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The Akt kinases: isoform specificity in metabolism and cancer

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Abstract

The Akt (PKB) protein kinases are critical regulators of human physiology that control an impressive array of diverse cellular functions, including the modulation of growth, survival, proliferation and metabolism. The Akt kinase family is comprised of three highly homologous isoforms: Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ). Phenotypic analyses of Akt isoform knockout mice documented Akt isoform specific functions in the regulation of cellular growth, glucose homeostasis and neuronal development. Those studies establish that the functions of the different Akt kinases are not completely overlapping and that isoform-specific signaling contributes to the diversity of Akt activities. However, despite these important advances, a thorough understanding about the specific roles of Akt family members and the molecular mechanisms that determine Akt isoform functional specificity will be essential to elucidate the complexity of Akt regulated cellular processes and how Akt isoform-specific deregulation might contribute to disease states. Here, we summarize recent advances in understanding the roles of Akt isoforms in the regulation of metabolism and cancer, and possible mechanisms contributing to Akt isoform functional specificity.

Keywords

Akt; isoforms; metabolism; cancer; glucose homeostasis; GLUT4; signaling specificity; cellular growth; Akt1; Akt2

Akt activation by extracellular stimuli

The serine/threonine protein kinase Akt is one of the most versatile kinases in the human kinome. Work from numerous laboratories over the last 10 years has elucidated the basic mechanisms underlying the activation of the Akt kinase and has identified a number of substrates that mediate Akt action (for review see references1,2). Akt activity is modulated downstream of phosphatidylinositol 3 (PI3) kinase in response to extracellular stimuli following a multistep process (Fig 1A). Akt, by virtue of an amino-terminal pleckstrin homology (PH) domain, is recruited to sites of the plasma membrane containing increased $PI(3,4,5)P_3$ or $PI(3,4)P_2$ produced by PI3-kinase. Once recruited to the plasma membrane, Akt is phosphorylated at two sites, one within the T-loop of the catalytic domain (Thr^{308}, Akt1 residue) by the phosphoinositide-dependent kinase 1 (PDK1) and within the carboxyl terminal hydrophobic domain (Ser^{473}, Akt1 residue) by the mammalian target of rapamycin complex 2 (mTORC2)3,4. Targeting of Akt to the plasma membrane, independent of external stimuli (or PI3 kinase activity), results in Akt activation, strongly suggesting that activation is limited predominantly by recruitment to the plasma membrane rather than the

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direct modulation of PDK1 and/or mTORC2 activities. Akt is transiently localized to the plasma membrane during activation 5,6 and once activated, Akt phosphorylates substrates distributed throughout the cell to regulate multiple cellular functions (Fig 1A)(see reference [2] for review).

Akt isoform-specific signaling

Akt kinases control an array of diverse functions including cell growth, survival, proliferation and metabolism, and one critical unresolved question is how Akt activity is specified to discrete cellular functions in response to extracellular stimulus. Recent studies of Akt isoform-specific knockout mice suggest that Akt signaling diversity might in part be due to different functions of the three Akt family members Akt1, Akt2 and Akt3 [7] (Fig 1B). Akt1 knockout mice are smaller than their wild-type counterparts and Akt1-null cells display higher rates of apoptosis, indicating a critical role for Akt1 in cell survival [8],9. Akt2 knockout mice develop a type 2 diabetes-like phenotype, and cells derived from those mice show impaired glucose utilization 9,10, suggesting a central role for Akt2 in the maintenance of glucose homeostasis. A role for Akt3 in brain development has been proposed based on the fact that Akt3 knockout mice display impaired brain development[11]. Although those data strongly support the hypothesis that different cellular processes are primarily under the control of the different Akt isoforms, phenotypic analyses of double Akt isoform knockout mice reveal some overlap (or compensation) among the isoforms. Simultaneous deletion of Akt1 and Akt2 causes lethality shortly after birth [12], Akt1 and Akt3 double knockout mice are embryonic lethal 13, whereas mice with a single functional allele of Akt1 ($Akt1^{+/-}Akt2^{-/-}Akt3^{-/-}$) are viable despite reduced body weight and insulin and glucose intolerance [14]. All together, these studies provide genetic evidence for overlapping as well as specific roles of the Akt family members. Further studies of Akt isoform conditional and tissue specific knockout mice, as well as in vitro studies of Akt isoform function in different cell types will be required to develop a complete and integrated understanding of the spectrum of common and isoform specific roles of Akt kinases.

The striking observation that despite their high homology, Akt isoforms regulate distinct physiological functions leads to the critical question of how Akt isoform-specific signaling is achieved. A priori, different mechanisms could dictate or contribute to Akt isoform function distinctions (Fig 1C). Those modes of regulation might include:

1. Distinct tissue distribution of the Akt isoforms.
2. Differential activation of the Akt isoforms by extracellular stimuli (that is, cues like the amplitude or timing of PI3 kinase activity triggered by different stimuli could be translated into differential activation/regulation of the Akt isoforms).
3. Distinct *intrinsic* catalytic activity of the Akt isoforms to phosphorylate substrates.
4. Cell context-specific factors. Examples of this mode of regulation include isoform specific subcellular compartmentalization that determines access to substrates and/or specific adaptor proteins that confer specificity to substrate selection.

Elucidating the individual and combinatorial contributions of these mechanisms to Akt isoform functional specificity will be required to understand the complexity of Akt-mediated signaling and to facilitate the development of therapeutic approaches directed to interfere with Akt isoform-specific functions. Akt signaling is at the center of growth and metabolic control, and alterations of Akt signaling are underlying causes of two of the most prominent diseases in developed countries, cancer and type 2 diabetes. Here we focus on these disease models to summarize some of the recent advances and remaining challenges regarding the roles and regulation of Akt isoform-specific signaling.

Akt isoform-specific signaling in metabolism

To date, the regulation of glucose homeostasis is one of the best-characterized Akt-mediated processes with strong isoform specificity. Insulin regulates whole body glucose homeostasis by inducing the uptake of glucose into muscle and fat cells and by inhibiting hepatic glucose output, both of which are under the regulation of Akt signaling [15]. To regulate glucose disposal insulin-induces the redistribution of the GLUT4 glucose transporter from intracellular compartments to the plasma membrane of fat and muscle cells [16]. The increase in plasma membrane GLUT4 promotes increased flux of glucose into those cells in a concentration dependent manner. As noted above, targeted deletion of Akt2 in mice, but not Akt1 or Akt3, results in fasting hyperglycemia, hyperinsulinimia, glucose intolerance and impaired glucose uptake by fat and muscle cells [10],[17]. Consistent with a requirement for Akt2 in control of glucose metabolism, a mutation in the catalytic domain of Akt2 causes severe insulin resistance and diabetes in humans [18]. In vitro studies showed that transient siRNA-mediated down-regulation of Akt2 inhibits insulin-induced GLUT4 translocation to the plasma membrane of 3T3-L1 adipocytes [19]–[21], suggesting that impaired glucose transport in Akt2-null adipocytes is at least in part explained by defective regulation of GLUT4 trafficking in those cells.

How is this Akt2 isoform signaling specificity achieved?

Two of the most likely mechanisms, tissue-specific isoform expression and isoform-specific activation, do not account for the selective requirement for Akt2 in the control of GLUT4 trafficking to the plasma membrane. Overexpression of Akt1 in Akt2-deficient brown fat adipocytes did not rescue the impairment in insulin-mediated glucose transport [22] nor does over-expression of Akt1 rescue defects in GLUT4 translocation to the plasma membrane of adipocytes induced by siRNA knockdown of Akt2 [21]. Those findings strongly argue that the distinct functional role for Akt1 and Akt2 in the regulation of glucose transport in fat cells is not determined by selective expression of Akt isoforms. Insulin activates both Akt1 and Akt2 in 3T3-L1 and primary rat adipocytes [21],[23], demonstrating that the failure of Akt1 to regulate GLUT4 translocation to the plasma membrane is not due to differential activation of the Akt isoforms by insulin. Thus, differences in Akt isoform-specific regulation of glucose transport in fat cells are determined post Akt activation, at the step of substrate selection/phosphorylation.

How is this substrate selectivity achieved?

One possibility is that Akt isoform substrate specificity is determined by cell context-specific factors. For example, isoform-specific subcellular compartmentalization could determine access to substrates. The first hints that differential localization might have a role in controlling isoform specific signaling were provided by biochemical fractionation of adipocytes in which Akt2 but not Akt1 was found associated with GLUT4 vesicles [24],[25]. Recent studies in intact cells provide compelling evidence that differences in subcellular localization are primary in determining Akt2-specific control of GLUT4 trafficking in fat cells (Fig 2). Using fluorescence reporters and total internal reflection fluorescence microscopy, we have observed that insulin induces a preferential accumulation of Akt2, relative to Akt1, at the plasma membrane of adipocytes. Changes that reduce the accumulation of the Akt2 kinase domain at the plasma membrane of insulin-stimulated adipocytes without affecting kinase activation, interrupted signaling to GLUT4, indicating that the selectivity is not intrinsic to the Akt2 kinase domain [21]. These data suggest that the greater accumulation of Akt2 at the plasma membrane is a contributing factor to isoform – specific signaling to GLUT4. In support of that hypothesis, expression of an Akt1 mutant with enhanced plasma membrane association, $Akt1^{E17K}$ [26], was sufficient to induce GLUT4 translocation and overcome Akt isoform specificity in the regulation of GLUT4 trafficking

[21]. These findings document that Akt1 can regulate substrates involved in GLUT4 translocation when it accumulates at the plasma membrane to a similar degree as Akt2, and thereby establish that stimulus-controlled differences in subcellular localization of Akt isoforms contribute to Akt isoform-specific signaling. Importantly, aberrant Akt subcellular localization, such as this caused by the E17K point mutation, can confer Akt2-like signaling to Akt1 resulting in the loss of isoform-specific signaling. These conclusions are in agreement with previous work in which Akt1 engineered to be constitutively targeted to membranes via addition of a myristoyl group [27] was shown to induce GLUT4 translocation.

The increased accumulation of Akt2 at the plasma membrane correlates with Akt2-specific substrate selection/phosphorylation. The best characterized downstream effector of Akt required for regulation of GLUT4 trafficking to the plasma membrane is the RabGAP AS160 (also known as TBCD1D4) [28]–30. Phosphorylation by Akt leads to inhibition of the AS160 GAP activity towards Rab proteins, including Rab8, Rab10, and Rab14 31. The subsequent activation of these Rab proteins facilitates the translocation of GLUT4 to the plasma membrane of adipocytes and muscle cells 32,[33]. siRNA-mediated Akt isoform specific down-regulation revealed a preferential role for Akt2 in the phosphorylation of AS160 in response to insulin, both in adipocytes [21] and muscle cells [34]. Furthermore, Akt2 but not Akt1 localizes at the plasma membrane with AS160, indicating that this might be the site of AS160 regulation by Akt2 in insulin-stimulated cells [21].

The requirement for Akt activity at the plasma membrane to regulate glucose transport is consistent with previous studies that proposed a role for Akt in the regulation of the late stages of GLUT4 vesicle exocytosis, including the docking and fusion of GLUT4 vesicles with the plasma membrane [35]–[37]. Although AS160 is the best characterized Akt substrate involved in GLUT4 trafficking, the phosphorylation of at least two other substrates believed to have roles in GLUT4 trafficking, Myosin Va and the syntaxin interacting protein Synip, have also been linked specifically to Akt2 [38] [39].

The molecular determinants of Akt2 preferential accumulation at the plasma membrane have not been elucidated. Akt plasma membrane targeting is mediated through interactions of its PH domain with $PI(3,4,5)P_3$ and $PI(3,4)P_2$ generated by PI3kinase. However, exchange of Akt1 PH domain by that of Akt2, does not confer Akt1 enhanced plasma membrane association, documenting that distinct insulin-induced Akt2 plasma membrane accumulation does not rely solely on Akt2 PH domain [21]. It is possible that differences in post-translational modifications in the Akt isoforms might contribute to the differences in compartmentalization. This type of regulation contributes to the discrete subcellular localization and functional specificity of the Ras isoforms and members of the Src family of kinases [40],[41]. However, to date Akt isoform-specific posttranslational modifications have not been described.

A growing number of proteins that interact with and regulate Akt function are being described (for review see references 42,43). However, Akt isoform specific binding partners that regulate subcellular location and substrate recognition are almost unknown. Schenck and colleagues 44 reported that Akt interaction with the Rab5 effector APPL1 regulates the activity of Akt and its downstream signaling specificity from an endosomal compartment with implications in vertebrate development. Interestingly, APPL1 interacts with Akt2 in unstimulated adipocytes, and down-regulation of APPL1 in adipocytes impairs insulin-induced GLUT4 translocation and glucose transport, indicating that APPL1 might facilitate Akt2 signaling to GLUT4 45. However, whether APPL1 also regulates Akt1-mediated signaling in adipocytes and muscle cells, and the mechanism by which APPL1 regulates Akt2-mediated GLUT4 translocation are unknown. Recently Ding and colleagues identified ClipR-59, a member of the Clip-170 family, as a scaffolding protein that interacts with Akt

and stabilizes it at the plasma membrane of insulin-stimulated adipocytes [46]. ClipR-59 preferentially binds to Akt2, and down-regulation of ClipR-59 impairs insulin-induced GLUT4 translocation and Akt-mediated phosphorylation of AS160. These studies, together with our recent observations underscore the role of insulin-mediated Akt2 plasma membrane accumulation in GLUT4 regulation and suggest ClipR-59 as a putative regulator of Akt isoform functional specificity in insulin-regulated glucose uptake.

These recent studies have started to uncover the molecular mechanisms that determine Akt isoform signaling specificity in the regulation of glucose homeostasis. The availability of cell-lines derived from mice lacking individual Akt isoforms, combined with the use of siRNA, proteomics, mass spectrometry and live-cell fluorescence microscopy techniques will facilitate the identification of Akt isoform-specific binding partners and signaling networks that regulate Akt-isoform signaling diversity in glucose homeostasis. Whether these modes of regulation will also function in non-insulin responsive cells or in response to other physiological stimulus will also need to be addressed. Due to the intricate relationship between insulin-responsive tissues in the maintenance of glucose homeostasis, the future development of tissue specific Akt isoform knock-ins and knock-outs will also be required to evaluate the contribution of Akt isoform signaling in different tissues to the systemic regulation of glucose homeostasis and insulin sensitivity.

Akt isoform-specific signaling in cancer

Akt is one of most frequently hyperactivated kinases in human cancers [47], which perhaps is not unexpected considering that Akt modulates cell proliferation, survival, intermediate metabolism, growth, invasiveness and angiogenesis, all of which are hallmarks of cancer. Interestingly, there is evidence for hyperactivation of specific Akt isoforms in certain tumors, suggesting that in some cases there is Akt isoform-specificity to cell transformation. Aberrant activation of Akt can occur by different mechanisms including amplifications, overexpression, mutations in Akt genes, and/or alterations in Akt upstream regulators [48], and as briefly discussed below, there are examples of all these modes of Akt hyperactivation in tumors.

Activation of specific Akt isoforms in cancer

The Akts are produced from three distinct genes and therefore one mechanism for isoform-specific increased expression is gene amplification. AKT2 gene amplification has been reported in ovarian and pancreatic cancers [49],[50], AKT1 amplification has been reported in gastric cancers [51], and AKT3 amplification in melanoma [52]. Overexpression of specific Akt isoforms independent of gene amplification has also been found to differ among different cancers. For example, Akt2 has been found to be upregulated in hepatocellular carcinomas and colorectal cancers [53],[54], Akt1 in breast cancers [55] and Akt3 mRNA has been shown to be upregulated in estrogen receptor-negative breast tumors [56] and melanoma [52]. Recently, a somatic activating mutation in Akt1 PH domain was identified in human ovarian, breast and colorectal cancers, in that study no changes in Akt2 or Akt3 were found [26]; however an equivalent mutation in Akt3 has recently been described in human melanoma cancers [57]. The correlation between specific tumors and the hyperactivation of specific Akt isoforms raises the possibility that Akt isoform-specific signaling might contribute to the development of certain tumors; however, additional studies are required to test this hypothesis.

Akt activity can also be altered by changes in upstream regulatory proteins. Examples include hyperactivation of the PIK3CA gene (which encodes a catalytic subunit of PI3-kinase), loss/down regulation of phosphatase and tensin homolog (PTEN, a lipid phosphatase that counteracts PI3-kinase activity), up-regulation of growth factor receptors or activation of Ras. These changes would/should result in activation of all three Akt isoforms,

therefore it is unknown whether they will translate to different contributions of the Akt isoforms to tumorigenesis. Interestingly, it has recently been shown that deficiency of Akt1 is sufficient to inhibit endometrial and prostate neoplasia in $PTEN^{+/-}$ mice [58], suggesting a predominant role of Akt1 in these cancers. Whether a similar Akt isoform dependency exists in PI3-kinase and/or Ras induced tumors is largely unknown.

How could differential activation of Akt isoforms contribute to tumorigenesis?

Although our understanding of the distinct roles of Akt isoforms in tumor formation, development and invasiveness is still limited, several studies have begun to address the differential roles for Akt1 and Akt2 in the regulation of cell cycle progression and cell migration. A series of recent studies provide compelling evidence for isoform-specific functions of Akt kinases in the regulation of cell migration (reviewed in reference59). In vivo and in vitro studies support that Akt1 attenuates while Akt2 enhances cancer cell migration. On the contrary Akt1 specifically promotes migration of non-transformed cells in certain cellular contexts 60, indicating that isoform specific regulation of cellular migration exist both in transformed and non-transformed cells although it might be dictated by distinct mechanisms. Several mechanisms have been proposed for Akt1-mediated inhibition of cancer cell migration including negative regulation of the extracellular-signal regulated kinase pathway 61, the tumor suppressor tuberous sclerosis complex 2 (TCS2) 62 and the nuclear factor of activated T cells (NFAT) a pro-migratory and pro-invasive transcription factor in breast cells 63. Akt1 down-regulation also caused phenotypic changes characteristic of an epithelial-mesenchymal transition in IGF-IR stimulated breast cancer cells, a phenotype inhibited by down-regulation of Akt2 61. Contrary to Akt1, overexpression of Akt2 promoted adhesion and invasion in eight human breast cancer cell lines that correlated with upregulation of β1 integrins 64.

Studies of transgenic mice further support Akt isoform-specific roles in tumor development and metastasis. Coordinated overexpression of activated Akt1 and ErbB-2 in the mammary gland of transgenic mice accelerated the appearance of multifocal mammary tumors and decreased the incidence of metastatic lesions compared to tumors initiated by ErbB-2 alone 65. Interestingly, coexpression of Akt2 with activated ErbB-2 in the mammary gland did not affect the latency of tumor development, but markedly increased the incidence of pulmonary metastases [66]. Consistent with those findings, ablation of Akt1 delayed mammary adenocarcinoma induction by polyoma middle T (PyMT) and Neu transgenes in mice, while ablation of Akt2 accelerates this process [67].

Apart from differences in cell migration, a differential role for Akt isoforms in cell cycle control has also been reported. Silencing Akt1 but not Akt2, in non-transformed mammalian cells results in reduction of cyclin A levels and S-phase entry, while over expression of Akt2 hindered cell cycle progression in M-G1 with increased nuclear p21 [68]. Analysis of Akt isoform function in early stages of oncogenesis showed that the number of mammary epithelial cells expressing cyclin D1 in PyMT/$Akt1^{-/-}$mice is lower than in the wild type mice, while PyMT/$Akt2^{-/-}$mice revealed a higher number of cyclin D1– expressing cellsin lesions than PyMT/wild type mice [67]. This work reveals differences in the regulation of the cell cycle progression by the Akt isoforms both in non-transformed and transformed mammalian cells.

Together these studies strongly suggest distinct functions of the Akt family members in the regulation of cell cycle progression, migration and invasion that might translate into differential roles in the modulation of tumor development and metastasis. Although the molecular mechanisms dictating Akt isoform specificity in these settings are largely unknown, some of the mechanisms identified in other cellular contexts, such as the regulation of glucose transport, could potentially be applied in these Akt-regulated

processes. Investigating how these modes of regulation contribute to Akt isoform specific signaling in cell growth, survival and migration, and their relevance in distinct types of tumors would provide the basis to understand the pluripotent role of Akt kinases in human tumorigenesis and determine how Akt inhibitors could be used most efficiently to treat human cancer.

Akt signaling in the metabolic reprogramming of cancer cells

Although the regulation of metabolism and cancer by the Akt kinases are very active areas of study, we still have a very limited understanding about the integration of these two processes by the Akt family members. High proliferating rates characteristic of cancer cells require the efficient conversion of nutrients into biomass. Indeed it is known for many years that cancer cells display a distinct intermediate metabolism from most normal tissues, favoring glycolysis and the production of high concentrations of lactate versus metabolizing glucose to carbon dioxide in the mitochondrial tricarboxylic acid (TCA) cycle as most differentiated cells. Although aerobic glycolysis is less efficient in terms of ATP production than oxidative phosphorylation, it provides cancer cells with an advantage in terms of production of ribose-5 phosphate, acetyl-CoA and NADPH required for macromolecular synthesis (reviewed in reference 69).

Akt signaling is at the crossroads of growth control and glucose metabolism. Akt regulates protein and long chain fatty acid synthesis via modulation of mTOR [70] and fatty acid synthase [71] respectively. The role of Akt in the modulation of glucose uptake into insulin-responsive tissues has been discussed above, however non-insulin responsive tissues also rely on Akt activity for glucose uptake and utilization. Growth factor-mediated Akt activation induces increased expression of the glucose transporter GLUT1 as well as an increase of GLUT1 in the plasma membrane [72],[73]. Both of these changes promote enhanced glucose flux into the cells. Akt also increases metabolism of glucose by modulating hexokinase association with mitochondria, thereby increasing the efficiency of glucose-6-phosphate production, and Akt stimulates phosphofructokinase activity increasing glycolysis [74],[75]. As a consequence, alterations in the PI3-kinase/Akt pathway leading to enhanced Akt activity correlate with the high glycolytic rates characteristic of cancer cells [76],[77]. Due to this metabolic reprogramming, glucose withdrawal induces cell death in a manner similar to that of growth factor withdrawal [78]. Indeed, glucose uptake by tumors containing mutations in PIK3CA is inhibited by small molecule inhibitors of PI3-kinase and mTOR, and correlates with tumor regression [79], documenting the role of the PI3-kinase/Akt signaling pathway in the control of glucose metabolism in cancer cells.

The role of the distinct Akt family members in the “glucose addiction” of cancer cells is an exciting and largely unexplored area of study. Although specific functions for these kinases might exist, it is also possible that under deregulation of Akt signaling, as in the presence of activating mutations of PI3-kinase or loss of PTEN, Akt isoform functional specificity might also be lost. Indeed, our recent data suggest that overexpression of $Akt1^{E17K}$, an Akt mutant with enhanced plasma membrane association identified in human cancers, is sufficient to induce GLUT4 translocation to the plasma membrane of adipocytes, a process that is under specific regulation of Akt2 [21].. Therefore, even if Akt isoforms have a distinct contribution to the regulation of glucose metabolism in “non-insulin responsive cells”, that specificity could be overridden by oncogenic mutations. Indeed, oncogenic transformation has been described by overexpression of all 3 myristoylated Akt isoforms [80]. Consequently, it is tempting to speculate that the loss of Akt isoform functional restrictions might further contribute to cellular transformation. The current effort to develop small molecule Akt inhibitors with high potency, improved pharmacokinetics, and isoform selectivity will certainly help to answer these questions.

Perspectives

The development of Akt isoform-specific null mice has proven a functional diversity within the Akt family of kinases in physiology and disease. Work over the last few years has begun to dissect specific roles of these kinases; however, we still have a limited knowledge about the cellular and molecular mechanisms that determine Akt isoform functional specificity. Exploring the contribution of differential expression patterns, substrate specificity, binding partners and subcellular localization to Akt functional specificity through the combination of genetic models, proteomics and live cell microscopy will be required in order to define Akt isoform specific regulatory and signaling networks. A challenge ahead is to understand how/if deregulation of specific Akt isoforms contributes to the development of disease such as cancer and type 2 diabetes. The answers to these questions will provide the basis for the development of efficient and selective pharmacological interventions of Akt function in disease states without globally perturbing the cellular functions modulated by Akt signaling.

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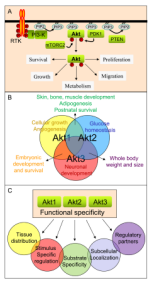

Figure 1.

Regulation of Akt signaling. A. Growth factor-mediated Akt activation. In response to numerous growth factors and cytokines Akt is activated downstream of PI3-kinase following a multistep mechanism. Activated PI3-kinase converts PIP2 into PIP3 providing sites of recruitment at the plasma membrane of proteins containing PH domains, including Akt and PDK1 kinases. Upon translocation to the plasma membrane Akt is phosphorylated by PDK1 within the catalytic domain and mTORC2 within the hydrophobic motif, which renders Akt catalytically active. Activated Akt, trough the phosphorylation of numerous downstream targets located throughout the cell, regulates a wide array of cellular functions. B. Overlapping and specific functions of the Akt family members. Summarized are common and distinct Akt isoform functions elucidated from the phenotypic analysis of single and double Akt isoform knockout mice. C. Potential mechanisms dictating Akt isoform functional specificity.

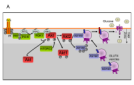

Figure 2.

Akt2 signaling specificity in insulin-mediated glucose uptake. Insulin binding to the insulin receptor at the surface of adipocytes leads the activation and the autophosphorylation of the receptor, followed by recruitment and phosphorylation of insulin receptor substrate proteins (IRS). Phosphorylated IRS create docking sites for the recruitment of PI3-kinase which converts PIP2 into PIP3, providing sites for the recruitment of Akt kinases to the plasma membrane. Both Akt1 and Akt2 translocate to the plasma membrane in response to PI3-kinase activation and both kinases are activated by phosphorylation trough PDK1 and the mTORC2. Using Akt1 and Akt2 reporters and total internal reflection fluorescence (TIRF) microscopy we found that upon insulin stimulation Akt2 accumulates at the plasma membrane environment (TIRF zone) to a larger degree than Akt1. This distinct subcellular distribution facilitates Akt2 phosphorylation and inactivation of the Rab GAP AS160 possibly located on GLUT4 containing vesicles. Inactivation of AS160 allows the subsequent docking and fusion of GLUT4 vesicles with the plasma, and consequently the entrance of glucose into the cells.