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Diverse mechanisms of AKT pathway activation in human malignancy

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Abstract

AKT/PKB (Protein Kinase B) are central proteins mediating signals from receptor tyrosine kinases and phosphatidylinositol 3-kinase. AKT kinases are involved in a number of important cellular processes including cell proliferation and survival, cell size in response to nutrient availability, tumor invasion/metastasis, and angiogenesis. Various components of the AKT signaling pathway are encoded by tumor suppressor genes and oncogenes whose loss or activation, respectively, plays an important role in tumorigenesis. The growing body of evidence connecting deregulated AKT signaling with sporadic human cancers and inherited cancer predisposition syndromes is discussed. We also highlight new findings regarding the involvement of activating mutations of *AKT1*, *AKT2*, and *AKT3* in somatic overgrowth disorders: Proteus syndrome, hypoglycemia with hypertrophy, and hemimegalencephaly, respectively. In addition, we review recent literature documenting the various ways the AKT signaling pathway is activated in human cancers and consequences for molecularly targeted therapies.

Keywords

AKT/PKB kinases; tumor suppressor genes; oncogenes; human malignancy; targeted therapies; Proteus syndrome; hypoglycemia; hemimegalencephaly

INTRODUCTION

The AKT/PKB (protein kinase B) family of kinases function as intermediate signaling molecules that regulate cell survival, proliferation, size and invasion as well as glucose metabolism and angiogenesis. The normal mechanisms by which AKT kinase is activated have been reviewed in detail elsewhere [1, 2]. A large body of literature has documented hyperactivated AKT signaling in human solid tumors and hematological malignancies [1]. In addition, genetically engineered mice have demonstrated the important role of AKT activation, alone or in combination with other genetic alterations, in promoting tumorigenesis [3, 4]. Since the AKT signaling pathway is important in tumor development, disease aggressiveness, and drug resistance, molecularly targeting components of the pathway have been an important focus in recent years.

AKT is known to be a central component in the signaling pathway composed of upstream phosphatidylinositol 3-kinase (PI3K) and PTEN (phosphatase and tensin homolog deleted on chromosome 10), and downstream tuberous sclerosis complex 2 (TSC2), forkhead box O (FOXO), and eIF4E (Figure 1). Several of these proteins (AKT, PI3K p110α catalytic

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CONFLICT OF INTEREST
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subunit, and eIF4E) can act oncogenically when overexpressed or mutated, while others (PTEN, FOXO, LKB1, TSC1/TSC2, NF1, and VHL) are tumor suppressors. Somatic mutations or epigenetic changes of the genes encoding these proteins have been identified in a number of cancers. Moreover, germline mutations of *PTEN*, *LKB1*, *TSC2/TSC1*, *NF1*, and *VHL* are involved in five dominantly inherited cancer predisposition syndromes [5–7]. Each of these genes encode tumor suppressors that negatively regulate the AKT-mTOR pathway, which when deregulated, results in aberrant translation of mRNA important in tumor development.

In this review, we summarize a large body of evidence implicating the AKT signaling pathway in cancers and in dominantly inherited cancer predisposition syndromes. We also summarize the various mechanisms by which the AKT signaling pathway is activated oncogenically (Table 1) and the implications for targeted therapies.

AKT REGULATES MANY CELLULAR PROCESSES IMPORTANT IN TUMORIGENESIS

Activated AKT is a well-established survival factor in cancer. It functions through the phosphorylation of a number of proteins involved in regulating apoptosis. AKT was shown to phosphorylate and inactivate the proapoptotic factors BAD and procaspase-9 (reviewed in [8]). Moreover, AKT phosphorylates and inactivates the FOXO transcription factor, which is involved in the expression of pro-apoptotic genes such as the FAS ligand gene, *TNFSF6*. AKT can activate IκB kinase (IKK), a positive regulator of NF-κB that transcribes anti-apoptotic genes (reviewed in [9]). AKT has also been shown to phosphorylate Mdm2, resulting in its translocation into the nucleus, where it downregulates p53 through protein degradation [10, 11]. Alternatively, AKT can negatively regulate p53 mRNA levels through phosphorylation of the PHF20/TZP transcription factor. Phosphorylation of PHF20/TZP results in its translocation from the nucleus to the cytoplasm, thereby attenuating the transactivation of the *p53* gene (*TP53*) [12].

AKT plays a role in cell cycle progression partly by phosphorylating and inhibiting glycogen synthase kinase 3β, which is a kinase responsible for phosphorylating and mediating cyclin D1 protein degradation. AKT can also phosphorylate the cell cycle inhibitors $p21^{WAF1}$ and $p27^{Kip1}$, resulting in the cytoplasmic retention of these proteins and precluding their binding and inhibition of the cyclin/CDK complexes (reviewed in [1, 13]).

AKT activation of downstream mTOR kinase signaling promotes cell growth. AKT accomplishes this through the inhibition of a complex consisting of TSC1 and TSC2, also known as hamartin and tuberin (reviewed in [14]). mTOR stimulates cell growth and proliferation through regulation of ribosomal biogenesis and mRNA translation. mTOR can act as a nutrient sensor to promote changes in cell size and cell cycling under optimal conditions (reviewed in [15, 16]). In brief, mTOR stimulates protein synthesis through the phosphorylation of p70S6 kinase (p70S6K) and eIF4E binding proteins 1, 2, and 3 (4E-BPs). Consequently, p70S6K phosphorylates ribosomal protein S6, resulting in increased translation of mRNA messages containing 5′ terminal oligopolypyrimidine (5′TOP) tracts. The phosphorylation of 4E-BPs leads to the release of the initiation factor eIF4E to promote cap-dependent translation of mRNA encoding proteins such as cyclin D1, MYC, and vascular endothelial growth factor (VEGF) [17, 18]. mTOR has been implicated in inducing expression of HIF1α and HIF2α, which in turn act as transcription factors to promote VEGF expression and angiogenesis under hypoxic conditions [19, 20]. mTOR is a component of two distinct protein complexes, mTORC1 and mTORC2, with the first complex being sensitive to rapamycin and related drugs [14]. siRNA targeting of components of each mTORC complex (raptor or rictor, respectively) have provided evidence that HIF1α

expression is dependent on both mTORC complexes, whereas HIF2α is dependent upon mTORC2 only [20]. Also, recent studies using p70S6K dominant negative or shRNA constructs have shown that p70S6K is the mTOR target responsible for the upregulation of HIF1α and VEGF expression [21, 22]. In addition to its role in promoting angiogenesis, HIF1α in combination with alterations in other transcription factors (i.e., c-myc upregulation and p53 loss), are responsible for transcribing genes responsible for aerobic glycolysis, a cancer hallmark also known as the Warburg effect [23].

AKT signaling is also involved in other known characteristics of tumors [24]. VEGF has been shown to have several biological effects on endothelial cells and autocrine survival effects on the tumor cells through the Flk1/VEGFR2-PI3K-AKT pathway [19, 25]. In addition, AKT can promote epithelial-mesenchymal transition (EMT) and induce cell metastasis and invasion through the secretion of matrix metalloproteinases [26, 27]. Furthermore, AKT can phosphorylate telomerase reverse transcriptase to stimulate telomerase activity and replication [28]. Collectively, the upregulation of the AKT signaling pathway plays an important role in many aspects of tumor development.

MECHANISMS OF AKT ACTIVATION IN CANCER

Amplification, overexpression, and mutations of AKT genes

AKT2 was the first AKT family member identified to have a recurrent genomic alteration [29]. *AKT2* was shown to be amplified and overexpressed in two of 15 primary ovarian tumors and two of eight ovarian carcinoma cell lines. A multicenter study confirmed the findings in a larger set of tumor specimens, wherein 16 of 132 (12%) of ovarian carcinomas and 3 of 106 (3%) breast carcinomas exhibited *AKT2* amplification [30]. Importantly, *AKT2* amplification was more frequent in undifferentiated ovarian tumors and correlated with poor patient prognosis. SNP-based copy number microarray studies followed by dual-color FISH analysis of high versus low grade ovarian serous carcinomas, upheld the importance of the role of AKT2 in advanced grade tumors [31]. Microarray data revealed that 27% and 9% of high grade tumors harbored *AKT2* or *PIK3CA* amplification, respectively, in contrast to few genomic alterations observed in low grade tumors. FISH analysis of a larger, different set of tumors using genomic probes encompassing the *AKT2* gene identified ~14% of high grade tumors with *AKT2* amplification [31]. Studies using RNA interference against *AKT2* in ovarian cancer cell lines provided evidence that AKT2 is involved in proliferation and chemotherapeutic drug resistance [32–34] while overexpression studies revealed the gene's role in invasion and metastasis [35].

Amplification and overexpression of *AKT2* has been reported in 10–20% of pancreatic tumors and cell lines [36–38]. In 1996, we reported that two pancreatic cancer cell lines, PANC1 and ASPC1, exhibited amplification of the *AKT2* gene in addition to increased mRNA and protein expression [36]. Most importantly, *AKT2* antisense transfection performed on these two cell lines decreased tumorigenicity in nude mice and reduced invasion in a rat tracheal xenotransplantation assay, the first evidence implicating AKT as a critical target for therapeutic intervention. In contrast, *AKT2* antisense had no effect in tumorigenicity compared to control constructs when transfected into COLO 357, a pancreatic cancer cell line without *AKT2* amplification or overexpression [36]. We also examined AKT2 activity in primary pancreatic carcinomas versus benign pancreatic tumors and normal pancreas [39]. An *in vitro* kinase assay revealed that 12 of 37 pancreatic tumors had greater than three fold increased AKT2 activity compared to normal pancreas [39]. Collectively, these data provided evidence for a role of AKT2 in tumor development in a subset of pancreatic carcinomas.

Unlike *AKT2*, amplification of *AKT1* is rarely reported in cancers. However, there are a growing number of publications implicating the importance of AKT3 upregulation in various cancers. CGH analysis revealed increased copy number of chromosomal region 1q44 (where *AKT3* is located) in 6 of 19 of hepatitis C virus-associated hepatocellular carcinomas [40] and in 2–4% of glioblastomas [41, 42]. Moreover, 40 to 60% of primary melanomas have been shown to have increased total or phosphorylated AKT3 protein compared to normal melanocytes [43]. Furthermore, siRNA-mediated knockdown of *AKT3* was shown to lower activated phospho-AKT levels in melanoma cell lines, which was not observable when *AKT1* or *AKT2* were downregulated by siRNA. Compared to a siRNA control, targeting of *AKT3* in the melanoma cell lines resulted in increased apoptosis in cell culture and in xenograft mouse studies [43]. In addition, approximately 20% of ovarian tumors of serous, endometrioid, and other subtypes exhibited increased AKT3 protein expression [44]. AKT3 appears to be involved in cell cycle progression in ovarian cancer cells, because knockdown of *AKT3* in cell lines decreased cell proliferation through G2/M cell cycle arrest [44].

Recently, Carpten et al. (2007) identified a recurrent activating mutation in *AKT1* in human breast, colorectal, and ovarian cancers [45]. The somatic mutation results in a lysine substitution for glutamic acid at residue 17 (E17K) of the pleckstrin homology (PH) domain. This mutation was observed in 8% of breast, 6% of colorectal, and 2% of ovarian cancers. Moreover, the E17K AKT1 mutant protein has increased cell membrane localization/activation under serum free and 10%-serum conditions and was capable of transforming Rat1 fibroblasts in soft agar assays and induced leukemia in mice [45]. Another study uncovered the E17K *AKT1* mutation in 6% of breast, 1% of colorectal, and less than 1% of lung cancers [46]. In addition, analysis of 137 melanoma clinical specimens identified one sample with the E17K *AKT1* mutation and another sample with the E17K *AKT3* mutation [47]. Among 65 melanoma cell lines analyzed, two had an E17K *AKT3* mutation, whereas no E17K mutations were identified in *AKT1* or *AKT2* [47]. Subsequent studies by others have identified the E17K *AKT1* mutation in a similarly small percentage of endometrial [48, 49], bladder [50], and prostate cancers [51]. Interestingly, Cohen et al found one particular endometrial tumor to have both the E17K *AKT1* mutation and an inactivating mutation in *PTEN* [49], suggesting that full activation of the PI3K pathway requires multiple mutations of genes in the same signaling pathway. It is noteworthy that *de novo* E17K mutations of *AKT1*, *AKT2*, and *AKT3* have also been reported in individuals with Proteus syndrome, hypoglycemia and hemimegalencephaly, respectively (see below).

Tyrosine kinase receptor mutation and/or overexpression

One of the common mechanisms for the activation of AKT in cancers is through the overexpression and/or mutation of upstream tyrosine kinase receptors. The identification of autocrine and paracrine activation of tyrosine kinase receptors in cancers has been extensively documented [52]. *EGFR*, *HER2/NEU* (*ERBB2*), *FGFR*, and *MET* (*HGFR*) have been found to be amplified in a number of different cancers, including breast, lung, ovarian, and colorectal carcinomas [52, 53]. A multi-institutional study of glioblastomas revealed amplification and mutation of *EGFR* in 45% of tumors, amplification of *PDGFRA* in 13%, mutations of *ERBB2* in 8%, and amplification of *MET* in 4% [42]. The frequent involvement of tyrosine kinase receptors in many different kinds of cancers has led to the development of a number of small molecule inhibitors and receptor-binding antibodies, which inhibit the downstream signaling pathways (reviewed in [52, 53]).

PIK3CA amplification and activating mutations

Downstream of tyrosine kinase receptors is PI3K, a kinase that is composed of regulatory and catalytic subunits. The *PIK3CA* gene, which encodes the p110α catalytic subunit of

PI3K, has been implicated as an oncogene in a number of carcinomas. Amplification of *PIK3CA*, increased expression of PIK3CA protein and increased PI3K activity has been reported in ovarian carcinomas [54]. *PIK3CA* amplification has also been reported in a number of other cancers such as head and neck squamous cell carcinomas [55], primary gastric carcinomas [56], and in endometrial carcinomas [57]. Especially noteworthy, *PIK3CA* amplification in endometrial and gastric cancers has been shown to correlate with poor prognosis [57, 58].

Activating mutations in *PIK3CA* were discovered as another mechanism by which PI3K can be constitutively activated. Mutations in certain 'hot spots', such as the commonly found E542K, E545K and H1047R, result in mutant proteins that can transform cells with high efficiency [59]. Sequencing of the *PIK3CA* gene in primary clear cell ovarian carcinoma specimens and cell lines revealed a 33% mutation rate [60]. Immunohistochemical analysis of clear cell ovarian cancer samples with a *PIK3CA* mutation revealed intense phospho-AKT staining. Of particular clinical interest, a screening of 54 breast cancer cell lines revealed that those harboring *PIK3CA* mutations are more sensitive to the PI3K inhibitor, GDC-0941 [61].

Mutation or deletions of the p85-alpha regulatory subunit gene, PIK3R1

There is increasing evidence that the *PIK3R1* gene, which encodes the p85α regulatory subunit of PI3K, is a tumor suppressor. Somatic mutations of this gene were identified in colon, colorectal, and ovarian cancer specimens and cell lines [62]. The Cancer Genome Atlas Research Network's comprehensive study of 91 glioblastomas revealed 9 tumors (10%) with somatic mutations in *PIK3R1* [42]. The group speculated that such mutations would affect the regulatory subunit's ability to inhibit PI3K activity based on known structural data of the encoded regulatory enzyme. An analysis of online microarray data indicated decreased *PIK3R1* mRNA expression in prostate, lung, bladder, ovarian, breast, and hepatocellular carcinomas [63]. An elegantly designed study showed that liver-specific *PIK3R1* deletion in mice results in liver carcinomas with metastasis to the lungs [63].

PTEN deletion and mutations

Working in opposition to PI3K is the PTEN tumor suppressor. As a lipid phosphatase, PTEN dephosphorylates phosphatidylinositol (3,4,5) triphosphate (PIP3) and phosphatidylinositol (3,4) diphosphate to inhibit AKT activation. PTEN expression can be lost through somatic mutations, deletions, promoter hypermethylation, and defects in protein stability [64]. Cancers commonly exhibiting loss of PTEN include endometrial and prostate carcinomas, high-grade glioblastomas, and melanomas (reviewed in [64]). Studies with knockout mice indicate that *Pten* haploinsufficiency contributes to tumorigenesis either alone or by cooperating with other genetic alterations. For example, prostatic intraepithelial neoplasia develops in heterozygous *Pten* knockout mice, although tumor incidence increases in mice with heterozygous knockout of both *Pten* and *Tp53*, even when the corresponding wild-type alleles are retained [65].

HSP90 overexpression

Heat shock protein 90 (HSP90) is a molecular chaperone involved in the proper folding of client proteins. HSP90 stabilizes various tyrosine kinases, including EGFR and BCR/ABL, as well as signaling molecules such as PI3K and AKT. Inhibition of HSP90 with 17-allylaminogeldanamycin (17-AAG) results in AKT protein ubiquitination and decreased half-life [66]. Furthermore, overexpression of HSP90 in melanomas, breast carcinomas, and gastrointestinal stromal tumors correlated with disease progression and/or poor prognosis (reviewed in [67]). Due to HSP90's role in regulating the stability of a number of oncoproteins, including ones in the AKT signaling pathway, HSP90 has presented as a good

molecular target as evidenced by the number of HSP90 inhibitors presently in clinical trials [67].

Loss and decreased expression of PHLPP

PHLPP (PH domain leucine-rich repeats protein phosphatase), consisting of two isoforms, PHLPP1 and PHLPP2, are serine/threonine phosphatases responsible for the dephosphorylation of AKT on ser473. Immunohistochemistry analysis of colorectal cancer specimens demonstrated loss or decreased expression of the two PHLPP isoforms in 78 to 86% of the samples analyzed [68]. Re-expression of either of the *PHLPP* genes in colon cancer cell lines resulted in decreased proliferation *in vitro* and reduced tumorigenicity in xenograft mouse studies [68]. Real-time mRNA quantitation of chronic lymphocytic leukemia (CLL) samples revealed loss of *PHLPP1* mRNA expression in ~50% of CLL characterized by deletion of chromosomal region 13q14; downregulation of *PHLPP1* expression was not due to copy number loss of *PHLPP1*, which is located at 18q21.33 [69]. DNA copy number analysis of primary and metastatic prostate cancer samples revealed that concurrent losses of *PHLPP1* and *PTEN* losses occurred only in metastatic cancers [70]. Similarly, Western blot analysis of gliomas indicated that increased phospho-AKT levels correlated with concurrent losses of PHLPP1 and PTEN expression [71]. In addition, high grade gliomas and glioblastomas had significantly less expression of PHLPP1, PTEN and the NHERF1 adaptor protein when compared to that observed in low grade gliomas [71].

MicroRNA dysregulation

MicroRNAs (miRNA) are small (~19–25 nucleotides) non-coding RNA molecules involved in post-transcriptional gene regulation. miRNAs function by binding to complementary sequences found on target mRNAs, leading to translational repression or mRNA degradation [72]. Certain miRNAs can act as oncogenes by targeting mRNAs encoding tumor suppressors. For instance, overexpression of MiR-21 results in increased targeting of *PTEN* mRNA (among others) and consequent hyperactivation of the AKT signaling pathway [72–74]. Other miRNAs have been classified as tumor suppressors because they can target oncogene encoding mRNAs. For example, MiR-145 and MiR-128 can target p70S6K, leading to the downregulation of HIF1α and VEGF [75, 76]. These and other studies (reviewed in [72]) have uncovered numerous dysregulated miRNAs important in tumorigenesis, and intriguingly, circulating levels of certain miRNAs can also act as diagnostic and prognostic markers in various cancers [77].

HEREDITARY CANCER SYNDROMES INVOLVING ACTIVATION OF THE AKT/mTOR PATHWAY

The phakomatoses are dominantly inherited disorders characterized by distinctive lesions and tumors of the skin and/or defects in the nervous system/eyes. This group includes tuberous sclerosis (TSC) 1 and 2, neurofibromatosis 1 and 2, Cowden Syndrome (CS), Peutz-Jeghers Syndrome (PJS), von-Hippel-Lindau (VHL) disorder, familial adenomatous polyposis, and juvenile polyposis [6]. Each of the genes involved in these disorders encode a tumor suppressor important in mammalian development. The mutations of the genes do not transform cells directly but instead lead to a proliferation stimulus in affected cells. As a result, these disorders are characterized by scattered hamartomatous or adenomatous lesions, which sometimes become malignant. It is now known that at least five of the phakomatoses are caused by mutations of genes encoding components of the AKT/mTOR pathway.

Germline *PTEN* mutations have been identified in individuals with CS, which is characterized by multiple benign hamartomas. Those affected have an increased risk for developing benign and malignant tumors of the breast, thyroid, and endometrium [7].

Approximately 80% of CS individuals were identified to have point mutations or deletions in the *PTEN* coding sequence [78]. Some individuals with CS have heterozygous mutations of the *PTEN* promoter region, which result in decreased PTEN protein expression [79]. Bannayan-Riley-Ruvalcaba Syndrome (BRRS) is another disorder in which approximately 60% of individuals harbor *PTEN* germline mutations [78]. Besides the congenital deformities associated with BRRS, those affected have benign hemangiomas and intestinal polyposis [7].

Germline *TSC1* and *TSC2* mutations give rise to TSC, a syndrome characterized by benign hamartomas and/or occasional malignancies of the central nervous system, kidney, heart, lung, and skin (reviewed in [80]). Somatic mutations of the *TSC1* gene have also been discovered in sporadic bladder cancers [81]. Tumor cells from TSC patients show biallelic activation of *TSC1* or *TSC2*, leading to AKT-independent activation of the mTOR signaling pathway. In clinical trials, use of a mTOR inhibitor, rapamycin (sirolimus), was shown to be effective in reducing the size of TSC-related tumors [82].

Hyperactivation of the mTOR signaling pathway is also a characteristic of PJS, which is characterized by multiple gastrointestinal hamartomatous polyps and increased risk for the development of other types of malignancies. Germline mutations of the *LKB1* tumor suppressor gene have been identified in approximately 70% of PJS patients in addition to frequent somatic mutations being discovered in sporadic lung cancers [83]. LKB1 is a serine/threonine kinase that phosphorylates AMPK (AMP-activated protein kinase), which in turn phosphorylates and activates TSC2 to negatively regulate the mTOR signaling pathway. An *Lkb1*+/− knockout mouse model has been developed which recapitulates PJS of humans. Rapamycin treatment of *Lkb1*+/− mice before or after polyp development significantly reduced the size and number of intestinal polyps [84, 85], thus providing preclinical evidence suggesting the possible efficacy of mTOR inhibitors for the treatment of patients with PJS. In another study, Shackelford et al. discovered increased levels of HIF1α and its targets (Glut1, Hexokinase II) under normoxic conditions in normal fibroblasts and tumor cells from *Lkb1*+/− mice when compared to cells from wild-type mice [86]. These HIF1α targets are responsible for aerobic glycolysis (Warburg effect) in the tumors, and the expression of these proteins was reduced with rapamycin treatment [86].

Neurofibromatosis type I is the most common of the phakomatoses (1 in 3000 live births) and is characterized by benign neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs), hamartomatous lesions of the iris, gliomas, pheochromocytomas, and myeloid malignancies [5]. Neurofibromatosis type I develops as a result of germline mutations in the tumor suppressor gene, *NF1*. The protein product of the gene, neurofibromin, is a Ras-GAP that inactivates the Ras protein. Thus, inactivation of *NF1* results in hyperactive Ras signaling and, as a result, activation of downstream PI3K/AKT/mTOR, MAP kinase and p21-activated kinase signaling [5]. Studies using a mouse model with heterozygous knockout of both *Nf1* and *Tp53*, which develops MPNST, has provided support for mTOR's essential role in *NF1*-related tumorigenesis. Rapamycin treatment of these mice resulted in decreased tumor growth with concomitant decreases in both phosphorylated ribosomal protein S6 and cyclin D1 protein levels [87].

Germline mutations of the *VHL* tumor suppressor gene results in the VHL syndrome. Affected individuals are predisposed to developing hemangioblastomas of the central nervous system and retina, clear cell renal cell carcinoma, pheochromocytoma, adenocarcinomas of the temporal bone, cystadenomas, and pancreatic tumors [88]. Somatic *VHL* mutations have also been identified in sporadic hemangioblastomas and clear-cell renal carcinomas. The VHL protein is involved in the ubiquitination and degradation of the HIFα transcription factor, which is downstream of the mTOR pathway. Loss of the *VHL*

gene results in HIFα stabilization and the upregulation of HIFα target genes (i.e., *VEGF*, *PDGF*, *TGF-α*), which play a role in angiogenesis, cell proliferation, and cell survival [89]. VEGF antibodies (bevacizumab) and certain small molecule inhibitors (sorafenib, sunitinib, and axitinib) have been shown to have efficacy in the treatment of *VHL* associated clear cell renal carcinomas [89, 90]. The importance of upstream mTOR signaling on the growth of *VHL*-null renal cancers has been demonstrated in clinical studies showing some effectiveness of the rapamycin analogs (rapalogs) temsirolimus and everolimus in inhibiting tumor growth (reviewed in [89, 90]). A preclinical study using WYE-125132, a mTOR inhibitor that is effective against both mTORC1 and mTORC2, shows even more efficacy compared to rapalogs that only inhibit mTORC1 [91]. The *in vivo* growth of two *VHL*-null renal cancer cell lines, A498 and 786-O, in xenograft mice was suppressed with WYE-125132 treatment. The inhibition of tumor growth was even more profound when the drug was combined with bevacizumab [91].

FROM MICE TO MEN; ANIMAL STUDIES WHICH FORESAW THE CAUSES FOR HUMAN GENETIC DISORDERS

The development of knockout mice models has led to tremendous advances in our understanding of gene functions in many human diseases. The phenotypes of Akt1, Akt2 and Akt3 knockout mice are dissimilar due to the each protein's specific expression and function in various tissues. Homozygous knockout of *Akt1* results in partial embryonic lethality, and surviving mice are approximately 20% smaller in size than wild-type littermates [92]. In contrast, insulin resistance and a diabetes type II-like phenotype is observed in Akt2 knockout mice [93], supporting earlier work first implicating this protein's role in glucose metabolism [94]. Thus, Altomare and colleagues reported that *Akt2* mRNA is highly expressed in mouse embryonic fat, skeletal muscle and liver, tissues that are highly responsive to insulin [94]. On the other hand, Akt3 was implicated in brain development, because knockout of this gene in mice resulted in reduction in overall brain size [95]. Quite remarkably, recent reports indicate that germline or mosaic activating mutations of these three AKT isoforms in humans are the cause of diseases with clinical features opposite of that observed when these genes were knocked out in mice, as discussed below.

Strikingly, mosaic activating mutations in *AKT1* have been identified as one cause of Proteus syndrome [96]. Individuals with this genetic disorder have asymmetric overgrowth of body parts (such as limbs, skull, and vertebrae), cerebriform connective tissue nevi of the skin, epidermal nevi, and dysregulated adipose tissues [97]. These patients also have an increased incidence of certain tumors such as ovarian cystadenomas, lipomas, and meningiomas [97]. Somatic mosaicism, in which a mutation occurs during early embryonic development, was hypothesized as the mechanism for the disease to explain the lack of germline, familial cases presumably due to embryonic lethality [97]. Studies by Lindhurst and colleagues provided elegant evidence that somatic mosaic *AKT1* mutations are responsible for a subset of Proteus syndrome patients [96]. Their study identified *AKT1* mutations (c.49G→A; with the predicted E17K protein substitution) initially through exome sequencing of eleven samples collected from affected and unaffected tissues from six Proteus syndrome patients. Subsequent Sanger sequencing and a PCR/restriction enzyme analysis of multiple tissue samples from these patients confirmed the mosaic nature of the mutation. As expected, Western blot analysis of affected samples or cell lines derived from single cell clones of affected tissues showed increased phospho-AKT levels [96]. Importantly, clinical trials are underway to test the effectiveness of targeted therapies in individuals with Proteus syndrome cases with *AKT1* mutations [98]. Germane to this, rapamycin was reported to be effective in controlling hamartoma growth in a patient diagnosed with Proteus syndrome and harboring a *de novo* germline *PTEN* mutation [98, 99]. However, there as been controversy about whether such individuals with germline

PTEN mutations are bona-fide cases of Proteus syndrome [100]. It has been argued that reports of such cases with hamartomatous tumors do not strictly fit the diagnosis of Proteus syndrome and are instead misdiagnosed by physicians inexperienced with this rare disease [100].

Increased insulin secretion due to pancreatic tumors or hyperactive pancreatic β cells results in hypoglycemia, increased weight gain and accelerated early growth. Incidentally, de novo germline or mosaic activating mutations in *AKT2* (leading to an E17K residue substitution) was observed in three individuals with hypoglycemia [101]. As noted earlier, Akt2 mRNA is highly expressed in embryonic brown fat and other insulin-responsive tissues, and the AKT2 kinase is activated by insulin [94]. Thus, it is not surprising that hyperactivating mutations of AKT2 results in overstimulation of the downstream PI3K pathway and consequent severely decreased levels of glucose in the patients [101]. The *AKT2* mutation was heterozygous in two of the individuals and mosaic in the third when DNA from lymphocytes and other cells were examined; in each case, the mutation appeared to be a *de novo* event, since none of the parents of the patients harbored the mutation. Besides hypoglycemia, these individuals also exhibited varying degrees of asymmetric, hemihypertrophy (asymmetrical somatic growth) of the face, arm, trunk, and/or legs, thus suggesting that AKT2 may also have some growth promoting function [101].

More recently, Poduri et al. found an activating mutation in *AKT3* in brain tissues, but not blood cells, in a patient with hemimegalencephaly (HMG) [102], a developmental brain disorder characterized by an enlarged cerebral hemisphere that is frequently associated with seizures. Remarkably, the observed somatic, mosaic mutation of AKT3 resulted in an E17K substitution precisely paralogous to the E17K mutations in AKT1 and AKT2 previously discovered in other somatic overgrowth syndromes [96]. Brain tissues from two of seven other HMG patients had trisomy of the long arm of chromosome 1, which encompasses many genes, including *AKT3*. [102]. Interestingly, mice with an activating mutation of *Akt3* showed an enlarged hippocampus [103], whereas Akt3 knockout mice have reduced brain size [95].

CONCLUDING REMARKS

The body of literature supporting the AKT signaling pathway's role in many aspects of tumorigenesis has grown enormously since the discovery of the *AKT* family members. Many of the cancer hallmarks can be attributed to the aberrant activation of this signaling pathway, and novel ways of activating this pathway oncogenically continue to be discovered. With the frequent activation of the AKT pathway in cancer and its role in tumorigenesis, molecularly targeting components of the pathway has seen progress in the clinic. However, due to the important role of the AKT2 protein in glucose homeostasis, hyperglycemia side effects (mimicking diabetes mellitus) was observed in some patients treated with certain inhibitors of the AKT signaling pathway. The discovery of new mechanistic insights involving the AKT pathway will help pave the way for other novel therapeutic strategies with fewer side effects to the patient.

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ABBREVIATIONS

4E-BP1,2 and 3	eIF4E binding proteins 1, 2, and 3
5′ TOP	5′ terminal oligopolypyrimidine
AKT/PKB kinases	Protein Kinase B
AMPK	AMP-activated protein kinase
BAD	Bcl2-associated death promoter
BRRS	Bannayan-Riley-Ruvalcaba Syndrome
CDK	cyclin dependent kinase
CGH	comparative genomic hybridization
CLL	chronic lymphocytic leukemia
CS	Cowden Syndrome
EGFR	epidermal growth factor receptor
eIF4E	eukaryotic translation initiation factor 4E
EMT	epithelial-mesenchymal transition
FGFR	fibroblast growth factor receptor
Flk1 (VEGFR2)	fetal liver kinase 1 (vascular endothelial growth factor receptor-2)
FOXO	forkhead box O
HER2/NEU (ERBB2)	human epidermal growth factor receptor 2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 protein)
HIF1a	hypoxia-inducible factor 1, alpha subunit
HIF2a	hypoxia-inducible factor 2, alpha subunit
HMG	hemimegalencephaly
HSP90	heat shock protein 90
IKK	IkB kinase
LKB1 (STK11)	liver kinase B1 (serine/threonine kinase 11)
MPNST	Malignant peripheral nerve sheath tumor
Mdm2	mouse double minute 2 homolog
MET (HGFR)	hepatocyte growth factor receptor
miRNA	microRNA
mTOR	mammalian target of rapamycin
mTORC1 and mTORC2	mTOR complex1 and mTOR complex 2
NF1, NF2	neurofibromatosis 1 and 2
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
P70S6K	p70S6 kinase
PDGF	platelet derived growth factor

PDGFRA	platelet derived growth factor receptor, alpha polypeptide
PHF20/TZP	PHD finger protein 20 / Tudor and zinc finger domain containing protein
PHLPP	PH domain leucine-rich repeats protein phosphatase
PI3K	phosphatidylinositide 3-kinase
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
PIP3	phosphatidylinositol (3,4,5) triphosphate
PJS	Peutz-Jeghers Syndrome
PTEN	phosphatase and tensin homolog deleted on chromosome 10
shRNA	short hairpin RNA
TGF-a	transforming growth factor alpha
TNFSF6	TNF receptor superfamily, member 6
TSC1/2	tuberous sclerosis complex 1/2
VHL	Von Hippel-Lindau

CITATIONS

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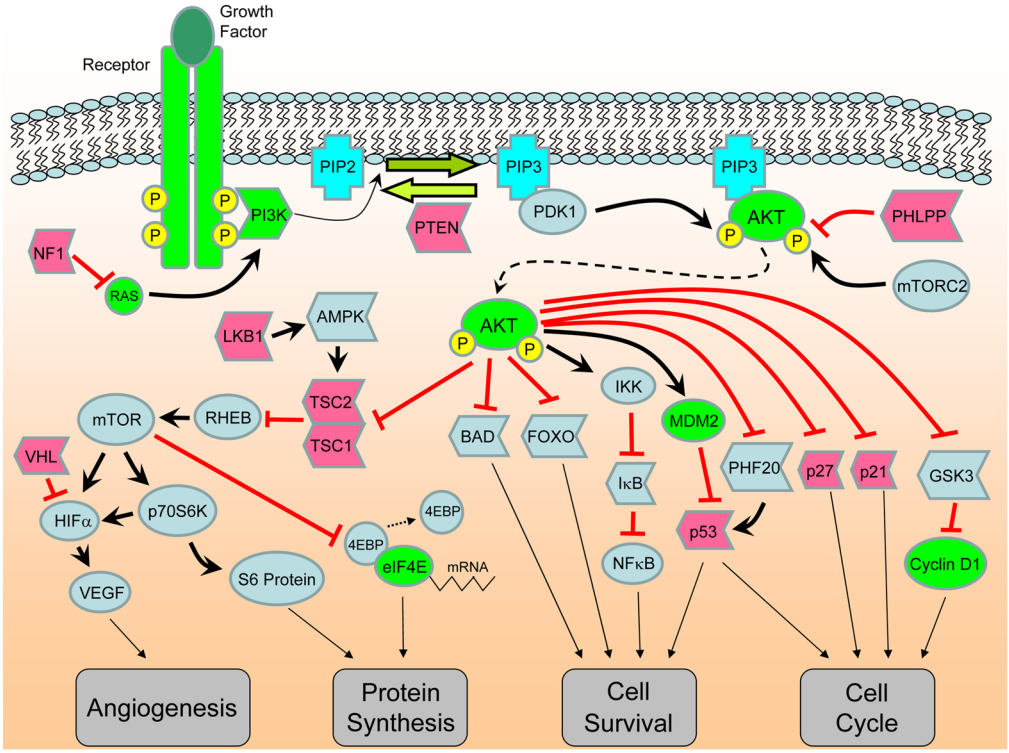

Figure 1.

Alterations of the AKT pathway in human cancer. Activation of tyrosine kinase receptors through growth factor stimulation or constitutive activation of the receptor through mutation/amplification leads to activation of the AKT signaling pathway. Other mechanisms observed in cancer resulting in AKT signaling include activation of proteins encoded by oncogenes (shown in green) and/or inactivation of tumor suppressors (shown in red). Germline mutations of genes encoding some of these tumor suppressors have been found to be responsible for various hereditary cancer syndromes. AKT-mediated phosphorylation of different downstream proteins converges on signaling pathways important in tumorigenesis, such as cell survival and cell cycle progression (convergence of pathways indicated with a thin, black arrow at the lower part of the figure).

Table 1

Perturbations of components of the AKT signaling pathway in human cancer

AKT Signaling Components	Aberrations Observed in Cancer
Receptor tyrosine kinase	Mutation, overexpression
Phosphatidylinositol 3-kinase (PI3K)	*PIK3CA* (p110α) amplification/overexpression and mutation *PIK3R1* (p85α) mutation, decreased expression
AKT1/2/3	Amplification/overexpression, mutation
PTEN	Germline mutation and somatic deletion, mutation, or promoter methylation
PHLPP1 and PHLPP2	Loss of expression
HSP90	Increased expression
TSC1 and TSC2	Germline and somatic (*TSC1*) mutation
LKB1	Germline and somatic mutation
NF1	Germline mutation
VHL	Germline and somatic mutation