

AKT: A novel target in pancreatic cancer therapy

Review Article

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Key words: pancreatic cancer therapy, Protein kinase B, PI3-kinase/AKT pathway, K-ras, Growth factor receptors, HER-2/*neu*, Downstream effectors of AKT, IKK/NF- κ B, PTEN, mTOR, cFLIP, cell cycle regulation, Apoptosis, Chemosensitization

Abbreviations: carboxyl-terminal modulator protein, (CTMP); cyclin dependent kinase, (CDK); FLICE-inhibitory protein, (cFLIP); insulin like growth factor receptor, (IGF-R); phosphatase and tensin homologue deleted on chromosome ten, (PTEN); phosphatidylinositol-3 kinase, (PI3K); phosphorylated, (activated) AKT, (pAKT); proliferating cell nuclear antigen, (PCNA); retinoblastoma protein, (Rb); SH2 containing phosphatases, (SHIPs); vascular endothelial growth factor receptor, (VEGF-R)

Received: 1 July 2004; Accepted: 12 July 2004; electronically published: July 2004

Summary

The serine/threonine kinase AKT (also known as Protein Kinase B) has been shown to mediate a potent survival signal in normal cells. There is increasing evidence of constitutive activation of AKT by various upstream signals in diverse cancer which confers the same survival signal but with the cellular consequence of resistance to the apoptotic effects of chemo- and radiotherapy. Given the lack of effective treatment for pancreatic cancer, new targets of therapy are desperately needed if any impact on this lethal malignancy is to be made. We review the signaling cascade of AKT (both upstream activators and downstream effectors) and the cellular consequences of AKT activation in pancreatic cancer. We further discuss the early experimental evidence that supports the concept that AKT is an appropriate molecular target for therapy in pancreatic cancer.

I. Introduction

Pancreatic adenocarcinoma is the fourth leading cause of cancer-related death in the United States today (Jemal et al, 2004). The diagnosis of pancreatic cancer carries a poor prognosis with a mean 5-year survival of only 3% (Bardeesy and DePinho, 2002) and a median survival of 5 months (Sakorafas and Tsiotou, 1999). Because of a lack of early symptoms, less than 20% of patients present with resectable disease and even then, the 5-year survival is a dismal 20% (Ahrendt and Pitt, 2002). Few effective treatment options are available when surgical resection is not possible. Gemcitabine is one of the few chemotherapeutic agents with any activity against pancreatic cancer and has become the standard of care, however, it only prolongs survival to 6-7 months with a response rate of 10% (Heineman, 2002). Ongoing clinical trials of gemcitabine in combination with other chemotherapeutics await analysis, but dramatic advances are unlikely (Heineman, 2002). Clearly, innovative treatment options are needed to make any progress in this lethal malignancy.

The understanding of the molecular biology of pancreatic cancer is rapidly expanding and hopefully can be utilized to develop better treatment strategies. A model

of the histological and genetic progression of pancreatic ductal adenocarcinoma has been developed (Sohn and Yeo, 2000; Bardeesy and DePinho, 2002; Schneider and Schmid, 2003) which may provide the background for the development of targeted therapy (Wolff, 2002; Gunzburg et al, 2003; Westphal and Kalthoff, 2003). Activating K-ras and HER-2/*neu* mutations occur early, followed by loss of p16 expression, and then later, inactivation of p53 and DPC4 (Hruban et al, 2001; Hansel et al, 2003). Each of these genetic defects has been shown to be involved in the biology of pancreatic cancer; however, targeting these events has been difficult. Ongoing research may identify additional genetic defects for which targeted therapy is effective.

II. AKT (Protein kinase B)

AKT (also known as protein kinase B) is a serine/threonine kinase involved in the regulation of cell proliferation, survival/apoptosis, angiogenesis, metabolism, and protein synthesis (Chang et al, 2003a; Luo et al, 2003; Fresno et al, 2004). Just in the last several years, there has been the consistent observation that activation of AKT is frequent among various types of cancer, initiating a potent survival/anti-apoptotic signal

(Sun et al, 2001). The first evidence supporting the role of AKT in cancer came with the isolation of v-AKT from the AKT8 retrovirus associated with a spontaneous T-cell lymphoma in a mouse model (Staal and Hartley, 1988; Bellacosa et al, 1991). Unlike other oncogenes, however, overexpression does not transform NIH3T3 cells; constitutive activation is required (Sun et al, 2001). Three separate subtypes of AKT have since been isolated (AKT1, AKT2 and AKT3) (Nicholson and Anderson, 2002). All three isoforms are ubiquitously expressed with AKT1 being the dominant isoform except in insulin-responsive tissues. AKT1 knockout mice are viable but smaller than wild-type littermates with increased apoptosis in various tissues (Chen et al, 2001; Peng et al, 2003). AKT2 knockout mice are also viable though insulin-resistant and prone to diabetes (Garofalo et al, 2003). The majority of research on AKT in cancer has focused on AKT1, which appears to be the primary mediator of the anti-apoptotic signal (Aoki et al, 1998).

The development of phospho-specific antibodies allows for determining whether a specific kinase is in the activated state. We have established a tumor bank of pancreatic adenocarcinomas and examined 78 tumor specimens for the presence of phosphorylated (activated) AKT (pAKT) (Schlieman et al, 2003). This heterogeneous group of tumors represented a mix of localized/resected tumors (N=35; 45%) and metastatic tumors (N=43; 55%). Of these 78 tumor specimens, 46 (59%) demonstrated activation of AKT by virtue of immunohistochemical staining for pAKT (**Figure 1**). Histologic grading demonstrated that almost one-half of tumors were moderately well differentiated, one-fifth was well differentiated and one-third was poorly differentiated. AKT activation correlated with histologic grade in that AKT was activated in 76% of the poorly differentiated tumors but only 38% of the well-differentiated tumors. This correlation suggests the involvement of AKT in more aggressive tumors as histologic grade remains one of the most significant prognostic variables in this cancer (Takahashi et al, 1997; Kedra et al, 2001). Our data demonstrate that AKT is activated in almost 60% of pancreatic adenocarcinoma tumors, placing it near the top of tumors that have been reported to harbor AKT activation.

These observations have been recently corroborated in 61 patients who had undergone curative resection of pancreatic adenocarcinoma (Yamamoto et al, 2004). Using the same immunohistochemical technique for detection of activated AKT by virtue of pAKT staining, 46% of the tumors demonstrated constitutive activation of AKT. Given that this group of patients was homogeneous, survival analysis was performed and demonstrated a significant correlation between activation of AKT and poorer survival. The 5-year survival rate was only 16.4 months for patients whose tumors demonstrated activation of AKT, but 50.6 months for patients whose tumors did not demonstrate activated AKT. The presence of activated AKT maintained its prognostic significance in a multivariate analysis suggesting a central role of AKT activation in the biology of pancreatic cancer.

III. Upstream regulators of PI3-kinase/AKT pathway

One of the main activators of AKT is phosphatidylinositol-3 kinase (PI3K). PI3K is activated by multiple upstream receptor tyrosine kinases and G-protein coupled receptors in response to a variety of growth stimuli (Chang et al, 2003b; Luo et al, 2003). PI3K activates AKT by generating phosphatidylinositol-3,4,5-triphosphate (PIP-3), which mediates translocation of AKT to the cell membrane (**Figure 2**). AKT is subsequently activated by phosphorylation on Thr308 by PDK1 (Vanhaesebroeck et al, 2000; Wick et al, 2000; Brazil et al, 2002). In addition, maximal activation of AKT requires a second phosphorylation at Ser473 by PDK2 (Chan and Tsichlis, 2001; Nicholson et al, 2002). The activity of AKT is further regulated by PTEN (phosphatase and tensin homologue deleted on

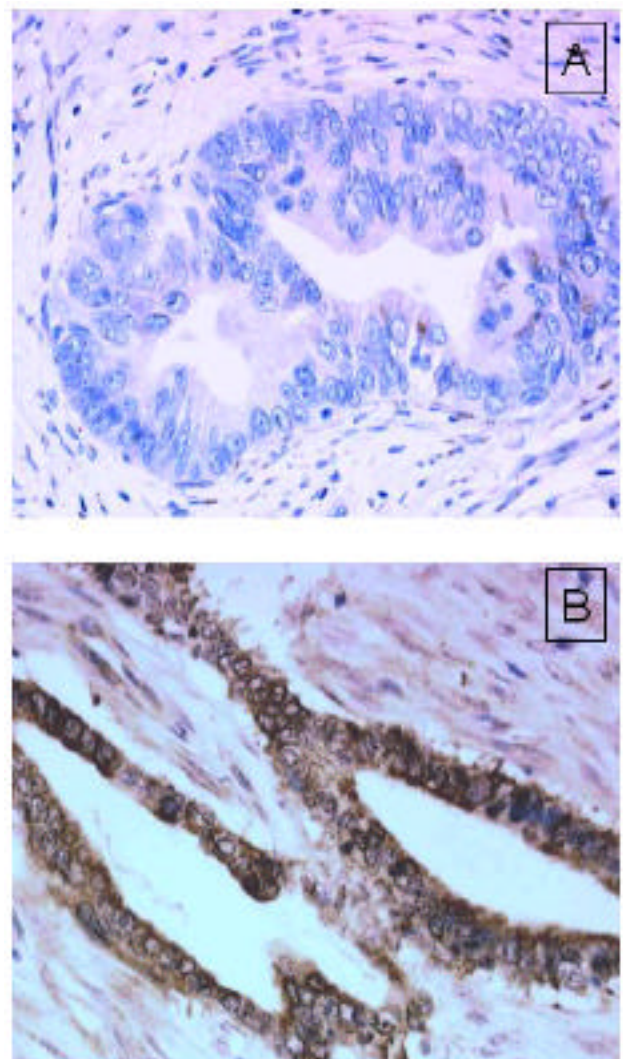


Figure 1. Representative immunohistochemical staining for phospho-AKT in pancreatic tumors. (A) tumor without detectable expression, (B) tumor with strong expression of phospho-AKT consistent with activation of AKT. Normal pancreatic ducts do not have detectable phospho-AKT (x40, original magnification).

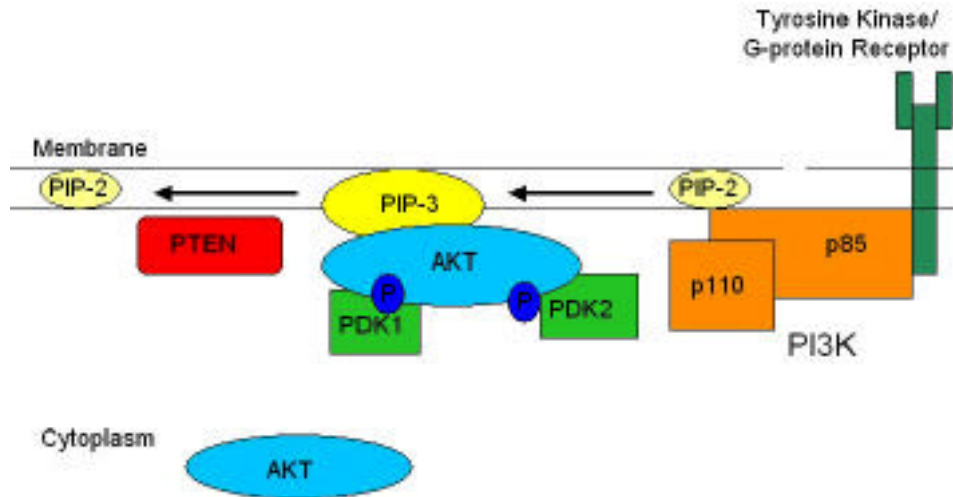


Figure 2. A primary mechanism of AKT activation is by phosphatidylinositol-3 kinase (PI3K). PI3K, a heterodimer of a p85 regulatory subunit and a p110 catalytic subunit, can be activated by various receptor tyrosine kinases or G protein-coupled receptors. PI3K phosphorylates phosphatidylinositol-4, 5-diphosphate (PIP-2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP-3), which binds to the pleckstrin homology domain of AKT, allowing for subsequent phosphorylation by PDK1 and/or PDK2. Negative regulation of AKT involves dephosphorylation of PIP-3 to PIP-2 by PTEN, disrupting the PDK1/PDK2 interaction.

chromosome ten). PTEN essentially functions as a tumor suppressor by dephosphorylating PIP-3, therefore blocking activation of AKT (Chan and Tsichlis, 2001). Mutations or deletions of PTEN (such as in glioblastoma multiforme or prostate cancer) lead to constitutively activated AKT (Davies et al, 1998, 1999). Other negative regulators of AKT include SH2 containing phosphatases (SHIPs), phosphatases effective against PI3K, and carboxyl-terminal modulator protein (CTMP), a negative regulator of AKT at the plasma membrane (Chang et al, 2003b).

A. K-ras

The ras gene family consists of GTPases, which translate extracellular signals from membrane coupled receptors into intracellular signal transduction pathways. Mutation of the K-ras gene is one of the earliest and most frequent genetic events observed in pancreatic cancer, occurring in over 95% of tumors (Hirai et al, 1995; Sakorafas et al, 2000; Ren et al, 2004). Mutation leads to constitutive activation with subsequent stimulation of downstream signal transduction pathways regulating cellular survival, proliferation, and invasion (Ellis and Clark, 1997; Katz and McCormick, 1997). Of the many effector pathways, the PI3K/AKT pathway has been implicated as a major mediator of constitutive ras activation, conferring various phenotypic consequences such as protection from anoikis and c-myc induced apoptosis (Kauffmann-Zeh et al, 1997; Khwaja et al, 1997; Downward et al, 1998; Shields et al, 2000). Inhibition of PI3K blocks cells from ras induced transformation, supporting the importance of PI3K/AKT pathway as a downstream effector of the survival signal of ras activation (Rodriguez-Viciano et al, 1997; Krasilnikov, 2000; Shao et al, 2004; Sheng et al, 2004).

B. Growth factor receptors

Among the many receptor tyrosine kinases capable of activating AKT, several have been shown to play a role in pancreatic cancer; these include the vascular endothelial growth factor receptor (VEGF-R), insulin like growth factor receptor (IGF-R), and c-erb2 (HER-2/*neu*). VEGF, a multifunctional protein involved in tumor angiogenesis and metastases, has been strongly implicated in the aggressive behavior of pancreatic cancer (Itakura et al, 1997; Itakura et al, 2000). Pancreatic tumors overexpress both VEGF and VEGF-R compared to normal pancreatic exocrine tissue. VEGF signals through AKT to maintain cell viability of endothelial cells and perhaps tumor cells (Larrivee and Karsan, 2000; Lie et al, 2000; Kliche and Waltenberger, 2001). Inhibition of VEGF receptors both in vitro and in xenograft models of pancreatic cancer decreased AKT activation, increased apoptosis and decreased tumor growth (Solorzano et al, 2001; Hoshida et al, 2002; Tseng et al, 2002; Buchler et al, 2003a).

Another receptor tyrosine kinase that has been selectively targeted in pancreatic cancer is the IGF1-R. Overexpression of IGF1-R confers resistance to apoptosis and increases invasiveness and metastatic capacity in pancreatic cancer cell lines (Bergmann et al, 1995; Min et al, 2003). Inhibition of IGF1-R decreases AKT activity, increases apoptosis in vitro and decreases tumorigenicity in vivo. Inhibition of IGF1-R is associated with a decrease in activated AKT, suggesting that AKT is involved in the downstream signaling of IGF1-R. Interestingly, overexpression of IGF1-R is dependent on AKT activation. This represents an interesting autocrine pathway in which AKT activation increases IGF1-R levels, which then further activates AKT and the coupled downstream pathways (Knuefermann et al, 2003).

C. HER-2/*neu*

The HER-2/*neu* oncogene is a member of the ErbB family of receptor tyrosine kinases. Multiple studies have shown that HER-2/*neu* is an upstream activator of the AKT pathway (Zhou et al, 2000, 2001a; Clark et al, 2002; Yakes et al, 2002). For example, in HER-2/*neu* overexpressing breast cancer cell lines, AKT is constitutively active in vitro (Zhou et al, 2000). In addition, the chemoresistance demonstrated in HER-2/*neu* overexpressing breast cancer cells has been linked to increased AKT activity (Clark et al, 2002; Knuefermann et al, 2003). In pancreatic cancer, HER-2/*neu* overexpression has been reported to vary between 7-82% (Hall et al, 1990; Williams et al, 1991; Yamanaka et al, 1993; Lei et al, 1995; Day et al, 1996; Dergham et al, 1997a; Dugan et al, 1997; Apple et al, 1999; Safran et al, 2001). The relationship between overexpression of HER-2/*neu* and activated AKT in pancreatic cancer has been studied in our laboratory. Of 78 human pancreatic tumor specimens examined, HER-2/*neu* was overexpressed in 67% of tumors and correlated with AKT activation (Schlieman et al, 2003). We furthermore demonstrated coupling of HER-2/*neu* overexpression to AKT activation using the Mia-PaCa-2 cell line, which has high levels of HER-2/*neu*. In vitro inhibition of HER-2/*neu* with the blocking monoclonal antibody trastuzumab (Herceptin[®]) decreased the degree of AKT activation.

Buchler et al. evaluated the cellular effect of trastuzumab treatment on several pancreatic cancer cell lines and noted growth inhibition in vitro in all cell lines that overexpressed HER-2/*neu* (Buchler et al, 2001). Tumor growth in vivo was similarly inhibited by trastuzumab treatment in the HER-2/*neu* overexpressing MIA-PaCa-2 cell line. Interestingly, we found this cell line to harbor the highest level of HER-2/*neu* overexpression as well as the greatest degree of AKT activation among 7 pancreatic cancer cell lines examined. The experimental observations of the potential significance of HER-2/*neu* in pancreatic cancer have led to clinical trials of trastuzumab alone or in combination with gemcitabine (Jacobs, 2002; Wolff, 2002). Preliminary data is promising in the subset of pancreatic cancer patients whose tumors overexpress HER-2/*neu*.

D. PTEN

PTEN mutations or deletions do not appear to be a major factor in the activation of AKT observed in pancreatic cancer (Sakurada et al, 1997; Okami et al, 1998; Matsumoto et al, 2002). However, the function of PTEN can be inhibited, with subsequent activation of the PI3K/AKT pathway. TGF- β has been recently shown to reduce transcription of PTEN (Li et al, 1997). Overexpression of TGF- β is common in pancreatic cancer and has been associated with a poorer prognosis (Friess et al, 1993). Whether this is causally related to the reduction of PTEN levels, and therefore indirectly activates AKT remains unclear. Immunohistochemical analysis of pancreatic tumors has correlated high levels of TGF- β with low levels of PTEN (Ebert et al, 2002). Therefore, the genetic defects in the TGF- β signaling pathway may be a

significant epigenetic cause of AKT activation in pancreatic cancer.

IV. Downstream effectors of AKT

There are multiple downstream signaling pathways that have been coupled to AKT (**Figure 3**). These include transcription factors (e.g. NF- κ B, forkhead/AFX, CREB and p53), mediators of apoptosis (e.g. bad and caspase 9), cell cycle regulators (e.g. p21, p27, mTOR and cyclin D1), and proteins involved in metabolism (i.e. GSK-3) (Franke et al, 2003). It remains unclear which of these mediate the various phenotypic consequences of AKT activation in cancer. A discussion of all the downstream signaling pathways regulated by AKT is beyond the scope of the current review; therefore only those downstream pathways that have been reported to have significance in pancreatic cancer will be discussed.

A. IKK/NF- κ B

NF- κ B represents a group of transcription factors composed of p65 (relA), p50, p52, relB, and c-rel, which function as homo- and heterodimers. NF- κ B transcriptionally regulates diverse genes including c-IAP-1, c-IAP-2, c-FLIP, Bcl-2, A1/bfl-1, cyclin D, p21, VEGF, and p53 with the end result of pro-survival signaling (Stehlik et al, 1998; Guttridge et al, 1999; Wang et al, 1999, 2003; Benoit et al, 2000; Kurland et al, 2001; Micheau et al, 2001; Basile et al, 2003; Tergaonkar et al, 2003; Xiong et al, 2004). The transcriptional activity of NF- κ B is initiated by nuclear translocation following release from the inhibitory protein I κ B. Phosphorylation of I κ B by IKK is one AKT-dependent mechanism responsible for the activation of NF- κ B (Madrid et al, 2001), though it is increasingly clear that there may be additional AKT-independent mechanisms of activation of NF- κ B (Pianetti et al, 2001). NF- κ B has been shown to have important biologic consequences in pancreatic cancer and may be an important biochemical sequelae of AKT activation (Gilmore, 1999; Madrid et al, 2000; Mayo and Baldwin, 2000). NF- κ B is constitutively activated in the majority of pancreatic tumors through a PI3K-dependent activation of IKK (Wang et al, 1999; Liptay et al, 2003; Arlt et al, 2003).

Of the various downstream targets of NF- κ B, one of the more relevant in pancreatic cancer is the antiapoptotic protein bcl-2 (Fahy et al, 2003; Fujioka et al, 2003). The bcl-2 family consists of both proapoptotic (bax, bak) and antiapoptotic (bcl-2, bcl-X_L) members which are critical in the regulation of induction of apoptosis through interactions at the mitochondrial membrane with subsequent effects on the release of cytochrome c (Tsujimoto and Shimizu, 2000; Reed, 1997). If the relative balance of antiapoptotic members predominates, the apoptotic threshold is raised and cells fail to undergo apoptosis even in the presence of powerful apoptotic signals.

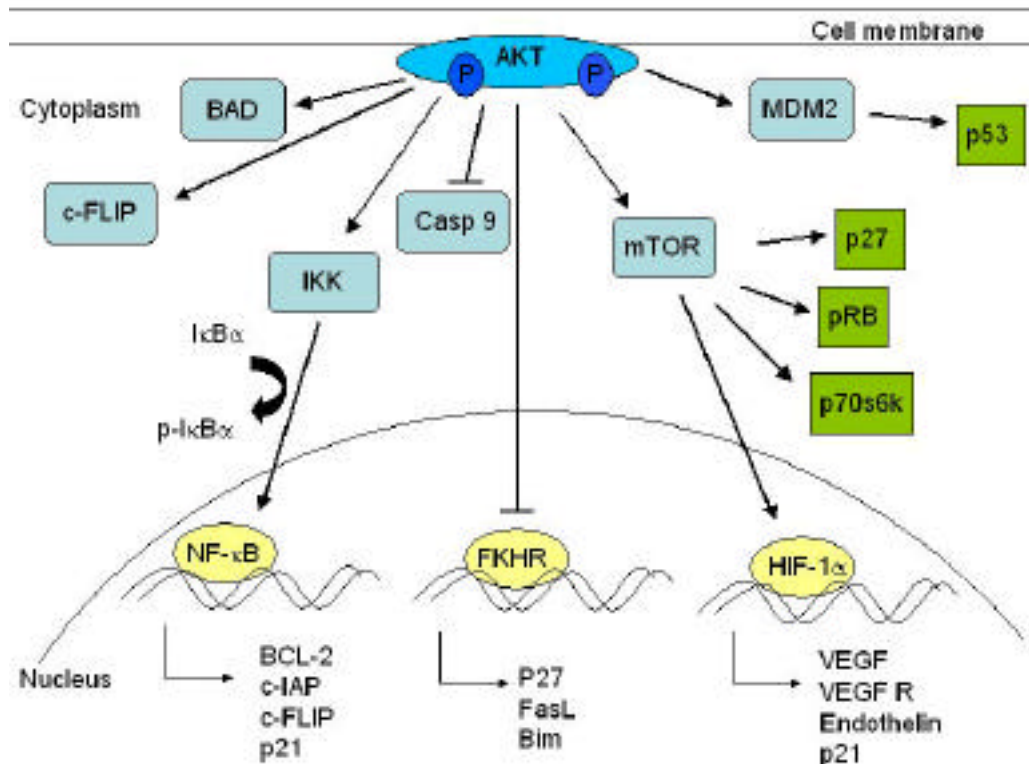


Figure 3. Downstream effectors of AKT include regulators of apoptosis (e.g. bad, caspase 9, c-FLIP), gene transcription (NF- B through IKK, FKHD, and HIF-1 through mTOR), cell cycle progression (p53 through MDM2, p27 and RB through mTOR). Representative genes regulated by transcription factors activated by AKT are shown.

We and others have shown that bcl-2 is frequently overexpressed in pancreatic tumors (Bold et al, 1999a; Campani et al, 2001), and this overexpression correlates with resistance to the cytotoxic effects of gemcitabine (Bold et al, 1999b; Shi et al, 2002). We have also shown that the PI3K/AKT/NF- B pathway is a central mediator of the bcl-2 overexpression in pancreatic cancer (Fahy et al, 2003).

B. mTOR

Another downstream effector of AKT is mammalian target of rapamycin (mTOR), a serine/threonine kinase that is directly phosphorylated by AKT (Nave et al, 1999; Sekulic et al, 2000). The substrates of mTOR are involved in regulation of gene transcription and cell cycle progression. These targets include the 40S ribosomal protein S6 kinase (p70s6k), cyclin D1/A, the cyclin dependent kinase (CDK) inhibitors p21 and p27, and the retinoblastoma protein (Rb) (Huang and Houghton, 2003; Abraham, 2004). Pancreatic cancers have been shown to have increased mTOR activity (Xu et al, 2004) and inhibition with rapamycin is sufficient to sensitize tumors to the apoptotic effect of gemcitabine (Grewe et al, 1999; Shah et al, 2001a).

An important target of mTOR is the transcription factor hypoxia-inducible factor, HIF-1 (Hudson et al, 2002). HIF-1 is responsible for activating transcription of multiple genes, such as VEGF, which are involved in neovascularization and metastasis (Semenza, 2002).

Preliminary data in pancreatic cancer specimens demonstrate elevated levels of HIF-1 mRNA and HIF-1 protein by IHC (Zhong et al, 1999; Buchler et al, 2003b; Kuwahara et al, 2004). Furthermore, overexpression of HIF-1 correlates with advanced tumor stage and poorer survival (Shibaji et al, 2003). Thus, the activation of AKT through upstream effectors may have an effect on HIF-1 and subsequently VEGF through mTOR leading to a more metastatic phenotype. Inhibitors of mTOR (e.g. rapamycin, CC1-779 and RAD001) have shown activity against a broad range of human cancers both in vitro and xenograft models including pancreatic (Shah et al, 2001a; Huang and Houghton, 2003; Xu et al, 2004; Boulay et al, 2004). Therefore, inhibition of the AKT/mTOR pathway may be another mechanism to target tumor angiogenesis, and achieve similar efficacy to that observed in experimental models that have inhibited VEGF (Hoshida et al, 2002; Buchler et al, 2003; Parikh et al, 2003; Solorzano et al, 2003).

C. cFLIP

FLICE-inhibitory protein (cFLIP) is a caspase-8 homologue that inhibits the death receptor pathway of apoptosis (Wajant, 2003). The expression of cFLIP has been shown to be regulated by PI3K/AKT pathway in several solid tumors (Panka et al, 2001); inhibition of AKT decreases cFLIP protein levels and increases sensitivity to apoptosis. The AKT-dependent regulation of cFLIP levels may be through the FKHR-L1 transcription factor, though the specific details remain unknown (Skurk

et al, 2004). Elnemr et al, found that despite Fas receptor expression, the Fas pathway of death receptor-induced apoptosis was not functional in pancreatic cancer (Elnemr et al, 2001). This may be due to concomitant overexpression of c-FLIP, which would block the effects of the Fas-FasL signaling (Ungefroren et al, 1998). Therefore, inhibition of the AKT pathway in pancreatic cancer may reduce cFLIP levels, and allow death receptor mediated apoptosis. It has already been demonstrated that inhibition of NF- B in pancreatic cancer cells decreases cFLIP levels and sensitizes cells to death receptor mediated apoptosis (Thomas et al, 2002).

V. AKT and cell cycle regulation

AKT can contribute to uncontrolled cellular division through effects on cell cycle progression with direct and indirect effects on p21, p27 and cyclin D (Figure 4). An important CDK inhibitor involved in regulation of the cell cycle is p21, which has been shown to both inhibit cell cycle progression and DNA synthesis as well as be a mediator of cell survival (Li et al, 2002). Elevated p21 is seen in a variety of tumors including pancreatic and has been correlated with chemoresistance (Dergham et al, 1997b; Li et al, 2002; Moller et al, 2002). AKT directly phosphorylates p21 with the overall consequence of promoting cell survival (Zhou et al, 2001b). First, phosphorylation at Thr145 disrupts binding of p21 with proliferating cell nuclear antigen (PCNA) allowing PCNA to complex with DNA polymerase allowing DNA synthesis (Rossig et al, 2001). This phosphorylation causes p21 to exit the nucleus and become retained in the cytoplasm (Zhou et al, 2001b) where it forms antiapoptotic complexes. Second, phosphorylated p21 is able to activate cyclin D1-CDK4 that allows for progression through the

G1-S phase of the cell cycle in response to mitogenic stimuli (Chang et al, 2003b). Whether p21 is a significant effector of AKT activation in pancreatic cancer is not completely clear, directly targeting p21 in pancreatic cancer has demonstrated efficacy (Shah et al, 2001b)

AKT also phosphorylates p27, another CDK inhibitor, which may function as a putative tumor suppressor. Cancers with low p27 levels demonstrate more aggressive behavior when compared to tumors with normal p27 levels (Sgambato et al, 2000). Phosphorylation of p27 by AKT causes cytoplasmic retention and loss of its inhibitory actions against Cdk-2 (Viglietto et al, 2002). Furthermore, through its actions on the Forkhead family of transcription factors, AKT indirectly decreases levels of p27 (Chandramohan et al, 2004). Immunohistochemical studies in pancreatic cancer have found decreased levels of p27, which may have prognostic significance (Feakins and Ghaffar, 2003; Juuti et al, 2003).

VI. Cellular effects following inhibition of AKT

Current experimental methods of inhibition of AKT primarily utilize inhibition of the upstream activator, PI3K. Two commercially available PI3K inhibitors are wortmannin, a fungal metabolite that binds to the p110 subunit of PI3K, and LY294002, a reversible small molecule inhibitor. Both have been demonstrated to decrease activation of AKT in vitro and in vivo (Powis et al, 1994; Cuenda and Alessi, 2000; Stein, 2001; Matsumoto et al, 2002). Furthermore, these agents inhibit tumor growth and induce apoptosis in multiple tumor models of pancreatic cancer (Ng et al, 2000, 2001; Perugini et al, 2000; Shah et al, 2001c; Bondar et al, 2002;

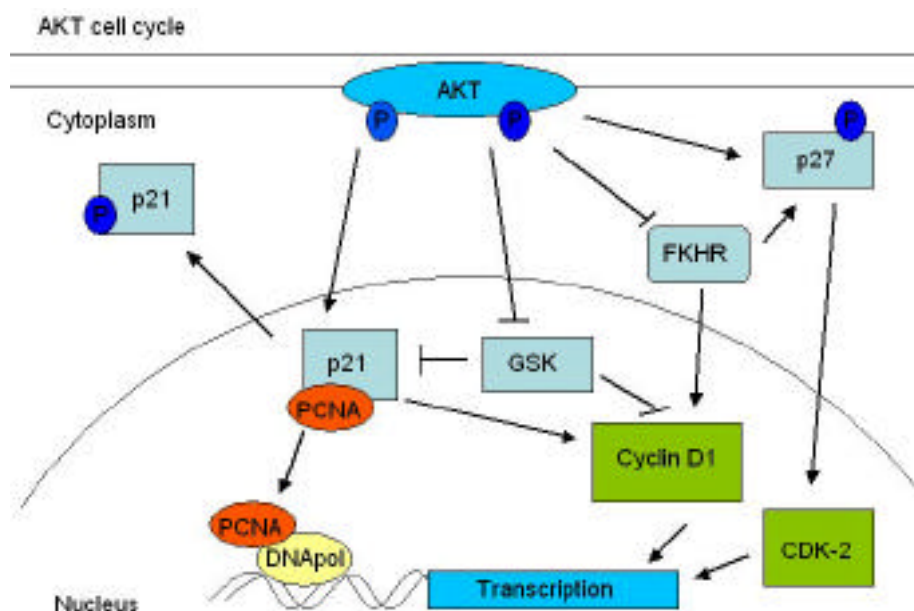


Figure 4. Activated AKT promotes cell cycle progression through multiple mechanisms, including direct phosphorylation of p21 which leads to release of PCNA with subsequent activation of DNA polymerase. Phosphorylation of another cdk inhibitor, p27, sequesters it in the cytoplasm thereby eliminating the inhibitory binding to CDK2. Cyclin D1 is further an indirect target of AKT through GSK-3 as well as FKHD-mediated transcription, both of which lead to increased cyclin D levels which then bind to CDK4/6 leading to G1 progression.

Yao et al, 2002; Fahy et al, 2003; Semba et al, 2003; Yip-Schneider et al, 2003; Takeda et al, 2004). Unfortunately, secondary to issues with toxicity as well as effective drug delivery, neither wortmannin nor LY294002 is currently suitable for use in clinical trials (West et al, 2002; Fresno et al, 2004). Other inhibitors of either PI3K or AKT directly are under development and preliminary data has shown acceptable toxicity in preclinical testing (Castillo et al, 2004).

A. Apoptosis

Inhibition of the PI3K/AKT pathway is sufficient to induce apoptosis in diverse tumor cell lines. However, PI3K/AKT inhibition is not effective in cell lines that have low levels of AKT or in normal cell lines (Jetzt et al, 2003). In pancreatic cancer, Bondar et al. observed apoptosis induced by both LY294002 and wortmannin in six of seven cell lines that displayed constitutive AKT activation but not in the two cell lines that did not (Bondar et al, 2002). Therefore, it appears that increased levels of phosphorylated AKT may be a requirement for effective use of PI3-kinase/AKT targeted therapies. This may be advantageous in terms of protecting non-neoplastic cells from effects of these therapies.

B. Chemosensitization

It is now fairly well established that some, if not all, of the cytotoxic effect of traditional chemo- and radiotherapy is through the initiation of endogenous apoptotic pathways. However, these agents also activate potent survival pathways, including PI3K/AKT, that can lead to chemoresistance (West et al, 2002). Perhaps one of the most dramatic results we have observed is chemosensitization in pancreatic cancer. Gemcitabine induces very little apoptosis but addition of LY294002 or dominant negative AKT combined with gemcitabine substantially increases the fraction of cells undergoing apoptosis, thereby converting gemcitabine into a potent apoptotic agent (Ng et al, 2001; Fahy et al, 2003; Fahy et al, 2004). We have further demonstrated the correlation of AKT-dependent chemoresistance with NF- κ B dependent activation of bcl-2 transcription (Fahy et al, 2004).

C. Metastasis

Increased metastatic potential in pancreatic cancer has been correlated with both NF- κ B and VEGF, both of which are targets of AKT. It has been observed that AKT may be linked to the development of metastasis through a variety of mechanisms. Tanno et al, activated AKT through a src pathway which led to an upregulation of IGF-IR expression in pancreatic cancer cell lines conferring higher invasive potential than control cell lines (Tanno et al, 2001). AKT activation may be important in preventing anoikis, a specific type of apoptosis resulting from loss of cell-cell or cell-cell matrix interaction that normally prevents cell survival in a site distant from its primary organ. Idogawa et al, demonstrated that AKT has the ability to induce resistance to anoikis through modulation of bcl-2 family members, I κ B, and caspase 9 (Idogawa et al, 2003). When constitutively activated AKT

is transfected into normal epithelial cells, there is a marked resistance to anoikis (Khawaja et al, 1997; Yu et al, 2001). In pancreatic cancer, many cell lines appear resistant to anoikis, which may be secondary to AKT activation (unpublished observations).

VII. Conclusions

Cancer arises out of a field of disturbed apoptotic regulation and/or uncontrolled proliferation. Through its myriad effects on proteins involved in cell proliferation, apoptosis and cell cycle, AKT certainly contributes to tumorigenesis in many tumor types and is important in the pathogenesis of pancreatic cancers. Targeted inhibition of AKT shows promise given its central position in cell survival signaling through diverse downstream pathways. Unfortunately, there is a lack of specific and tolerable inhibitors of the AKT pathway. Currently, small molecule inhibitors are under investigation, however, none are currently in clinical use. Targeted therapy of the AKT pathway may be an important novel target in the future treatment of pancreatic cancer.

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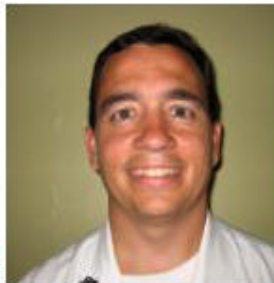
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