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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.

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