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Review

Phosphatidylinositide 3-kinase/AKT in radiation responses

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Summary. Ionizing or ultraviolet radiation-induced cellular survival signaling pathways induce development of cancer and insensitivity of tumor cells to radiation therapy. Accumulating evidence suggests that the phosphatidylinositide 3-kinase (PI3K)/AKT signal pathway is a major contributor to radioresistance. In many cell types PI3K/AKT signaling is a key cytoprotective response downstream of the EGFR family receptors and mediated carcinogenesis. Cytokines, such as HGF, IGF-I, and IL-6 also protects cells against apoptosis induced by radiation through PI3K/AKT pathway. The mechanics by which PI3K/AKT signaling functions in radiation responses may include its regulation of mitochondrial proteins, transcription factors, translation machinery, and cell-cycle progression. In addition, cross-talk between the PI3K/AKT pathway and mitogen-activated protein kinases, protein kinase A, and protein kinase C signal pathway may also play an important role.

Key words: Radiation, Phosphatidylinositide 3-kinase, Signal transduction, Cross-talk

The phosphatidylinositide 3-Kinase/AKT signal pathway

Phosphatidylinositide 3-Kinase (PI3K) enzymes consist of two subunits, a catalytic P110 subunit and a regulatory and localizing subunit, P85. Several different classes of PI3K enzymes exist (Wymann and Pirola, 1998; Vanhaesebroeck and Alessi, 2000). The P85 subunit of PI3K enzymes contains a phosphotyrosine (SH2)-binding domain (Ching et al., 2001). The major catalytic function of the PI3K is in the P110 subunit that acts to phosphorylateinositol phospholipids (PIP2: phosphatidyl inositol 4,5 bisphosphate), in the plasma membrane at the 3-position within the inositol sugar ring.

The proto-oncogene *c-akt*, encoding a 57-kDa serine/threonine protein kinase, is the cellular homolog of the viral oncogene v-akt (Bellacosa et al., 1991). AKT, also known as protein kinase B, is catalytically activated by phosphorylation at Thr308 and Ser473. Binding of cytokines to its receptor triggers activation of PI3K, enabling PI3K to phosphorylate phosphoinositides (Chan et al., 1999). Phosphorylated phosphoinositides bind to the pleckstrin homology domains of AKT and PDK1, resulting in their plasma membrane translocation, and phosphorylate AKT at Thr308 and Ser473 (Bellacosa et al., 1998). Dually-phosphorylated active AKT is then able to phosphorylate and thereby inactivate the pro-apoptotic protein Bad (Datta et al., 1997) and the pro-apoptotic FOXO transcription factor FKHRL1 (Brunet et al., 1999) as well as to phosphorylate Iκ B kinase, promoting the expression of anti-apoptotic genes through activation of nuclear factor-κ B (Romashkova and Makarov, 1999).

The PI3K/AKT signal pathway in radiation responses

Ionizing radiation has been previously shown to rapidly activate kinases, and contributes to tumor cell viability. Sensitivity of tumor cells to radiation therapy is a critical determinant of the probability of local control and, ultimately, of cure (Peters and Brock, 1993; West et al., 1993).

A number of studies have shown a positive relationship between epidermal growth factor receptor (EGFR) expression and tumor resistance to radiation (Sheridan et al., 1997). The EGFR family consists of four closely-related growth factor receptors, including EGFR or HER-1 (*erb*-B1), HER-2 (*erb*-B2/*neu* or p185neu), HER-3 (*erb*-B3), and HER-4 (*erb*-B4). EGFR binds several distinct ligands, including EGF, transforming growth factor-α, and ampheregulins.

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EGFR signaling leads to radiation resistance. In some cell types, the antiapoptotic effects of EGFR receptor signaling have been attributed to activation of the PI3K/AKT pathway (Kainulainen et al., 2000). EGFR signaling to PI3K/AKT has been proposed to enhance the expression of the mitochondrial anti-apoptosis proteins Bcl-xL and Mcl-1 and caspase inhibitor proteins such as c-FLIP isoforms (Leverrier et al., 1999; Kuo et al., 2001; Panka et al., 2001). Enhanced expression of Bcl-xL and Mcl-1 will protect cells from apoptosis via the intrinsic/mitochondrial pathway, whereas expression of c-FLIP isoforms will block killing from the extrinsic pathway via death receptors (Suhara et al., 2001). In addition, AKT has been shown to phosphorylate Bad and human procaspase 9, thereby rendering these proteins inactive in apoptotic processes (Li et al., 2001). Inhibitors of EGFR signaling have been shown to decrease the activity of the PI3K/AKT pathway in a variety of cell types and to increase the sensitivity of cells to a wide range of toxic stresses including cytotoxic drugs and radiation (Pianetti et al., 2001). Activation of AKT was shown to protect cells from death in the presence of EGFR receptor inhibition (Cuello et al., 2001). These findings strongly argue that PI3K/AKT signaling is a key cytoprotective response in many cell types downstream of the EGFR family receptors.

Ultraviolet (UV)-initiated signal transduction pathways in some circumstances have tumor promotion effects (Staberg et al., 1983). It has been reported that exposure of mammalian cells to UV radiation including short (UVC, 200–280 nM), long (UVA, 320–400 nM), and mid- (UVB, 280– 320 nM) wavelengths leads to a large number of changes in cells such as activation of transcription factors and protein kinases, and leads to the expression of genes that are observed to be up-regulated in different types of cancer in addition to skin cancer (Ronai and Weinstein, 1988; Matsui and DeLeo, 1990; Devary et al., 1991; Huang et al., 1999).

While UV activates cell survival pathways to fight against UV-induced cell death, the cell survival of mutated cells could lead to overexpression of certain oncogenes thereby causing skin cancer. One possible mechanism for UVB-induced carcinogenesis involves its ability to induce COX-2 expression. It has been reported that up-regulation of COX-2 in respones to UV radiation may be mediated by the PI3K/AKT pathway (Tang et al., 2001). Induction of COX-2 causes increased prostaglandin synthesis, a phenomenon associated with UV-induced tumorigenesis (Grewe et al., 1993; Fischer et al., 1999).

Hepatocyte growth factor/scatter factor (HGF/SF) not only protects cells against apoptosis induced by UV and ionizing X-rays (Fan et al., 1998), but also stimulates DNA repair activity. HGF/SF induced the phosphorylation of AKT, and stabilization of the expression of Bcl-xL. These biological effects of HGF/SF could be inhibited by wortmannin, suggesting that these activities of HGF/SF are due, in part, to a PI3K- and AKT-dependent signaling pathway. Another major survival factor, insulin-like growth factor I (IGF-I) is also able to protect cells from apoptosis under a wide variety of circumstances, including radiation with UVB. Kulik and his colleagues (1998) reported that although signal transduction pathways used by the IGF-I receptor in protecting cells from apoptosis includes PI3K/AKT, mitogen-activated protein kinase (MAPK), p38/HOG1, and p70S6 kinase, only the activation of PI3K and its effector AKT did correlate with the regulation of apoptosis in Rat-1 fibroblasts system induced by radiation with UVB.

Furthermore, radiation of the vascular endothelium alone is sufficient to induce AKT phosphorylation through a PI3K-dependent mechanism, and PI3K contributes to endothelial cell viability (Edwards et al., 2002). Mutations or down-regulation of tumor suppressor gene Phosphatase and Tensin (PTEN), which directly antagonizes PI3K, have been observed in a number of human cancers (Dahia et al., 1999), and the mutation is associated with AKT activation (Suzuki et al., 1998; Davies et al., 1999). The alteration of PTEN causes elevated phosphorylation of AKT. Wick et al. (1999) have shown that expression of PTEN, the phosphatase that counteracts the effects of PI3K, radiosensitizes glioma cells lacking a functional copy of this gene.

However, some evidence also suggests that radiation-induced activation of AKT is partially independent of PI3K. Examples of PI3K-independent activation of AKT have been described previously. Expression of upstream oncogenes such as Src and Ras produce AKT activity that is not completely abolished by PI3K inhibitors (Liu et al., 1998) and some authors have demonstrated that the calmodulin kinase kinase is a PI3K-independent mechanism for AKT activation (Yano et al., 1998).

The mechanisms by which PI3K/AKT signaling functions in radiation responsesPI3K/AKT and reactive oxygen species (ROS)

Some UV-induced genes are believed to be regulated by an oxidative mechanism (Tyrrell, 1996). Naturally occurring free radicals typically include ROS and reactive nitrogen species (Lander, 1997). In addition to inducing cellular injury such as DNA damage and lipid peroxidation, free radicals also function as intracellular messengers (Sen and Packer, 1996; Lander, 1997). UV radiation leads to the generation of ROS, especially H_2O_2, which is responsible for an increase in AKT phosphorylation at Ser473 and Thr308 in mouse epidermal Cl 41 cells. Data are accumulating which indicate a vital role of ROS in mediating cellular responses to various extracellular stimuli. It has been reported that free radicals are involved in the production of cytokines, growth factors, and hormones in the activation of nuclear transcription factors, gene transcription, neuromodulation, and apoptosis (Tyrrell, 1996; Sen and Packer, 1996; Lander, 1997). For

example, it has been reported that the generation of H_2O_2 is required for platelet-derived growth factor signal transduction (Sundaresan et al., 1995).

The role of PI3K/AKT in the regulation of mitochondrial proteins

In vivo cooperation between Bcl-xL and the PI3K/AKT signaling pathway for the protection of epidermal keratinocytes from apoptosis induced by UVB radiation has been observed in animal models (Umeda et al., 2003). Contribution of the PI3K/AKT signaling pathway to the protection of Bcl-xL-deficient keratinocytes from apoptosis was clearly demonstrated by in vitro inhibition experiments using wortmannin. Upon activation by PI3K, AKT induces phosphorylation of Bad at Ser136 (Datta et al., 1997). Bad phosphorylation results in sequestration in the cytoplasm in association with 14–3-3 proteins leaving from a mitochondrial location after dissociation with antiapoptotic Bcl-2 members (Zha et al., 1996).

Alternatively, besides Bad phosphorylation, recent studies have found that AKT can directly regulate caspase activation either at a premitochondrial level (Kennedy et al., 1999) or at a postmitochondrial level downstream of cytochrome c release and before activation of caspase-9 (Zhou et al., 2000). A recent report demonstrated that the PI3K/AKT pathway was required for keratinocyte survival independent of Bcl-xL expression (Jost et al., 2001).

Epicutaneous treatment with wortmannin of Bcl-xL$^{-/-}$ mice resulted in a marked sensitization to UVB radiation, control mice were not significantly affected by this treatment, suggesting that dependency on PI3K/AKT was reciprocal to Bcl-xL expression. UVB radiation resulted in translocation of phosphorylated AKT from the basal cell layer to throughout the epidermis in wild-type and Bcl-xL$^{-/-}$ mice, although the underlying mechanism remains to be elucidated. Since Bcl-xL is expressed predominantly in the suprabasal keratinocytes, the redistribution of active AKT over the suprabasal layer might represent the spatial compensation for Bcl-xL deficiency upon UVB radiation. Thus, these data provide compelling evidence that AKT can compensate for Bcl-xL deficiency to form a "fail-safe" system against apoptotic stimuli (Umeda et al., 2003).

Mcl-1 is an antiapoptotic protein of the Bcl-2 family. Experimentally, the PI3K/AKT signaling pathway is important for IL-6-elicited anti-apoptotic signaling and Mcl-1 expression in human keratinocyte cells when exposed to UV radiation (Petit-Frere et al., 1998). Unlike the phosphorylation of Bad or procaspase 9, the PI3K/AKT pathway upregulates the Mcl-1 expression at the level of transcription. Interestingly, unlike the situation in the hematopoietic cells, the PI3K pathway is commonly activated and necessary for Mcl-1 upregulation in a wide array of epithelial cancer cells, including hepatoma cells (Kuo et al., 2001), prostatic cancer cells (Chung et al., 2000), cervical cancer cells (Wei et al., 2001), and basal cell carcinoma cells (Jee et al., 2002).

The role of PI3K/AKT in the regulation of translation machinery

Recent studies have shown that low-energy laser radiation (LELI) significantly enhanced the regeneration process. LELI promotes cell proliferation by inducing translation of early G1-phase regulatory proteins (Ben-Dov et al., 1999). Previous studies have shown that induction of early G1-phase regulatory proteins, such as c-myc (Mendez et al., 1996) and cyclin D1 (Barbet et al., 1996) requires de novo mRNA and protein synthesis, resulting from translation of pre-existing mRNAs (Brown and Schreiber, 1996). Eukaryotic initiation factor 4E (eIF4E) is a major regulator of cap-dependent mRNA translation in response to proliferative stimuli (Polunovsky et al., 1996). One of the mechanisms known to regulate eIF4E is phosphorylation-dependent dissociation of a translational-repressor protein, i.e. protein heat and acid stable (PHAS-I), also referred to as eIF4E-binding protein-1 (4EBP1) (Lin et al., 1994). The non- or partially phosphorylated form of PHAS-I, which strongly interacts with eIF4E, limits the latter's availability of eIF4E to the translation process. LELI induced the phosphorylation of PHAS-I, which was abolished by the addition of the PI3K inhibitor wortmannin, suggesting this phosphorylation to be PI3K-dependent. Moreover, LELI induced the phosphorylation of mammalian target of rapamycin (mTOR), which was directly mediated by AKT, and in turn induced PHAS-I phosphorylation (Raught and Gingras, 1999). The fully phosphorylated PHAS-I dissociated from eIF4E, allowing the latter to form the initiation complex and translation to proceed (Gingras et al., 1999, 2001). Taken together, it was suggested that PI3K-dependent phosphorylation of AKT mediates the effect of LELI on PHAS-I phosphorylation and eIF4E availability to the translation machinery.

The role of PI3K/AKT in the regulation of transcription factors

Activator Protein-1 (AP-1), a protein complex consisting of members of the Fos and Jun protein families, is one of the major transcription factors that are upregulated in response to UVB radiation (Barthelman et al., 1998). There is a direct correlation between UVB-induced AP-1 activation and increased c-fos gene expression (Sheridan et al., 1997). Specific properties of PI3K suggest that it is likely to function as a mediator molecule in the UVB-induced signaling pathways that upregulate c-Fos and AP-1 expression. Studies in the JB6 murine epidermal cell line demonstrated that insulin or EGF-induced AP-1 transactivation required PI3K activity (Huang et al., 1996). Other studies in insulin-responsive rat fibroblasts (HIRc-B cells) demonstrated

that microinjection of the SH2 domain of the regulatory subunit p85 could inhibit the insulin-induced expression of c-Fos (Jhun et al., 1994). In another case, NIH3T3 cells that were transfected with a constitutively active mutant p110 construct displayed a marked increase in c-fos transactivation (Hu et al., 1995). UVB radiation induces the PI3K signaling pathway which is also involved in the upregulation of c-Fos in the HaCaT cell line.

The role of PI3K/AKT in the regulation of cell cycle checkpoint

AKT is not only a "cell survival" kinase but it may play an important role in regulation of cell-cycle progression (Muise-Helmericks et al., 1998; Medema et al., 2000). The classical pathway coupling DNA damage to cell-cycle arrest involves up-regulation of p53 and its transcriptional targets. In response to DNA damage, p53 induces the expression of growth-inhibitory genes, such as p21cip1/waf1 and GADD45 (Levine, 1997). However, the existence of p53-independent mechanisms resulting in cell-cycle arrest has been demonstrated in lymphoid cells derived from p53-/- mice (Strasser et al., 1994). Similarly, hematopoietic cell lines lacking p53 protein arrest in the G1 and G2/M phases after treatment with ionizing radiation (Quelle et al., 1998).

The activation of PI3K/AKT may be a general requirement for cytokines to override the growth arrest at G1 and G2/M checkpoints induced by γ-radiation. PI3K/AKT can overcome p53-independent cell cycle arrest. For example, Rat1a cells in which the p21cip1/waf1 promoter is inactive and which are thus deficient in p53-dependent cell-cycle checkpoints still respond to γ-radiation by transient G2 arrest, which is alleviated by activated AKT. In addition, it was recently shown that activation of AKT also has the potential of alleviating the p53-mediated cell-cycle checkpoints. AKT may exert its effect through the inhibition of the FOXO transcription factors that are downstream phosphorylation targets of AKT. It was recently shown that FOXO3a modulates the expression of several genes that regulate response to stress at the G2/M cell-cycle checkpoint (Tran et al., 2002). AKT may also exert its effect through phosphorylation and sequestration of p21cip1/waf1 and through enhanced degradation of p53 (Zhou et al., 2001).

Evidence also suggests a general role for the PI3K signaling pathway in regulating cell-cycle progression. Notably, PI3K activity can be sufficient to induce G1 transit in fibroblasts (Klippel et al., 1998) and is required for IL-2–dependent activation of E2F in T-cells (Brennan et al., 1997). Thus, downstream targets of PI3K/AKT–dependent pathways that regulate normal cell-cycle progression may also participate in overriding γ-radiation–induced checkpoints. PI3K activity has been shown to contribute to induced expression of D-type cyclins (Gille and Downward, 1999) and to increase cyclin D1 stability through AKT-dependent phosphorylation of GSK-3ß (Diehl et al., 1998). Alternatively, PI3K effects on G1-phase progression may be mediated through the downregulated expression of the Cdk inhibitor, p27kip1, because various inhibitors of the PI3K pathways have been shown to cause enhanced expression of p27kip1 protein (Brennan et al., 1997). Although PI3K activation does not appear to be required for EPO- or IL-3-dependent proliferation of non-irradiated cells, lack of PI3K activity in DNA-damaged cells could impair some or all of these events, resulting in growth arrest.

Cross-talk with other signal pathways in radiation responses

Cross-talk with MAPK superfamily

MAPK belongs to a large family of serine/threonine protein kinases and include extracellular signal-regulated protein kinases (ERKs), p38 kinase, and c-Jun N-terminal kinases (JNKs). ERKs are involved in survival signaling in response to a variety of growth factors, whereas activation of JNKs or p38 kinase is suggested to play decisive roles in the control of cell death (Xia et al., 1995). However, the activation of JNKs and p38 kinase and overexpression of MAP kinase kinase 6, an upstream kinase of p38 kinase, have been reported to protect cells from apoptosis (Roulston et al., 1998; Zechner et al., 1998).

ERKs are also critical for radiation-induced signal transduction (Dent et al., 2003). In some cell types, ERK and AKT signaling can cooperate to reduce the apoptotic threshold in cells. In some cases, the proapoptotic protein Bad is phosphorylated and inactivated by both ERK signaling (Serine112) and PI3K/AKT (Serine136) (Hayakawa et al., 2000). In addition, it is also possible that ERKs and AKT, via the P70 S6 kinase, may cooperate to inhibit Bad function (Harada et al., 2001). Recently, ionizing radiation has been shown to activate the P70 S6 kinase in an EGFR-, PI3K- and MEK1/2-dependent fashion (Carter et al., 1998; Contessa et al., 2002). Thus, radiation-induced P70 S6 kinase signaling may alter the apoptotic response of irradiated cells via the modulation of Bad phosphorylation.

JNK are strongly activated by diverse cell stresses, many of which induce cell death. UV radiation is a strong stimulator of JNK activity in PC12 cells, and JNK activation is associated with programmed cell death. It has been reported that AKT inhibits activation of JNK by cytotoxic stimuli in a manner correlated with induction of JNK interacting protein-1 (JIP-1), suggesting that the JNK pathway represents an additional point of antiapoptotic signaling by AKT (Levresse et al., 2000).

p38 MAP kinase plays an important and unique role in signal transduction pathways in response to UV radiation (Dent et al., 2003). Rane et al. (2001) recently showed that the p38 kinase pathway regulates AKT activation in human neutrophils. UV activates AKT via a

ROS-sensitive pathway in the early phase and UV-induced release of proinflammatory cytokines, such as TNF-α and IL-1ß leads to the feedback activation of p38, which in turn contributes to the prolonged activation of AKT in cultured human keratinocytes and in human skin in vivo (Strickland et al., 1999; Kulms et al., 2000). It seems then that a balance between AKT and p38 signal pathways may well be an important modulator between cell death and survival in response to UV radiation.

Cross-talk between different protein kinase pathways is often more complex than according to the above observations. For example, in JB6 mouse epidermal cells, it was demonstrated that the activation of AKT induced by UVB radiation is mediated by ERKs and p38 kinase, but not JNKs, through their downstream kinase, mitogen- and stress-activated protein kinase 1 (MSK1), in addition to the PI3K/PDK pathway (Nomura et al., 2001). In HaCaT cells, the activity of both PI3K and p38 kinases is required for UVB-induced AKT activation (Tang et al., 2001). In other studies, PI3K inhibitors repressed ERK activation in several cell types after various modes of stimulation (Cross et al., 1994; Welsh et al., 1994; Hawes et al., 1996). Overexpression of DN-p85 also impaired UVB-induced ERK phosphorylation, although the blocking of ERK activation has been suggested to be independent of PI3K activity (Scheid and Duronio, 1996; Ferby et al., 1996).

The results that ERKs and p38 kinase mediate AKT activation suggest a novel role for MAPKs in signal transduction. Investigators have speculated that the members of the MAPK family might not directly phosphorylate AKT. Activated MAPKs are translocated to the nucleus, where they phosphorylate several different transcription factors (Seger and Krebs, 1995; Gupta et al., 1995; Zinck et al., 1995; Chow et al., 1997; Deak et al., 1998). In addition to phosphorylating nuclear proteins, several cytoplasmic proteins (e.g. RSKs and MSKs), phospholipase A2, and the EGFR have been shown to be substrates for ERKs (Lin et al., 1993; Seger and Krebs, 1995; Deak et al., 1998; Frodin and Gammeltoft, 1999), and p38 kinase has been shown to phosphorylate cytoplasmic proteins (e.g. MAPKAP-Ks and MSKs) (Stokoe et al., 1992; McLaughlin et al., 1996; Deak et al., 1998). In contrast to ERKs and p38 kinase, which appear to have substrates outside the nucleus, substrates for JNKs are believed, to date, to be transcription factors exclusively. Moreover, UVB-activated MSK1 phosphorylated AKT at both Thr308 and Ser473, whereas UVB-activated MAPKAP-K2 phosphorylated AKT at only s Ser473, as previously demonstrated by Alessi et al. (1996). These facts may account for the differences between MAPK family kinases in the regulation of AKT.

Cross-talk with protein kinase A (PKA)

It has been reported that AKT could be activated in a PI3K-independent manner (Sable et al., 1997; Filippa et al., 1999). An agonist of the PKA pathway can activate AKT by increasing cytoplasmic calcium levels (Sable et al., 1997; Filippa et al., 1999). The increased calcium binds to calmodulin, and the Ca^{2+}/calmodulin complex activates the calcium/calmodulin-dependent kinase kinase, which then activates AKT by directly phosphorylating AKT at Thr308 (Yano et al., 1998). Although the details of the molecular mechanism for involvement of signal transduction pathways are not clear, PKA and calcium/calmodulin-dependent kinase kinase also play an important role in H_2O_2-mediated AKT phosphorylation by UV radiation. This hypothesis is supported by a previous finding that UV radiation induced a rapid increase in intracellular free calcium and transactivation of nuclear factor of activated T-cells, which is believed to depend on Ca^{2+}/calmodulin complex formation and activation of calcium/calmodulin-dependent kinase kinase (Huang et al., 2000).

Cross-talk with protein kinase C (PKC)

Recently, a cross-talk between the PI3K/AKT pathway and PKC activity has been observed. Overexpression of PKC stimulated AKT activity and suppressed cytokine-dependent apoptosis. On the other hand, the phorbol ester phorbol 12-myristate 13-acetate, an activator of PKC, down-regulates growth factor-induced AKT activation, and specific isoforms of PKC directly interact as negative regulators of AKT (Doornbos et al., 1999; Li et al., 1999; Zheng et al., 2000). Thus, PKC inhibitors might be potential modulators of this survival pathway. PKC inhibitor STP and clinically relevant antineoplastic derivatives, such as PKC412, down-regulate the activity of the PI3K/AKT-survival pathway in otherwise treatment-resistant cancer cells and sensitizes cancer cells to chemotherapy and radiotherapy.

Conclusions

The cellular response to radiation is complex; the balance between death, arrest, and survival is tipped by the presence or absence of signaling through specific pathways. Emerging studies have shown that the PI3K/AKT cell survival pathway is activated post ionizing radiation and UV radiation. PI3K/AKT-mediated survival pathways may fight imminent cell death and possibly induce development of cancer and insensitivity of tumor cells to radiation therapy. Because activation of PI3K/AKT may influence tumor response to therapy, the status of AKT might act as a prognostic marker and be a valid target to overcome an apoptotic threshold in efforts to improve the outcome of the associated disease.

References

Alessi D.R., Andjelkovic M., Caudwell B., Cron P., Morrice N., Cohen P.

and Hemmings B.A. (1996). Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 15, 6541-6551.

Barbet N.C., Schneider U., Helliwell S.B., Stansfield I., Tuite M.F. and Hall M.N. (1996). TOR controls translation initiation and early G1 progression in yeast. Mol. Biol. Cell 7, 25-42.

Barthelman M., Chen W., Gensler H.L., Huang C., Dong Z. and Bowden G.T. (1998). Inhibitory effects of perillyl alcohol on UVB-induced murine skin cancer and AP-1 transactivation. Cancer Res. 58, 711-716.

Bellacosa A., Chan T.O., Ahmed N.N., Datta K., Malstrom S., Stokoe D., McCormick F., Feng J. and Tsichlis P. (1998). Akt activation by growth factors is a multiple-step process: the role of the PH domain. Oncogene 17, 313-325.

Bellacosa A., Testa J.R., Staal S.P. and Tsichlis P.N. (1991). A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. Science 254, 274-277.

Ben-Dov N., Shefer G., Irintchev A., Wernig A., Oron U., Halevy O. and Irinitchev A. (1999). Low energy laser irradiation affects cell proliferation and differentiation in vitro. Biochim. Biophys. Acta 1448, 372-380.

Brennan P., Babbage J.W., Burgering B.M., Groner B., Reif K. and Cantrell D.A. (1997). Phosphatidylinositol 3-kinase couples the interleukin-2 receptor to the cell cycle regulator E2F. Immunity 7, 679-689.

Brown E.J. and Schreiber S.L. (1996). A signaling pathway to translational control. Cell 86, 517-520.

Brunet A., Bonni A., Zigmond M.J., Lin M.Z., Juo P., Hu L.S., Anderson M.J., Arden K.C., Blenis J. and Greenberg M.E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96, 857-868.

Carter S., Auer K.L., Birrer M., Fisher P.B., Schmidtt-Ulrich R.K., Valerie K., Mikkelsen R. and Dent P. (1998). Inhibition of the mitogen activated protein (MAP) kinase cascade potentiates cell killing by low dose ionizing radiation in A431 human squamous carcinoma cells. Oncogene 16, 2787-2796.

Chan T.O., Rittenhouse S.E. and Tsichlis P.N. (1999). AKT/PKB and other D3 phosphoinositide-regulated kinases: kinase activation by phosphoinositide-dependent phosphorylation. Annu. Rev. Biochem 68, 965-1014.

Ching T.T., Lin H.P., Yang C.C., Oliveira M., Lu PJ. and Chen C.S. (2001). Specific binding of the C-terminal Src homology 2 domain of the p85alpha subunit of phosphoinositide 3-kinase to phosphatidylinositol 3,4,5-trisphosphate. Localization and engineering of the phosphoinositide-binding motif. J. Biol. Chem 276, 43932-43938.

Chow C.W., Rincon M., Cavanagh J., Dickens M. and Davis R.J. (1997). Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway. Science 278, 1638-1641.

Chung T.D.,Yu J.J., Kong T.A., Spiotto M.T. and Lin J.M. (2000). Interleukin-6 activates phosphatidylinositol-3 kinase, which inhibits apoptosis in human prostate cancer cell lines. Prostate 42, 1-7.

Contessa J.N., Hampton J., Lammering G., Mikkelsen R.B., Dent P., Valerie K. and Schmidt-Ullrich R.K. (2002). Ionizing radiation activates Erb-B receptor dependent Akt and p70 S6 kinase signaling in carcinoma cells. Oncogene 21, 4032-4041.

Cross D.A.E., Alessi D.R., Vandenheede J.R., McDowell H.E., Hundal H.S. and Cohen P. (1994). The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletal muscle cell line L6 is blocked by wortmannin, but not by rapamycin: evidence that wortmannin blocks activation of the mitogen-activated protein kinase pathway in L6 cells between Ras and Raf. Biochem. J 303, 21-26.

Cuello M., Ettenberg S.A., Clark A.S., Keane M.M., Posner R.H., Nau M.M., Dennis P.A. and Lipkowitz S. (2001). Down-regulation of the erbB-2 receptor by trastuzumab (herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2. Cancer Res. 61, 4892-4900.

Dahia P.L., Aguiar R.C., Alberta J., Kum J.B., Caron S., Sill H., Marsh D.J., Ritz J., Freedman A., Stiles C. and Eng C. (1999). PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanismsin haematological malignancies. Hum. Mol. Genet. 8, 185-193.

Datta S.R., Dudek H., Tao X., Masters S., Fu H., Gotoh Y. and Greenberg M.E. (1997). Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91, 231-241.

Davies M.A., Koul D., Dhesi H., Berman R., McDonnell T.J., McConkey D., Yung W.K. and Steck P.A. (1999). Regulation of Akt/PKB activity, cellular growth, and apoptosis in prostate carcinoma cells by MMAC/PTEN. Cancer Res 59, 2551-2556.

Deak M., Clifton A.D., Lucocq J.M. and Alessi D.R. (1998). Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J. 17, 4426-4441.

Dent P., Yacoub A., Fisher P.B., Hagan M.P. and Grant S. (2003). MAPK pathways in radiation responses. Oncogene 22, 5885-5896.

Devary Y., Gottlieb R.A., Lau L.F. and Karin M. (1991). Rapid and preferential activation of the c-jun gene during the mammalian UV response. Mol. Cell. Biol. 11, 2804-2811.

Diehl J.A., Cheng M., Roussel M.F. and Sherr C.J. (1998). Glycogen synthase kinase-3b regulates cyclin D1 proteolysis and subcellular localization. Genes Dev. 12, 3499-3511.

Doornbos R.P., Theelen M., van der Hoeven P.C.J., van Blitterswijk W.J., Verkleij A.J. and van Bergenen Henegouwen P.M.P. (1999). Protein kinase Czeta is a negative regulator of protein kinase B activity. J. Biol. Chem 274, 8589-8596.

Edwards E., Geng L., Tan J., Onishko H., Donnelly E. and Hallahan DE. (2002). Phosphatidylinositol 3-Kinase/Akt signaling in the response of vascular endothelium to ionizing radiation. Cancer Res. 62, 4671-467.

Fan S., Wang J-A., Yuan R-Q., Rockwell S., Andres J., Zlatapolskiy A., Goldberg I.D. and Rosen E.M. (1998). Scatter factor protects epithelial and carcinoma cells against apoptosis induced by DNA-damaging agents. Oncogene 17, 131-141.

Ferby I.M., Waga I., Hoshino M., Kume K. and Simizu T. (1996). Wortmannin inhibits mitogen-activated protein kinase activation by platelet-activating factor through a mechanism independent of p85/p110-type phosphatidylinositol 3-kinase. J. Biol. Chem. 271, 11684-11688.

Filippa N., Sable C.L., Filloux C., Hemmings B. and Van Obberghen E. (1999). Mechanism of protein kinase B activation by cyclic AMP-dependent protein kinase. Mol. Cell. Biol. 19, 4989-5000.

Fischer S.M., Lo H.H., Gordon G.B., Seibert K., Kelloff G., Lubet R.A. and Conti C.J. (1999). Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. Mol. Carcinog. 25, 231-240.

Frodin M. and Gammeltoft S. (1999). Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol. Cell. Endocrinol. 151, 65-77.

Gille H. and Downward J. (1999). Multiple ras effector pathways contribute to G(1) cell cycle progression. J. Biol. Chem. 274, 22033-22040.

Gingras A.C., Raught B. and Sonenberg N. (1999). eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu. Rev. Biochem. 68, 913-963.

Gingras A.C., Raught B. and Sonenberg N. (2001). Regulation of translation initiation by FRAP/mTOR. Genes. Dev. 15, 807-826.

Grewe M., Trefzer U., Ballhorn A., Gyufko K., Henninger H. and Krutmann J. (1993). Analysis of the mechanism of ultraviolet (UV) B radiation-induced prostaglandin E2 synthesis by human epidermoid carcinoma cells. J. Invest. Dermatol. 101, 528 -531.

Gupta S., Campbell D., Derijard B. and Davis R.J. (1995). Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science 267, 389-393.

Harada H., Andersen J.S., Mann M., Terada N. and Korsmeyer S.J. (2001). p70S6 kinase signals cell survival as well as growth, inactivating the pro-apoptotic molecule BAD. Proc. Natl. Acad. Sci. USA 98, 9666-9670.

Hawes B.E., Luttrell L.M., Biesen T.V. and Lefkowitz R.J. (1996). Phosphatidylinositol 3-kinase is an early intermediate in the G beta gamma-mediated mitogen-activated protein kinase signaling pathway. J. Biol. Chem. 271, 12133-12136.

Hayakawa J., Ohmichi M., Kurachi H., Kanda Y., Hisamoto K., Nishio Y., Adachi K., Tasaka K., Kanzaki T. and Murata Y. (2000). Inhibition of BAD phosphorylation either at serine 112 via extracellular signal-regulated protein kinase cascade or at serine 136 via Akt cascade sensitizes human ovarian cancer cells to cisplatin. Cancer Res. 60, 5988-5994.

Hu Q., Klippel A., Muslin A.J., Fantl W.J. and Williams LT. (1995). Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase. Science 268, 100-102.

Huang C., Ma W.Y. and Dong Z. (1996). Requirement for phosphatidylinositol 3-kinase in epidermal growth factor-induced AP-1 transactivation and transformation in JB6 P+ cells. Mol. Cell. Biol. 16, 6427-6435.

Huang C., Ma W.Y., Maxiner A., Sun Y. and Dong Z. (1999). p38 kinase mediates UV-induced phosphorylation of p53 protein at serine 389. J. Biol. Chem. 274, 12229-12235.

Huang C., Mattjus P., Ma W.Y., Rincon M., Chen N.Y., Brown R.E. and Dong Z. (2000). Involvement of nuclear factor of activated T cells activation in UV response. Evidence from cell culture and transgenic mice. J. Biol. Chem. 275, 9143-9149.

Jee S.H., Chiu H.C., Tsai T.F., Tsai W.L., Liao Y.H., Chu C.Y. and Kuo M.L. (2002). The Phosphotidyl inositol 3-kinase/Akt signal pathway is involved in interleukin-6-mediated Mcl-1U pregulation and anti-apoptosis activity in basal cell carcinoma cells. J. Invest. Dermatol. 119,1121-1127.

Jhun B.H., Rose D.W., Seely B.L., Rameh L., Cantley L., Saltiel A.R. and Olefsky J.M. (1994). Microinjection of the SH2 domain of the 85-kilodalton subunit of phosphatidylinositol 3-kinase inhibits insulin-induced DNA synthesis and c-fos expression. Mol. Cell. Biol. 14, 7466-7475.

Jost M., Huggett T.M., Kari C., Boise L.H. and Rodeck U. (2001). Epidermal growth factor receptor-dependent control of keratinocyte survival and Bcl-xL expression through a MEKdependent pathway. J. Biol. Chem. 276, 6320-6326.

Kainulainen V., Sundvall M., Maatta J.A., Santiestevan E., Klagsbrun M. and Elenius K. (2000). A natural ErbB4 isoform that does not activate phosphoinositide 3-kinase mediates proliferation but not survival or chemotaxis. J. Biol. Chem 275, 8641-8649.

Kennedy S.G., Kandel E.S., Cross T.K. and Hay N. (1999). Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. Mol. Cell. Biol 19, 5800-5810.

Klippel A., Escobedo M.A., Wachowicz M.S., Apell G., Brown T.W., Giedlin M.A., Kavanaugh W.M. and Williams L.T. (1998). Activation of phosphatidylinositol 3-kinase is sufficient for cell cycle entry and promotes cellular changes characteristic of oncogenic transformation. Mol. Cell Biol. 18, 5699-5711.

Kulik G. and Weber M.J. (1998). Akt-dependent and -independent survival signaling pathways utilized by insulin-like growth factor I. Mol. Cell Biol. 18, 6711-6718.

Kulms D., Pöppelmann B. and Schwarz T. (2000). Ultraviolet radiationinduced interleukin 6 release in HeLa cells is mediated via membrane events in a DNA damage-independent way. J. Biol. Chem. 275, 15060-15066.

Kuo M.L., Chuang S.E., Lin M.T. and Yang S.Y. (2001). The involvement of PI 3-K/Akt-dependent up-regulation of Mcl-1 in the prevention of apoptosis of Hep3B cells by interleukin-6. Oncogene 20, 677-685.

Lander H.M. (1997). An essential role for free radicals and derived species in signal transduction. FASEB J. 11, 118-124.

Leverrier Y., Thomas J., Mathieu A.L., Low W., Blanquier B. and Marvel J. (1999). Role of PI3-kinase in Bcl-X induction and apoptosis inhibition mediated by IL-3 or IGF-1 in Baf-3 cells. Cell. Death. Differ. 6, 290-296.

Levine A.J. (1997). p53, the cellular gatekeeper for growth and division. Cell 88, 323-331.

Levresse V., Butterfield L., Zentrich E. and Heasley L.E. (2000). Akt negatively regulates the cJun N-terminal kinase pathway in PC12 cells. J. Neurosci. Res 62, 799-808.

Li W., Zhang J., Flechner L., Hyun T., Yam A., Franke T.F. and Pierce J.H. (1999). Protein kinase C-alpha overexpression stimulates Akt activity and suppresses apoptosis induced by interleukin 3 withdrawal. Oncogene 18, 6564-6572.

Li Y., Tennekoon G.I., Birnbaum M., Marchionni M.A. and Rutkowski J.L. (2001). Neuregulin signaling through a PI3K/Akt/Bad pathway in Schwann cell survival. Mol. Cell. Neurosci 17, 761-767.

Lin L.L., Wartmann M., Lin A.Y., Knopf J.L., Seth A. and Davis R.J. (1993). cPLA2 is phosphorylated and activated by MAP kinase. Cell 72, 269-278.

Lin T.A., Kong X., Haystead T.A., Pause A., Belsham G., Sonenberg N. and Lawrence J.C. Jr. (1994). PHAS-I as a link between mitogenactivated protein kinase and translation initiation. Science 266, 653-656.

Liu A., Testa J., Hamilton T., Jove R., Nicosia S. and Cheng J.Q. (1998). AKT2, a member of the protein kinase B family, is activated by growth factors, v-Ha-ras, and v-src through phosphatidylinositol 3-kinase in human ovarian epithelial cancer cells. Cancer Res. 58, 2973-2977.

Matsui M.S. and DeLeo V.A. (1990). Induction of protein kinase C activity by ultraviolet radiation. Carcinogenesis 11, 229-234.

McLaughlin M.M., Kumar S., McDonnell P.C., Van Horn S., Lee J.C., Livi G.P. and Young P.R. (1996). Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of

CSBP p38 MAP kinase. J. Biol. Chem. 271, 8488-8492.

Medema R.H., Kops G.J., Bos J.L. and Burgering B.M. (2000). AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. Nature 404, 782-787.

Mendez R., Myers M.G Jr., White M.F. and Rhoads R.E. (1996). Stimulation of protein synthesis, eukaryotic translation initiation factor 4E phosphorylation, and PHAS-I phosphorylation by insulin requires insulin receptor substrate 1 and phosphatidylinositol 3-kinase, Mol. Cell. Biol. 16, 2857-2864.

Muise-Helmericks R.C., Grimes H.L., Bellacosa A., Malstrom S.E., Tsichlis P.N. and Rosen N. (1998). Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. J. Biol. Chem. 273, 29864-29872.

New L., Zhao M., Li Y., Bassett W.W., Feng Y., Ludwig S., Padova F.D., Gram H. and Han J. (1999). Cloning and characterization of RLPK, a novel RSK-related protein kinase. J. Biol. Chem. 274, 1026-1032.

Nomura M., Kaji A., Ma W.Y., Zhong S., Liu G., Bowden G.T., Miyamoto K.I. and Dong Z. (2001). Mitogen- and stress-activated protein kinase 1 mediates activation of Akt by ultraviolet B irradiation. J. Biol. Chem. 276, 25558-25567.

Panka D.J., Mano T., Suhara T., Walsh K. and Mier J.W. (2001). Phosphatidylinositol 3-kinase/Akt activity regulates c-FLIP expression in tumor cells. J. Biol. Chem. 276, 6893-6896.

Peters L.J. and Brock W.A. (1993). Cellular radiosensitivity as predictors of treatment outcome: where do we stand? Int. J. Radiat. Oncol. Biol. Phys. 25, 147-148.

Petit-Frere C., Clingen P.H., Grewe M., Krutmann J., Roza L., Arlett C.F. and Green M.H. (1998). Induction of interleukin-6 production by ultraviolet radiation in normal human epidermal keratinocytes and in a human keratinocyte cell line is mediated by DNA damage. J. Inves. Dermatol. 111, 354-359.

Pianetti S., Arsura M., Romieu-Mourez R., Coffey R.J. and Sonenshein G.E. (2001). Her-2/neu overexpression induces NF-kappaB via a PI3-kinase/Akt pathway involving calpain-mediated degradation of IkappaB-alpha that can be inhibited by the tumor suppressor PTEN. Oncogene 20, 1287-1299.

Polunovsky V.A., Rosenwald I.B., Tan A.T., White J., Chiang L., Sonenberg N. and Bitterman P.B. (1996). Translational control of programmed cell death: eukaryotic translation initiation factor 4E blocks apoptosis in growth-factor-restricted fibroblasts with physiologically expressed or deregulated Myc. Mol. Cell. Biol 16, 6573-6581.

Quelle F.W., Wang J., Feng J., Wang D., Cleveland J.L., Ihle J.N. and Zambetti G.P. (1998). Cytokine rescue of p53-dependent apoptosis and cell cycle arrest is mediated by distinct Jak kinase signaling pathways. Genes. Dev. 12, 1099-1107.

Rane M.J., Coxon P.Y., Powell D.W., Webster R., Klen J.B., Ping P., Pierce W. and McLeish K.R. (2001). p38 Kinase-dependent MAPKAPK-2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in human neutrophils. J. Biol. Chem. 276, 3517-3523.

Raught B. and Gingras A.C. (1999). eIF4E activity is regulated at multiple levels. Int. J. Biochem. Cell Biol. 31, 43-57.

Romashkova J.A. and Makarov S.S. (1999). NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature 401, 86-90.

Ronai Z.A. and Weinstein I.B. (1988). Identification of a UV-induced transacting protein that stimulates polyomavirus DNA replication. J. Virol. 62, 1057-1060

Roulston A., Reinhard C., Amiri P. and Williams L.T. (1998). Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor alpha. J. Biol. Chem 273, 10232-10239.

Sable C.L., Filippa N., Hemmings B. and Van Obberghen E. (1997). cAMP stimulates protein kinase B in a Wortmannin-insensitive manner. FEBS Lett. 409, 253-257.

Scheid M.P. and Duronio V. (1996). Phosphatidylinositol 3-OH kinase activity is not required for activation of mitogen-activated protein kinase by cytokines. J. Biol. Chem. 271, 18134-18139.

Seger R. and Krebs E.G. (1995). The MAPK signaling cascade. FASEB J. 9, 726-735.

Sen CK. and Packer L. (1996). Antioxidant and redox regulation of gene transcription. FASEB J. 10, 709-720.

Sheridan M.T., O'Dwyer T., Seymour C.B. and Mothersill C.E. (1997). Potential indicators of radiosensitivity in squamous cell carcinoma of the head and neck. Radiat. Oncol. Invest. 5,180-186.

Staberg B., Wulf H.C., Klemp P., Poulsen T. and Brodthagen H. (1983). The carcinogenic effect of UVA irradiation. J. Invest. Dermatol 81, 517-519.

Stokoe D., Campbell D.G., Nakielny S., Hidaka H., Leevers S.J., Marshall C. and Cohen P. (1992). MAPKAP kinase-2; a novel protein kinase activated by mitogen-activated protein kinase. EMBO J. 11, 3985-3994.

Strasser A., Harris A.W., Jacks T. and Cory S. (1994). DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhabitable by Bcl-2. Cell 79, 329-339.

Strickland F.M., Darvill A., Albersheim P., Eberhard S., Pauly M. and Pelley R.P. (1999). Inhibition of UV-induced immune suppression and interleukin-10 production by plant oligosaccharides and polysaccharides. Photochem. Photobiol. 69, 141-147.

Suhara T., Mano T., Oliveira B.E. and Walsh K. (2001). Phosphatidylinositol 3-kinase/Akt signaling controls endothelial cell sensitivity to Fas-mediated apoptosis via regulation of FLICE-inhibitory protein (FLIP). Circ. Res. 89, 13-19.

Sundaresan M., Yu Z.X., Ferrans V.J., Irani K. and Finkel T. (1995). Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. Science 270, 296-299.

Suzuki A., de la Pompa J.L., Stambolic V., Elia AJ., Sasaki T., del Barco Barrantes I., Ho A., Wakeham A., Itie A., Khoo W., Fukumoto M. and Mak T.W. (1998). High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr. Biol 8, 1169-1178.

Tang Q.B., Gonzales M., Inoue H. and Bowden G.T. (2001). Roles of Akt and glycogen synthase kinase 3ß in the ultraviolet B induction of cyclooxygenase-2 transcription in human keratinocytes. Cancer. Res. 61, 4329-4332.

Tran H.A., Brunet J.M., Grenier S.R., Datta A.J., Fornace Jr P.S., DiStefano L.W. and Chiang M.E Greenberg. (2002). DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science 296, 530-534.

Tyrrell R.M. (1996). Activation of mammalian gene expression by the UV component of sunlight--from models to reality. Bioessays 18, 139-148.

Umeda J., Sano S., Kogawa K., Motoyama N., Yoshikawa K., Itami S., Kondoh G., Watanabe T. and Takeda J. (2003). In vivo cooperation between Bcl-xL and the phosphoinositide 3-kinase-Akt signaling pathway for the protection of epidermal keratinocytes from apoptosis. FASEB J. 17, 610-620.

Vanhaesebroeck B. and Alessi D.R. (2000). The PI3K-PDK1

connection: more than just a road to PKB. Biochem. J. 346, 561-576.

Wei L.H., Kuo M.L., Chen C.A., Chou .CH., Cheng W.F., Chang M.C., Su J.L. and Hsieh C.Y. (2001). The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 though a PI 3-kinase/ Akt pathway. Oncogene 20, 5799-5809.

Welsh G.I., Foulstone E.J., Young S.W., Tavare J.M. and Proud C.G. (1994). Wortmannin inhibits the effects of insulin and serum on the activities of glycogen synthase kinase-3 and mitogen-activated protein kinase. Biochem. J. 303, 15-20.

West C.M., Davidson S.E., Roberts S.A. and Hunter R.D. (1993). Intrinsic radiosensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. Br. J. Cancer 68, 819-823.

Wick W., Furnari F., Naumann U., Cavanee W. and Weller M. (1999). PTEN gene transfer in human malignant glioma: sensitization to irradiation and CD95L-induced apoptosis. Oncogene 18, 3936-3943.

Wymann M.P. and Pirola L. (1998). Structure and function of phosphoinositide 3-kinases. Biochim. Biophys. Acta 1436, 127-150.

Xia Z., Dickens M., Raingeaud J., Davis R.J. and Greenberg M.E. (1995). Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270, 1326-1331.

Yano S., Tokumitsu H. and Soderling T. (1998). Calcium promotes cell survival through CaM-K kinase activation of the protein-kinase-B pathway. Nature 396, 584-587.

Zechner D., Craig R., Hanford D.S., McDonough P.M., Sabbadini R.A. And Glembotski C.C. (1998). MKK6 activates myocardial cell NF-kappaB and inhibits apoptosis in a p38 mitogen-activated protein kinase-dependent manner. J. Biol. Chem. 273, 8232-8239.

Zha J., Harada H., Yang E., Jockel J. and Korsmeyer S.J. (1996). Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCLX(L). Cell 87, 619-628.

Zheng W.H., Kar S. and Quirion R. (2000). Stimulation of protein kinase C modulates insulin-like growth factor-1-induced Akt activation in PC12 cells. J. Biol. Chem 275,13377-13385.

Zhou B.P., Liao Y., Xia W., Spohn B., Lee M.H. and Hung M.C. (2001). Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat. Cell Biol. 3, 245-252.

Zhou H., Li X.M., Meinkoth J. and Pittman R.N. (2000). Akt regulates cell survival and apoptosis at a postmitochondrial level. J. Cell Biol. 151, 483-494.

Zinck R., Cahill M.A., Kracht M., Sachsenmaier C., Hipskind R.A. and Nordheim A. (1995). Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. Mol. Cell. Biol. 15, 4930-4938.

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