The Akt pathway in oncology therapy and beyond (Review)

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Abstract. Protein kinase B (Akt), similar to many other protein kinases, is at the crossroads of cell death and survival, playing a pivotal role in multiple interconnected cell signaling mechanisms implicated in cell metabolism, growth and division, apoptosis suppression and angiogenesis. Akt protein kinase displays important metabolic effects, among which are glucose uptake in muscle and fat cells or the suppression of neuronal cell death. Disruptions in the Akt-regulated pathways are associated with cancer, diabetes, cardiovascular and neurological diseases. The regulation of the Akt signaling pathway renders Akt a valuable therapeutic target. The discovery process of Akt inhibitors using various strategies has led to the identification of inhibitors with great selectivity, low side-effects and toxicity. The usefulness of Akt emerges beyond cancer therapy and extends to other major diseases, such as diabetes, heart diseases, or neurodegeneration. This review presents key features of Akt structure and functions, and presents the progress of Akt inhibitors in regards to drug development, and their preclinical and clinical activity in regards to therapeutic efficacy and safety for patients.

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1. Structure and function

Akt is a serine/threonine kinase previously known as protein kinase B (PKB), consisting of three isoforms (Akt1, Akt2 and Akt3), with a crucial role in major cellular functions including cell size, cell cycle progression, regulation of glucose metabolism, genome stability, transcription, protein synthesis and neovascularization. Akt promotes cell survival by mediating the cellular growth factors and blocking apoptosis by the inactivation of pro-apoptotic proteins (1-5).

From a structural point of view, Akt/PKB is characterized by its similarity to protein kinases A (PKA) and C (PKC), as well as to the retroviral oncoprotein viral akt (v-akt) (6-9). Structurally, Akt comprises three domains: An amino-terminal (N-terminal), a central and a carboxyl-terminal fragment (C-terminal). The N-terminal domain, a pleckstrin homology (PH) one, consists of 100 amino acids and is similar to others found in 3-phosphinositide binding molecules, interacting with membrane lipid products such as phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and phosphatidylinositol 4,5-bisphosphate (PIP2). The kinase domain is highly similar to the AGC protein kinases sharing a regulatory threonine residue, Thr308. The phosphorylation of this residue activates Akt. The C-terminal groove consists of 40 amino acids forming a hydrophobic region, containing a regulatory serine residue, Ser473 (6).

Upstream of molecular signaling pathways of Akt, are found systems generating PIP3 as an effect of the action of phosphoinositide 3-kinase (PI3K) (10-12). Three types of intracellular lipid kinases are described (class I, II and III) based on their structure and substrate selectivity. Class I is divided into class IA (PI3Kα, β and δ) and class IB (PI3Kγ). The PI3Kγ kinases are heterodimers with a catalytic subunit (p110α, β, γ or δ) and a regulatory subunit (p85α, β or γ). Class IA PI3Ks (PIK3CA, PIK3CB and PIK3CD), are constituted from a p110 catalytic unit and a p85 regulatory one, and are the most important isoforms in cancer studies. PI3Ks are activated by different agents; PI3Kα, β and δ are activated when the extracellular ligands bind to one of the transmembrane glycoproteins with enzymatic activity called receptor tyrosine kinases (RTKs), while PI3Kγ is activated by G-protein-coupled receptors (GPCRs) and by the RAS family of GTPases (13-15).

The Akt cascade is activated by various signals including RTKs, integrins, B and T cell receptors, cytokine receptors and GPCRs throughPIP3 produced by PI3Ks. Akt is not directly activated by PIP3, which alters Akt configuration by binding to its PH region and recruits it to the plasma membrane allowing phosphoinositide-dependent kinase-1 (PDK1) to phosphorylate at Thr308 residue in the kinase domain (6,16-18). The full activation of Akt requires a second phosphorylation at regulatory Ser473 (Fig. 1).

A number of kinases are known to be capable of phosphorylating Akt at Ser473. These include PDK-1, integrin-linked kinase (ILK) or an ILK-associated kinase and Akt itself (19). Binding proteins such as actin, Erk1/2, Hsp90, Hsp27 or Posh have been found to regulate the activity of Akt (20). Members of the PI3K-related kinase (PIKK) family, including DNA-dependent protein kinase (DNA-PK), can also phosphorylate Akt at Ser473 (2,10,11).

Phosphorylated Akt contributes to the phosphorylation of different proteins located either in the plasma membrane, in the nucleus or the cytosol, supporting cell growth and survival, among other cellular effects. Akt phosphorylates a large number of targets on RxRxxS/T consensus motifs (21). Such downstream targets of Akt phosphorylation are PRAS40, a component and regulator of mTOR complexes, the actin-associated protein palladin, the cell cycle inhibitors p21 (Cip1) and p27, and vimentin, all enhancing tumor motility, invasion and metastasis growth (22,23).

One of the key elements of the Akt network is the serine/threonine protein kinase known as mammalian target of rapamycin (mTOR). mTOR can form two functionally distinct multiprotein complexes, mTOR complex 1 (mTORC1) by joining with regulatory-associated protein of target of rapamycin (RAPTOR) and mammalian lethal with SEC13 protein 8 (mLST8). mTOR complex 2 (mTORC2) contains the protein RICTOR which is insensitive to rapamycin, mLST8 and mammalian stress-activated protein kinase interacting protein (mSIN1) (24,25). mTOR is a downstream member of Akt and a key regulator of cell growth and metabolism, but also an activator, mTORC2 directly phosphorylating Akt's hydrophobic motif Ser473 (26). Ser473 phosphorylation enhances Akt kinase activity and facilitates the Thr308 phosphorylation by PDK1 (27).

The Akt pathway intercedes cell growth and survival by influencing the tuberous sclerosis complex (TSC) 1/2 along mTORC signaling and, respectively, by inhibiting pro-apoptotic

proteins or signals (22,23,28,29). Activated Akt reduces the formation of TSC1/2 and thus blocks the activation of Rheb, a RAS family protein that activates mTORC1, which in turn, induces the phosphorylation of ribosomal protein S6 kinase (S6K) and the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). Phosphorylated 4E-BP1 stimulates the release of eukaryotic translation initiation factor 4E (eIF4E). Both S6K and eIF4E promote protein translation and cell proliferation (30-32). The phosphorylation of TSC2 by Akt can dissociate the TSC1-TSC2 complex, thereby activating mTORC1. When mTORC1 is activated, autophagy is inhibited (33).

Akt directly regulates cell survival by inhibiting pro-apoptotic signals, such as Bad and Forkhead box O (FOXO) transcription factors. FOXO proteins activate or suppress the transcription of target genes through a highly conserved winged helix DNA-binding domain and are regulated by several posttranslational modifications, such as phosphorylation, acetylation and ubiquitination (34). FOXOs activate the transcription of target genes to promote cell cycle arrest, cell death and cellular oxidative stress to maintain metabolic stability. Akt phosphorylates and inactivates the FOXO transcription factors, resulting in their nuclear exclusion and degradation in the cytoplasm, triggering cell survival. The decreased activity of FOXO blocks the transcription cyclin-dependent kinase (CDK) inhibitors p27 and p21, resulting in cell cycle progression (35,36). It also inhibits the extrinsic apoptotic pathway mediated by the transcription of proapoptotic factors such as Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) (16,37).

There are some systems responsible for turning off Akt. Akt is dephosphorylated by protein phosphatase 2A (PP2A) and the PH domain leucine-rich repeat-containing protein phosphatases (PHLPP). PP2A preferentially dephosphorylate Akt at Thr308 residue, but under certain conditions it can also dephosphorylate the Ser473 residue (38). Although PHLPP1 and PHLPP2 both dephosphorylate the Ser473 residue, they differentially stop the signaling by regulating distinct Akt isoforms; PHLPP1 specifically modulates the phosphorylation of HDM2 and glycogen synthase kinase (GSK)-3α by Akt2, whereas PHLPP2 regulates the phosphorylation of p27 by Akt3 (39).

Phosphatase and tensin homolog (PTEN) is the most important negative regulator of Akt function and its metabolic downstream effects. The phosphatase activity of PTEN functions as an antagonist of PI3K, dephosphorylating PIP3 in position 3' to form PIP2 (40,41). Mutations leading to the amplification of genes in the receptor-PI3K pathway, and also the loss of function of PTEN are found frequently in cancer tissues, resulting in pathologically enhanced PI3K signaling and the loss of cell growth control by decreased apoptosis (36).

The PI3K/Akt/mTOR pathway is dysregulated in different diseases, such as solid tumors, immune-mediated disease, idiopathic pulmonary fibrosis, cardio-vascular disease, metabolic impairments such as diabetes mellitus, and constitutes a promising therapeutic target (29,42,43).

2. Akt in malignant disease

The PI3K/Akt pathway has been intensively investigated in cancer due to its significant role in cell survival and anti-apoptotic mechanisms. Multiple pathogenic mechanisms, including
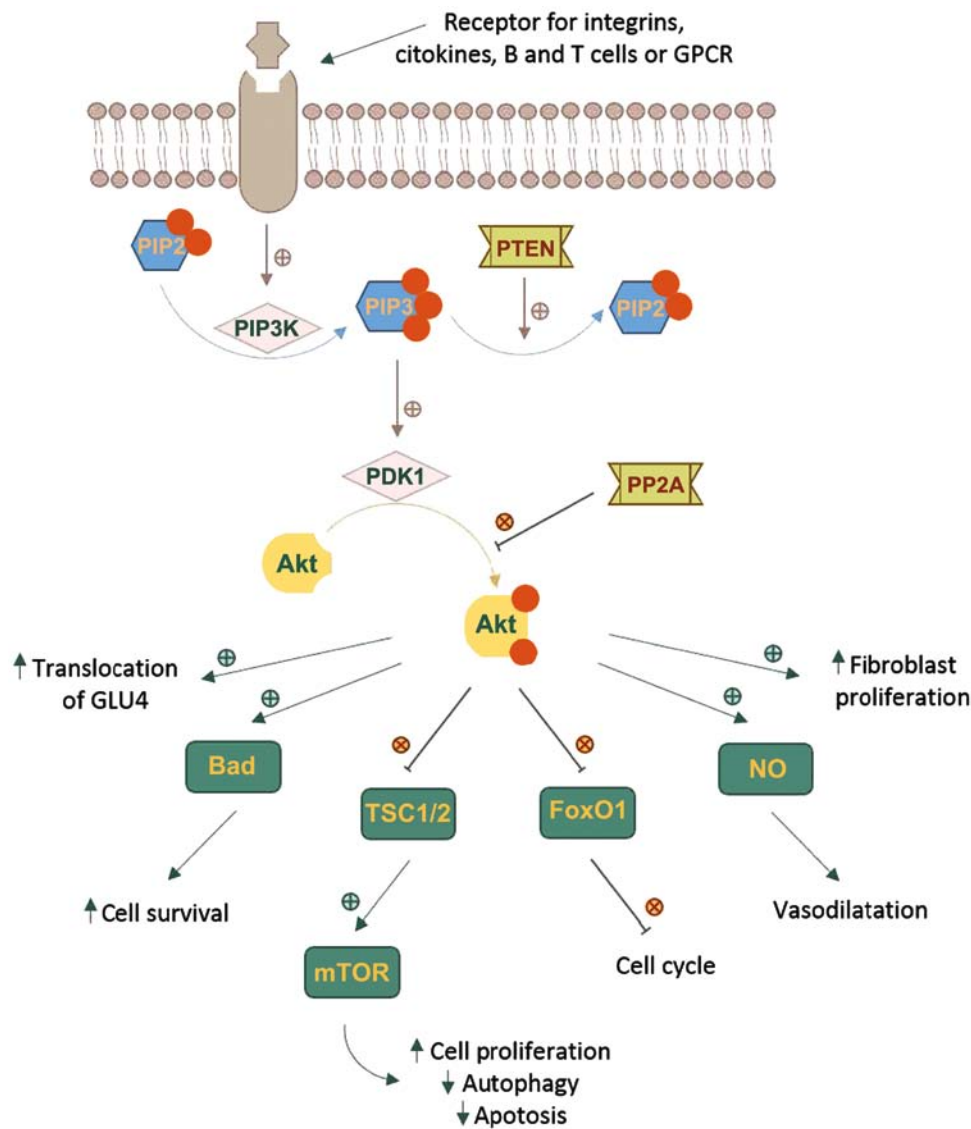
Figure 1. Regulation and downstream effects of the Akt signaling pathway.

the loss of PTEN, mutations that activate the catalytic subunit of PI3K, the activation of RAS and growth factor receptors, or tge amplification of the genes encoding PI3K or Akt can lead to Akt hyperactivation in cancer cells (44).

Studies have proven that the Akt signaling pathway frequently malfunctions in various types of cancer and, in some cases, is associated with tumor aggressiveness. Many types of human cancer are associated with the upregulation of Akt. Anomalies of Akt genes were described for a varie ty of human cancers; Akt1 gene amplifications have been reported in gastric carcinoma, glioblastomas and gliosarcomas, whereas Akt2 amplification has been identified in head and neck squamous cell carcinoma, pancreatic, ovarian and breast cancers (45). Several studies have reported upregulated Akt3 expression in androgen resistant prostate cancer cells, estrogen receptor-deficient breast cancer cells, and in primary ovarian cancers (46,47).

Several of the proteins involved in the Akt signaling pathway (eIF4E, periostin, both the p110α and p85α subunits of PI3K), when overexpressed, can function as oncoproteins, while the ones involved in quenching this pathway (PTEN, FOXO and TSC1/2) may constitute tumor suppressors (6,16,48). PTEN

activity can be impaired by various mechanisms, including but not limited to, somatic mutations, homozygous deletions, epigenic silencing through gene promoter methylation, or post-transcriptional modifications (49). PTEN somatic mutations are described in a large percentage of human cancers, with the highest frequency in endometrial cancers, glioma, colorectal cancers, melanoma and prostate cancers (45).

Autophagy is one mechanism through which cells can move towards programmed cell death. Literature data indicate that there are multiple links between impaired autophagy and cancer, autophagy being a mechanism of tumor suppression. The Akt activated mTOR signaling pathway negatively regulates autophagy; this constitutes a pharmacological target, as anti-cancer molecules blocking the PI3K/Akt/mTOR signal promote autophagy progression, and also reduce angiogenesis (33,50). In this context, recent research has proven that anti-inflammatory molecules (aspirin, celecoxib, meloxicam, or indomethacin) may be useful tools in dealing with tumors as they have the ability to interact with these signaling pathways (50-52).

In addition, the overexpression of Akt is linked to resistance to chemotherapeutic agents such as cisplatin, methotrexate or

paclitaxel (53). Cisplatin-induced DNA damage causes the phosphorylation of BAD via Akt, suppressing its apoptotic effect (54). This observation can be capitalized as a promising strategy for synergic cancer therapy. The Akt inhibitor, MK-2206, has been shown to improve the effectiveness of cisplatin in the gastric cancer cell line, AGS (55), and against the ovarian cancer cell line, SKOV3 (56). MK-2206 demonstrated similar synergic effect in combination with paclitaxel in SKOV3 cells (56). A low PTEN expression is a good predictor of poor responses to human epidermal growth factor receptor (HER2) antagonist therapies and it has been hypothesized that Akt inhibitors can overcome the development of resistance (57,58).

The importance of Akt in cancer pathologies renders it a much pursued target for anticancer therapy, and multiple drug discovery programs focus on finding selective and potent inhibitors. One of the mains issues counteracting drug design efforts consists of achieving selectivity over structurally similar protein kinase, particularly towards the AGC kinase family. The existence of three isozymes with distinct function, tissue distribution and ligand affinity also obstructs the research. The target-based drug development of Akt-specific and isoform-selective inhibitors using the catalytic domain has been predicted to be difficult due to high sequence homology, determining alternative and novel approaches to identify allosteric inhibitors (29,44).

In an extensive review, we proposed a new classification of the Akt inhibitors based on the binding mechanism related to adenosine triphosphate (ATP) and on their main chemical scaffold (29). The Akt inhibitors advanced from ATP-competitive agents to ATP non-competitive inhibitors binding to allosteric sites in order to solve the high structural similarity in the catalytic domain between Akt isoforms and considerable structural analogy to the AGC kinase family (29,59).

The majority of Akt inhibitors targeting the ATP binding site are non-selective against the three isoforms, and poorly selective against structurally similar kinases (60). Akt1-3 share a high ATP-binding site homology with S6K1 (84%), PKA (81%), PKC (78%), SGK (78%), PRKX (75%), PKN1 (75%) and Aurora A (72%) (43). The ATP-competitive inhibitors of Akt can be chemically described as isoquinoline-5-sulfonamides, azepane derivatives, aminofurazans, heterocyclic 6-5 fused rings, phenylpyrazoles, thiophene carboxamides and thiazole carboxamides (29).

The ATP non-competitive Akt inhibitors are allosteric modulators, the binding mechanism offering important advantages, as greater specificity, reduced side-effects and lower toxicity (43). Chemically, they are categorized as 2,3-diphenylquinoxaline and analogs, alkylphospholipids, derivatives of indole-3-carbinol, sulfonamides, thiourea deratives and purine derivatives. A special class of ATP non-competitive are the irreversible inhibitors that interact covalently with the enzyme (29).

Current pre-clinical and clinical data suggest that the use of an intermittent high-dose posology is more effective than a continuous low dose by daily administration. The high doses are required for induction of apoptosis and pauses can overcome the toxicological risks. Also, data shows a delay in treatment resistance mechanisms. The combination with other targeted therapeutic agents further enhances the antitumor activity of Akt inhibitors (42,61).

3. Neurological diseases

Early observation associated with increased amounts of glutamate with excitotoxicity and neural cell death have opened and facilitated the development of treatments for glutamate-related disorders, such as Alzheimer's disease (AD), parkinsonism, epilepsy, or multiple sclerosis (62). The overstimulation of glutamate receptors (GluRs) and other post-synaptic signaling components results in excitotoxicity. It is considered that *N*-methyl-D-aspartate (NMDA) subtypes play a major role, and other GluR subtypes such as acid 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic (AMPA) or kainate receptors play a critical role in the excitotoxic neuronal cell death process (63). Reversely, the stimulation of metabotropic GluR1 (mGluR1) exerts neuroprotectives effect by reducing nerve cell death induced by exposure to NMDA (64,65).

AD is a progressive neurodegenerative disease characterized by the alteration of memory and cognitive functions, and is caused by the damage of neurons. Post-mortem histopathological examinations have revealed extracellular amyloid β (Aβ) plaques and intracellular neurofibrillary tangles as hallmark lesions of AD. It has been demonstrated that an elevated GSK-3β activity is directly linked to increased levels of Aβ production and deposits, tau hyperphosphorylation and the formation of neurofibrillary tangles (66,67). GSK-3β is rendered inactive when it is phosphorylated at Ser9 by phosphorylated Akt, and therefore an upregulation of Akt may contribute to a decrease in AD progression. This hypothesis is associated with the neurotoxic effect of wortmannin, an inhibitor of PI3K that induces tau hyperphosphorylation similar to that observed in AD following incubation in hippocampus slice culture. Lithium chloride, a GSK-3β inhibitor, prevents the neurotoxic effects of wortmannin (68).

The treatment of hippocampal and cerebral cortical cell cultures with toxic doses of NMDA has been shown to cause a significant decline in phosphorylated Akt levels and phosphorylated GSK-3β, without caspase-3, caspase-7, or poly(ADP) ribose polymerase (PARP) cleavage; the total levels of Akt and GSK-3β were not shown to be affected (69). Excessive NMDA activity has been demonstrated to cause excitotoxicity, promoting cell death, a probable mechanism of neurodegeneration in AD, in agreement with the GSK-3β hyperactivation hypothesis (70). The role of Akt hypofunction in AD has been demonstrated by several studies on PTEN regulation (71,72). Okadaic acid leads to PTEN activation in SH-SY5Y cells associated with an increase in tau phosphorylation, an effect that can be blocked by PTEN knockdown (73).

The elevation of Akt activity has emerged as a effective strategy with which to prevent progressive neuronal death in neurological diseases. The 4*H*-chromene-3-carboxylate derivative, SC79, was identified as a specific pan-Akt activator. SC79 specifically binds to the PH domain of Akt, leading to a conformation favorable for phosphorylation by upstream protein kinases, without altering total Akt levels. The treatment of cultured cortical neurons with SC79 has been shown to markedly enhance Akt phosphorylation and reduce neuronal death elicited by glutamate excitotoxicity in hippocampal neurons. SC79 effectively reduced the neocortical lesion size by 35% after a single dose of 0.04 mg/g of body weight in a murine ischemic stroke model (74).

The inhibition of PTEN function in conditions associated with neurodegeneration, tissue injury or ischemia may be a valuable pharmacological intervention with which to activate Akt function. Bisperoxovanadium compounds are reversible and relatively specific small PTEN inhibitors, with nanomolar affinity and have been experimentally explored as a potential therapeutic approach in AD and other neurological diseases (75).

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder characterized by the premature death of dopaminergic neurons in the substantia nigra (76). Oxidative stress is a major cause of PD pathogenesis, inducing neuronal cell death and apoptosis by intracellular calcium overload, lipid peroxidation, DNA damages and excitotoxicity.

The activation of Akt has been shown to efficiently protect neurons or neuronal cells from oxidative stress and is an established target of drug design in PA. Pre-treatment of human dopaminergic neuronal cells with the specific Akt activator, SC79 (10 μM), was previously shown to markedly attenuate hydrogen peroxide-induced toxicity. The neuroprotective effect of SC79 was abolished by Akt inhibitors (77).

Sulfuretin, a trihydroxyaurone isolated from the bark of the lacquer tree (*Toxicodendron vernicifluum*), was previously shown to significantly decrease apoptotic cell death induced by the 1-methyl-4-phenylpyridinium (MPP⁺) in an experimental model of PD. Sulfuretin reduced caspase-3 and PARP activity accompanied by a reduction of intracellular reactive oxygen species (ROS) production and recovered the normal mitochondrial membrane potential. The mechanism involved the augmentation of the phosphorylation of Akt, GSK-3β and Erk pathway, confirmed by the disappearance of the cytoprotective effects of sulfuretin following the administration of PI3K/Akt and Erk inhibitors (78). Vitexin, a glucoside derivative of apigenin and several other flavonoids had similar protective effects on cell and mouse models of PD (79).

4. Insulin signaling and diabetes

Akt is involved in the insulin signaling pathway; the activation of the insulin receptor triggers a phosphorylation cascade, initiated by receptor autophosphorylation and the activation of insulin receptor substrate proteins (IRS-1 and IRS-2), recruiting PI3K that phosphorylates PIP2 to PIP3. Membrane-anchored PIP3 activates Akt, thus contributing to the translocation of the glucose transporter 4 (GLUT4) at the membrane level (80,81). Similarly to insulin, members of the family of insulin-like growth factors (IGF) activate PI3K to produce PIP3, which, in turn, recruits two protein kinases to the plasma membrane via their PH domains Akt and PDK1, leading to Akt activation (81). Akt2 and its downstream effectors control the insulin-stimulated translocation of GLUT4 to the plasma membrane. The most important are AS160, a Rab GTPase-activating protein, CDP138, a C2 domain-containing phosphoprotein, and an actin-capping protein, tropomodulin 3 (82,83).

The downstream steps determining the effects of insulin on target tissues, including glucose uptake in skeletal muscle and adipose tissue, and hepatic gluconeogenesis are controlled by Akt2. Glycogen synthesis is regulated by Akt phosphorylation and the inactivation of GSK-3β, resulting in glycogen synthase activation (81).

Akt2 is highly expressed in insulin-responsive tissues and is an important regulator of glucose metabolism. The deletion of Akt2 in knockout mice was previously shown to result in insulin resistance, hyperinsulinemia and glucose intolerance, whereas Akt1 or Akt3 knockout mice have a normal glucose metabolism (84). Leptin was shown to increase insulin sensitivity in Akt-deficient mice (85). Consistent with these studies, defects in the kinase domain of Akt2 are associated with insulin resistance and diabetes in humans (86). Conversely, an activating mutation of Akt2 in humans was reported and was shown to be associated with severe insulin-independent hypoglycemia and asymmetric overgrowth (87) and a lower risk of diabetes was observed in individuals with PTEN haplo-insufficiency (81).

Insulin is also a potent neurotrophic factor and the activation of the insulin receptors, highly expressed in sensory neurons, enhances axon growth through the stimulation of the PI3K/Akt pathway and counteracts diabetic polyneuropathy symptoms. Recent research considers that an important strategy for reversing the neuropathic deficits of diabetic neuropathy may be either the activation of intrinsic PI3K/Akt signaling or the inhibition of the PTEN effect (80,81). As in the case of AD, various vanadium compounds have been proven to reduce blood glucose in diabetic rats (88,89). Although he systemic long-term PTEN pharmacological inhibition is likely to promote cancer, it is possible that a tissue-specific inhibition may be beneficial in type 2 diabetes treatment (75).

Another strategy which can be used to enhance Akt signaling to potentially overcome insulin resistance, is the inhibition of PHLPP. The strategy is enforced by the observation that the levels of PHLPP1 are highly elevated in the skeletal muscle of obese humans, and are associated with the increased body mass index and insulin resistance. Additionally, PHLPP1 mRNA levels are elevated in muscle cells from diabetic patients (40). Two specific inhibitors of PHLPP2 were identified in a virtual screening coupled with biochemical assays, a derivative of aminoanthraquinone and a diazenyl derivative of salicylic acid. Both compounds inhibit the dephosphorylation of Akt on Ser473 at micromolar doses and have the potential to improve insulin secretion (90).

Statins are HMG-CoA reductase inhibitors intensively used to reduce cholesterol levels in a wide range of patient populations. Several studies have demonstrated that statin use is associated with an increased risk of developing type 2 diabetes mellitus by the reduction of insulin secretion and the induction of insulin resistance. These effects are produced by decreasing GLUT-4 levels and the inhibition of GSK-3β, p38 MAPK and Akt phosphorylation (91,92). Statin treatment profoundly affects signaling through Akt in several cell models. Simvastatin and atorvastatin have been shown to inhibit Akt activation and to be cytotoxic in mouse myoblast cell line at doses of 10 μM, while for rosuvastatin the dose is 50 μM (92,93). These studies highlight the importance of the Akt signaling pathway in both statin-induced myotoxicity and diabetes, as well as the anticancer effects of statins.

5. Involvement of Akt in cardiovascular events

In a number of physiological and pathological cardiovascular processes, such as vessel remodeling, atherosclerosis and

vascular injury, apoptotic cell death was identified as a major common factor leading to a close analysis of the Akt network involvement. Akt activation stimulates downstream anabolic signaling, favoring cell growth, proliferation and survival; this is a desired effect for cardiac cells, whose metabolic improvement following the stimulation of Akt may also alleviate cardiac cellular damage (94).

Akt1 plays an important role in the regulation of cardiac hypertrophy and angiogenesis, as the absence of Akt1 leads to severe atherosclerosis and occlusive coronary arterial disease, associated with significant reduction in NO production and endothelial cell viability. It was observed that atherosclerosis results in the reduction of Akt expression and phosphorylation in plaque intimal vascular smooth muscle cells and an increased expression of active FOXO3a. An ectopically activated Akt was used to demonstrate that Akt activation is sufficient for the survival of vascular smooth muscle cells in response to oxidative stress. The PI3K/Akt pathway can stimulate NO production by endothelial cells and increase the synthesis of prostacyclin, thus leading to vasodilation and retarding atherogenesis (94-96).

Akt2 is also involved in multiple functions in the cardiovascular system due to its abundance in insulin-responsive tissues. Mice lacking Akt2 present with hyperglycemia and dyslipidemia with high levels of triglycerides and cholesterol, although the absence of Akt2 does not influence the progression of atherosclerosis in mice (95).

The PI3K/Akt pathway is involved in cardioprotection by various mechanisms, such as the heat shock protein (HSP) 90-mediated protection of cardiomyocytes subjected to hypoxia or the estrogen receptor (97). Studies using mouse models have shown that a reduced activation of the PI3K p110α catalytic subunit increases the risk of atrial fibrillation. Conversely, the augmentation of Akt activity in murine models of heart failure has been shown to improve cardiac function (98,99). These findings explain why some anticancer protein kinase inhibitors, such as ibrutinib and nilotinib, increase the occurrence of atrial fibrillation and drug-induced long QT syndrome (98,100).

It is hypothesized that the PI3K/Akt signaling pathway may provide a solution for addressing proarrhythmia by regulating cell proliferation and survival through the PIP3 activation of downstream signals; nevertheless, an increased activity of PI3K/Akt signaling may lead to cancer development (100).

6. Pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease, one of the most common types of interstitial pneumonia associated with high morbidity and mortality. In normal lung fibroblasts, the interaction with type I collagen, suppresses the PIP3K/Akt pathway, through the activation of PTEN, thus inhibiting cell proliferation and promoting fibroblast apoptosis. Pathologically, in fibroblasts from patients with IPF, PI3K/Akt activity is enhanced due to the suppression of PTEN, rendering the cells highly proliferative and resistant to apoptosis (101-103). It has been demonstrated that the low activity of PTEN is caused by the decline of the caveolin-1 and FOXO3a levels (104,105).

Previously, a model of pulmonary fibrosis was used in rats following the intratracheal administration of bleomycin, and it was demonstrated that the high levels of phosphorylated Akt were involved in the progression of pulmonary fibrosis and

contributed to fibrogenesis (106). Tubastatin, a known histone deacetylase 6 (HDAC6) inhibitor, has been shown to protect mice against bleomycin-induced fibrosis by decreasing Akt phosphorylation and increasing Akt association to PHLPP, independent of its HDAC6 effects (107).

7. Akt in inflammation and autoimmune diseases

Autoimmune diseases occur when the immune system mistakenly recognizes self-tissues as foreign and triggers an immune response. The autoreactivity of the immune system is multifactorial and may lead to pathogenic autoimmunity and is related autoimmune diseases (108). Although the exact mechanisms of the pathogenesis of autoimmune diseases remain unclear, the over activation and abnormal function of T cells, B cells and myeloid cells has been extensively studied (109-111). Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the high hyperactivity of T cells and B cells, which result in the production of antinuclear, anti-double-stranded DNA and anti-glomerular antibodies, nephritis, renal failure and mortality (112).

Patients with SLE are characterized by the activation of mTORC1 and reduced mTORC2 levels, promoting the expression of pro-inflammatory cytokines, and the deletion of the regulatory T cell subtype that normally regulates inflammation (113). To further explore the underlying mechanisms of SLE, researchers have used mice that are characterized by the constitutive activation of the PI3K pathway in T lymphocytes and found that these mice develop SLE-like diseases (114). The increased activation of mTORC1 has also been reported in the T cells of patients suffering from multiple sclerosis (115). In a study on patients with rheumatoid arthritis, Zhang *et al* found significantly higher levels (~4-fold) of p-Akt in fresh synovial tissue isolated from these patients (116). These findings highlight the central role of the Akt pathway in autoimmune diseases. It has been suggested that alterations in the PI3K/Akt axis found in highly active immune cells are mediated through metabolic changes in these cells (117).

Studies now focus on the therapeutic potency of molecules that target the PI3K/Akt pathway for the treatment of autoimmune diseases. Indeed, rapamycin, which inhibits the antigen-induced proliferation of T cells through the inhibition of mTOR, has been shown to exert beneficial effects in experimental models and patients with autoimmune diseases (118,119). Although there are several challenges that need to be tackled, the further exploration of this pathway may lead to the identification of disease biomarkers and therapeutic targets (120).

In Table I the main pathways that could be modulated by different molecules interfering with Akt signaling are reviewed.

8. Repurposing studies

Drug repositioning is a highly studied alternative using available drugs for the treatment of various conditions in order to discover novel therapeutic opportunities. This strategy is also used on the Akt pathway, capitalizing the lower toxicological risk of known drugs. Ivermectin is a macrocyclic lactone used as an antiparasitic drug that has been shown to significantly decrease Akt phosphorylation at Ser473. This result could allow its clinical investigation for cancer patients (121). Another

Table I. Possible mechanism for Akt modulation used in clinical and preclinical settings.

Pathology models associated with Akt impairment	Signaling pathway injury	Molecules involved in modulating intracellular pathways	(Refs.)
Malignant disease (carcinomas, glioblastoma, hematological malignancies, gastric carcinoma, glioblastomas and gliosarcomas, head and neck squamous cell carcinoma, pancreatic, ovarian, skin, prostate and breast cancers)	Loss of PTEN activity, including mutations involving PTEN mutations activating the catalytic subunit of PI3K, activation of RAS and growth factor receptors, amplification of the genes encoding PI3K and Akt impairment of proteins involved in the Akt signaling pathway impairment of PI3K/Akt/mTOR regulated autophagy	Isoquinoline-5-sulfonamides, azepane derivatives, aminofurazans, heterocyclic 6-5 fused rings, phenylpyrazoles, thiophenecarboxamides and derivatives 2,3-diphenylquinoxaline and analogs, alkylphospholipids, indole-3-carbinol derivatives, sulfonamides, thiourea deratives, and purine derivatives anti-inflammatory molecules (aspirin, celecoxib, meloxicam, or indomethacin) MK-2206	(30,45,46, 49-51,52-56)
Neurological disorders (Alzheimer's, Parkinson's, and Huntington's disease, epilepsy and multiple sclerosis)	Elevated GSK-3β levels (due to Akt inhibition) overactivation of PTEN	Lithium chloride okadaic acid 4H-chromene-3-carboxylate derivative (SC79), bisperoxovanadium compounds sulfuretin and vitexin	(63-65,68,69, 70,75,76)
Insulin resistance, hyperinsulinemia, and glucose intolerance, diabetic polyneuropathy symptoms	Deletion of Akt2 inhibition of PI3K-Akt signaling PTEN activation inhibition of PHLPP	Vanadium compounds	(41,85,86,87)
Cardiovascular disease (vessel remodeling, atherosclerosis, etc.)	Inhibition of Akt1 reduction of Akt expression and phosphorylation overexpression of active FOXO3a	Anticancer protein kinase inhibitors (ibrutinib and nilotinib)	(91-93,95-97)
Idiopathic pulmonary fibrosis	PTEN suppression Activation of mTORC1	Tubastatin	(98-100, 101,103)

antiparasitic drug, artesunate, may be useful in nasopharyngeal carcinoma by inhibiting Akt (122). Recent research has demonstrated the potential of propofol, an intravenous anesthetic drug, to be repurposed to overcome resistance to imatinib in chronic myeloid leukemia treatment (123). Cimetidine, a drug used in the treatment of peptic ulcers, induces the apoptotic death of cholangiocarcinoma cells by the suppression of Akt phosphorylation and may be an effective candidate for future treatments (124). A well-known antifungal agent, itraconazole, has demonstrated anticancer activity in various cell lines by inhibiting Akt/mTOR signaling (125). Quetiapine, an atypical antipsychotic, decreases the level of certain pro-inflammatory cytokines, such as interleukin (IL)-17, IL-6 and IL-1β in mouse model of arthritis by suppressing the Akt and Erk pathways (126).

9. Natural products targeting the Akt pathway

Plants and natural products are used as complementary or alternative therapies for cancer treatment, as a large number of patients consider them safer and less toxic (127,128). Curcumin,

the main curcuminoid found in *Curcuma longa*, has showed beneficial results regarding the inhibition of human malignant glioma cells by inducing autophagic cell death. The mechanisms implicated are the inhibition of the Akt/mTOR/p70S6K pathway and the activation of Erk1/2 pathway, and these have been demonstrated in *in vitro* and *in vivo* experiments (129). Lycopene, a carotenoid pigment found in tomatoes, has been shown to exert antitumorgenic effects on human colon cancer HT-29 cell lines, partly through the inhibition of Akt phosphorylation (130). Lupeol is a triterpenoid compound found in mango, dandelion and several other species of *Acacia visco* and *Abronia villosa*. Lupeol and its acetyl derivative have been shown to inhibit the proliferation and induce the apoptosis of human pancreatic cancer and chemoresistant prostate cancer cells by decreasing the levels of p-Akt and p-Erk (131). The chemically similar tirucallic acids isolated from the oleogum resin of *Boswellia carterii* inhibit Akt in prostate cancer cells by bonding within the PIP3 binding pocket (132).

As mentioned above, several flavonoid compounds, such as sulfuretin and apigenin, have been proven to target Akt. Jaceosidin

is a flavone from *Artemisia princeps*, a traditional medicinal herb, with a marked effect on oral squamous cell carcinoma by Akt downregulation (133). The antiproliferative and antiinflammatory effect of luteolin via the inactivation of PI3K/Akt pathway has also been demonstrated by several studies (134,135).

Euplotin C is a secondary metabolite of the marine ciliate *Euplotes crassus* that has demonstrated marked cytotoxic effects on human cutaneous melanoma cells via the inhibition of the Erk and Akt pathways (136). A large diversity of plant extracts have demonstrated promising anticancer properties in various cell lines, such as *Libidibia ferrea* and *Celastrus orbiculatus* in colorectal cancer (137,138), *Astragalus membranaceus*, *Anthriscus sylvestris* and *Vernonia amygdalina* in breast cancer cells (139-141), *Azadirachta indica* in prostate cancer (142), and *Fallopia aubertii* and *Fallopia convolvulus* in cervical cancer cells (143), and these may represent future sources of leading Akt inhibitors. The usefulness of the natural sources is not limited to oncology. Bamboo leaf extract administered for a period of 12 weeks to diabetic rats has been shown to improve body weight and biochemical markers, and to alleviated renal injury by increasing p-Akt levels (144,145). Similar properties were observed for sea buckthorn fruit oil extract and the traditional Chinese medicine Liuwei Dihaung (146,147).

10. Akt inhibitors in clinical studies

Several compounds have been proven to inhibit Akt in *in vitro* and *in vivo* models, but only a small number have entered clinical evaluation, and yet no Akt inhibitor has been approved for anticancer therapy (148). Miltefosine is approved for the oral treatment of both visceral and cutaneous leishmaniasis, but not for oncological use (149).

GSK690693 was the first clinically evaluated Akt inhibitor. In preclinical experiments, GSK690693 was shown to inhibit all three isoforms at nanomolar concentrations and to significantly inhibit the growth of various tumors in mouse xenograft models. Despite the encouraging preclinical data, the clinical development of the agent was halted due to drug-related hyperglycemia and peripheral insulin resistance (150,151).

AZD5363 is a pyrrolopyrimidine derivative that inhibits all Akt isoforms with nanomolar affinity, and inhibits the proliferation of a large number of solid and hematological tumor cell lines, with the highest therapeutical potential in breast cancer cells (152). The safety and tolerability of AZD5363 as oral monotherapy was evaluated in an open-label study (NCT01226316) on 90 patients with advanced solid malignancies. The patients received 320, 480 or 640 mg in a continuous or intermittent schedule. The most frequently reported adverse effects in all dosing schedules were diarrhea, nausea and vomiting. A total of 56 (62%) patients experienced grade 3 adverse events, such as hyperglycemia (20%), diarrhea (14%) and rash (11%). An adverse event leading to discontinuation was reported in 23% of the patients (153).

AZD5363 is under clinical evaluation as a short-term monotherapy in estrogen receptor-positive breast cancers (NCT02077569), and in non-small cell lung cancer (NCT02664935). It has been evaluated in combination with other anticancer drugs in various clinical trials (153,154).

Ipatasertib belongs to the heterocyclic 6-5 fused rings class of ATP-competitive inhibitors. In a phase I study (NCT01090960), the safety and tolerability in patients with refractory solid tumors was evaluated following orally administration of ipatasertib at daily doses from 25 to 800 mg. Ipatasertib was well tolerated, the most frequently reported adverse events of grade 2 or above were diarrhea (35%), nausea (27%), asthenia (25%), hyperglycemia (10%), decreased appetite (6%), rash (6%) and vomiting (6%). The maximum tolerated dose (MTD) for ipatasertib is 600 mg on a once daily, 21/7 dosing schedule (155).

The combination of ipatasertib (400 mg daily) and paclitaxel (80 mg/m^2 weekly) was well-tolerated in a phase II study (NCT02301988) designed to estimate the efficacy in women with triple-negative breast cancer (42). A double-blind placebo controlled randomized phase II trial (NCT02162719) demonstrated that the combination modestly improved the progression-free survival endpoint, the results being prominent in the patients with PIK3CA/Akt1/PTEN alterations (156).

Afuresertib (GSK2110183) is a thiophenecarboxamide derivative, orally bioavailable Akt inhibitor, with higher potency against Akt1 compared to Akt2 and Akt3 (157). An open-label phase I clinical study (NCT00881946) evaluated afuresertib in 73 patients with advanced hematologic malignancies. All patients enrolled in the study experienced at least one adverse event, the most frequent being nausea, diarrhea, dyspepsia, fatigue, gastrointestinal reflux disease and anorexia. Treatment-related adverse events of grade 3 had a frequency under 10% and included neutropenia, rash, odynophagia, fatigue and asthenia, abnormal liver function test and thrombocytopenia. No grade 4 or 5 adverse events were observed. Afuresertib produced a small impact on glycaemia, due to the selectivity towards PKC (158). In a phase IIa study (NCT1395004), 17 patients with Langerhans cell histiocytosis received daily oral doses of 125 mg. The majority of the reported adverse events were grade 1 or 2 and consisted on nausea (59%), fatigue (53%), diarrhea (47%) and upper respiratory infection (47%) (159).

Uprosertib (GSK2141795) is closely related to afuresertib, the main difference being the replacement of the thiophene with a bioisostere furan ring (29). The safety, tolerability, pharmacokinetics, and pharmacodynamics of the drug were evaluated in a phase I, open-label study in patients with solid tumors. Uprosertib was safe and well-tolerated, most treatment-related adverse events were low grade and included diarrhea, fatigue, vomiting and decreased appetite (160).

MK-2206 is an orally effective, highly potent and selective allosteric pan-Akt inhibitor (150). Based on a phase I study of MK-2206 (NCT00670488) the MTD value was established at 60 mg for an alternate days administration. Most frequent drug-related toxicities recorded were skin rash (51.5%), nausea (36.4%), pruritus (24.2%), hyperglycemia (21.2%) and diarrhea (21.2%). The main dose limiting toxicity is a dose-dependent, generalized erythematous, nonblistering, maculopapular rash. This rash was observed also in other clinical trials with MK-2206 (161) and is similar to that produced by mTOR or PI3K inhibitors, but unlike those caused by epidermal growth factor receptor inhibitors indicating a mechanism-based toxicity, and not an off-target effect (162). It was investigated in several clinical trials. The potential use of MK-2206 goes beyond oncology, demonstrating a significant reduction of plasma LDL-cholesterol levels in cultured hepatic cells (163).

Perifosine is an alkylphospholipid derivative that interferes with the PH domain of Akt rendering it incapable of phosphorylation and activation. Chemically, it resulted by replacing the choline moiety of miltefosine with a piperidine scaffold, leading to higher metabolic stabilityand better gastrointestinal tract tolerance because perifosine is not able to generate phosphocholine, responsible for the parasympathomimetic effects (29,164). Clinical trials using oral perifosine as a single therapy for various types of cancer have produced disappointing results. Several combinations of perifosine are yielded promising results and are undergoing clinical development (165).

SR13668 was designed as an Akt activation inhibitor based on the naturally occurring indole-3-carbinol. In preclinical safety testing, no toxic effects were observed in rats after a single oral dose of 1,000 mg/kg or multiple doses of 25 up to 600 mg/kg/day for 14 days. In mice, doses up to 500 mg/kg had no effects on fasting glucose levels and body weight. It has a very low solubility which may hinder the future development of the drug (166,167).

Triciribine (API-2, TCN, NSC 154020) is a tricyclic nucleoside that is metabolically activated inside cells by adenosine kinase to triciribine phosphate (TCN-P) which binds to the PH domain near PIP3 binding pocket, preventing PDK1 phosphorylation. TCN is highly selective towards Akt with no significant effect on PI3K, PKA or PKC (168,169). The antitumor effects of TCN-P were known before the discovery of the Akt inactivation and were tested in several clinical trials. The therapeutic development of TCN-P limited by dose-limiting toxicities, including reversible hyperglycemia, hepatic toxicity, thrombocytopenia, hypocalcaemia and high triglyceride levels (170,171). In order to circumvent the toxicities associated with TCN-P, the clinical development has focused on tumors that express high p-Akt levels (172).

Miransertib (ARQ 092) binds to the inactive Akt and inhibits non-competitively all three isoforms at nanomolar concentrations and has excellent selectivity over other kinases (173). It is analyzed in several clinical trials for treatment as single therapy or combinations of advanced solid tumors and recurrent malignant lymphoma. Positive results were obtained with miransertib in cells and tissues harboring Akt1 E17K mutations and is being investigated for patients with overgrowth diseases and/or vascular anomalies generated by genetic alterations of the PI3K/AKT pathway (174). Miransertib is under study as a potential therapy for Proteus syndrome, a disease characterized by progressive and typically asymmetric overgrowth caused by the Akt1 E17K somatic activating mutation during development (175).

BAY 1125976 is a highly selective, potent allosteric Akt inhibitor by blocking the PDK1 phosphorylation after binding in the pocket formed by kinase and PH domain. Chemically it is a imidazo[1,2-b]pyridazine derivative, closely related to miransertib. It inhibits cell proliferation in a panel of human cancer cell lines, especially in hormone dependent breast and prostate cancer cell lines. It is under investigation is a phase I study (NCT01915576) in patients with advanced solid tumors (176).

11. Conclusions

Akt is the core of a complex signaling pathway that is one of the most intensively investigated cell signaling networks due to its crucial involvement in cell metabolism, growth, proliferation, motility, survival and apoptosis. These plethora of effects render Akt a most valuable target of drug discovery. Even if cancer treatment is at the center of the research, the potential applications are numerous other major diseases, such as diabetes, heart diseases, or neurodegenerative diseases. The position of Akt at the crossroads between life and death rises multiple challenges, but increasing understanding of cell biology and accumulating promising results are raising hope to find personalized treatments.

Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

GMN, DMdV and AT were involved in the conception of the study. GMN, MVdV, GN, AU, PJ, QP, OTO, DG, AT, DT, DAS and DM were involved in the acquisition of the data and study design. GMN, MVdV, GN, AU, PJ, QP, OTO, DG, AT, DT, DAS and DM were involved in the writing of the article. GMN, DM, MVdV, PJ and DAS critically revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article.

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