Switching Akt: from survival signaling to deadly response

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Akt, a protein kinase hyperactivated in many tumors, plays a major role in both cell survival and resistance to tumor therapy. A recent study,[1] along with other evidences, shows interestingly, that Akt is not a single-function kinase, but may facilitate rather than inhibit cell death under certain conditions. This hitherto undetected function of Akt is accomplished by its ability to increase reactive oxygen species and to suppress antioxidant enzymes. The ability of Akt to down-regulate antioxidant defenses uncovers a novel Achilles' heel, which could be exploited by oxidant therapies in order to selectively eradicate tumor cells that express high levels of Akt activity.

Keywords: Akt; apoptosis; oncogenes; oxidative stress; senescence

Akt and tumorigenesis

Until recently, the serine–threonine kinase Akt, also called protein kinase B, has been regarded solely as a survival kinase. Akt activity is triggered by various signals that activate upstream phosphatidylinositol 3′-kinase (PI3K), resulting in the activation of multiple downstream effectors.[2–4] Several mechanisms are involved in oncogenic Akt activation. For example, more than 30% of prostate cancers have a mutational loss of PTEN, the phosphatase counteracting PI3K-dependent Akt activation. Constitutive Akt activity in tumors may also arise from mutations in the catalytic PI3K subunit, receptor tyrosine kinase activation, or Ras activation.[2–4] Even though Akt is rarely mutated itself, it is frequently hyperactivated in tumors.

Akt may foster tumorigenesis by multiple means.[5,6] By stabilizing Myc and cyclin D1 or by inducing degradation of the cyclin-dependent kinase (Cdk) inhibitor $p27^{Kip1}$, it promotes cell cycle progression. Akt is also a profound inhibitor of apoptosis due to its ability to inactivate pro-apoptotic molecules, including caspase-9 and the BH3-only protein Bad, and by triggering the activity of the transcription factor NF-κB (Fig. 1). In addition, Akt promotes nuclear translocation of the ubiquitin ligase MDM2, which counteracts p53-mediated apoptosis. An important aspect of Akt's promotion of cell survival involves alterations in cellular energy metabolism.[5,6] Akt modulates the function of glycogen synthase kinase-3 (GSK3) and mammalian target of rapamycin (mTOR). Akt also enhances energy production by stimulating nutrient transporters, which in turn supports mTOR-dependent protein translation. Thus, by preventing apoptosis and increasing oxidative metabolism, Akt lies at the hub of complex signaling networks that integrate a multitude of potentially oncogenic signals.

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Akt and induction of cell death

In contrast to these well-established tumorigenic properties, Akt activation does not always appear to be advantageous for cellular proliferation. In a recent study, Nogueira *et al.*[1] showed that, surprisingly, strong Akt activation increases oxidative stress and renders cells susceptible to reactive oxygen species (ROS)-triggered cell death. Not only does this finding have intriguing implications for tumor biology, but it also might create an impact on the development of novel therapeutic strategies for cancer treatment. The observed increase in pro-oxidant conditions is caused by the Akt-dependent upregulation of oxidative phosphorylation and oxygen consumption. Perhaps more importantly, Akt hyperactivation leads to a sustained inhibition of FoxO transcription factors, which normally up-regulate the expression of antioxidant proteins. In addition to MnSOD and catalase, Nogueira *et al.*[1] identified sestrin-3 as an important target of FoxO3a. Sestrins were originally found to be target genes of p53, which confer resistance to oxidative stress by regenerating oxidized peroxiredoxins. As a consequence of FoxO inhibition, and owing to the depletion of antioxidants, cells with active Akt should be more vulnerable to oxidative stress. Indeed, the authors found that both hydrogen peroxide and β-phenylethylisothiocyanate, a drug that depletes glutathione, induces potent apoptosis in Akt-expressing cells.[1] Furthermore, rapamycin, an mTOR inhibitor already in clinical

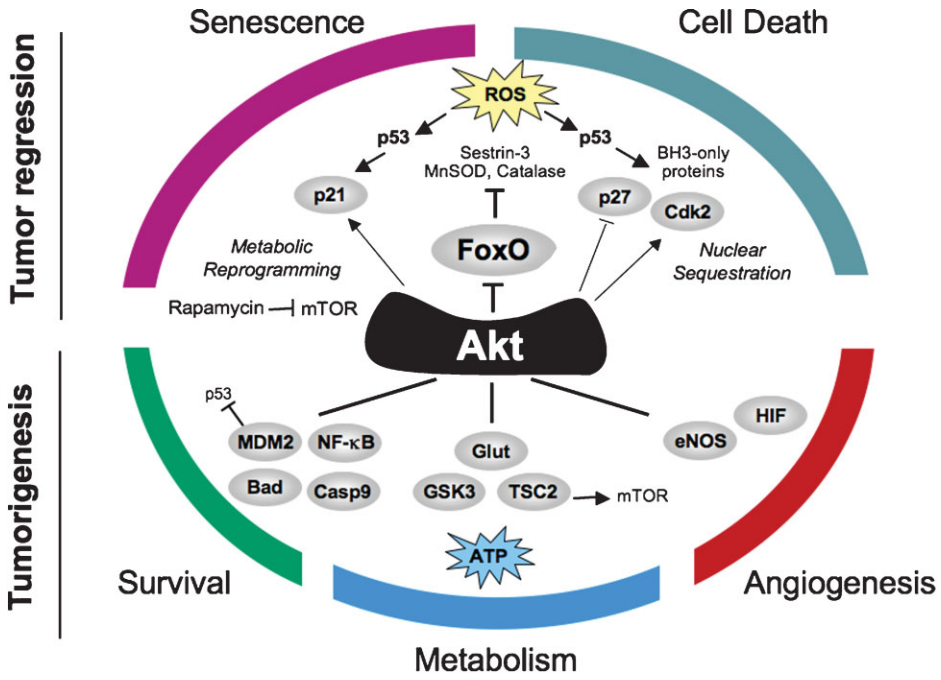

Figure 1. Simplified scheme of the double-edged role of Akt in tumor development. Moderate or unsustained levels of Akt activity inhibit apoptosis and promote cell growth and angiogenesis through various downstream effectors. Inhibition of caspase-9 and the Bcl-2 protein Bad, or activation of MDM2 and NF-κB prevent apoptosis. In addition, stimulation of amino acid and glucose transporters (Glut), or inhibition of GSK3 and tuberous sclerosis complex-2 (TSC2), along with the modulation of other not-indicated enzymes, exert growth-promoting effects by activating the mTOR pathway and oxidative metabolism. Akt can stimulate angiogenesis by activating endothelial nitric oxide synthase (eNOS) and HIF, which stimulate the expression of endothelial mitogens such as VEGF. Hyperactivation of Akt, in contrast, triggers senescence and apoptotic cell death, thereby preventing tumor progression. These effects involve increased ROS production as well as inhibition of FoxO transcription factors and the subsequent down-regulation of antioxidant enzymes, including sestrin-3, MnSOD, and catalase. Pro-oxidant conditions can activate p53, resulting either in senescence by induction of the Cdk inhibitor p21 or in apoptosis by activation of BH3-only proteins. In addition, the atypical nuclear expression of Akt can induce apoptosis by triggering unscheduled activation of the cell cycle-regulatory kinase Cdk2, either by direct phosphorylation or by induction of p27 degradation. Several interfering pathways and downstream effectors of Akt are omitted for clarity.

application, strongly sensitizes Akt-expressing cells to apoptosis, since it activates Akt *via* the inhibition of a negative feedback loop.[4] In conclusion, this new study by Nogueira *et al.* provides intriguing data demonstrating that cells with enhanced Akt activity can be selectively killed due to their hypersensitivity to ROS.

The induction of both cell death and survival begs the question of how Akt is able to mediate such opposing responses. There are many other examples of molecules that can trigger both pathways. Particularly, oncogenes such as Myc, Ras, and E2F1, which deliver strong mitogenic signals, have also been reported to cause cell death. Although sometimes interpreted as a conflict of growth signals, the most widely held view of oncogene-induced apoptosis is that cell-cycle entry sensitizes cells to apoptosis.[7,8] This dual-signal requirement provides an inbuilt tumor-suppressor function and ensures that oncogenic mutations also activate a suicide program in order to prevent uncontrolled proliferation. Interestingly, similar to Akt signaling, Myc, E2F1, and Ras-induced signaling are also linked to ROS accumulation, which, in the case of Ras, is reduced by sestrin-3.[9,10] Thus, obviously both proliferative and cell death pathways triggered by certain oncogenes engage overlapping signaling routes. It is noteworthy that not only do oncogenes possess pro-apoptotic activities but, conversely, many death regulators, including caspases, p53, and others, seem to exert additional vital functions unrelated to cell death.[11,12]

Additional support for a role of Akt in cell death

There are additional, still unappreciated indications for a pro-cell death activity of Akt. For instance, cells over-expressing the oncoprotein Bcr–Abl are also hypersensitive to ROS.[13] Abl kinase that is constitutively active as a result of its fusion to BCR phosphorylates PI3K and causes Akt hyperactivation.[14] Bcr–Abl also activates Ras, which favors a pro-oxidant state and, like up-regulated Akt, sensitizes cells to ROS-triggered apoptosis.[15] A pro-apoptotic activity of Akt

has also been reported in studies investigating the mechanism of action of apoptin, a viral protein that selectively kills cancer cells upon nuclear translocation.[16,17] Apoptin was found to trigger hyperactivation of Akt, which led to an unexpected activation of the proliferative kinase Cdk2. This activation of Cdk2 was required for apoptin-induced cancer cell death and was mediated by a direct phosphorylation event, as well as by apoptin-induced degradation of the Cdk inhibitor $p27^{Kip1}$.[17] Cdk2 is, therefore, another example of a cell cycle regulator that normally drives proliferation but, when activated abnormally, might also induce cell death.

Despite its established role at the plasma membrane, all components of the PI3K/Akt cascade can also be found in the nucleus. Although a nuclear role of Akt has been largely unexplored, nuclear Akt may phosphorylate substrates different from its targets in the cytoplasm. It has been reported that nuclear translocation of Akt is not harmful by itself, but strongly potentiates cell death induced by apoptin or certain anticancer drugs.[16] There are also other data supporting a pro-cell death function of Akt in the nucleus. For example, the pro-myelocytic leukemia tumor suppressor PML prevents tumor onset by inactivating Akt inside the nucleus.[18] Furthermore, nuclear, but not cytoplasmic, Akt interacts with Ebp1 (an inhibitor of caspase-activated DNase-dependent DNA fragmentation) and modulates its antiapoptotic action.[19] Thus, the net outcome of Akt activation apparently varies depending on signaling context and temporospatial characteristics. Whether activation of Akt in the nucleus is also linked to increased ROS production has not yet been explored, but remains an intriguing question.

Akt and senescence

Energy metabolism is tightly linked to aging, and calorie restriction has been shown to extend the life span of several organisms. As a paradigm, reduced insulin signaling through the PI3K–Akt pathway is associated with a decline in energy metabolism and prolonged life span.[20] In *Caenorhabditis elegans*, the insulin/IGF-1 → AGE-1/PI3K → Akt → DAF-16/FoxO pathway is considered the main regulator of life span. In particular, Akt phosphorylation prevents nuclear translocation of the FoxO transcription factor DAF-16, thereby reducing expression of antioxidant and growth-promoting target genes. In contrast, reduced Akt signaling permits DAF-16 promotion of increased lifespan.

In analogy to aging, Nogueira *et al.*[1] demonstrated that hyperactive Akt can also induce cellular senescence, which is characterized by a state of permanent cell cycle arrest. In addition to apoptosis, cellular senescence acts as an important barrier to neoplastic transformation.[20] Several stimuli, among them DNA-damaging agents and oncogenes, induce senescence and thereby prevent tumorigenesis. p53 serves as an integrator of these signals by controlling the expression of numerous target genes, presumably the most relevant of which in this context, is the CDK inhibitor $p21^{Waf1}$.[21] In many cases, induction of senescence appears to involve elevated ROS levels. Support for the involvement of ROS comes from experiments showing impaired senescence in the presence of low oxygen or antioxidants.[20] In agreement with the role of ROS in cellular aging, Nogueira *et al.*[1] showed that ROS production predisposes various tumor cell lines to senescence, whereas Akt-deficient cells are resistant to senescence. A similar mechanism is presumably responsible for induction of senescence upon mutation of PTEN.[22] Whether Akt-induced senescence requires p21-mediated cell cycle arrest is unknown. On the one hand, Akt can inhibit p21 expression through MDM2 activation and subsequent down-regulation of p53. In contrast, it was reported that the Akt2 isoform can bind and stabilize p21, thereby blocking cell cycle progression.[23] Thus, by increasing intracellular ROS levels, Akt appears to be a novel regulator of cellular senescence.

Future implications and clinical perspectives

The pro-apoptotic and senescent functions of Akt seem to be at odds with the well-established activity of Akt, namely the maintenance of cell survival and energy regulation. Why would Akt induce senescence or death in the same cell that it is trying to maintain? While some of the answers clearly lie in cell-type dependent differences, an interesting hypothesis is that the strength of Akt activation influences the cell's fate. In response to low levels of Akt activation, cells can remove ROS, and Akt allows for normal growth and development. In contrast, the anti-proliferative activities of Akt are presumably triggered in response to strong kinase activity, increased ROS production, and sustained inhibition of FoxO in order to prevent uncontrolled cellular proliferation.

Given the prevalent upregulation of Akt in cancer, there is much interest in the development of inhibitors that target this signaling pathway. The now-uncovered role of Akt in the induction of senescence and cell death is certainly highly interesting in terms of future therapeutic applications for tumors with dysregulated Akt activity. However, both Akt's ubiquitous expression and the multitude of pathways that are influenced by this molecule certainly pose pharmacological challenges. This presumably explains why attempts to develop Akt inhibitors for anticancer therapy have so far been largely unsuccessful.[3,24] Interestingly, the study by Nogueira *et al.*[1] suggests a new approach to target cancer, which exploits the ability of Akt to promote ROS accumulation. Indeed, the authors showed that high levels of Akt activity and

ROS predispose murine and human cancer cells to selective killing by oxidant stimuli, such as hydrogen peroxide or antioxidant-depleting drugs. No less remarkable is the finding that treatment with rapamycin, which augments Akt activation, specifically sensitizes cells to pro-oxidant drugs but not to traditional chemotherapeutics, such as etoposide.[1] The authors have therefore provided an important proof-of-principle for a treatment approach exploiting the hypersensitivity of tumors to oxidants. Thus, a combination of rapamycin and ROS may constitute a strategy for the selective eradication of cancer cells *via* Akt activation.

An important question is whether these findings, which have been generated in cell culture or animal models, can be extended to human tumors and how they are influenced by frequently observed alterations, such as p53 mutations or the constitutive activation of hypoxia-inducible factor (HIF). Normal Akt activity can counteract p53-controlled apoptosis by several mechanisms. Interestingly, however, p53 is also appreciated as a potent inducer of antioxidant genes, including sestrins and others.[25] Moreover, as PTEN is a p53 target gene, tumors with p53 mutations should have increased Akt activity. It will thus be intriguing to see whether loss of p53 synergizes with Akt in the induction of oxidative stress and cell death. One potential roadblock for an oxidant-induced treatment may be the presence of hypoxic conditions in established tumors. Thus, regardless of Akt activation, the therapeutic induction of oxidative stress could be problematic, as hypoxic tumors might be refractory to oxidative treatments. Therefore, it will be important to investigate how the particular tumor environment interferes with the growth-suppressive properties of Akt. Nevertheless, the newly uncovered role of Akt as a regulator of cell death and senescence provides important progress in our understanding of tumor biology and presents another example of the tight link between cell death and survival. Future research in this direction might identify novel treatment regimens for tumors with increased Akt activity that have so far been proven to be therapy-resistant.

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