

## Review

# Functional specificity of Akt isoforms in cancer progression

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## Abstract

Akt/PKB kinases are central mediators of cell homeostasis. There are three highly homologous Akt isoforms, Akt1/PKB $\alpha$ , Akt2/PKB $\beta$  and Akt3/PKB $\gamma$ . Hyperactivation of Akt signaling is a key node in the progression of a variety of human cancer, by modulating tumor growth, chemoresistance and cancer cell migration, invasion and metastasis. It is now clear that, to understand the mechanisms on how Akt affects specific cancer cells, it is necessary to consider the relative importance of each of the three Akt isoforms in the altered cells. Akt1 is involved in tumor growth, cancer cell invasion and chemoresistance and is the predominant altered isoform found in various carcinomas. Akt2 is related to cancer cell invasion, metastasis and survival more than tumor induction. Most of the Akt2 alterations are observed in breast, ovarian, pancreatic and colorectal carcinomas. As Akt3 expression is limited to some tissues, its implication in tumor growth and resistance to drugs mostly occurs in melanomas, gliomas and some breast carcinomas. To explain how Akt isoforms can play different or even opposed roles, three mechanisms have been proposed: tissue-specificity expression/activation of Akt isoforms, distinct effect on same substrate as well as specific localization through the cytoskeleton network. It is becoming clear that to develop an effective anticancer Akt inhibitor drug, it is necessary to target the specific Akt isoform which promotes the progression of the specific tumor.

**Keywords:** Akt/PKB isoforms; chemoresistance; intermediate filaments cytoskeleton; invasion; tumor growth.

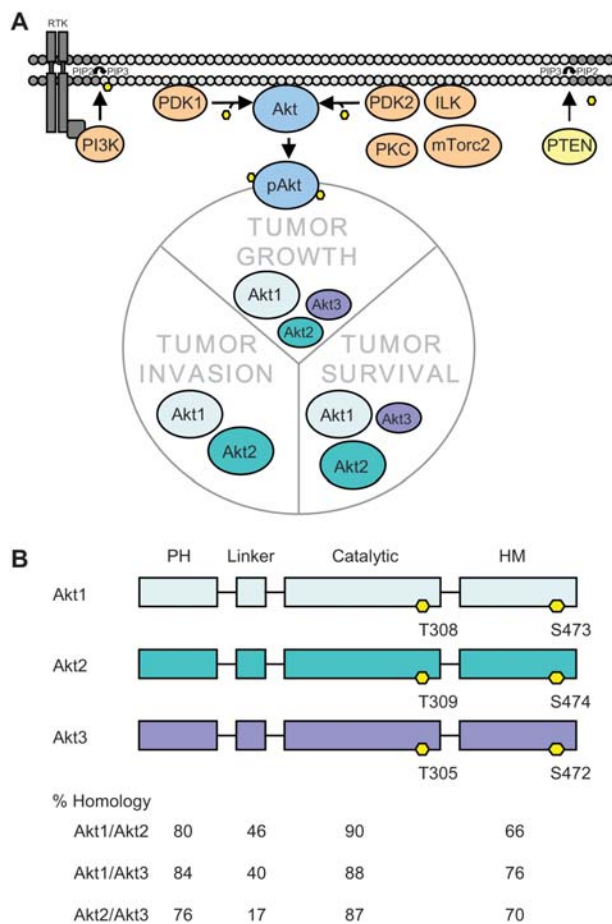
## Introduction

Cancer development results from the acquired capacity of normal cells to escape from their tightly regulated tissue-specific microenvironment signaling pathway and proliferate

without control. The serine-threonine kinase Akt or protein kinase B (Akt/PkB) family is a member of the oncogene families involved in tumorigenesis. The Akt signaling pathway plays a central role in cell fate by linking together normal cellular physiological processes such as glucose metabolism, cell growth, protein synthesis, cell cycle control, cell survival and apoptosis, cell migration and angiogenesis. As a fundamental mediator of these crucial signaling cascades, alterations in Akt activity, by a gain of function, results in cellular perturbations that are the hallmark of cancer [reviewed in (1–3)].

Akt represent a family of three proteins that are encoded by distinct genes: Akt1/PKB $\alpha$ , Akt2/PKB $\beta$  and Akt3/PKB $\gamma$  located, respectively, on chromosomes 14, 19 and 1 in human. Akt isoforms are highly related homologous proteins (4) (Figure 1). Following receptor tyrosine kinase activation, phosphatidylinositol-3 kinase (PI3K) phosphorylates phosphoinositides (PtdIns) at the 3-position of the inositol ring, generating PtdIns3P, PtdIns(3,4) $P_2$  and PtdIns(3,4,5) $P_3$  (PIP3), which specifically associates with the pleckstrin homology (PH) domains of several proteins, including Akt. Recruitment of Akt to the membrane by PIP3 allows the full activation of the kinase through its phosphorylation on threonine residue in the catalytic domain (T308 on Akt1, T309 on Akt2 and T305 on Akt3) by 3-phosphoinositide-dependent kinase 1 (PDK1) (5) and on serine residue in the C-terminal domain (S473 on Akt1, S474 on Akt2 and S472 on Akt3) by both autophosphorylation and phosphorylation by other kinases, such as PDK2, mammalian target of the rapamycin complex 2 (mTORc2), integrin-linked kinase (ILK) and protein kinase C (5–10). In a later step, active Akt is translocated, through an unknown mechanism, to different subcellular compartments such as mitochondria and nucleus where its substrates are located. This process and the production of PIP3 are negatively regulated by the lipid protein phosphatase tensin homolog deleted on chromosome 10 (PTEN) (Figure 1).

Many studies completed so far have examined Akt by using a general approach, without focusing on functional specificity of each isoforms. It is now clear that to understand the mechanisms on how Akt affects specific cancer cells, it is necessary to consider the relative importance of each of the three Akt isoforms in the altered cells. The specific physiological functions of the individual Akt isoforms were well demonstrated on studying mice deficient in Akt1, Akt2 or Akt3. All three phenotypes were viable but presented specific deficiencies. Disruption of Akt1 showed growth retardation with proportional decrease in the sizes of all organs, increased apoptosis and neonatal mortality (11–13).



**Figure 1** (A) Stimulation of receptor tyrosine kinase (RTK) recruits phosphatidylinositol-3 kinase (PI3K) to the cell membrane to phosphorylate phosphoinositide diphosphates (PIP<sub>2</sub>) to phosphoinositide triphosphates (PIP<sub>3</sub>), a process that is reversed by the phosphatase and tensin homolog (PTEN). PIP<sub>3</sub> allows the recruitment of Akt to the cell membrane where it is phosphorylated on the threonine 308 (T308) by 3-phosphoinositide-dependent kinase 1 (PDK1) and on the serine 473 (S473) by other kinases such as 3-phosphoinositide-dependent kinase 2 (PDK2), integrin-linked kinase (ILK), protein kinase C (PKC) and mammalian target of the rapamycin complex 2 (mTORc2). After full activation, Akt translocates to cytosol and subcellular compartments to phosphorylate different substrates which are involved in tumor growth, cancer cell invasion and chemoresistance. Importance of each Akt isoforms in these processes is represented by the size of the draw. (B) Akt isoforms sequence composed of a conserved N-terminal pleckstrin homology (PH) domain, a linker domain, a central catalytic domain and a C-terminal regulatory hydrophobic motif (HM) domain, with percentage of homology and specific phosphorylation sites.

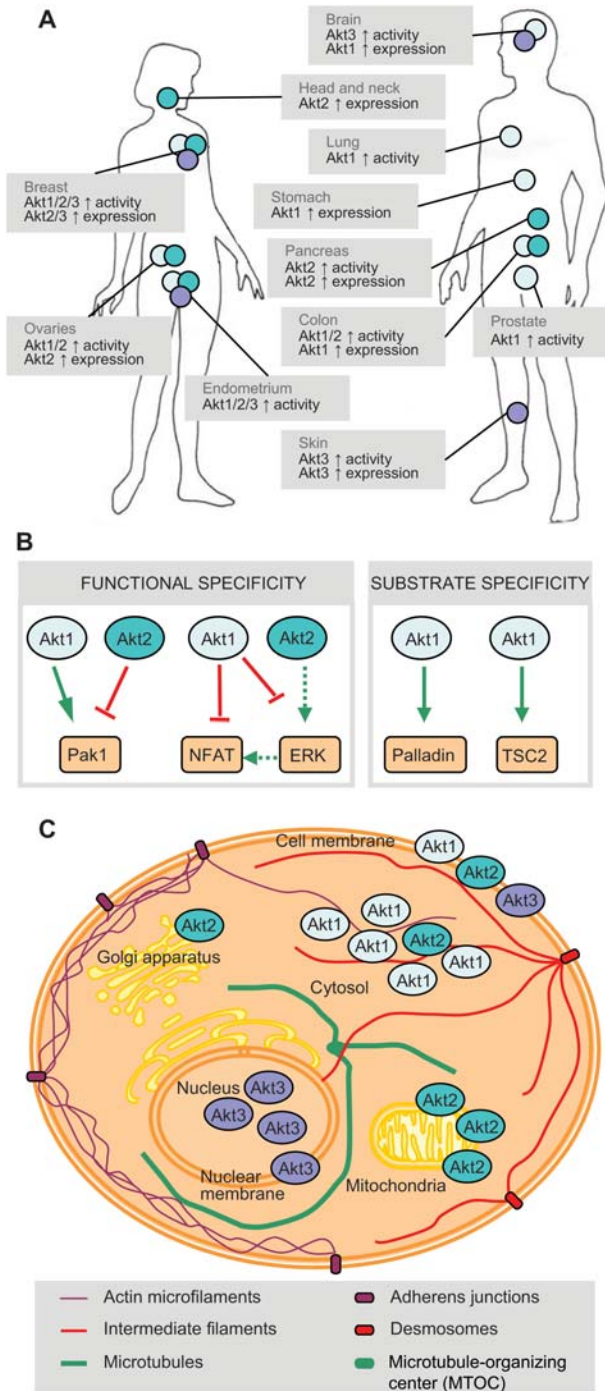
Akt2 knockout mice exhibited a diabetes-like syndrome with insulin resistance and, depending on the genetic background, mild growth retardation (14, 15). The absence of Akt3 in mice led to a selective decrease in brain size, without increased perinatal mortality, growth retardation or altered glucose metabolism (16, 17). The relatively subtle phenotypes of mice lacking individual Akt isoforms as well as the viability of the animals suggested that for many functions,

Akt isoforms are able to compensate for each other. To address the issue of isoform redundancy, mice with combined Akt deficiencies have been generated. Mice lacking both Akt1 and Akt2 isoforms died shortly after birth, and the newborns displayed impaired skin and bone development, severe skeletal muscle atrophy and reduced adipogenesis (18). Akt1/Akt3 double-knockout induced embryonic lethality from severe developmental defects in the cardiovascular and nervous systems (19). Interestingly, mice lacking Akt2 and Akt3 were viable despite reduced body size and impaired glucose metabolism (20). The viability of this phenotype suggests that Akt1 isoform is sufficient to perform all essential Akt functions in postnatal survival.

In the present review, we will highlight the current knowledge about the roles of the different Akt isoforms in tumor growth, chemoresistance and cancer cell migration, invasion and metastasis. For instance, from an analysis of the literature, there is a trend showing that Akt isoforms specificity in definite cancer could be explained by differential relative expression/activation of all isoforms, specific substrates and subcellular distribution. There is increasing evidence that the distinct localization of Akt isoforms is controlled by their interaction with the cytoskeleton network. In accordance with others, our findings also suggest that the cytoskeleton could play a role as a regulatory element by sequestering and liberating Akt proteins depending on the cellular context.

### Alterations of Akt isoforms activities in tumor

Increasing evidence suggests that hyperactivation of Akt signaling plays a central role in a variety of human cancers. Most tissue expresses the three Akt isoforms but the level of their expression is variable. For instance, Akt1 level is generally higher than Akt2 except in insulin-responsive tissues including liver, skeletal muscle and adipose tissue (21, 22). Akt3 is usually expressed at the lowest level especially in intestinal and muscle tissue. Its highest levels are found in brain, testes, lungs and kidneys (13, 23). Multiple reports describe an increased or constitutive activity of one of the three Akt isoforms in different cancers (Figure 2A). Indeed, approximately 40% of breast and ovarian cancers, more than 50% of prostate carcinomas and approximately 20% of gastric adenocarcinomas exhibited increased Akt1 expression and kinase activity (24). Elevated kinase activity of Akt2 has been demonstrated in 25% of primary breast carcinomas (25) and in approximately 30% of ovarian cancer specimens (26), the majority of cases being late-stage tumors. A similar ratio of pancreatic carcinomas showed markedly elevated levels of Akt2 activity compared with normal and benign pancreatic tumors (27). Elevated expression of Akt2 protein was reported in nearly 40% of hepatocellular carcinomas (28). Akt3 is the predominant active Akt isoform in approximately 60% of advanced-stage melanomas compared with melanocytes or early-stage lesions that have relatively low levels of Akt activity (29). Increased Akt3 activity has also been reported in estrogen receptor-deficient breast cancer and



**Figure 2** Mechanisms involved in the functional specificity of Akt isoforms during carcinoma progression.

(A) Tissue-specific alterations of Akt isoform expression and activity in different types of carcinomas. (B) Highlight of functional specificity of Akt isoforms on common substrates and distinct interaction of Akt isoforms with substrates. (C) Schematic representation of specific subcellular localization of Akt isoforms.

androgen-insensitive prostate cancer cell lines (30). Gliomas, bowel tumors, lung cancer and the majority of primary human lymphomas are also linked to Akt hyperactivity (31).

Interestingly, Akt isoforms constitutive activation appears to be associated with tumor progression rather than initiation. For instance, in different types of tumors, Akt2 kinase activity and/or expression level are increased with the pathological grade of malignancy (25–27, 32). The same correlation is observed for Akt3 in advanced-stage of metastatic melanomas (29).

The causes of Akt isoforms hyperactivation in tumors are multiple. Constitutive phosphorylation of Akt in cancers can be explained by gene mutation, amplification and/or mRNA overexpression. However, the most common aberrant Akt activation occurs through numerous mechanisms that affect elements upstream of Akt. These include amplification of oncogenes such as PI3K and Ras, loss of tumor suppressor genes such as PTEN, NF1 (neurofibromin) and threonine kinase LKB1, and aberrant activation or overexpression of growth factor receptors such as the epidermal growth factor receptor (2).

Many cancers arise from mutations in regulatory proteins that confer growth advantage. Although Akt proteins are key regulatory proteins in the pathogenesis of many cancers, mutations of Akt genes are rarely observed in human cancers (33). To date, one mutation on Akt1 has been described (34). This mutation results in a substitution of a glutamic acid to a lysine at amino acid 17 (E17K) in the PH domain of Akt1. This mutation activates Akt1, by favoring its binding to the plasma membrane stimulating its activation. Even though the E17K mutation activates Akt1 pathway, and has been reported to be responsible for the development of various cancers (breast, colorectal, ovarian, lung, endometrial cancer, leukemia and retinoblastoma) (34–38), the E17K mutation is rare and accounts for a very small percentage of increased activation of Akt1 signaling pathway in cancer. As observed for Akt1, mutations on Akt2 and Akt3 are also rare (39–41). In conclusion, to date, mutations of Akt genes are not considered as a major event in cancer development and other mechanisms need to be taken into account to explain Akt misregulation in cancer.

Analysis of multiple tumor types has indicated that amplification of Akt genes and increase Akt mRNA expression occurs only in a small number of cancer cells and is not a frequent event in tumorigenesis. Indeed, Akt1 gene amplification was initially detected in only a single over five gastric carcinomas and one over 255 cases of different human tumors (42). Moreover, a recent investigation revealed amplification and overexpression of Akt1 in a single case of gliosarcoma in a series of 103 cases (43). Amplification of Akt2 is relatively more frequent than amplification of other isoforms and was found in a subset of 16 over 32 ovarian, 7 over 72 pancreatic and 3 over 106 breast carcinomas (44–46). Little has been reported on Akt3 amplification except for a recent study that clearly demonstrated using fluorescence *in situ* hybridization that Akt3 was amplified in a significant subset of estrogen receptor-positive breast carcinomas, whereas Akt1 and Akt2 were shown to be more likely to be deleted (47). Overexpression of Akt3 mRNA has also been shown to be increased in hormone-independent breast and prostate carcinomas (30). In melanomas, elevated Akt3

signaling occurs as a result of a combination of increased Akt3 gene copy number and PTEN loss (29).

### Akt isoforms in tumor growth

Akt isoforms are important regulators of cell proliferation. For instance, although mutant mice lacking any one of the three Akt isoforms presented minor deficits (11–17), double mutants Akt1<sup>-/-</sup> Akt2<sup>-/-</sup> or Akt1<sup>-/-</sup> Akt3<sup>-/-</sup> and Akt2<sup>-/-</sup> Akt3<sup>-/-</sup> presented growth defects (18–20). Interestingly, the different mutants showed organ-specific deficiency (18–20). The analysis of these mice clearly demonstrated that *in vivo*, in a normal physiological situation, Akt isoforms are involved in cell proliferation and that their role is isoform-specific and tissue-specific.

In several recent studies, researchers have attempted to dissect the mechanisms by which specific isoforms of Akt are regulating cell proliferation in different cell types. For instance, the analysis of mouse embryo fibroblasts lacking Akt1 showed that the absence of Akt1 reduced cell proliferation and caused a delay in G1/S transition by decreasing accumulation of cyclin D1 (48). Embryonic fibroblasts lacking Akt2 showed normal proliferation and G1/S transition (48). The importance of Akt1 PH domain in regulating Akt1 effect on cell proliferation was shown by swapping Akt1 PH domain with that of Akt2. This substitution had a similar effect as Akt1 deletion. Finally, normal regulation of cell proliferation and G1/S transition time was recovered by reintroducing in the Akt1-deficient cells Akt1 or a chimeric protein containing the PH domain of Akt1 (48). Thus, the PH domain of Akt1 provides full kinase activity and is necessary for the G1/S transition. Moreover, this set of experiments demonstrated that Akt isoforms do not have identical function in cells. Downregulation of Akt1 by small interfering RNA (siRNA) in an ovarian cancer cell line has also been shown to be sufficient to interfere with cell proliferation. In these cells, downregulation of Akt1 was shown to affect cell cycle regulatory proteins (49). For instance, the reduction in the amount of Akt1 induced a decrease in the expression of cyclin D1, cyclin-dependant kinase 4, cyclin E, p21 and in phosphorylated retinoblastoma, and in this way reduced cell proliferation (49). The effect was similar to that of inhibiting PI3K by siRNA (49). However, the specificity of Akt1 isoform inhibition on abrogation of cell cycle in ovarian cancer cells is not that clear because downregulation of Akt2 was shown by other researchers to have a similar effect (50). The use of stable knockdown (49) instead of transient siRNA transfections (50) could explain the discrepancy between the two studies. Akt1 was shown to be required for anchorage-independent cell growth in a subset of cancer cell types: colon cancer cell line (HCT-15), lung carcinoma cell line (H460), pancreatic cancer cell line (MiaPaCa-2), cervix adenocarcinoma cell line (HeLa) and a fibrosarcoma cell line (HT1080) (51).

Adding to the complexity in understanding the role of individual Akt isoforms in tumor growth, the regulation of cell growth in cancer cells could involve two Akt isoforms.

For instance, Ericson et al. (52) demonstrated that both Akt1 and Akt2 were required for *in vitro* and *in vivo* growth of human colon cancer cell lines HCT116 and DLD1. They showed that knockout of either Akt1 or Akt2 in these cells had minimum effects on cell growth or downstream signaling, whereas double-knockout (Akt1 and Akt2) resulted in markedly reduced proliferation *in vitro* when growth factors were limiting. Indeed, the role of Akt1 and Akt2 in tumor growth was evident only in more challenging microenvironments, when cells were triggered by starvation or in experimental metastasis in mice (52). Thus, this particular set of experiments shows that increases in activation of Akt1 together with Akt2 provide cells with a growth advantage that is necessary for tumor growth. The synergistic effect of Akt isoforms has also been observed in different malignant glioma cell lines. In these cells, the knockdown of Akt2 and Akt3 resulted in a stronger cell growth inhibition than knockdown of individual isoforms (32). These results suggest that Akt2 and Akt3 isoforms can play redundant functions in the regulation of cell growth.

Akt3-specific activity in cell growth has been less studied than Akt1 and Akt2. However, Akt3 is the most active Akt isoform in 70% of cases of advance-stage melanoma. The reduction in these cells of Akt3, but not Akt2, activity is done by using siRNA diminished anchorage-independent cell growth (53) and increased apoptosis in tumors growing in nude mice. Thus, Akt3 activity can be linked to cellular growth related to tumorigenicity in advance-stage melanoma (29, 53). As for melanoma, the elevated Akt3 activity that has been reported in estrogen receptor-deficient breast cancer suggests that Akt3 could contribute to the aggressiveness of breast carcinomas. In the hormone-responsive breast cancer cell line MCF7, expression of active Akt3 abolishes the hormone-responsiveness and the MCF7-Akt3 cells grow in an estrogen-independent manner. Moreover, Akt3-expressing cells were found to produce tumors in mice in the absence of estradiol (54).

In conclusion, there is accumulating evidence that the relative importance of Akt isoforms in cell growth is cell line-specific (55). Although in some cases the predominant active isoform is the one which is involved in promoting cell growth, it was also demonstrated that Akt isoforms could compensate for others in signal transduction. Thus, only combined knockdown led to a reduction in the phosphorylation level of the substrates and cell survival. Therefore, to obtain the most specific effect on tumor cell growth inhibition, it is necessary to predetermine which Akt isoform is the most responsible for tumor cell growth and inhibit this specific isoform. The importance of selecting the proper Akt isoform to inhibit cell growth is exemplified by a study from Maroulakou et al. (56), who showed that inhibition of Akt1 and Akt2 can have opposite effects on tumors. For instance, using a mouse breast cancer model, Maroulakou et al. showed that the ablation of Akt1 or Akt2 inhibited or accelerated PyMT- and Neu-driven tumor formation, respectively (56). An antisense oligonucleotide against Akt1 which shows growth inhibitory activity in several cancer cell lines and inhibits the tumor formation in two different xenograft mice models is currently used in clinical trials (57).

## Akt isoforms in chemoresistance

Deregulations in the apoptotic and/or survival pathways are responsible for resistance to chemotherapeutic agent-induced apoptosis. Akt is well known to play a major role in cell survival, a function shared by all three Akt isoforms.

Akt1 forced expression in human lung cancer cells was sufficient to render the cells resistant to cisplatin. Similarly, Akt1 inhibition by its dominant negative mutant (58) or by siRNA targeting Akt1 (59) reversed the cisplatin-resistant phenotype through the inhibition of mTOR signaling and the MEK/ERK pathway, respectively. Analysis of Akt2 and Akt3 mRNA levels showed that they were not induced in cisplatin-resistant human non-small-cell lung cancer (NSCLC) cells A549 (58). Moreover, Akt2 siRNA or Akt3 siRNA transfection only marginally affected cisplatin-induced NSCLC cell death compared with cisplatin treatment alone (59). These results imply that Akt1 plays an essential role in cell survival and chemosensitivity in lung cancer cells. However, in uterine cancer cells, overexpression of constitutively active Akt1 isoform failed to protect cells from cisplatin-induced apoptosis, but was sufficient to decrease taxol-induced apoptosis (60). Therefore, simultaneous overexpression of multiple Akt isoforms is necessary to confer resistance to cisplatin in these cells (61).

Akt2 could confer cancer cells resistance to paclitaxel. In fact, Akt2 has been shown to mediate the resistance to paclitaxel of the breast cancer cells overexpressing the prosurvival transcription factor Twist. Knockdown of Akt2 significantly reduced the prosurvival effect of Twist on MCF7-Twist cells treated with paclitaxel (62). Akt2 activity can also counteract docetaxel-induced apoptosis in ovarian and breast cancer cells by regulating survivin levels (63, 64). The inhibitor of apoptosis proteins (IAPs) such as survivin and XIAP block the execution phase of apoptosis (65). Their expression in cancer cells is associated with resistance to various pro-apoptotic agents such as TGF- $\beta$  (66) and TNF- $\alpha$  (67), TRAIL (68, 69) and to chemotherapeutic drugs (60, 69–71). Some studies have shown that Akt and particularly Akt2 regulate IAP levels in cancer cells (72, 73). For instance, expression of a constitutively active Akt2 prevented cisplatin-mediated downregulation of XIAP and apoptosis in A2780 ovarian cancer cells (74). Indeed, Dan and coworkers (75) demonstrated that Akt1 and Akt2 phosphorylate XIAP and protect it from ubiquitination and degradation in response to cisplatin. XIAP downregulation by siRNA largely abrogated constitutively active Akt2 protection of the cells from cisplatin-induced apoptosis (75). Moreover, Akt2 has been shown to render A2780 ovarian cancer cells resistant to cisplatin-induced apoptosis through regulation of the ASK1/JNK/p38 pathway (76). In fact, Akt2 inhibits JNK/p38 and Bax activation through phosphorylation of ASK1 and thus plays an important role in chemoresistance.

Akt3 can also affect resistance to chemotherapeutic agents. For instance, in melanoma cell lines, reduced Akt3 protein expression by siRNA increased cleaved caspase-3 levels following staurosporine exposure (53). Elevated Akt3 activity has been reported in estrogen receptor-deficient breast cancer

tumors. A model of estrogen-responsive MCF7 cells expressing Akt3 has demonstrated a role for this isoform in estrogen receptor function. Indeed, the MCF7-Akt3 cells were found to be resistant to tamoxifen-induced cell death (54).

Akt isoforms have a distinct impact on cellular resistance to a given drug and Akt activity does not confer equal resistance to the different chemotherapeutic agents. Regarding the regulation of cell growth, studies demonstrate that in some cases the expression pattern of Akt isoforms could indicate which isoform is responsible for drug resistance. Chemoresistance is a multifactorial phenomenon which implies several signaling pathways downstream of Akt activation, which could account for Akt isoform-specific roles in chemoresistance.

## Akt isoforms in cell migration, invasion and metastasis

Akt isoforms have been shown to play a role in cell migration, invasion and metastasis of many cancer cells. Regarding growth control, a specific Akt isoform can have different effects on these cellular processes in different cell types. Cell migration and invasion are generally regulated by activating proteins involved in the modulation of actin cytoskeleton organization, extracellular matrix (ECM) degradation, cellular interaction with the ECM, expression of proinvasive genes and establishment of cellular polarity.

### Akt1 as an enhancer of cell invasion

In fibroblasts and endothelial cells, Akt1 has repeatedly been found to promote invasion (77–79). The differences between the effect of Akt1 and Akt2 activity in fibroblast migration was highlighted by Zhou et al. (78) who reported that Akt2 knockout mouse embryo fibroblasts migrated through ECM faster than wild-type cells. Moreover, Akt1 knockout cells migrated slower than wild-type cells. Consistently, Akt2 knockout cells had elevated levels of activated p21-activated kinase (Pak1) and Rac. These two proteins are implicated in a molecular cascade that leads to the rearrangement of actin cytoskeleton, the regulation of lamellipodium extension dynamic, as a cell spreading in response to adhesion. The inhibitory effect of Akt2 on migration was confirmed by showing that although both Akt2 and Akt1 formed a complex with Pak1, only Akt2 inhibited Pak1 (80). This suggests the existence of a different underlying mechanism that gives rise to the two isoform-specific phenotypes. Phosphorylation of the actin binding protein girdin by Akt1 also enhances fibroblast migration by promoting actin stress fibers and lamellipodia formation (81). Taken together, these results indicate that Akt1 and Akt2 play opposite roles in the regulation of Rac/Pak signaling in fibroblasts (80). Akt1 has also been shown to promote invasion of fibrosarcoma cell line HT1080 (82). In this case, increased cell migration was associated with ECM degradation through metalloproteinase-9 (MMP-9) production, in a manner that was highly dependent on Akt1 kinase activity and membrane-translocation ability. The increase in MMP-9 production was mediated by

Akt1 activation of nuclear factor- $\kappa$ B transcriptional activity. However, Akt1 did not affect the cell-cell or cell-matrix adhesion properties of HT1080 (82). Although it is well established that Akt1 promote fibroblasts and endothelial cell migration, some studies have shown that Akt1 can also induce motility in epithelial cancer cells. Regarding fibroblast cell lines, Akt1 activation in mouse mammary epithelial cells enhances MMP-2 activity, thereby increasing invasion potential (83). Moreover, Akt1 deficiency in Neu/Her2/erbB2-induced mammary epithelial cancer (MEC) cells was found to reduce lung metastases. The *in vivo* proinvasive role of Akt1 was mediated through the tumor suppressor tuberous sclerosis complex 2 (TSC2) phosphorylation (84). Indeed, Akt1 mediated MEC migration through paracrine signaling via induction of promigratory membrane-anchored chemokine CXCL16 and chemoattractant MIP1 $\gamma$ . Thus, Akt1 governs invasion of breast cancer originally induced by Neu/Her2/erbB2 oncogene by controlling cell polarity, migration and directionality. Similarly, Akt1 downregulation by siRNA in ovarian cancer cell lines is sufficient to reduce cell migration and invasion (49).

### Akt2 as an enhancer of cell invasion

Although there is clear evidence that Akt1 stimulates migration in some cancer cells, Akt2 also stimulates migration in other cell types. Even though Akt1 governs migration of some breast and ovarian cancer cells, a trend seems to emerge from *in vitro* studies that Akt1 generally stimulates migration and invasion of fibroblasts and endothelial cells, whereas Akt2 promotes the same phenomenon in epithelial cells. Interestingly, in cells where one isoform stimulates motility, the other isoform usually has a limited or even opposite role.

The Toker group has shown that active Akt1 limits breast cancer cell migration. Indeed, Akt1 decreased the promigratory activity of the transcription factor NFAT (85) and actin remodeling by its association with Akt1-phosphorylated p130Cas (86). In a similar way, silencing Akt1 expression increased migration and epithelial-mesenchymal transition (EMT) of the breast MCF10A cells overexpressing IGF-I receptor via the ERK pathway. Interestingly, downregulation of Akt2 suppressed the EMT-like morphological conversion induced by Akt1 downregulation and inhibited migration in EGF-stimulated cells (87). Consistent with these findings, some studies have shown that reduction of Akt2 expression by siRNA inhibited EGF-induced chemotaxis of MDA-MB-231, T47D and MCF7 breast cancer cells (88) and glioblastoma cells (89). Expression of a wild-type Akt2 in Akt2 siRNA-transfected cells rescued migration properties of the cells. Indeed, Akt2-depleted MDA-MB-231 cells showed a marked reduction in metastasis to mouse lungs, demonstrating the biological relevancy of Akt2 activity in cancer metastasis *in vivo* (88). In glioma cells, Akt2 phosphorylates girdin and ACAP1 which promotes integrin recycling and increases MMP-9 expression (89). Regulation of girdin by Akt1 and subsequent lamellipodia formation has already been shown to occur in fibroblast migration (81). Different roles for Akt isoforms are also observed in A549 lung cancer cells. For

instance, Akt2 siRNA remarkably downregulated migration and invasion, whereas Akt1 and Akt3 siRNA had little effect on these cellular events (90). Using an opposite approach where ectopic expression of Akt isoforms was achieved, it was demonstrated that in human breast and ovarian cancer cells Akt2, but not Akt1 or Akt3, increased migration and invasion by upregulating  $\beta$ 1-integrins and subsequently rendering the cells more metastatic (91). Consistent with these results, Cheng et al. observed that Twist-mediated induction of Akt2 expression, but not Akt1, promoted migration/invasion of breast cancer cells (62). Likewise, ILK preferentially mediated phosphorylation of Akt2 which induced mTOR activation that resulted in MMP-2 expression and promoted endothelial cell wound-induced or chemotactic migration (92). Suppression of Akt2 expression in highly metastatic colorectal carcinoma cells inhibited their ability to metastasize in an experimental liver metastasis model. Akt1 overexpression did not restore metastatic potential of colorectal carcinoma cells with downregulated Akt2, suggesting non-redundant roles for both isoforms in these cells (93). Another study explained the Akt1 inhibiting role in cell motility by the phosphorylation of TSC2 and its targeting for degradation (94). For instance, proteasome-dependent degradation of TSC2 in mammary epithelial cell lines leads to reduced Rho-GTPase activity, decreases actin stress fibers and focal adhesions, and reduces motility and invasion (94). By contrast, *in vivo* experiments demonstrated that Akt1-induced phosphorylation of TSC2 leads to its activation rather than its degradation. This promoted cell migration through the signaling cascade of Rho-GTPase/actin remodeling (84). Discrepancy between *in vitro* and *in vivo* roles of Akt1 in cell migration and invasion could be due to a TSC2 stabilization mechanism that prevents its ubiquitin-mediated degradation (95, 96). Iliopoulos et al. (97) showed that the opposing roles of Akt1 and Akt2 on the migratory and invasive phenotype in breast cancer cell lines and primary tumors were due to the differential effects of Akt1 and Akt2 on the abundance of the tumor suppressor miR-200 microRNA family. Thus, a decreased ratio of Akt1 to Akt2 is crucial for the downregulation of microRNA gene expression and the induction of invasiveness (97). Maroulakou et al. (56) reported the specificity of Akt isoforms by focusing on the effects of their ablation on mice mammary oncogenesis driven by polyoma virus middle T antigen (PyMT) and Neu/Her2/erbB2 oncogene (56). Whereas Akt1<sup>-/-</sup>PyMT and Akt2<sup>-/-</sup>PyMT tumors were both invasive, Akt1<sup>-/-</sup>Neu-induced tumors exhibited the highest degree of invasiveness. However, invasiveness did not always correlate with metastasis in that Akt1<sup>-/-</sup>PyMT and Akt2<sup>-/-</sup>PyMT tumors were significantly less metastatic than wild-type PyMT tumors. The Muller group (98) has reported that crossing MMTV/activated Akt1 transgenic mice with an activated Neu strain resulted in fewer metastatic lesions than the activated Neu strain alone. A subsequent study showed an increase in the incidence of pulmonary metastases following coexpression of Akt2 with the activated oncogene Neu in the mammary glands of transgenic mice. Thus, Akt1 and Akt2 might have opposite roles in tumor metastasis formation induced by the oncogene Neu (99).

### How Akt isoforms could play opposite roles in cell migration?

It is now clear that Akt isoforms could play different roles in migration, invasion and metastasis. The different properties of the three highly homologous Akt isoforms could be related to tissue-specific expression/activation of all isoforms, distinct effectors or substrate specificity and/or differential compartmentalization and localization of the isoforms (Figure 2).

The relative abundance or activation of Akt isoforms could be dynamic and change depending on different cellular contexts. For instance, Akt3 is the predominant active isoform in the majority of advanced-stage melanomas and thus is involved in tumor growth, survival and metastasis (29, 53). Also, the invasive or metastatic potential of breast cancer cell lines could depend not on the expression and activity of Akt but on the balance between Akt1 and Akt2 and their specific downstream signaling (85, 97).

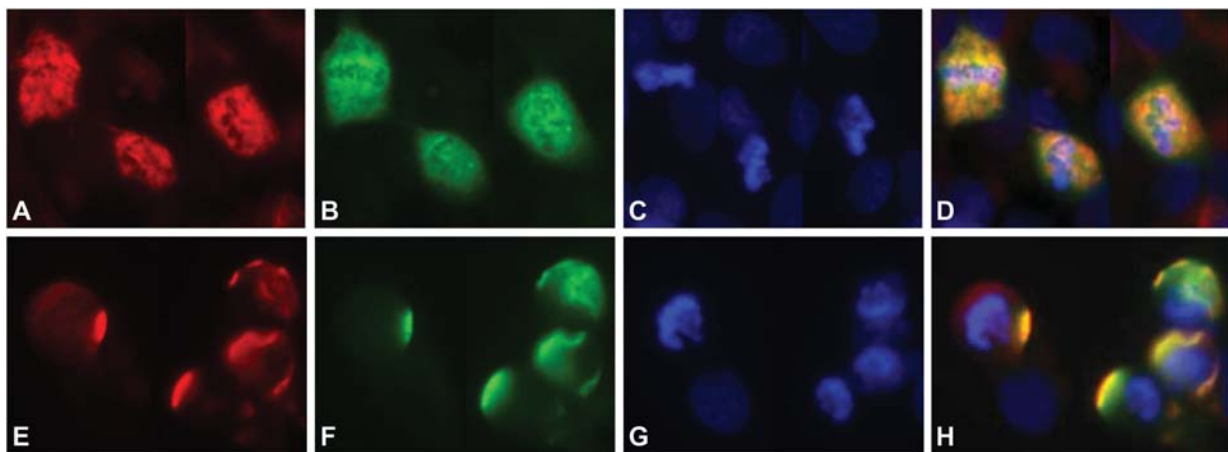
Moreover, the specificities of closely related Akt isoforms in cell invasion and metastasis involve their interaction with distinct effectors, such as TSC2/RhoGTPase (84, 94), NFAT (85) and palladin (86), which are specifically related to Akt1 isoform activity. Despite common preferred substrate motifs, Akt isoforms could have different impacts on the same substrate. For instance, Akt1 activates Pak1, whereas Akt2 inhibits its kinase activity (80).

Together with the two precedent mechanisms, the distinct subcellular localization of Akt isoforms could explain their differential behavior. Santi and Lee (100) have demonstrated, by doing an exhaustive immunocytochemistry analyses in a large range of cell types, that each Akt isoform is present, in most cases, at a unique subcellular location. According to the currently accepted model, activation of Akt is mediated by its recruitment and phosphorylation at the plasma membrane, in response to PI3K activation. Akt activation is followed by its release in the cytosol to accomplish its kinase function. As expected, active Akt1 and Akt2 were mainly localized in the cytoplasm. However, Akt2 showed some

cluster staining around the nucleus, which colocalized with mitochondria and to a lesser extent with the Golgi apparatus. Akt3 mainly localized in the nucleus and nuclear membrane. Moreover, because mitochondrial Akt2 and nuclear Akt3 were activated, it is possible that Akt2 and Akt3 did not need to reach the plasma membrane to be phosphorylated (100). The formation of a stable fluorescent complex of Akt1 and PDK1 fused proteins demonstrated that Akt1 T308 phosphorylation was independent of PtdIns (101). With regard to the common functions mediated by Akt isoforms, Santi and Lee asked the question whether Akt isoform localization could be altered by the ablation of another one. Single as well as double isoform knockdown did not alter the subcellular localization of the remaining isoform. These data are consistent with the concept that all three Akt isoforms have their own unique functions that are not shared by other isoforms.

### Akt isoforms moving through cytoskeleton

There is increasing evidence that the cytoskeleton network could modulate the localization of Akt isoforms. There are three types of cytoskeletal filamentous structures: actin microfilaments, microtubules and intermediate filaments (IFs). The cytoskeletal components constitute an extensive filamentous network which interacts with the different cellular organelles, adhesion complexes and non-cytoskeletal proteins (e.g., kinases, phosphatases, etc.) and participates to maintain cellular homeostasis by organizing the cytoplasm of the cell. A wide variety of cellular processes including movement of mitochondria, cell adhesion and cell migration are dependent on the dynamic of the cytoskeleton. Akt has been shown to associate with actin, tubulin and IFs (102). Indeed, it was shown in cultured human HaCaT keratinocytes that Akt was sequestered by the IF protein keratin 10, which impaired its translocation to the cell membrane and its activation. The authors reasoned that keratin 10 could directly inhibit cell proliferation via sequestration of Akt (103). We also observed that phosphorylated Akt colocalized



**Figure 3** Colocalization of phosphorylated Akt with cytoskeletal intermediate filaments. Immunofluorescence staining of keratin 18 (A, E), phosphorylated Akt (T308) (B, F), nuclei with Hoechst dye (C, G) and merge (D, H) in hepatocellular carcinoma HepG2 cells during mitosis (A–D) and upon toxic stress (E–H).

with keratin IFs in HepG2 cells during mitosis and upon toxic stress (Figure 3) (104). In the context of cell migration, keratin IFs which are linked to the  $\beta 4$  integrin has been shown to be necessary to convey signals from the  $\beta 4$  integrin to Akt (105). Thus, the cytoskeletal network could act as a scaffolding platform to convey signals up to Akt kinases. Other studies have demonstrated a similar role for another IF protein, vimentin, in the sequestration of cell migration mediators (106). RhoA-binding kinase  $\alpha$ , implicated in the reorganization of actin filaments, has been shown to be associated with the vimentin IF network in HeLa cells. Upon vimentin IF collapse during the formation of actin stress fibers and focal adhesion complexes, RhoA-binding kinase  $\alpha$  translocate to the cell periphery (107). In the same cells, we demonstrated that Akt2 overexpression induced a perinuclear collapse of vimentin network which was associated with an increase in cell invasion (108). Other studies have reported associations between vimentin perinuclear collapse and enhanced cell migration and invasion (109–111) and proposed that vimentin collapse contributed to the mechanisms of cell movement by the liberation of vimentin IF-sequestered proteins. All these studies support the hypothesis that the cytoskeleton plays a role in the regulation and the localization of Akt isoforms by sequestering or acting as a scaffold for the kinase and in this way modulates downstream signaling involved in cell migration and invasion.

## Highlights

We have summarized recent advances in the functional specificity of Akt isoforms in cancer progression. This review highlights the need for a better understanding of tumor expression profiles of Akt isoforms, substrate specificity and interaction partners or scaffolds. In some cases, the most active isoform is the one being considered as the most susceptible to be involved in promoting tumor growth, chemoresistance and migration, invasion and metastasis. However, in other cases, Akt isoforms could compensate for others in signaling. Thus, targeting multiple isoforms might be necessary to inhibit cancer progression. It is crucial to determine which isoform(s) is(are) accountable for each tumor to develop the proper treatment. The non-universality of the Akt signaling pathway is an emerging topic in the development of specific drug targeting in cancer research.

## Acknowledgments

The authors would like to thank the Natural Sciences and Engineering Research Council of Canada for grants to M.C. and E.A. E.A. holds a Canada Research Chair in Molecular Gyneco-Oncology.

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