomes and transduces migratory signals through phospho-regulation of its substrates that are required for cell movement.

mediating crosstalk between affiliated signaling axes. Akt binds and phosphorylates endothelial nitric oxide (NO) synthase (eNOS) on serine 1177 (S1177) (70). S1177 phosphorylation enhances eNOS catalytic activity and leads to NO production, which is necessary for endothelial cell migration (71,72). Consistent with the activity of NO in angiogenesis, VEGFR activation induces NO production, vasculature formation, and cell migration. Indeed, VEGFR/eNOS pathway–regulated cell migration is dependent on Akt-mediated S1177 phosphorylation (73). Dephosphorylation of S1177 attenuates migratory potential (74), demonstrating the importance of the PI3K/Akt signaling axis in crosstalk with the VEGFR/eNOS pathway in the regulation of endothelial cell motility (75). This appears to increase in statistical significance, as accumulating observations have pointed out the importance of NO in pathological situations, particularly in malignant tumor development (76,77).

In parallel, VEGFR signaling often interacts with the G-protein-coupled receptor, sphingosine-1-phosphate (S1P) receptor 1 (S1PR1), also known as endothelial differentiation gene 1 (EDG-1). S1P is a platelet-derived sphingolipid that binds to EDG-1 and activates eNOS. S1P/EDG-1 activation leads to VEGFR phospho-activation mediated through activated Src kinase, which subsequently activates the PI3K/Akt/eNOS axis (78). Notably, EDG-1 activation can be further enhanced by Akt-mediated phosphorylation on threonine 236 (T236), promoting cortical actin assembly, angiogenesis, and directed cell migration (79). These data define Akt as the central node in looping signaling routes between VEGFR and S1P/EDG-1, actively regulating cell motility.

Akt and Ephrin Receptor Tyrosine Kinase Signaling

Ephrin receptor tyrosine kinases (Eph) are probably the largest tyrosine kinase family, including about 14 receptors and 8 ligands. Eph signaling is rather complex and paradoxical: it was reported to be both pro- and antitumorigenic, most likely in a cellular context–dependent manner (80). One receptor family member, EphA2, was shown to inhibit cancer cell growth through a receptor-ligand signaling route, but promote cancer cell invasion through intracellular signaling crosstalk, for example with PI3K/Akt, independent of ligand binding. Interestingly, in the case of ligand binding–independent activation, activated Akt physically associates with and phosphorylates EphA2 on serine 897 (S897). In human brain cancer cells, S897 phosphorylation was shown to be responsible for Akt-promoted cell migration and invasion due to effects on dendritic actin cytoskeleton assembly and lamellipodia formation at the leading edge of migrating cells (81). Consistent with a pro-oncogenic role of EphA2, the S897 phosphorylation level was shown to positively associate with malignancy in human glioma samples, indicating that Akt/EphA2 plays an essential role in brain tumor progression.

Akt, ACAP1/ARF6, and Integrin Recycling

Integrin recycling directed by small GTPases, particularly Rab family members, has been increasingly reported (82–84). At present, it is well established that membrane redistribution of integrin upon extracellular signaling is an important mediator of cell movement. The arf-GAP, with a coiled-coil, ANK repeat, and pleckstrin homology domain–containing protein 1 (ACAP1), is a GTPase-activating protein (GAP) for ADP ribosylation factor 6 (ARF6) and has been shown to participate in integrin β1 recycling. In the investigations of whether Akt is able to phosphorylate ACAP1 on serine 554 (S554), which is required for its interaction with integrin β1 in response to serum stimulation, specific knockdown of Akt or the application of an Akt inhibitor abolished S554 phosphorylation and blocked induced integrin β1 recycling and, consequently, cell migration (85). Another GAP protein, RhoGAP22, was also reported to regulate cytoskeleton remodeling upon phosphorylation on serine 16 by activated Akt (86), further illustrating a functional involvement of Akt signaling in small GTPase-modulated cell motility.

Indeed, Akt-dependent cell migration in association with many other signaling axes such as GTPases has also been reported but by as-yet-unknown mechanisms (22,23). Given that Akt is at a central signaling node, Akt activation–promoted cell motility through transducing signals from upstream signalosomes is apparently a global event.

Akt and EMT Inducers

EMT is an embryonic program important for organogenesis in normal development, but its dysfunction can favor the survival and dissemination of cancer cells (87). Several transcription factors have been discovered that can initiate and maintain this process, including Snail, Twist, and Zeb. Although these transcription factors are apparently deregulated in many types of invasive cancer, the precise signaling mechanisms by which they are normally regulated at the molecular level are poorly understood. Evidence is emerging of a connection between Akt and the EMT-inducing transcription factor signaling axis. The first observation was that Snail is phosphorylated by GSK3β in normal epithelial cells. Snail is a rather unstable protein and hardly detectable in normal cells. Its expression in epithelial cells strongly induces morphological change associated with increased migratory capacity. Further experimental data showed that Snail, when phosphorylated by GSK3β, undergoes continuous degradation (88,89). However, when GSK3β is phosphorylated by hyperactivated Akt, which leads to its inactivation, the Snail protein is stabilized and activates the EMT program. This is probably consistent with the situation in invasive cancers in which Snail is overexpressed, positively associated with high Akt phosphorylation but negatively with GSK3β expression. Akt-promoted activation of Snail1 was also observed when YB-1 was phosphorylated by Akt and subsequently translocated into the nucleus (90). Moreover, upregulated Snail1 could, in turn, enhance Akt activity (91). This signaling loop apparently favors escape of cancer cells from oncogene-induced senescence and their re-establishment in distant organs. A direct interaction was reported recently when Akt2 was shown to stabilize Snail1 binding to the CDH1 (which encodes E-cadherin) promoter through direct protein–protein interaction (92). It is not yet clear whether Akt2 mediates formation of a specific transcriptional complex that leads to inhibition of Snail-induced senescence gene expression but activation of proinvasive gene expression. However, a further EMT-inducer, Twist, was shown to bind directly to and specifically

activate Akt2 transcription in invasive breast cancer cells (93); inactivation of Akt downregulates Twist (68). Furthermore, Akt phosphorylates and activates Twist1, which results in increased Akt phosphorylation, at least in part because of enhanced TGFβ signaling (94–96). Recent data also suggests that the polycomb group protein Bmi-1 is a downstream target of Twist1 and is indispensable for EMT and cancer metastasis (97). Interestingly, Akt directly phosphorylates Bmi-1 in human high-grade prostate tumors (98). Taken together, these results indicate an important interaction between Akt and EMT inducer–associated signaling (Figure 3). This synergistic interplay has crucial and unfortunate pathological effects: 1) it prevents stress-induced cell cycle arrest in cancer cells, 2) it promotes proinvasive/metastatic gene expression, and 3) it maintains hyperactivation of PI3K/Akt signaling, which further enhances the antiapoptotic potential of cancer cells.

Akt Isoforms and Differential Regulation of Cell Motility

Mammalian genome Akt encodes three isoforms, and studies of germ line depletion have revealed nonredundant activities of Akt isoforms in development. Akt1-depleted mice exhibit severe growth retardation and partial perinatal lethality, Akt2-depletion leads to a diabetic phenotype, and Akt3 knockout mice show defects in brain development (4). Although high Akt phosphorylation is known to drive malignant cancer progression, it is not easy to dissect any Akt isoform–specific roles in cancer because of their very high homology and their shared substrates. Over the past 10 years, accumulating results from cultured cancer cells and mouse models have begun to reveal distinct roles for Akt1 and Akt2 in different cancer types.

Impacts of Akt1 in Maintenance of Primary Tumor Growth and Breast Cancer Cell Dissemination

Most studies on Akt1 and Akt2 in cancer are based on mouse mammary tumor virus (MMTV)–polyoma virus middle T antigen (PyMT) and MMTV–ErbB2 transgenic mice that exhibit metastatic phenotypes. Maroulakou and colleagues showed that genetic ablation of Akt1 in both MMTV–PyMT and MMTV–ErbB2 mice statistically significantly inhibited transgene-driven tumor formation (99), led to increased apoptosis of tumor cells, and thus substantially delayed tumor growth. A similar effect was observed by Ju and colleagues in the same genetic background of the model animal (100). This was consistent with observations in MMTV–ErbB2 mice with constitutive Akt1 (myr-Akt1) or Akt1–T308D/S473D knock-in of accelerated primary tumor formation (101,102). In another mouse model expressing the MMTV–PyMTmut transgene that uncouples the PI3K pathway, expression of Akt1–T308D/S473D rescued MMTV–PyMTmut-induced apoptosis, thus supporting tumor growth (103). Interestingly, expressing Akt1–T308D/S473D in MMTV–ErbB2 mice statistically significantly inhibited transgene-promoted lung metastasis (102,104), but this was not apparent in MMTV–PyMTmut transgenic mice (103).

In addition to the transgenic models, studies in cultured cells and in xenograft models have offered further information. For example, Toker and colleagues showed that expression of Myr–Akt1 or Akt1–T308D/S473D in breast cancer cell lines inhibited invasive potential but, conversely, increased invasion upon knockdown of endogenous Akt1 (105,106). Bissell and colleagues showed decreased motility of breast cancer cells expressing activated Akt1 due to decreased Rho GTPase activity (55). Downregulation of Akt1 strongly promoted EMT in a mammary epithelial cell line, indicating its antimigratory activity (107). These results established the anti-invasive influence of Akt1 in breast cancer. However, Akt1 promotes cell motility and metastasis in other cell types and animal models of different cancer types. In a transgenic mouse model expressing a mutant thyroid hormone receptor ($TR\beta^{PV/PV}$), ablation of Akt1 inhibited metastasis of thyroid cancer (108,109). In Dictyostelium, Akt1-null mutant has reduced migratory potential toward the chemoattractant cAMP (110). Knockdown of Akt1 in the colon cancer cell line HCT116 substantially reduced liver metastasis in a xenograft mouse model of colorectal cancer (111), and Akt1 knockout in MEF cells resulted in slower migration (112). Finally, introducing Akt1 into a transformed mouse mammary epithelial cell line, COMMA-1D, elevated its motility in association with upregulation of MMP-2 (113). In addition, several studies have reported the proinvasive influence of Akt1 in other cancer types, such as pancreatic cancer (114), fibrosarcoma (115), soft-tissue sarcoma (58), and ovarian cancer (116).

Impacts of Akt2 in Cell Motility and Cancer Metastasis

Unlike the profound influence of Akt1 on cell proliferation and primary tumor initiation and maintenance, Akt2 has advantages in promoting cell migration and invasion. Knocking in Akt2–T308D/S473D in both MMTV–PyMTmut and MMTV–ErbB2 transgenic mice led to massive lung metastases with no impact on mammary tumor development (104). In a xenograft model of colorectal cancer, knockdown of Akt2 in colon cancer cells KM20 inhibited liver metastasis; the converse was observed when Myr–Akt2 was expressed (117). Knockdown of Akt2 induced reversion of the EMT process in mammary epithelial cell lines (107). Activation of Akt2 increased cell invasion and metastasis of breast and ovarian cancer cells through upregulated integrin signaling (118). Inactivation of Akt2 inhibited glioma cell invasion (119), and knockdown of Akt2, rather than Akt1, in the lung adenocarcinoma cell line A549 dramatically abolished its invasive potential (120). Interestingly, in contrast with Akt1, Akt2 inhibits MEF cell migration (112), indicating that Akt1 and Akt2 differentially impact on cell motility in different cell types (Figure 4).

Conclusions

The PI3K/Akt pathway not only regulates cell proliferation and survival and increases protein synthesis and metabolism, but it is also actively involved in cancer metastasis, in particular in cancer cell motility. Because cancer metastasis is the major cause of death of cancer patients, preventing cancer cell movement would be beneficial in the clinic. Given the high sequence homology of Akt isoforms, it is surprising that each isoform has distinct activities in different contexts and they are not intercomplementary. This provokes the critical question of how individual Akt isoforms transduce specific signals. One way to answer this question would be to search for isoform-specific substrates, and recent studies have started to shed light in this direction. The finding that palladin, the actin-binding protein that regulates cell motility by tethering

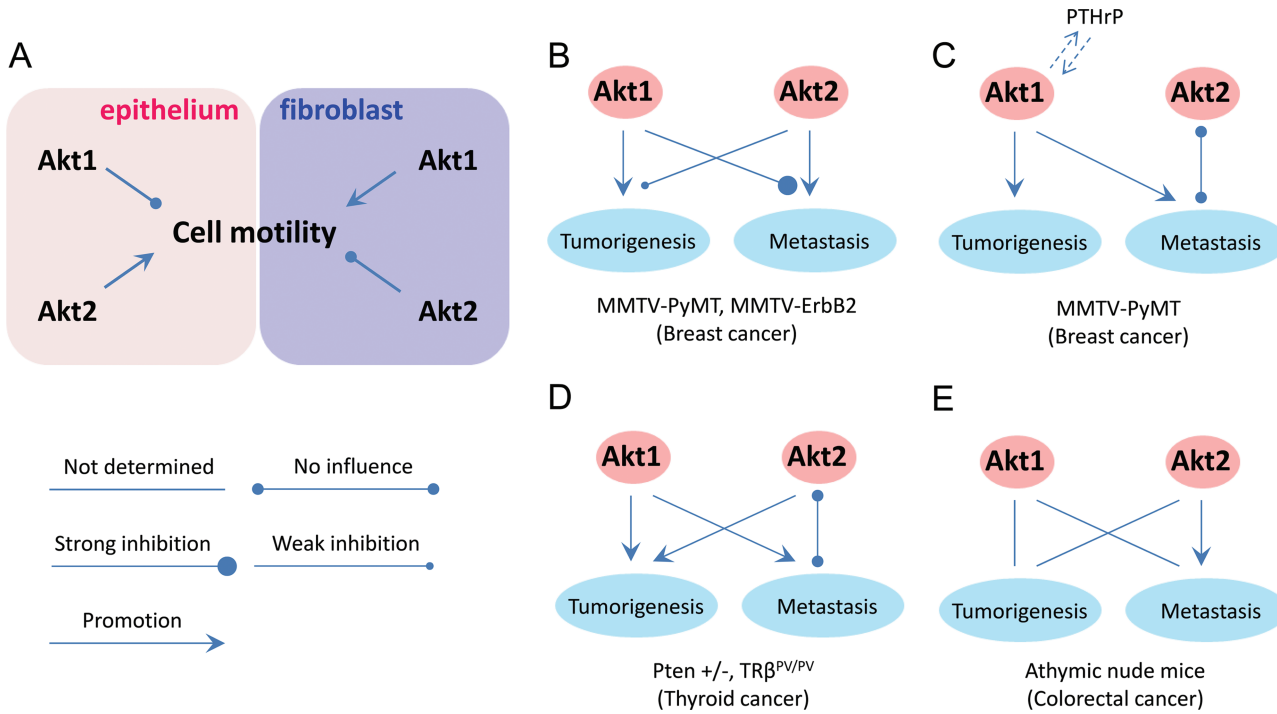

Figure 4. The impacts of Akt isoforms on cell migration and invasion. **A**) Akt1 and Akt2 promote cell motility in a cell type–dependent manner. Depending on genetic mouse models generated for study of different cancers, Akt1 and Akt2 knockout mice show different tumorigenetic and metastatic potential (**B, C, D, E**).

F-actin bundles, is preferentially phosphorylated by Akt1 (121) indicated a molecular mechanism by which Akt1 directly inhibits epithelial cell motility. Interestingly, palladin is also associated with Akt2 in a phosphorylation-independent manner (122), giving rise to the important concept that the ratio of Akt1 and Akt2 may have a temporal and spatial influence on cell movement (123). A further cytoskeletal component, the intermediate filament protein vimentin, was shown recently to be phosphorylated by Akt1 (58). Akt1-mediated vimentin phosphorylation promotes migration and invasion of soft-tissue sarcoma cells. As summarized above, for several cytoskeleton-associated proteins that were recently identified as Akt substrates, such as Girdin, Kank, and Filamin, it is not known so far whether they are selectively phosphorylated by individual Akt isoforms. However, it should be noted that the kinase domains of all three isoforms are highly conserved and many of the known Akt substrates may be indiscriminately phosphorylated by all isoforms. Therefore, apart from the isoform-specific substrates, Akt isoform–regulated cell motility may be mediated by isoform-specific interaction proteins (4). It is conceivable that the selectivity of substrates is due to sequence-determined specific interactions and that it is actually this interaction that plays the crucial role. To test this, it would be important to use nonphosphorylatable substrates that are nevertheless able to bind Akt to interfere with the phosphorylation process. Given the relatively clear phenotypes of the transgenic mouse models, ideally catalytically inactive or binding-deficient Akt mutants could be used to try to rescue these phenotypes, especially in models in which Akt isoforms are genetically deleted.

In summary, extensive evidence suggests that Akt-transduced signals directly influence cell motility in normal development and in disease. An increasing number of "good drugs do bad things" have been reported in the clinic. For example, the $BRAF^{V600E}$ inhibitors mistakenly also target another RAF isoform, CRAF, and unfavorably create more malignant phenotypes in melanoma (124,125). Therefore, it is worth paying extra attention to the performance of the many Akt inhibitors being tested in preclinical trials. Several such inhibitors have been shown to attenuate cancer cell invasion (126,127), and it is, therefore, important to test whether they exhibit priorities in targeting individual Akt isoforms. This approach arises from recent advances in the field showing that the three highly homologous Akt isoforms have nonoverlapping activities and even exert opposing effects in different cancer types. Thus, given that metastasis causes 90% of death of cancer patients (128) and Akt is the major signaling node that integrates membrane, cytoplasmic, and nuclear signals determining cell fate, dissection of Akt isoform–specific signaling pathways to gain more insight into the identified Akt downstream substrates that affect cell motility will contribute to targeted cancer therapies in the clinic.

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