REVIEW ARTICLE

Induction of Akt Activity by Chemotherapy Confers Acquired Resistance

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Resistance to chemotherapy is a major cause of treatment failure in human cancer. Accumulating evidence has indicated that the acquisition of resistance to chemotherapeutic drugs involves the activation of the PI3K/Akt pathway. Modulating Akt activity in response to chemotherapy has been observed often in chemoresistant cancers. The potential molecular mechanisms by which chemotherapeutic agents activate the PI3K/Akt pathway are emerging. Activation of this pathway evades the cytotoxic effects of chemotherapeutic agents via regulation of essential cellular functions such as protein synthesis, antiapoptosis, survival and proliferation in cancer. How chemotherapeutic agents induce Akt activation and how activated Akt confers chemoresistance through regulation of signaling networks are discussed in this review. Combining PI3K/Akt inhibitors with standard chemotherapy has been successful in increasing the efficacy of chemotherapeutic agents both *in vivo* and *in vitro*. Several small molecules have been developed to specifically target PI3K/Akt and other components of this pathway, which in combination with chemotherapy may be a valid approach to overcome therapeutic resistance. We propose several feedback and feedforward regulatory mechanisms of signaling networks for maintenance of the Akt activity for cell survival. These regulatory mechanisms may limit the efficacy of PI3K/Akt-targeted therapy; therefore, disruption of these mechanisms may be an effective strategy for development of novel anti-cancer therapies. [*J Formos Med Assoc* 2009; 108(3):180–194]

Key Words: Akt, antiapoptosis, cancer, drug resistance

Phosphoinositide 3-kinase (PI3K)/Akt has emerged as an important pathway in regulating the signaling of many fundamental biological processes, such as cell proliferation, survival and apoptosis, glucose metabolism, ribosomal function, gene transcription, and cell migration, via phosphorylation of a variety of substrates by the crucial serine/threonine kinase Akt. Dysregulation of the PI3K/Akt pathway is associated with the development of many human malignancies. Constitutive activation of the PI3K/Akt pathway occurs in tumor cells via several mechanisms, such as oncogene activation, gene amplification, and inactivation of tumor suppressors. Activated Akt contributes to tumor progression by modulating the function of numerous substrates related to the regulation of cell proliferation, antiapoptosis and angiogenesis, and therefore, is a valid target for anti-cancer therapy. PI3K/Akt signaling plays a major role not only in tumor growth, but also in the potential response of tumors to anti-cancer treatment. Accumulating evidence has indicated that activation of Akt promotes acquired resistance to treatment with radiation, chemotherapy,

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and/or targeted therapy. Therefore, specific inhibition of Akt activation may be a valid approach to treat human malignancies and overcome the resistance of cancer cells to anti-cancer therapy. Here, we discuss mainly the underlying mechanism of Akt activation for the acquisition of chemoresistance.

PI3K/Akt Activation in Tumors

The PI3K/Akt pathway is activated initially at the cell membrane, where the signals for pathway activation are propagated through PI3K. PI3K is composed of a catalytic subunit (p110) and an adapter/regulatory subunit (p85). It is activated by receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R), cell adhesion molecules such as integrins, G-protein-coupled receptors (GPCRs), and oncogenes such as Ras. Once activated, PI3K is responsible for the phosphorylation of plasma membrane lipid phosphatidylinositol-4,5-bisphosphate at the 3 position of its inositol ring, to generate the biologically active molecule phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P3]. Upon generation, PI (3,4,5)P3 recruits 3′-phosphoinositide-dependent kinase 1 (PDK1) and serine/threonine kinase Akt to the cell membrane, where they are subsequently activated, via binding to their pleckstrin homology (PH) domains. The tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10) antagonizes PI3K by dephosphorylating PI(3,4,5)P3, thereby blocking translocation and activation of PDK-1 and Akt.

The Akt kinase family consists of three structurally similar isoforms, Akt1, -2 and -3, which are expressed in most tissues.[1] Activation of Akt1 occurs through two crucial phosphorylation events. Upon recruitment to the cell membrane via binding to PI(3,4,5)P3 with its PH domain, Akt1 is initially activated through phosphorylation at T308 in the catalytic domain by PKD1.[2,3] For its full activation, a subsequent phosphorylation at S473 in the hydrophobic motif is required, which can be mediated by several kinases such as PDK1,[4,5] integrin-linked kinase,[6,7] Akt itself,[8] DNA-dependent protein kinase,[9,10] or mammalian target of rapamycin (mTOR).[11,12] Akt2 and Akt3 are also phosphorylated at the homologous residues, and thereby are activated by the same mechanism. Structural features of phosphorylated and non-phosphorylated Akt proteins have been reviewed previously.[13–15]

Aberrant activation of PI3K/Akt signaling pathways has been implicated in many cancers through several mechanisms.[14–16] As described above, PI3K/Akt signaling is initiated as a result of activation of RTKs or GPCRs. Since cell surface receptors are commonly overexpressed or constitutively activated in a large number of human cancers, downstream PI3K/Akt signaling is often activated as a result. One of the most extensively studied examples is the overexpression of erbB2 that is achieved by strong activation of PI3K/Akt in breast and other cancers. In addition to RTKs, other upstream molecules, such as protein kinase C (PKC), SHP1, Rac, Ras, Rho and Src, have been found to enhance PI3K/Akt activity in some human cancers.[16,17]

Dysregulation of the PI3K/Akt pathway in cancer cells also occurs because of gene amplification or mutations in the components of the pathway. The gain function of PI3K mutations has been frequently observed in many human cancers, such as ovarian, breast, gastric and hepatocellular carcinoma.[18] Active mutations of PI3KCA, the gene that encodes the p110a catalytic subunit of PI3K, have been observed in many cancers including, colorectal, gastric, breast, lung, ovarian, hepatocellular and thyroid cancer, glioblastoma, and acute leukemia.[19–26] Amplification and overexpression of PI3KCA have also been found in several other types of cancer, including cervical, gastric, ovarian and breast cancer, through a large-scale mutational analysis.[27–31] The amplification of Akt isoforms has been detected in a number of human cancers, including gastric carcinoma, glioblastoma, head and neck squamous carcinoma, pancreatic, ovarian, prostatic and breast cancer.[32–38] The tumor suppressor PTEN, an antagonist of

PI3K, is also frequently mutated or lost in primary glioblastoma, breast, lung and melanoma biopsies.[28,39–42] The elevated Akt activity caused by these mechanisms is correlated with progressive outcome and poor prognosis in some human cancers.[41,43,44]

Akt and PTEN Status Determines Intrinsic Sensitivity to Chemotherapy

Akt is considered to be a molecular "crutch" that cancer cells rely on early to escape cell death once they are exposed to toxic stimuli. Aberrant activation of Akt in most types of cancer has been found to correlate with poor prognosis and resistance to various anti-cancer therapies.[45] In a series of studies in human pancreatic adenocarcinoma and breast cancer cells that have constitutively active high Akt activity, treatment with gemcitabine, a nucleoside analog, failed to affect Akt activity or induce cell death.[46–48] Activation of Akt during preoperative chemotherapy for esophageal cancer has been observed and is correlated with poor prognosis.[49] Moreover, a retrospective review of a cohort of cervical squamous carcinoma patients showed that immunohistochemical staining of Akt phosphorylation was significantly more frequent in the radiation-resistance compared with the radiation-sensitive group.[50] pAkt signal is inversely correlated with the clinical benefit of hormone therapy in breast cancer patients.[51,52] Furthermore, ectopic expression of the constitutively active form of Akt decreases the sensitivity of NSCLC A549 and prostate cancer DU145 cells to cisplatin treatment. Conversely, expression of the Akt dominant negative mutant renders cisplatin-resistant NSCLC A549 and human ovarian cancer A2780 cells susceptible to cisplatin treatment.[53] These clinical correlations between Akt activation and poor treatment outcome and *in vitro* studies suggest that the Akt signaling pathway is a common mechanism for cellular insensitivity or acquired resistance to various anti-cancer therapies. However, some studies have indicated that PI3K/Akt activation is a favorable prognostic factor in acute myelogenous leukemia and cholangiocarcinoma patients.[54,55] The opposite functions of different Akt isoforms may explain this discrepancy.[56] A survival analysis of early-stage breast cancer has also revealed that Akt phosphorylation does not correlate with prognosis, nor predict resistance to anthracyclines,[57] and PTEN status provides the possible explanation for this discrepancy. Terakawa et al reported that the loss of PTEN, accompanied with Akt phosphorylation, is a poor prognostic factor for patients with endometrial cancer.[58] Among patients undergoing chemotherapy, PTEN-positive cases showed significantly better survival rates than negative or heterogeneous cases.[58] Overexpression of the PTEN gene promotes the chemosensitivity of bladder cancer cells and enhances the sensitivity of malignant glioma cells to irradiation.[59,60] These findings show that PI3K/Akt activity and PTEN status may have a major impact on the sensitivity and resistance to these treatments.

Chemotherapeutic Agents Induce Akt Activation

In addition to the implication of aberrant Akt activation in determining the intrinsic resistance of malignancies to chemotherapy, as described above, several lines of evidence also indicate that Akt activity can be modulated by chemotherapeutic agents, which contributes to the development of acquired resistance.

Cisplatin

As measured by Akt Thr308 and/or Ser473 phosphorylation and the phosphorylation state of its endogenous substrate GSK3, activation of Akt can be induced by cisplatin in several cancer cell lines, including ovarian,[61–63] breast,[62] glioma and pancreatic[62] cancer cells. Constitutively active Akt has also been found in cisplatin-selected chemoresistant lung,[53] glioma[63] and ovarian[63,64] cancer cell lines, compared with their sensitive parental counterparts. Combined treatment with Ly294002,

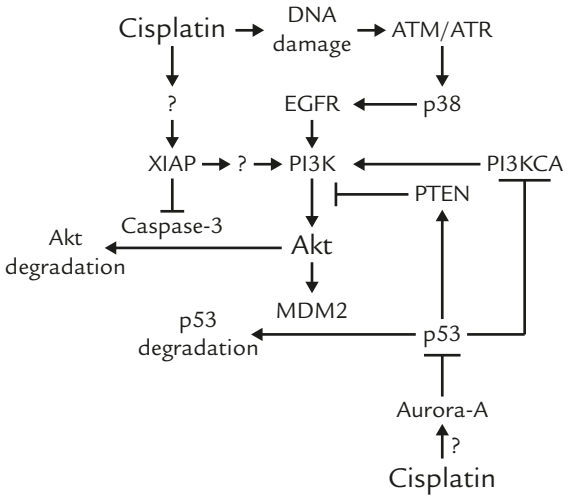

Figure. Signaling network of cisplatin-induced Akt activation.

a PI3K inhibitor, not only attenuates cisplatin-dependent Akt activity, but also enhances cisplatin-induced cytotoxicity, which indicates that Akt activation is caused by upstream activation of PI3K and renders cancer cells more resistant to cisplatin.[62] Several mechanisms have been proposed for mediating the cisplatin-induced PI3K/Akt activation, as illustrated in the Figure. The cisplatin-resistant NSCLC A549 cells, established by stepwise exposure to increasing concentrations of cisplatin, shows increases in Akt1 activity, protein and mRNA levels, and gene amplification compared with its parental cells.[53] The levels of pAkt signals in human lung tumor tissues are inversely related to the cisplatin sensitivity of primary cultured lung cancer cells from the same tumor tissues.[53]

In addition to Akt gene alterations, Tsang's group has shown that cisplatin is able to induce both X-linked inhibitor of apoptosis (XIAP) and Akt cleavage by activating caspase-3 in chemosensitive (A2780-s and OV2008) but not in cisplatin-resistant (A2780-cp and C13*) ovarian cancer cells.[61] Their data have also shown that overexpression of XIAP by sense adenoviral XIAP cDNA infection increases pAkt content, which is associated with a decrease in cisplatin-induced apoptosis. Since XIAP is able to bind to and inhibit caspase 3,[65] the increased level and activity of Akt in cisplatin-resistant cells may result from XIAP-mediated inhibition of caspase 3. However, in the presence of LY294002, overexpression of XIAP fails to increase Akt phosphorylation and block cisplatin-induced apoptosis.[61] These findings indicate that upregulation of Akt activity by XIAP may result from, not only the prevention of caspase-3-mediated Akt cleavage, but also modulation of upstream PI3K activity. However, it remains to be investigated whether XIAP upregulates this pathway by activating kinases (such as PI3K or PDK1), deactivating phosphatases (such as SH2-containing inositol polyphosphate-5-phosphatase or PTEN), or both. Interestingly, this group has also found that downregulation of XIAP by antisense adenovirus increases the sensitivity of p53 wild-type C13* cells to cisplatin, but is unable to induced apoptosis or increase cisplatin sensitivity in cisplatin-resistant, p53-mutated A2780cp cells.[66] This suggests that p53 status is a determinant of XIAP- and Akt-mediated cisplatin resistance in ovarian cancer cells.[67] It has been demonstrated that the induction of PTEN and reduction of PI3KCA expression by p53 at the transcriptional level leads to inhibition of Akt activity.[68,69] Our previous study has also shown that mouse double minute 2 (MDM2)-dependent ubiquitination and degradation of p53 is mediated by Akt, and is required for resistance to DNA-damaging agents.[70] This raises the possibility that the increased levels of XIAP and subsequent Akt activity in response to cisplatin downregulate p53 expression, to form a feedforward Akt activation loop via downregulation of PTEN but upregulation of PI3KCA expression (Figure).

Similar to this hypothetic model, Aurora-A serine/threonine protein kinase, which is frequently activated in epithelial malignancies, has also been found to induce cell survival and chemoresistance by activation of Akt through a p53-dependent mechanism in ovarian cancer cells. Aurora-A kinase can attenuate p53 DNA binding and transcriptional activity via phosphorylating its Ser215 residue.[71] Ectopic expression of Aurora-A abrogates p53-induced PTEN promoter activity, and thereby leads to Akt activation that confers cisplatin resistance.[64] The involvement of

XIAP and Aurora-A kinase in the regulation of p53 seems to be important for the Akt-mediated acquired resistance of ovarian cancer to cisplatin. However, the cross-talk among these molecules in the cisplatin-resistant cancer cells remains to be clarified.

Cisplatin-induced Akt activation has been shown to rely on EGFR activity, which is upstream of PI3K.[62] Interestingly, cisplatin-induced phosphorylation of EGFR is accompanied by EGFR internalization and ATM- or ATR-dependent activation of p38, which is known to occur as a result of cisplatin-induced DNA damage.[62,72,73] Inhibition of p38 can attenuate EGFR internalization. Direct phosphorylation of EGFR at T699 by p38 has been identified as essential for cisplatin-induced internalization of EGFR. Internalized EGFR can form signaling complexes in endosomes, and induces tyrosine phosphorylation and activation of the p85 subunit of PI3K,[74] which suggests that p38-dependent internalization of EGFR induced by cisplatin may drive PI3K/Akt activation through phosphorylation of its p85 subunit.

Etoposide

Etoposide, a podophyllotoxin, has pleiotropic actions within cells, including inhibition of topoisomerase II, generation of reactive oxygen species, and induction of DNA damage. Treatment with etoposide can induce PI3K/Akt activity in gastric cancer cells[75,76] and NIH 3T3 fibroblast cell lines,[77] which decreases the sensitivity to chemotherapy via prevention of apoptosis. However, a mechanistic connection between PI3k/Akt activation and the actions of etoposide remains to be established. EGFR activation signaling may contribute to etoposide-induced Akt activity and chemoresistance. Elevated peptide level of a heparin-binding EGF-like growth factor (HB-EGF), a ligand of EGFR, has been observed in etoposide-exposed HeLa cells.[78] A subset of EGF system ligands but not receptors, including amphiregulin, HB-EGF and epiregulin, has also been induced by etoposide in bladder cancer cell lines.[79] Etoposide increases the expression levels of these ligands via stabilizing their mRNA by a mechanism that involves the 3′ untranslated region,[78] but the precise mechanism awaits clarification. Activation of EGFR by these ligands in response to etoposide treatment may drive Akt signaling to override etoposide-induced apoptosis. Combined therapy with etoposide and EGFR inhibitors has been reported to have synergistic effects on induction of apoptosis, further supporting this hypothetical mechanism.[80]

In addition to EGF–EGFR autocrine activation, increased adhesion to the local environment may also account for the protection of tumor cells from etoposide-induced apoptosis via PI3k/Akt signaling. Activation of β1 integrin by extracellular matrix[81] and progenitor cell marker NG2/MPG[82] has been found to activate PI3k/Akt signaling, and to prevent etoposide-induced caspase-3 activation and subsequent apoptosis in small cell lung cancer (SCLC) and glioblastoma cells. Although the elevated Akt activity and integrin-dependent adhesion have been observed in etoposide-resistant SCLC cells,[83] whether and how etoposide activates integrin remain unclear.

Several other molecules that can drive PI3k/Akt activation have also been implicated in chemoresistance to etoposide. High levels of plasminogen activator inhibitor (PAI-1) in tumors are associated with poor prognosis in several cancer types.[84,85] PAI-1 has been shown to stimulate angiogenesis,[86] mediate/stimulate cell migration,[87] and modulate cell adhesion.[88] Silencing of PAI-1 by RNA interference in wild-type fibrosarcoma cells decreases the level of active Akt. This is accompanied by sensitization of the cells to etoposide-induced cell death, which suggests that PAI-1 influences sensitivity to etoposide-induced apoptosis through the PI3K/Akt cell survival pathway by acting upstream of PI3K and Akt.[89] Secretion of neuropeptide calcitonin (CT) from prostate cancer and activation of CT receptor (CTR) are associated with tumor progression. Addition of CT significantly induces Akt activation and attenuates etoposide-induced apoptosis in prostate cancer cell lines. Pretreatment with PI3K inhibitor LY 294002 can resensitize tumor cells to etoposide, which indicates that CTR activation confers

chemoresistance to etoposide via activating PI3k/Akt and downstream pathways.[90] Although autocrine regulation of PAI-1 and CT has been demonstrated to be involved in etoposide resistance through activation of the PI3k/Akt pathway, whether etoposide has a role in the secretion of PAI-1 and CT remains to be examined.

Paclitaxel

Activation of Akt by paclitaxel was observed firstly in ovarian cancer cells. Induction of activity and phosphorylation of Akt in response to paclitaxel treatment was detected at 30 minutes and, reached a plateau at 3 hours, and declined thereafter. This transient activation of Akt by paclitaxel mediates the following inhibitory regulation and phosphorylation of Raf-1, and prevents taxol-induced apoptosis.[91] Constitutive activation of Akt has also been found in acquired taxol-resistant HNE1-T3 cells, which are derived from human nasopharyngeal carcinoma (NPC) cell line HNE1 by exposure to increasing concentrations of taxol.[92] Interestingly, upregulation of TWIST, a basic helix–loop–helix transcription factor, has been found to mediate the development of acquired resistance to paclitaxel in an Akt-dependent manner.[93] Inactivation of TWIST by siRNA can lead to the suppression of Akt phosphorylation and decreased cell viability in response to taxol,[92] which suggests that the increased TWIST in NPC cells may be able to activate the Akt pathway, which in turn, attenuates paclitaxel-induced apoptosis. Moreover, the increased TWIST protein levels are also associated with another microtubule-targeting anticancer drug, vincristine, in several types of human cancer, including nasopharyngeal, bladder, ovarian and prostate cancer.[93] This indicates that TWIST/Akt may play a central role in the resistance to microtubule-disrupting agents. The TWIST-induced transcriptional expression of Akt2 is further proposed to mediate acquired resistance to taxol.[94] Although the precise molecular mechanism is not clear, EGFR activation may be involved in the paclitaxel-induced increase in TWIST level and subsequent Akt expression. A concomitant increase in EGFR and Akt activity has been observed in paclitaxel-treated lung and pancreatic cancer cells,[95,96] and determines the response to combined gefitinib/chemotherapy treatment.[95] A recent study has indicated that activation of EGFR induces STAT3-mediated TWIST gene expression,[97] which suggests that paclitaxel-induced EGFR activation, in addition to directly driving downstream Akt activation signaling, may also indirectly increase Akt protein level via TWIST-dependent gene upregulation.

Doxorubicin and daunorubicin

Elevated Akt activity in response to the anthracyclines doxorubicin and daunorubicin has been observed in gastric cancer,[75] leukemic cells,[98,99] and breast cancer.[100] Pretreatment with PI3K inhibitors or overexpression of PTEN attenuates the Akt activity and apoptosis induced by doxorubicin, which indicates that the PI3K/Akt pathway mediates the acquisition of resistance to doxorubicin.[75,98,100] Doxorubicin-induced Akt phosphorylation, in contrast to paclitaxel-induced transient Akt activation, occurs slowly at 6 hours after treatment, and is sustained continuously for 48 days,[75,99] which implies that PI3K/Akt activity is regulated at the transcriptional level.

Increased expression of forkhead transcription factor FOXO3a is involved in Akt activation and confers resistance to the cytotoxic effects of doxorubicin.[99] The mammalian FOXO family of transcription factors mediates expression of numerous genes that are related to cell proliferation, apoptosis and differentiation, and phosphorylation by Akt results in their nuclear exclusion and prevents their transcriptional activity. Hu and Hung provided evidence that chronic exposure of chronic myelogenous leukemia (CML) cells to doxorubicin increases nuclear accumulation of FOXO3a.[101] Nuclear FOXO3a targets gene expression of the PI3K catalytic subunit p110α, and knockdown of p110α reduces Akt activation and sensitizes tumor cells to doxorubicin. The results of Hu and Hung suggest that chronic activation of FOXO3a by doxorubicin in CML cells can enhance survival through feedback hyperactivation

of Akt by increasing p110α expression.[101] Since FOXO3a is inactivated and excluded from the nucleus by Akt, the nuclear accumulation of FOXO3a and induction of PI3K/Akt activity are transient. However, this homeostatic mechanism seems to be disrupted in doxorubicin-resistant cells, which results in constitutive nuclear expression of FOXO3a and consequent PI3k/Akt activation. The mechanism by which doxorubicin treatment allows FOXO3a to evade Akt-dependent cytoplasmic sequestration and inactivation remains to be explored. Nuclear FOXO3a can induce apoptosis via mediating expression of several proapoptotic genes, such as Fas ligand, Bim and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL),[101] which contrasts with the pro-survival effect of induced p110α expression and subsequent Akt activation in doxorubicin-resistant cells. It is not yet clear whether this non-traditional Akt/FOXO3a relationship causes the doxorubicin-resistant phenotype.

More recently, two peaks of Akt activation in response to doxorubicin and γ-irradiation have been observed: a more prominent peak after 30 minutes treatment with doxorubicin treatment and a smaller peak at 4 hours. The doxorubicin-induced Akt activation was abolished in DNA-PKcs–/– MEFs over time, which suggests that DNA-PK activated by DNA double strand breakage induces Akt activation for cell survival after exposure to DNA-damaging agents.[102,103]

Molecular Mechanism of Akt Conferring Drug Resistance

The action of most chemotherapeutic agents is thought to be mainly associated with their ability to induce apoptosis, which involves cytochrome c release and the apoptosome-catalyzed caspase cascade. The failure of drug-induced apoptosis appears to be a critical cause of chemoresistance. Upon activation, PI3K/Akt signaling confers acquired resistance to chemotherapy through its antiapoptotic effects, via phosphorylation of several downstream targets.

p53-independent antiapoptosis

Most directly, active Akt is thought to inhibit apoptosis by phosphorylating molecules upstream and downstream of mitochondrial perturbation. Phosphorylation of proapoptotic Bcl-2 family members such as Bad at Ser136,[104,105] Bax at Ser184,[106] and Bim(EL) at Ser87[107] has been shown to decrease their ability to hold mitochondria in an open configuration, and thereby reduce cytochrome c release. Downstream to cytochrome c release, Akt can phosphorylate caspase-9 at Ser196 to inhibit its protease activity, which activates executioner caspase 3.[108] In contrast, Akt also reduces cytochrome c release through regulation of antiapoptotic Bcl-2 homologous Mcl-1 at the post-translational level.[109] Phosphorylation of Mcl-1 at Ser159 by GSK-3 leads to ubiquitinylation and degradation of Mcl-1, inducing Bax and Bak oligomerization in a Bim-dependent manner for mitochondrial outer membrane permeabilization and release of cytochrome c. Increased Akt activity can abrogate this apoptotic process through inhibitory phosphorylation of GSK-3. Moreover, upregulation and phosphorylation at Thr34 of survivin, an inhibitor of apoptosis protein (IAP), in an Akt-dependent manner has also been observed in cisplatin-resistant SCLC cells, and protect cells from cisplatin-induced apoptosis.[110] The survivin phosphorylation at Thr34 is known to be mediated by the cdc2–cyclin B1 complex.[111] Therefore, whether Akt phosphorylates survivin directly or by indirectly activating cdc2–cyclin B1 or other kinases awaits investigation.

p53-dependent antiapoptosis

Akt exerts indirect inhibition of apoptosis through regulation of transcription factors. Transcription factor p53 is the most important regulator of apoptosis in response to DNA-damaging agents such as cisplatin.[112] p53 induces apoptosis and controls cytochrome c release via transcriptional upregulation of gene products such as Bax, p53-upregulated modulator of apoptosis (PUMA) and NOXA,[113–116] and transcriptional repression of gene products such as Bcl-2 and survivin.[115,117,118] Rapid stabilization and activation of p53 in

response to DNA-damaging agents occur through its site-specific phosphorylation, which attenuates bindings of p53 to proto-oncoprotein MDM2, and E3 ligase mediates the ubiquitination of p53 and facilitates its proteasomal degradation. We, and others, have reported that activated Akt physically associates with and phosphorylates MDM2 at Ser166 and Ser186, in response to HER2 overexpression and other growth factors.[70,119–124] Phosphorylation of MDM2 enhances its nuclear localization, and thus increases p53 ubiquitination and degradation.[70,124] Furthermore, upregulation of PTEN by the synthetic compound 3,5-bis-(2-fluorobenzylidene)piperidin-4-one can resensitize cisplatin-resistant ovarian cancer cells through induction of G2M arrest and apoptosis by attenuating Akt- and MDM2-mediated p53 downregulation.[125] Inhibition of p53 phosphorylation and nuclear accumulation and subsequent p53-mediated PUMA upregulation by Akt have also been observed in cisplatin-resistant ovarian cancer cells.[126] These findings indicate that Akt activation confers chemoresistance, in part, through downregulation of p53 via enhancing MDM2 activity and subsequent control of p53-regulated gene expression. p53 also regulates apoptosis through a transcription-independent mechanism. p53 protein can directly induce permeabilization of the outer mitochondrial membrane by forming complexes with the protective Bcl-xL and Bcl2 proteins. This results in cytochrome c and Smac release,[127,128] which was observed in cisplatin-treated cells.[129] Akt has also been reported to attenuate mitochondrial p53 accumulation and Smac/cytochrome c/Omi release, thereby contributing to cisplatin resistance.[129] However, whether the inhibition of p53 accumulation in mitochondria by Akt is also mediated in an MDM2-dependent manner remains to be examined.

Other transcriptional functions in antiapoptosis

In addition to p53, Akt also regulates other transcription factors such as cAMP-responsive element-binding protein (CREB), members of the Forkhead family, and nuclear factor (NF)-κB to increase antiapoptotic and pro-survival gene expression or decrease proapoptotic gene expression. Akt activates CREB via phosphorylation of its Ser133 residue.[130] CREB can induce Bcl-2 expression in a myriad of cell types.[131,132] Loss of PTEN or overexpression of Akt has also been found to induce CREB-mediated Bcl2 expression, which contributes to chemoresistance of advanced prostate cancer cells.[133] Akt also inhibits the expression of proapoptotic Bcl2 family members through inactivation of FOXO proteins. Akt-dependent phosphorylation of FOXO proteins occurs in the nucleus, and facilitates their interactions with 14-3-3, which displace FOXO transcription factors from target genes and trigger their export from the nucleus. Through this mechanism, Akt attenuates FOXO-mediated transcription of target genes that promote apoptosis, cell-cycle arrest and metabolism.[134] Akt-dependent control of NF-κB gene transcription has also been implicated in Akt-mediated antiapoptotic or survival signaling.[135–138] The involvement of Akt-mediated NF-κB activation in chemoresistance has been observed in various human cancers[75,79,139–143] The antiapoptotic activities of NF-κB result from transactivation of antiapoptotic factors such as IAP proteins, cFLIP and Bcl-xL.[110,144–149] In addition to the regulation of antiapoptotic gene expression, Akt-dependent NF-κB activation also mediates the transactivation of transmembrane transport pump P-glycoprotein, which causes efflux of chemotherapeutic agents from cells and is an important system that secures multidrug resistance of neoplastic cells.[139,142,143]

Caspase-independent antiapoptosis

Akt activation also mediates the inhibition of caspase-independent apoptosis. Cisplatin induces the mitochondrial release and nuclear translocation of apoptosis-inducing factor (AIF), a mediator of caspase-independent apoptosis, and AIF-dependent apoptosis. Activation of Akt attenuates the cisplatin-induced mitochondrial release and accumulation of AIF and apoptosis in chemosensitive ovarian cancer cells, whereas inhibition of Akt activity facilitates these effects and sensitizes

chemoresistant cells to AIF-dependent, cisplatin-induced apoptosis.[150]

Cell growth

Another important function of Akt in chemoresistance is its role in promoting cell growth. Akt is linked to the survival pathway of mammalian target of rapamycin (mTOR), a serine/threonine kinase that is implicated in protein synthesis control, which can be potently inhibited by the immunosuppressant rapamycin. Activation of mTOR enhances tumor growth and chemoresistance via inhibiting cell death pathways such as apoptosis and autophagy.[151–153] Akt activates mTOR complex 1 (mTORC1; or mTOR-raptor complex) indirectly by inhibiting phosphorylation of tuberous sclerosis complex 2 (also known as tuberin), thereby allowing Ras-related small G protein (Rheb)-GTP to activate mTORC1 signaling.[134] Survival results from mTORC1, which drives translation by activation of ribosomal S6 kinase (p70S6K) and release of eukaryotic initiation factor 4E by phosphorylating its inhibitor binding protein 4E-BP1.[154,155] By controlling protein synthesis, p70S6K and 4E-BP1 also control cell growth and hypertrophy,[156] which are important processes for tumor growth.

Combined Therapy with Chemotherapy and PI3K/Akt/mTOR Inhibitors, and Possible Limitations

Since deregulated signaling through activated PI3K/Akt/mTOR pathways contributes to the development of cancer resistance to chemotherapy, targeting PI3K/Akt/mTOR activation has emerged as a promising combinatory approach with conventional chemotherapy for successful treatments of cancer. Combinations of pathway inhibitors, including PI3K inhibitors LY294002 and wortmannin, Akt inhibitors perifosine and triciribine, and PIAs, and mTOR inhibitors rapamycin and its analogs, with various types of chemotherapy have been investigated extensively in preclinical studies, and have shown synergistic efficacy with chemotherapeutic agents *in vivo*. An update on the clinical progress and efficacy of combinatory therapies with PI3K/Akt/mTOR inhibitors has been presented in a review.[157] Only a few clinical trials with Akt inhibitor perifosine and mTOR inhibitors rapamycin, CCI-779 and RAD-001 have been reported, while no clinical trials using PI3K inhibitors have been published. Whether any of these compounds might be truly useful in the management of patients with cancer awaits further study. Here, several feedback and feedforward regulatory mechanisms have been proposed to maintain homeostatic Akt activation for cell survival. These regulatory mechanisms may limit the efficacy of PI3K/Akt-targeted therapies. Therefore, disruption of this regulation of Akt activity may be an effective strategy for the development of novel cancer therapies. Another possible limitation result from the development of cross-talk with other pathways. Hyperactivation of mitogen-activated protein kinase (MAPK) signaling has been observed when cells are treated with the PI3K inhibitor LY294002, which indicates that, even with inhibition of PI3K/Akt pathways, MAPK activation compensates the survival signaling while Akt signaling is inhibited.[158] The other predicted compensator will be IKK, since our own studies and those of others have indicated that these three oncokinases, including Akt, ERK and IKK, exert a concerted regulation on several substrates such as TSC and FOXO3a, and jointly regulate the survival-related signaling network.[159] This suggests that signaling cross-talk between the PI3K/Akt and other pathways occurs during treatment, which highlights the potential pitfall of targeting the PI3K/Akt pathway in cancer therapy.

Conclusion

The PI3K/Akt pathway orchestrates multiple cell responses including mitogenic signaling, cell survival and growth, metabolic control, and tumor metastasis. Aberrant PI3K/Akt activation, which is believed to be a major component contributing to the intrinsic insensitivity of cancer cells to

chemotherapy, is caused by genetic alterations. Accumulating evidence indicates that activation of the PI3K/Akt pathway is also a consequence of administration of many types of chemotherapeutic agents. The chemotherapy-induced PI3K/Akt activation may account for the major limitation of successful and durable clinical responses to chemotherapy, and for the development of acquired resistance. Therefore, knowledge of PI3K/Akt activation by chemotherapeutic agents may have important clinical implications in cancer chemotherapy. The important issues to be addressed are whether PI3K/Akt activation is a general determinant of acquired resistance to various chemotherapeutic agents, and whether activation of other oncokinases in concert with Akt also participates in the development of chemoresistance. Such clinical data are almost missing nowadays. Future work may identify the mechanisms involved in activation of PI3K/Akt by chemotherapy, and provide new targets for combination therapy aimed at circumventing or decreasing chemoresistance.

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