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Akt: A NEW APPROACH FOR CANCER TREATMENT

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ABSTRACT

Direct genetic alterations leading to deregulated PI3K/Akt signaling are common in a significant fraction of human malignancies. Both Akt and Aurora A kinase have been shown to be important targets for intervention for cancer therapy. Nuclear Akt1 expression and Akt activation are common in cancer invasion. However, the mechanisms for this association and its causal role in invasion are uncertain. It is a key downstream effector of phosphoinositide 3'-kinase (PI3K) and directly modulates a wide range of pro-apoptotic and metabolism regulating proteins. Inhibition of Akt is a significant therapeutic goal due to the prevalence of activating mutations in the PI3K/Akt pathway.

INTRODUCTION: AKT protein family, which members are also called protein kinases B (PKB) plays an important role in mammalian cellular signaling. The serine/threonine kinase Akt, or protein kinase B, has recently been a focus of interest because of its activity to inhibit apoptosis. It mediates cell survival by acting as a transducer of signals from growth factor receptors that activate phosphatidylinositol 3-kinase. Akt1 is involved in cellular survival pathways, by inhibiting apoptotic processes. Akt1 is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth.

Since it can block apoptosis, and thereby promote cell survival, Akt1 has been implicated as a major factor in many types of cancer. Akt (now also called Akt1) was originally identified as the oncogene in the transforming retrovirus, AKT8 [1]. AKT8 was isolated from an AKR mouse spontaneous thymoma cell line by cocultivation with an indicator mink cell line. The transforming cellular sequences, v-akt, were cloned from a transformed mink cell clone and these sequences were used to identify Akt1 and Akt2 in a human clone library. AKT8 was isolated by Stephen Staal in the laboratory of Wallace P. Rowe; he subsequently cloned v-akt and human AKT1 and AKT2 while on staff at the Johns Hopkins Oncology Center [2].

Akt2 is an important signaling molecule in the Insulin signaling pathway. It is required to induce glucose transport. These separate roles for Akt1 and Akt2 were demonstrated by studying mice in which either the Akt1 or the Akt2 gene was deleted, or "knocked out". In a mouse which is null for Akt1 but normal for Akt2, glucose homeostasis is unperturbed, but the animals are smaller, consistent with a role for Akt1 in growth. In contrast, mice which do not have Akt2, but have normal Akt1, have mild growth deficiency and display a diabetic phenotype (insulin resistance), again consistent with the idea that Akt2 is more specific for the insulin receptor signaling pathway [3]. The role of Akt3 is less clear, though it appears to be predominantly expressed in brain. It has been reported that mice lacking Akt3 have small brains [4]. The name Akt does not refer to its function. Presumably, the "Ak" in Akt was a temporary classification name for a mouse strain developing spontaneous thymic lymphomas. The "t" stands for ‘transforming’; the letter was added when a transforming retrovirus was isolated from the Ak strain, which was termed "Akt-8". When the oncogene encoded in this virus was discovered, it was termed v-Akt. Thus, the later identified human analogues were named accordingly.

Signal Transduction Mediated by the PI3K/Akt Pathway: The lipid kinase PI3K (Phosphoinositide 3- kinase) is involved in the regulation of a number of cellular processes, like transcription, migration, angiogenesis, cell growth, proliferation, apoptosis and glucose metabolism. PI3K is activated by several hormones including insulin, growth factors such as EGF, IGF(Insulin like growth factor), PDGF, NGF, HGF, by signals derived from receptors for extracellular matrix molecules such as integrins, by several forms of cellular stress such as oxidative stress or cell swelling, PI3K phosphorylates phosphatidylinositols of the cell membrane, thus generating, e.g., phosphatidylinositol- 3, 4, 5- trisphosphate (PIP3) from phosphatidylinositol-4,5-bisphosphate (PIP2).

PIP3 at the cell membrane recruits protein kinases such as PKB/Akt and PDK1 which bind with their PH-(pleckstrin homology)-domain to PIP3. Full activation of PKB/Akt requires the phosphorylation by the Ser473-Kinase (PDK2:

integrin-linked kinase-1: (ILK-1) or DNA-dependent protein kinase (DNA-PK)) and by PDK1 at Thr-308. PDK1 further activates SGK and and aPKC. PKB/Akt, aPKC and SGK in turn phosphorylate a wide variety of cellular signaling molecules relevant for the regulation of cell growth, cell cycle and cell proliferation, including FKHR, GSK3ß, mTOR and p70S6K, for apoptosis, including Bad, caspase 9, IκB, FKHR, Mdm2, or for transport, including several transporters and channels (**Fig. 1**). PKB/Akt further activates mTOR, a kinase stimulating the uptake of nutrients such as glucose, amino acids, cholesterol and iron. mTOR regulates the phosphorylation of p70S6K which can similarly be activated by PDK1. mTOR further activates eIF4E-binding protein-1 and thus is involved in the regulation of translation.

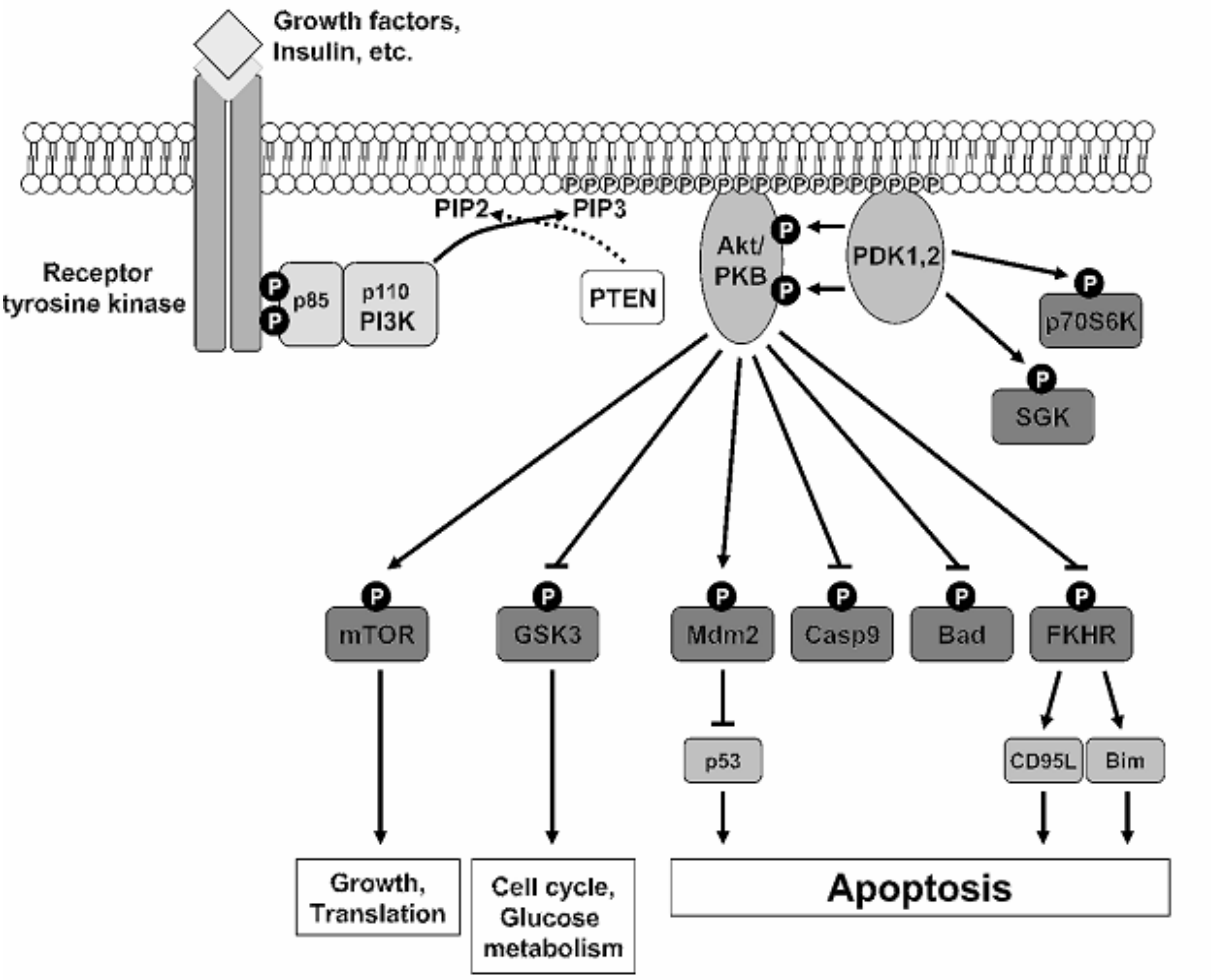


FIG. 1: REGULATION OF THE PI3/AKT SIGNALING PATHWAYACTIVATION OF PI3K BY RECEPTOR TYROSINE KINASES MEDIATES THE PHOSPHORYLATION OF PIP2 TO PIP3 AND THEREBY THE RECRUITMENT OF PKB/AKT AND PDK1 TO THE PLASMA MEMBRANE. PKB/AKT IS SUBSEQUENTLY ACTIVATED BY THE SER473-KINASE (PDK2) AND THE PHOSPHORYLATION AT THR308 BY PDK1. PTEN FUNCTIONS AS AN ANTAGONIST OF PI3K. PKB/AKT, BY PHOSPHORYLATION OF A NUMBER OF INTRACELLULAR PROTEINS, REGULATES DIFFERENT CELLULAR PROCESSES LIKE CELL GROWTH, CELL CYCLE, APOPTOSIS AND GLUCOSE METABOLISM

Activation: Akt is activated as a result of inactivation of tumor suppressor PTEN (Phosphatase and tensing homolog), a lipid phosphatase mutated or deleted in >50% of human cancers .Both Akt1 and Akt2 are commonly overexpressed or constitutively active in a large number of human cancers including brain, gastric, colon, breast, lung, and prostate carcinomas and their activation correlates to cancer progression. Activation of Akt is a multi-step process involving both membrane binding and phosphorylation. Upon PI3K activation and production of PtdIns-3,4,5-P3 and PtdIns-3,4-P2, Akt is recruited to the plasma membrane where it binds to the these phosphoinositides through its PH domain [5]. Activation is then thought to involve a conformational change and phosphorylation on two residues. One such phosphorylation site lies within the kinase domain activation loop (Thr 308 in Akt1) and is phosphorylated by another PH-domain containing protein, PDK1. In addition, a second phosphorylation site in the C-terminus (Ser 473 in Akt1) is required for full or maximal activity. Growth factor stimulation of PI3K activity leads to Akt activation. Conversely, PI3K inhibition (i.e. using chemical inhibitors such as wortmannin or LY294002) and PTEN mediated dephosphorylation of PtdIns-3, 4, 5-P3 and PtdIns-3,4-P2 results in inhibition of Akt. After activation, Akt can phosphorylate a number of substrates both in the cytoplasm and in the nucleus.

Regulation:

1. **Binding Phospholipids**: Akt possesses a protein domain known as a PH domain, or Pleckstrin Homology domain, named after Pleckstrin, the protein in which it was first discovered. This domain binds to phosphoinositides with high affinity. In the case of the PH domain of Akt, it binds either PIP_3 (phosphatidylinositol (3, 4, 5) trisphosphate, PtdIns(3,4,5)P_3) or PIP_2 (phosphatidylinositol (3,4)-bisphosphate, PtdIns(3,4)P_2) .This is useful for control of cellular signaling because the di-phosphorylated phosphoinositide PIP_2 is only phosphorylated by the family of enzymes, PI 3-kinases (phosphoinositide 3-kinase or PI3-K), and only upon receipt of chemical messengers which tell the cell to begin the growth process. For example, PI 3-kinases may be activated by a G protein coupled receptor or receptor tyrosine kinase such as the insulin receptor. Once activated, PI 3-kinase phosphorylates PIP_2 to form PIP_3.

2. **Phosphorylation:** Akt phosphorylates a variety of substrates involved in the regulation of key cellular functions including cell growth and survival, glucose metabolism and protein translation. These targets include GSK3, IRS-1 (insulin receptor susbtrate-1), PDE-3B (phosphodiesterase-3B), BAD, human caspase 9, Forkhead and NF-kB transcription factors, mTOR, eNOS, Raf protein kinase, BRCA1, and p21.

3. **Lipid Phosphatases and PIP3:**PI3K dependent Akt activation can be regulated through the tumor suppressor PTEN, which works essentially as the opposite of PI3K mentioned above .PTEN acts as a phosphatase to dephosphorylate PtdIns (3, 4, 5) P3 back to PtdIns (4, 5) P2. This removes the membrane-localization factor from the Akt signaling pathway. Without this localization, the rate of Akt activation decreases significantly, as do the all the downstream pathways that depend on Akt for activation. PIP3 can also be de-phosphorylated at the "5" position by the SHIP family of inositol phosphatases, SHIP1 and SHIP2. These

poly-phosphate inositil phosphatases dephosphorylate PtdIns (3, 4, 5) P3 to form PtdIns (3, 4) P2.

4. **Protein Phosphatases:** The phosphatases in the PHLPP family, PHLPP1 and PHLPP2 have been shown to directly de-phosphorylate, and therefore inactivate, distinct Akt isoforms. PHLPP2 dephosphorylates Akt1 and Akt3, whereas PHLPP1 is specific for Akt 2 and Akt3.

Function: Akt implication in different processes characteristic of cancer which summerised as follows:

- Growth signal autonomy process: Akt overexpression or activation may lead to increased response to ambient levels of growth factors.
- Insensitivity to antiproliferative signals: Akt induces nuclear entry of Mdm2, which leads to inhibition of p53 regulated processes.Induces cytoplasmic localization of $p21^{Cip/Waf1}$ and p^{27Kip}, promoting proliferation and stabilizes Cyclin D1.
- Inhibition of apoptosis: Akt inactivates the proapoptotic factors Bad and Procaspase-9. Activates IKK, activating the transcription of NF-jB regulated antiapoptotic genes.Inactivates Forkhead family transcription factors, inhibiting proapoptotic gene expression, such as Fas ligand.
- Unlimited replicative potential Angiogenesis Invasion and metastasis: Akt increases telomerase activity by phosphorylation of hTERT and Pomotes angiogenesis through eNOS activation and also contributes to invasiveness by inhibiting anoikis and stimulating MMP secretion

Akt inhibitors as Anticancer: These are the novel agents which inhibit proliferation, and reverse the repression of apoptosis and the resistance to cytotoxic therapy in cancer cells. Inhibitors of proteins that are involved in several PI3K/Akt signalling pathways have been under development for some time, and some have now entered clinical trials. These include inhibitors that target both upstream regulators of PI3K/Akt, such as growth factor receptors, and downstream effectors, such as the components of the mTOR pathway. Drug falling these category are summarized in the **Table 1.**

Table 1: AKt Inhibitor as Anticancer

DRUGS	CLASS	TARGET	INDUSTRY
RH3	Antibody	Upstream targets EGFR	York Medical Bioscience
EMD 72000	Antibody	Upstream targets EGFR	Merck KgaA Darmstadt
ABX-EGF	Antibody	Upstream targets EGFR	Abgenix
MDX-447	Antibody	Upstream targets EGFR	Merck KgaA
PKI116	Kinase inhibitor	Upstream targets EGFR	Novartis
CI-1033/PD183805	Kinase inhibitor	Upstream targets EGFR	Pfizer
EKB-569	Kinase inhibitor	Upstream targets EGFR	Wyeth–Ayerst
GW2016/572016	Kinase inhibitor	Upstream targets EGFR	GlaxoSmithKline
Trastuzumab (registered)	Antibody	HER-2/Neu	Herceptin-Genentech
UCN-01	Staurosporine analogue	PDK1	Kyowa Hakko Kogyo
Wortmannin	PI3K Inhibitor	PI3K	-
LY294002	PI3K Inhibitor	PI3K	-
Rapamycin	Inhibits mTOR kinase by binding to FKBP12	Dowstream targets mTOR	Wyeth

Akt in Ovarian Cancer: A study of 91 ovarian cancer specimens revealed elevated levels of p-Akt 2 (Ser 474) in 33 cases (36.3%). Most tumors displaying activated Akt 2 were high grade and in stages III and IV. p-Akt 2 in tumor specimens localized to the cell membrane and cytoplasm but not the nucleus. Five cases displayed elevated p-Akt 1 (Ser 473) but not p-Akt 2 [6]. In another investigation of human ovarian cancer, a tumor tissue microarray with a pan–p-Akt (Ser 473) antibody revealed elevated staining in 21 (68%) of 31 ovarian carcinomas. p-Akt staining was associated with activated phospho-mTOR in 27 (87%) of 31 ovarian tumors [7].

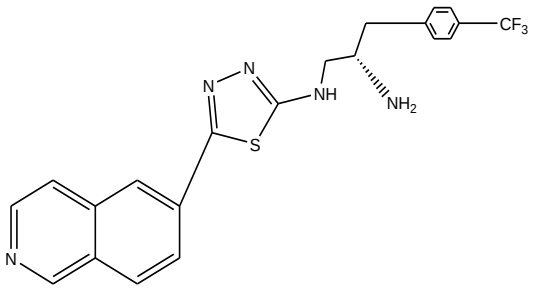
Akt in Prostatic and Renal Adenocarcinomas: Immuno- histochemical staining of p-Akt (Ser 473) in a study of 74 biopsy specimens of resected prostate cancer was significantly greater in cases with Gleason grades 8 to 10 (92% of such cases staining strongly) compared with prostatic intraepithelial neoplasia (PIN) and all other grades of prostate cancer. Only 10% of the latter cases stained strongly [8].

Akt in Gastrointestinal and Pancreaticobiliary Carcinomas: In a study of 65 patients with pancreatic ductal adenocarcinomas, a significant positive correlation between high p-Akt and Akt 2 expression and low 5-year survival rate was observed; suggesting p-Akt was a useful prognostic indicator [9].

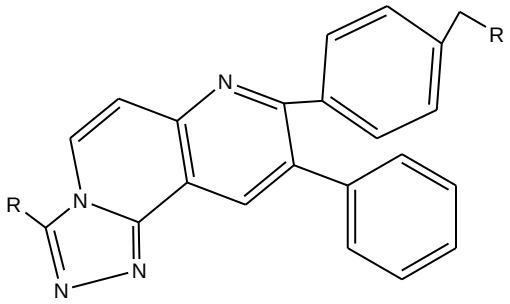
Recent Advances:

- Recently in 2010, Cadrin et al., investigated that investigated the role of Akt (1 and 2) in regulating IF expression in different epithelial cancer cell lines. Over-expression of Akt1 increases K8/18 proteins. Akt2 up-regulates K18 and vimentin expression by an increased mRNA stability [10].

- Recently Bourbeau et al., described the series of 2-aminothiadiazole of inhibitors of AKT1. Moderate selectivity observed in several compounds for AKT1 versus PKA is rationalized by X-ray crystallographic analysis. Key compounds showed activity in cellular assays measuring phosphorylation of two AKT substrates, PRAS40 and FKHRL1 [11].



- In 2009, Li et al., synthesis a series of [1, 2, 4] triazolo [3, 4- f] [1, 6] naphthyridine allosteric dual inhibitors of Akt1 and 2 have been developed. These compounds have been shown to have potent dual Akt1 and 2 cell potency [12].



- In the year 2008, Wu et al., reports a new synthetic route of pyridopyrimidines to facilitate their structural optimization in a library fashion and describes the development of pyridopyrimidines that have excellent enzymatic and cell potency against Akt1 and Akt2 [13].

- Again in 2009, Cho et al.,studied Akt1 silencing efficiencies in lung cancer cells by sh/si/ssiRNA transfection using a reductable polyspermine carrier [14].

- In 2008, Hartnett et al., shows inhibitor SAR on a pyridine series of allosteric Akt inhibitors to optimize enzymatic and cellular and also highlight the pharmacokinetic profile of an optimized inhibitor that has low clearance and long half life in dogs [15].

- In 2008 Bilodeau et al., synthesis series of naphthyridine and naphthyridinone allosteric dual inhibitors of Akt1 and 2 have been developed. These compounds have been optimized to have potent dual activity against the activated kinase as well as the activation of Akt in cells [16].

CONCLUSION: In conclusion, direct genetic alterations leading to deregulated PI3K/Akt signaling are common in a significant fraction of human malignancies. The phosphoinositide 3-kinase (PI3K)/AKT pathway has been shown to be central in the promotion of cell survival since the alteration of this signalling cascade is a frequent event in human malignancies. Cellular processes regulated by AKT include cell proliferation and survival, cell size and response to nutrient availability, intermediary metabolism, angiogenesis, and tissue invasion. All these processes represent hallmarks of cancer.

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