Cell. Mol. Life Sci. 64 (2007) 2512 – 2521
1420-682X/07/192512-10
DOI 10.1007/s00018-007-7072-4

Cellular and Molecular Life Sciences

Visions & Reflections (Minireview)

Inhibition of pancreatic cancer cell growth

T. E. Adrian

Department of Physiology, Faculty of Medicine and Health Sciences, United Arab Emirates University, PO Box 17666, Al Ain (United Arab Emirates), Fax: +971 3 767 1966, e-mail: tadrian@uaeu.ac.ae

Received 12 February 2007; received after revision 25 June 2007; accepted 18 July 2007
Online First 6 August 2007

Abstract. Pancreatic cancer cells are resistant to the growth-inhibitory and apoptosis-inducing effects of conventional chemotherapeutic agents. There are multiple genetic and epigenetic events during the process of carcinogenesis that enable the cancer cells to avoid normal growth constraints and apoptosis. Investigation of the mechanisms involved has led to multiple strategies that encourage cell death and apoptosis to occur. The pathways involved are summarized in this review, together with some recently developed strategies to promote cell death in this cancer.

Keywords. Growth inhibition, pancreatic cancer, apoptosis.

Pancreatic cancer and its resistance to chemotherapy

Pancreatic adenocarcinoma is a devastating disease with the worst survival outcome of any human cancer. Symptoms occur late in the development of pancreatic malignancy and conventional chemotherapy has little impact on the outcome [1]. Less than 20 % of patients are candidates for potentially curative resection and the vast majority of even this pre-selected group of patients eventually succumbs to metastatic disease [1]. It is claimed that 20 % 5-year survival after surgery is responsible for an overall 5-year survival of 2–4 %. However, even this dismal figure has been disputed recently [2].

Pancreatic cancers are resistant to the effects of available drugs. The reasons for this are hard to define and evidence suggests that they are complex [3, 4]. Meaningful studies on the mechanisms of resistance to therapy can only be performed once a highly effective drug has been found and at present that appears to be a distant hope. Resistance to chemotherapeutic drugs is partly due to the intrinsic properties of the cancer cells, such as their ability to overcome normal cellular growth constraints, to resist apoptosis and partly due to resistance acquired from therapy, such as the up-regulation of multi-drug resistance proteins, which are pumps that lower the intracellular drug concentrations. In the case of pancreatic cancers, there are a host of genetic and epigenetic changes that enable them to avoid normal growth constraints [3, 5, 6]. These include up-regulation and activation of growth pathways and loss of function of tumor suppressor proteins. These pathways provide a number of potential targets for inhibition of pancreatic cancer cell growth and induction of apoptosis. With application of therapies targeted to these pathways, we can hopefully improve on the dismal outcome of this disease in the future.

Targeting the apoptosis pathways in pancreatic cancer

Programmed cell death, known as apoptosis plays a pivotal role in cellular homeostasis. Abnormal cells are eliminated by apoptosis, but cancer cells develop mechanisms to prevent this process just as they manage to avoid cellular growth constraints [7, 8]. There are two pathways through which apoptosis can

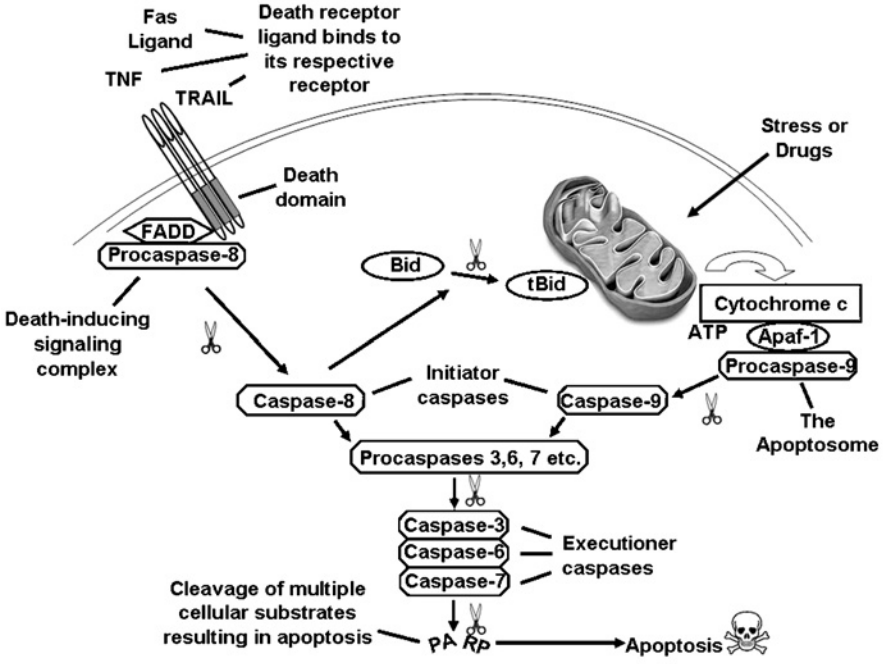

Figure 1. Simplified scheme for induction of apoptosis through the death receptor and mitochondrial pathways. For description of the mechanism refer to the text. Apaf-1, apoptosis activating factor; Bid, BH3-interacting death domain agonist; DISC, death inducing signaling complex; FADD, Fas-associated death domain protein; Fas ligand, the ligand for tumor necrosis receptor superfamily member 6; tBid, truncated Bid; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis inducing ligand; PARP, poly-ADP ribose polymerase.

be triggered (Fig. 1). The first involves death receptors belonging to the tumor necrosis factor (TNF) super family. The second is triggered by environmental stresses or drugs that result in permeabilization of the mitochondrial outer membrane and release of apoptotic factors into the cytoplasm [7, 8]. Apoptosis is mediated by activation of a cascade of serine proteases, called caspases. There are initiator caspases (caspases 8 and 9) that trigger the cascade and effector or executioner caspases (caspases 3, 6, 7, *etc.*) that are responsible for cleavage of substrates that directly or indirectly lead to cell death. In contrast to necrosis, cellular contents remain membrane bound and subsequently undergo phagocytosis in apoptosis, thus preventing inflammation. In some cells, activation of caspase-8 through the death receptors is sufficient to directly activate the central effector caspase, caspase-3 and to trigger apoptosis directly. In other cells, the mitochondrial pathway has to be involved in the apoptotic process. In pancreatic cancer cells, regardless of whether apoptosis is triggered by death receptors or by the stress or drug-activated mechanism, the mitochondrial pathway is always involved.

The death receptor pathway, involves binding of TNF-α, Fas ligand, or TNF-related apoptosis-inducing ligand (TRAIL) to their respective specific receptors, which share a common internal region called the death domain. Ligand binding causes receptor trimerization and recruitment of Fas-associated death domain protein (FADD) and caspase-8 to form the death-inducing signaling complex (DISC), then caspase-8 is activated by cleavage. In pancreatic cancer cells the signal-enhancing effect of mitochondria is needed, and the Bcl family member, Bid, mediates the activation of mitochondria in response to death receptor activation. Bid is cleaved by caspase-8 to its truncated form (tBID), which becomes integrated into the mitochondrial membrane and induces release of cytochrome c and other apoptogenic factors. In the cytoplasm, cytochrome c forms a complex with apoptotic protease-activating factor (Apaf-1), ATP and caspase-9, called the apoptosome. Caspase-9 is another initiator caspase. It is activated by cleavage at the apoptosome and in turn activates the executioner caspases, caspases-3, -6 and -7. These executioner caspases then cleave substrates that result in DNA fragmentation, cleavage of cytoskeletal proteins and cell death. Most studies have shown that, while the Fas ligand and receptor are produced in pancreatic cancer cells, they are resistant to apoptosis through this pathway. This may be due to non-functional receptors or to changes in the downstream pathway, with up-regulation of Bcl-x_L (anti-apoptotic member of the Bcl protein family) or other family members [9]. The secreted Fas ligand may be important in evasion of the immune response to the tumor. Similarly, resistance to TRAIL is not related to lack of receptors, but rather to up-regulation of anti-apoptosis proteins such as Bcl-$x_{L,}$ X-linked inhibitor of apoptosis protein (XIAP), and the short form of FADD-like ICE inhibitory proteins ($FLIP_S$) [10, 11]. Furthermore, inhibition of

production of these proteins sensitizes the cells to the apoptosis-inducing effects of TRAIL [10, 11].

Key factors in the cell's balance of apoptosis or survival are the Bcl family of apoptosis regulators. This is a large family of at least 16 members including anti-apoptotic (*e.g.*, Bcl-2, Mcl-1, Bcl-x_L) and pro-apoptotic proteins (*e.g.*, Bax, Bak, and Bad). These regulators interact with other proteins through a helical region, called the Bcl homology domain. This interaction is important for regulation of apoptosis. The pro-apoptotic Bcl proteins activate permeabilization of the mitochondrial membrane, either by forming tetrameric channels themselves or by interaction with the mitochondrial permeability pore complex, allowing cytochrome c to pass into the cytoplasm. The anti-apoptotic members of the family prevent cytochrome c release, and it is probably the balance between these effectors that determines whether apoptosis will proceed or not. Bcl-x exists in two molecular forms, one long (Bcl-x_L) that is anti-apoptotic and a short form (Bcl-x_S) that is pro-apoptotic. There is some evidence in pancreatic cancer cells that Bcl-x_L is more important than Bcl-2 in protecting against apoptosis triggered by the Fas ligand or TRAIL [12]. Bcl-x_L is constitutively over-expressed in pancreatic cancer cells. Like Bcl-2, Bcl-x_L prevents cytochrome c release, but it also binds to Apaf-1 and thereby prevents association of caspase-9 with Apaf-1 and activation of this caspase [13] (Fig. 2). However, Bcl-2 itself and Mcl-1 appear to be important in resistance to drug-induced apoptosis [14, 15]. Bcl-x_L or other family members might be useful targets for pancreatic cancer therapy and certainly knockout of Bcl-x_L function results in an increase in apoptosis and sensitivity to gemcitabine, the cytotoxic agent that is the mainstay of therapy in pancreatic cancer [16, 17]. Similarly, over-expression of Bax increases the sensitivity of pancreatic cancer cells to drug-induced apoptosis [18]. Expression of another family member, Bak and apoptosis occur in the areas of inflammation surrounding the cancer, but not in the cancer cells themselves. This may actually promote tumor growth. There is considerable effort being aimed at restoring the function of the pro-apoptotic Bcl-2 family members in the tumor cells using peptide mimetics and drugs that mimic the function of these proteins [19].

The complex control mechanisms of apoptosis also involve a series of caspase inhibitors. One class of these is the FLIP. Long ($FLIP_L$) and short ($FLIP_S$) forms of FLIP have been characterized [20]. $FLIP_L$, which is a homologue of caspase-8 but lacks the amino acids necessary for caspase activity, competes with caspase-8 for binding to FADD at the DISC, preventing activation of the caspase [20]. $FLIP_L$ is over-expressed in pancreatic cancer cells that are resistant of Fas-mediated apoptosis, and thus is a potential target for therapy [9]. Indeed, peroxisone proliferator-activated receptor (PPAR) γ agonists sensitize tumor cells to apoptosis by decreasing FLIP expression [21]. FLIP is also involved in activation of NF-κB by recruiting adaptor proteins such as TNF-α receptor-associated factor (TRAF), which in turn activates expression of several genes involved in tumor growth and progression [22, 23].

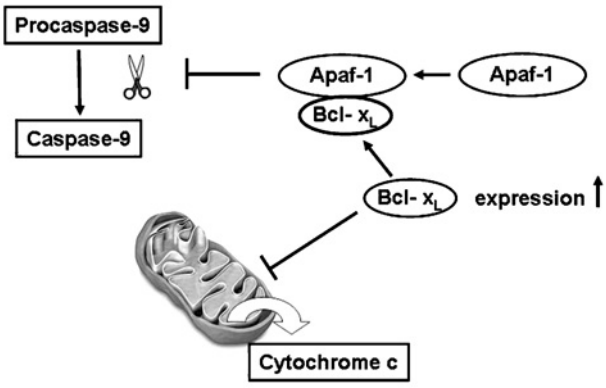

Figure 2. Illustration of the central role for BCL-X_L in the regulation of apoptosis. BCL-X_L inhibits release of cytochrome c from the mitochondria into the cytoplasm and also associates with Apaf-1 to prevent activation of the initiator pro-caspase-9. BCL-X_L, pro-apoptotic member of the Bcl-2 protein family.

Another group of caspase inhibitors is the inhibitor of apoptosis (IAP) family, including cIAP, XIAP and survivin [24]. These proteins contain a region called the baculoviral IAP repeat domain, through which they bind to caspases. The cellular function of the IAPs is not clear, but their expression is increased in cancer cells resistant to Fas and TRAIL-induced apoptosis. Forced expression of cIAP1, cIAP2 and XIAP suppresses apoptosis [24]. Survivin is a member of the IAP family, but has a unique structure that discriminates it from the other family members [24]. It is expressed in the G2/M phase of the cell cycle in a regulated manner. The induction of G1 cell cycle arrest and retinoblastoma (Rb) activation in pancreatic cancer cells treated with a CDK4 inhibitor results in increased sensitivity to TRAIL induced by down-regulation of survivin expression [25]. Knockdown of hypoxia-inducible factor alpha (HIF-1α) also results in decreased survivin expression and increased sensitivity to the apoptotic effects of chemotherapeutics, indicating that the up-regulated HIF-1α is also involved in the increased survivin expression in pancreatic cancer [26]. Survivin binds and inhibits both caspase-3 and caspase-7, which are the major executioner caspases [27]. It is not expressed in normal differentiated cells from any organ in adults,

but is highly expressed in a wide range of cancers, including pancreatic cancer. Survivin expression steadily increases through the developmental stages of pancreatic intraepithelial neoplasias (PanINs), which are the precursor lesions of pancreatic cancer [28]. Since survivin is such a potent caspase inhibitor, its over-expression in cancer is implicated in the resistance to different apoptotic stimuli, including chemotherapy. This appears to be a major reason for the resistance of pancreatic cancer to therapeutic agents. Several studies have shown that knockdown of survivin, XIAP or cIAP-2 expression using small inhibitory RNA (siRNA) can induce apoptosis in a number of different cancer cell types, including pancreatic cancer and increase sensitivity to gemcitabine, 5-fluorouracil, paclitaxel and doxyrubicin [29–32]. That is why these proteins are of such interest as targets in cancer therapy and also for chemoprevention. A recent report showed that small molecule inhibitors of XIAP (XAntags 1396–11 and 1396–12) increased caspase-3 and caspase-7 activity with effects in the low nM range [33]. These inhibitors also produced marked apoptosis of mouse xenografts of human pancreatic cancers and showed marked synergy with TRAIL or radiation therapy [33].

The function of IAPs is inhibited by a protein called SMAC [34]. The precursor form of SMAC is localized to mitochondria and it is released into the cytosol by cellular stress [34]. Its release, like that of cytochrome c, is suppressed by Bcl-2 [34]. Up-regulation of SMAC or its function may be valuable in pancreatic cancer therapy. A small fragment of the N-terminal sequence of SMAC coupled to a carrier peptide is able to enhance the anti-proliferative effect and apoptosis induction stimulated by a wide range of anti-neoplastic agents [35].

Procaspase-3 expression is increased in pancreatic cancer and levels are related to the invasiveness of the cancer cells [36]. Attempts to increase its expression would, therefore, be futile. Recently, it came to light that the dormancy of the proenzyme is maintained by a regulatory "safety catch", which prevents accidental apoptosis [37]. The safety catch comprises of a triplet of aspartic acid residues contained within the proenzyme itself that blocks access to the Ile-Glu-Thr-Asp (IETD) proteolytic activation site. It appears that the safety catch becomes disabled under the low cellular pH conditions that accompany apoptosis, enabling activation of this executioner caspase by caspase-9 or autoactivation [37] (Fig. 3). This "safety catch" clearly represents an important drug target, since procaspase-3 is up-regulated in pancreatic cancer and its activation results in apoptosis. These observations triggered the search for a small molecule that would directly activate this executioner caspase and bypass the upstream apoptosis regulatory pathways. A screen of more than 20 000 diverse small molecules revealed four compounds that could increase hydrolysis of a peptidic caspase-3 substrate [38]. One compound in particular showed a strong dose-dependent effect on procaspase-3 activation. This compound, named procaspase-activating compound 1 (PAC-1) induces apoptosis in a wide range of cell lines and retarded growth of tumors in three different mouse models of cancer [38]. PAC-1 is active when administered orally [38]. Hopefully, PAC-1 or a similar compound can be developed as an anti-cancer agent.

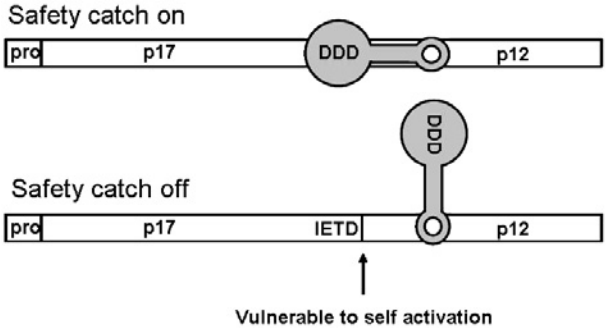

Figure 3. Schematic diagram showing how activation of procaspase-3 is suppressed. A triplet of aspartic acid residues (DDD) overlays the Ile-Glu-Thr-Asp (IETD) proteolytic cleavage site. Low pH within the apoptotic cell causes a conformational change, which renders this activation site vulnerable to cleavage by initiator caspases (caspase-8 and -9) or by self activation.

Targeting the growth pathways in pancreatic cancer

Genetic mutations in oncogenes and tumor suppressor genes leads to up-regulation of growth factors, their receptors and downstream signaling pathways in pancreatic cancer. These tumors are largely independent of extrinsic growth factors, giving them a distinct growth advantage. Removal of autocrine growth factor support triggers apoptosis and many targeted therapy strategies are focused on these pathways. Important targets include the phosphatidylinositol-3 kinase (PI3K) and protein kinase B (AKT) pathway, the epidermal growth factor receptor (EGFR)-mediated pathway, the NF-κB pathway, the p53 tumor suppressor gene and the lipoxygenase and cyclooxygenase pathways (Fig. 4).

The *k*-ras gene is activated by mutations in more than 90% of pancreatic cancers. The vast majority of activating point mutations in this gene occur at codon 12, with a few other mutations targeting codons 13 or 61 [39]. These mutations occur early in the development of pancreatic neoplasia, giving the cells a growth advantage even before invasive cancer develops [39].

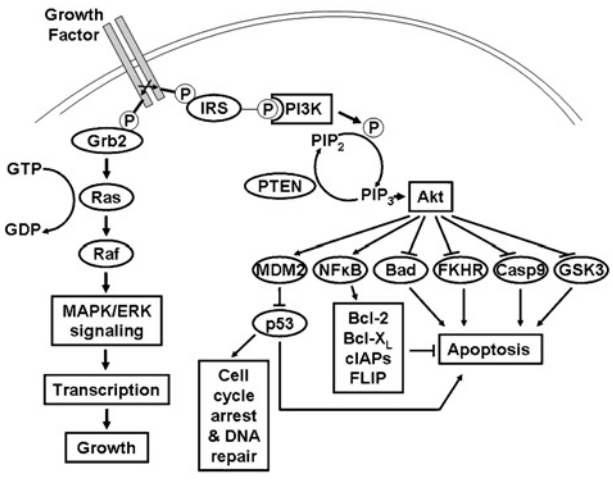

Figure 4. Simplified scheme showing the interaction between different intracellular signal transduction components of the growth factor pathways. Growth factor receptors, such as the epidermal growth factor receptor (EGFR), are membrane-spanning glycoprotein dimers with an N-terminal ligand binding domain, a hydrophobic transmembrane domain and a cytoplasmic domain containing tyrosine kinase and receptor regulatory motifs. The growth factor binds to its respective receptor and induces homodimerization (or heterodimerization with another member of the ErbB receptor family) and autophosphorylation of the opposite chain. The resulting phosphorylated tyrosines serve as binding and activation sites for signal transducers and adaptor proteins, such as Grb2 and IRS-1/2. Two major signaling routes are the Ras-Raf-MAPK pathway and the PI3K/Akt pathway. Akt triggers several events that suppress apoptosis and promote cell survival. These include inactivation of pro-apoptotic proteins such as Bad, FKHR, caspase-9, and GSK3, activation of MDM2 that results in inactivation of the p53 tumor suppressor protein, and activation of NF-κB that increases transcription of anti-apoptotic proteins such as Bcl-2, Bcl-X_L, the cIAPs and FLIP. PTEN, which is inactivated in many cancers is a natural inhibitor of the PI3K/Akt pathway. ErbB, erythroblastic leukemia viral (v-erb-b) oncogene homolog; Grb2, growth factor receptor-bound protein; IRS, insulin receptor substrate; Ras, rat sarcoma viral oncogene homolog; Raf, v-raf-1 murine leukemia viral oncogene homolog; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3 kinase; Akt, v-akt murine thymoma viral oncogene homolog; Bad, Bcl-2 antagonist of cell death; FKHR, forkhead in rhabdomyosarcoma; GSK3, glycogen synthase kinase 3; MDM2, murine double minute 2 protein; NF-κB, nuclear factor κB; Bcl-2, B cell lymphoma 2 protein; cIAPs, cellular inhibitor of apoptosis proteins; FLIP, FLICE inhibitory protein; PTEN, phosphatase and tensin homolog.

The PI3K/AKT pathway is activated by growth factor receptors, such as the EGF and IGF-1 receptors. This pathway is important for cellular survival as well as growth. Activation of AKT induces up-regulation of anti-apoptotic Bcl family members, MDM2 and NF-κB, as well as inactivation of pro-apoptotic proteins such as Bad, forkhead, caspase-9, and glycogen synthase kinase 3 (GSK3) [40, 41]. AKT also phosphorylates and thereby inactivates the pro-apoptotic Bcl family member, Bad as well as caspase-9 [42, 43]. The activity of PI3K is inhibited by a protein called phosphatase and tensin homolog (PTEN). In pancreatic cancer cells, PI3K, AKT, growth factors and their receptors are over-expressed, while expression of PTEN is often suppressed. Because of the survival advantage that this pathway renders, it provides an important target for sensitizing cells to apoptosis. Wortmannin, a potent PI3K inhibitor blocks proliferation, induces apoptosis and enhances the effects of gemcitabine in pancreatic cancer cells [44, 45]. Other PI3K inhibitors are currently under investigation for cancer therapy. GN963 is a tyrosine kinase inhibitor with activity against Akt as well as platelet-derived growth factor receptor and Src. This inhibitor markedly reduced growth of pancreatic cancer xenografts in nude mice. It was even more effective and completely blocked hepatic metastases, when used in combination with gemcitabine [46].

The EGFR is over-expressed in the majority of pancreatic cancer cells and several of the ligands for this receptor are also up-regulated, including EGF itself, transforming growth factor-α (TGF-α), amphiregulin, and heparin-binding EGF [47]. High-affinity binding of these ligands to the receptor triggers a growth-stimulatory signaling cascade that involves *k*-ras, which is mutated and constitutively active in about 95 % of pancreatic cancers. This pathway is, therefore, extremely important for growth and survival of this cancer and several therapeutic strategies targeting the pathway are being pursued. These include various strategies to normalize the function of *k*-ras. Previous studies have shown that protein kinase C (PKC) activation by tumor-promoting phorbol esters cause a paradoxical inhibition of pancreatic cancer cell proliferation [48]. A recent study showed that phosphorylation of the *k*-ras oncogene on Ser^{181} results in translocation from the plasma membrane to intracellular membranes, including the outer mitochondrial membrane, where it interacts with Bcl-xL to promote apoptosis [49]. The PKC partial agonist bryostatin-1 inhibited the growth *in vitro* and *in vivo* of cells transformed with oncogenic *k*-ras in a Ser^{181}-dependent mechanism (Fig. 5). These findings provide a direct link between PKC activation and apoptosis, and provide a possible explanation for PKC activation-mediated growth inhibition. Furthermore, since *k*-ras is mutated in almost all pancreatic cancers, these findings suggest that bryostatin may be effective in the treatment of these tumors [49]. Another important approach to targeting the EGF growth pathway is the use of EGF receptor tyrosine kinase inhibitors, the first of which (Erlotinib) has been approved by the FDA for treating the disease [50, 51]. However, it is now clear that a small subset of patients with EGFR mutations are the ones that respond well to the effects of these agents [52]. Another kinase inhibitor under investigation is

ZD6474, which is known to inhibit the tyrosine kinase activity of vascular endothelial growth factor and EGFR. In a recent study in pancreatic cancer cells, ZD6474 inhibited growth and induced apoptosis and showed synergistic effects with gemcitabine and radiation [53]. The drug inhibited phosphorylation of Akt as well as EGFR [53]. In the athymic mouse xenograft model, ZD6474 inhibited tumor growth and significantly enhanced the effects of gemcitabine and radiation [53]. Other approaches to targeting this pathway include chimeric or humanized monoclonal antibodies that target the EGFR, such as Cetuximab and Matuzumab [54, 55], as well as inhibitors of the extracellular regulated kinases (ERKs) [56]. Curcumin inhibits expression of EGFR *via* a pathway that involves inhibition of cyclooxygenase-2 (COX-2) expression [57]. Another member of the EGFR family, ErbB2, also known as Her-2/neu, has also been targeted with a monoclonal antibody marketed as Herceptin [58].

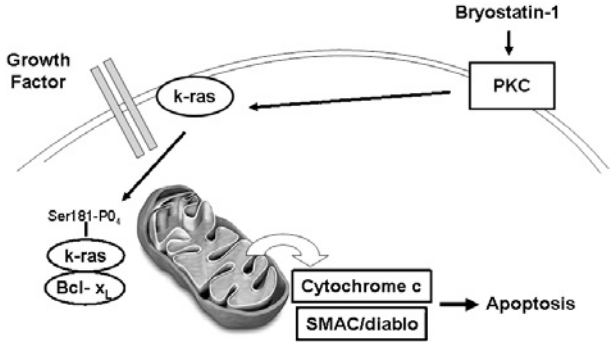

Figure 5. Activation PKC by bryostatin-1 induces phosphorylation of active oncogenic *k*-ras by a farnesyl-electrostatic switch with causes tranlocation of serine[181] *k*-ras from the inner plasma membrane to inner cell membranes, including that of the mitochondria, where it becomes associated with BCL-X_L and induces apoptosis. *k*-ras, Kersten rat sarcoma 2 viral oncogene homolog; SMAC/diablo, second mitochondrial second-mitochondria-derived activator of caspase also known as direct AIP binding protein with low pI; PKC, protein kinase, C.

NF-κB is a transcription factor involved in diverse cellular activities including inflammation and the immune response. NF-κB induces transcription of multiple target genes, including Bcl-x_L, cIAPs, and FLIPs [59]. Normally, NF-κB is kept in an inactive state in the cytoplasm by another protein called IκBα. Cytokines, growth factors and cellular stress activate NF-κB by degradation of IκBα, whereupon it is translocated to the nucleus to increase expression of several anti-apoptotic genes listed above. NF-κB appears to be constitutively activated in pancreatic cancer cells and is further activated by several chemotherapeutic agents, indicating that this transcription factor contributes to resistance to apoptosis in this cancer [23]. Inhibitors of NF-κB, such as genestein, gliotoxin, sulfasalazine, and gabexate mesilate sensitize pancreatic cancer cells to chemotherapeutic drugs and to TNF-α with an increase in apoptosis [60–62]. Such agents are likely to be valuable in enhancing the effect of chemotherapeutic drugs in pancreatic cancer. Recent reports show that inhibition of NF-κB activation by lidamycin or curcumin markedly inhibits growth of human pancreatic cancer xenografts in athymic mice and also suppresses angiogenesis [63, 64]. Notch signaling plays a critical role in maintaining the balance between cellular proliferation, differentiation and apoptosis, and recent reports suggest that Notch-1 is an upstream regulator of NF-κB [65, 66]. Indeed, the inhibitory effects of genistein and curcumin on NF-κB activation appear to be mediated by the down-regulation of Notch-1 [65, 66]. GSK and tissue transglutaminase (TG2) are critical regulators of NF-κB and so they are also potential therapeutic targets [67, 68].

Interferons (IFN-α, -β and -γ) have anti-proliferative and apoptotic effects in pancreatic cancer cell lines expressing the respective receptors [69]. The apoptotic effects are blocked by a caspase-8 inhibitor, indicating that IFNs induce apoptosis by triggering the caspase cascade [69]. Enhanced cytotoxicity is seen when IFNs are combined with gemcitabine or 5-fluorouracil.

Activation of sonic hedgehog (shh) signaling is seen in the majority of pancreatic cancers. Shh increases proliferation of the cancer cells and protects them from apoptosis by activation of PI3K and stabilization of Bcl-2 and Bcl-X_L [70]. In addition, Shh enhances *k*-Ras-induced tumorigenesis by reducing dependence of the tumor cells on the sustained activation of the ERK and PI3K/Akt growth pathways [70]. Thus, Shh is another important target for inhibition of growth and induction of apoptosis.

In normal cells, the p53 tumor suppressor protein inhibits growth by triggering cell cycle arrest and apoptosis (Fig. 6). It acts directly by binding and inactivating Bcl-2 and Bcl-x_L, and indirectly by increasing the expression of pro-apoptotic Bcl family members such as Bax, Bid, and Bim [6]. This tumor suppressor gene is inactivated in more than 60% of pancreatic cancers, giving the cells a growth advantage [6]. Forced expression of wild-type p53 suppresses growth of pancreatic cancer cells and sensitizes them to apoptosis. Thus, an important therapeutic approach for pancreatic cancer is to normalize p53 function [71].

Lipoxygenases (particularly 5-LOX, 12-LOX) and cyclooxygenases (COX-2), which are key enzymes of arachidonic acid metabolism, play a major role in

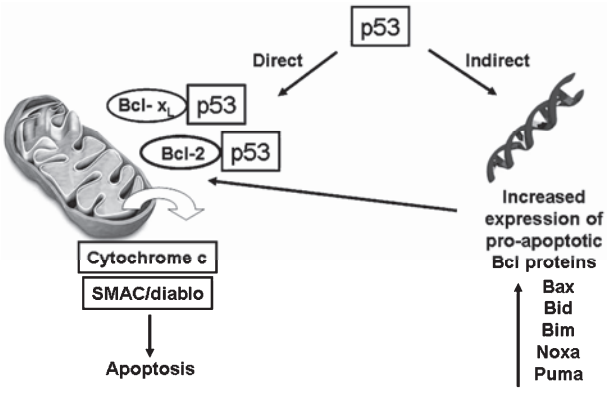

Figure 6. Scheme showing the direct and indirect mechanisms by which the tumor suppressor protein, p53 promotes apoptosis. Bax, Bid, Bim, Noxa and Puma, pro-apoptotic Bcl-2 related proteins.

development and progression of pancreatic cancers [15, 72, 73]. These enzymes are not expressed in normal ductal cells, but are expressed in pancreatic cancers as well as in the precursor PanIN lesions [74, 75]. LOX and COX metabolites stimulate growth of pancreatic cancer cells, while inhibitors of these enzymes block growth both *in vitro* and *in vivo* [15, 72, 73, 76–79]. The LOX enzymes are up-regulated in response to growth factor stimulation, while inhibitors of these enzymes block growth factor-induced growth and mitogen-activated protein kinase (MAPK) activation, indicating interaction of these pathways. COX and LOX inhibitors, and receptor antagonists for the downstream metabolite, leukotriene B4 induce apoptosis *in vitro* and *in vivo* and enhance the effectiveness of gemcitabine against these cancer cells [15, 73, 78, 79]. These pathways are clearly valuable targets for pancreatic cancer therapy and chemoprevention.

Novel agents recently shown to inhibit pancreatic cancer growth with apoptosis

There are many naturally occurring substances that have been recently shown to potently inhibit growth of pancreatic cancer cells with induction of apoptosis. These include genistein, a flavonoid from soy beans [61]; resveratrol, a phytoalexin found in high concentrations in grape skins and red wine [80]; apigenin, a flavonoid found in herbs such as parsley, thyme and peppermint [81]; gingerol a major phenolic compound in root ginger [82]; frondoside A., a triterpine glycoside from the skin of the edible Atlantic sea cucumber [83]; and sansalvamide, a small cyclic depsipeptide from a marine fungus [84]. All of these compounds appear to have low toxicity and may be developed into therapeutics themselves or as adjuncts to conventional chemotherapeutic drugs.

Conclusions

The complex pathways involved in apoptosis are controlled by a variety of pro- and anti-apoptotic factors, the balance of which ensures tissue homeostasis. Activation or down-regulation of pro- and anti-apoptotic genes can influence cancer cell viability and sensitivity to chemotherapy or radiotherapy, tumor development and progression. There are a number of potential targets for inducing and enhancing apoptosis in this disease, including enhancing or mimicking the effects of pro-apoptotic Bcl family members; blocking the effects of caspase inhibitors; up-regulating SMAC/diablo function; inhibition of the PI3K/Akt pathway; blocking growth factor (*e.g.*, EGF) pathways; inhibiting NF-κB; normalizing p53 function; inhibiting 5-LOX, 12-LOX, and or COX-2 activity or blocking the downstream pathways for their metabolites. Such therapeutic strategies, perhaps in combination may help to improve outcome of this devastating disease.

1 Cardenes, H. R., Chiorean, E. G., DeWitt, J., Schmidt, M. and Loehrer, P. (2006) Locally advanced pancreatic cancer: Current therapeutic approach. Ocologist 11, 612 – 623.
2 Carpelan-Holmström, M., Nordling, S., Pukkala, E., Sankila, R., Lüttges, J., Klöppel, G. and Haglund, C. (2005) Does anyone survive pancreatic ductal adenocarcinoma? A nationwide study re-evaluating the data of the Finnish Cancer Registry. Pancreas 54, 385 – 387.
3 Giovannetti, E., Mey, V., Nannizzi, S., Pasqualetti, G., Del Tacca, M. and Danesi, R. (2006) Pharmacogenetics of anticancer drug sensitivity in pancreatic cancer. Mol. Cancer Ther. 6, 1387 – 1395.
4 Nakano, Y., Tanno, S., Koizumi, K., Nishikawa, T., Nakamura, K., Minoguchi, M., Izawa, T., Mizukami, Y., Okumura, T. and Kohgo, Y. (2007) Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. Br. J. Cancer 96, 457 – 463.
5 Talar-Wojnarowska, R. and Malecka-Panas, E. (2006) Molecular pathogenesis of pancreatic adenocarcinoma: Potential clinical implications. Med. Sci. Monit. 2, RA186-RA193.
6 Schneider, G. and Schmid, R. M. (2003) Genetic alterations in pancreatic carcinoma. Mol. Cancer 2, 15.
7 Okada, H. and Mak, T. W. (200) Pathways of apoptotic and non-apoptotic death in tumor cells. Nat. Rev. Cancer 4, 592 – 603.
8 Ghobrial, I. M., Witzig, T. E. and Adjei, A. A. (2005) Targeting apoptosis pathways in cancer therapy. CA Cancer J. Clin. 55, 178 – 194.
9 Elnemr, A., Ohta, T., Yachie, A., Kayahara, M., Kitigawa, H., Fujimura, T., Ninomiya, I., Fushida, S., Nishimura, G. I., Shimizu, K. and Miwa, K. (2001) Human pancreatic cancer cells disable function of Fas receptors at several levels in Fas signal transduction pathway. Int. J. Oncol. 2, 311 – 316.
10 Matsuzaki, H., Schmied, B. M., Ulrich, A., Standop, J., Schneider, M. B., Batra, S. K., Picha, K. S. and Pour, P. M. (2001) Combination of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and actinomycin D induces apoptosis even in TRAIL-resistant human pancreatic cancer cells. Clin. Cancer Res. 2, 407 – 414.
11 Vogler, M., Durr, K., Jovanovic, M., Debatin, K. M. and Fulda, S. (2007) Regulation of TRAIL-induced apoptosis by XIAP in pancreatic cancer cells. Oncogene 26, 248 – 257.

12 Hinz, S., Trauzold, A., Boenicke, L., Sandberg, C., Beckmann, S., Bayer, E., Walczak, H., Kalthoff, H. and Ungefroren, H. (2000) Bcl-x_L protects pancreatic adenocarcinoma cells against CD95- and TRAIL-receptor-mediated apoptosis. Oncogene 19, 5477 – 5486.

13 Hu, Y., Benedict, M. A., Wu, D., Inohara, N. and Numez, G. (1998) Bcl-x_L interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. Proc. Natl. Acad. Sci. USA 95, 4386 – 4391.

14 Li, X., Ding, X. and Adrian, T. E. (2003) Arsenic trioxide induces apoptosis in pancreatic cancer cells via changes in cell cycle, caspase activation, and GADD expression. Pancreas 27, 174 – 179.

15 Tong, W. G., Ding, X. Z., Witt, R. C. and Adrian, T. E. (2002) Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. Mol. Cancer Ther. 1, 929 – 935.

16 Xu, Z., Friess, H., Solioz, M., Aebi, S., Korc, M., Kleeff, J., and Büchler, M. W. (2001) Bcl-x_L antisense oligonucleotides induce apoptosis and increase sensitivity of pancreatic cancer cells to gemcitabine. Int. J. Cancer 94, 268 – 274.

17 Shi, X., Liu, S., Kleeff, J., Friess, H. and Büchler, M. W. (2002) Acquired resistance of pancreatic cancer cells towards 5-fluorouracil and gemcitabine is associated with altered expression of apoptosis-regulating genes. Oncology 62, 354 – 362.

18 Xu, Z. W., Friess, H., Büchler, M. W. and Solioz, M. (2002) Overexpression of Bax sensitizes human pancreatic cancer cells to apoptosis induced by chemotherapeutic agents. Cancer Chemother. Pharmacol. 49, 504 – 510.

19 Baell, J. B. and Huang, D. C. (2002) Prospects for targeting the Bcl-2 family of proteins to develop novel cytotoxic drugs. Biochem. Pharmacol. 64, 851 – 863.

20 Krueger, A., Baumann, S., Krammer, P. H. and Kirchoff, S. (2001) FLICE-inhibitory proteins: Regulators of death receptor-mediated apoptosis. Mol. Cell Biol. 21, 8247 – 8254.

21 Kim, Y., Suh, N., Sporn, M. and Reed, J. C. (2002) An inducible pathway for degradation of FLIP protein sensitizes tumor cells to TRAIL-induced apoptosis. J. Biol. Chem. 277, 22320 – 22329.

22 Kataoka, T., Budd, R. C., Holler, N., Thorne, M., Martinon, F., Irmler, M., Burns, K., Hahne, M., Kennedy, N., Kovacsovics, M. and Tschopp J. (2000) The caspase-8 inhibitor FLIP promotes activation of NFκB and Erk signaling pathways. Curr. Biol. 10, 640 – 648.

23 Bharti, A. C. and Aggarwal, B. B. (2002) Nuclear factor-κ-B and cancer: Its role in prevention and therapy. Biochem. Pharmacol. 64, 883 – 888.

24 Deveraux, Q. L. and Reed, J. C. (1999) IAP family proteins-suppressors of apoptosis. Genes Dev. 13, 239 – 252.

25 Retzer-Lidl M., Schmid, R. M. and Schneider G. (2007) Inhibition of CDK4 impairs proliferation of pancreatic cancer cells and sensitizes towards TRAIL-induced apoptosis via downregulation of survivin. Int. J. Cancer. 121, 66 – 75.

26 Chang, Q., Qin, R., Huang, T., Gao J. and Feng, Y. (2006) Effect of antisense hypoxia-inducible factor 1alpha on progression, metastasis, and chemosensitivity of pancreatic cancer. Pancreas 32, 297 – 305.

27 Tamm, I., Wang, Y., Sausville, E., Scudiero, D. A., Vigna, N., Oltersdorf, T. and Reed, J. C. (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res. 58, 5315 – 5320.

28 Bhanot, U., Heydrich, R., Moller, P. and Hasel, C. (2006) Survivin expression in pancreatic intraepithelial neoplasia (PanIN): Steady increase along the developmental stages of pancreatic ductal adenocarcinoma. Am. J. Surg. Pathol. 30, 754 – 759.

29 Shrikhande, S. V., Kleeff, J., Kayed, H., Keleg, S., Reiser, C., Giese, T., Büchler, M. W., Esposito, I. and Friess, H. (2006) Silencing of X-linked inhibitor of apoptosis (XIAP) decreases gemcitabine resistance in pancreatic cancer cells. Anticancer Res. 26, 3265 – 3273.

30 Li, Y., Jian, Z., Xia, K., Li, X., Lv, X., Pei, H., Chen, Z. and Li J. (2006) XIAP is related to the chemoresistance and inhibited its expression by RNA interference sensitize pancreatic carcinoma cells to chemotherapeutics. Pancreas 32, 288 – 296.

31 Guan, H. T., Xue, X. H., Dai, Z. J., Wang, X. J., Li, A. and Qin, Z. Y. (2006) Down-regulation of survivin expression by small interfering RNA induces pancreatic cancer cell apoptosis and enhances its radiosensitivity. World J. Gastroenterol. 12, 2901 – 2907.

32 Lopes, R. B., Gangeswaran, R., McNeish, I. A., Wang, Y. and Lemoine, N. R. (2007) Expression of the IAP protein family is dysregulated in pancreatic cancer cells and is important for resistance to chemotherapy. Int. J. Cancer. 120, 2344 – 2352.

33 Karikari, C. A., Roy, I., Tryggestad, E., Feldmann, G., Pinilla, C., Welsh, K., Reed, J. C., Armour, E. P., Wong, J., Herman, J., Rakheja, D. and Maitra, A. (2007) Targeting the apoptotic machinery in pancreatic cancers using small-molecule antagonists of the X-linked inhibitor of apoptosis protein. Mol. Cancer Ther. 6, 957 – 966.

34 Verhagan, A. M., Ekert, P. G., Pakusch, M., Silke, J., Connolly, L. M., Reid, G. E., Moritz, R. L., Simpson, R. J. and Vaux, D. L. (2000) Identification of DIABLO., a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 102, 43 – 53.

35 Arnt, C. R., Chiorean, M. V., Heldebrant, M. P., Gores, G. J. and Kaufmann, S. H. (2002) Synthetic Smac/DIABLO peptides enhance the effects of chemotherapeutic agents by binding XIAP and cIAP *in situ.* J. Biol. Chem. 277, 44236 – 44243.

36 Satoh, K., Kaneko, K., Hirota, M., Toyota, T. and Shimosegawa, T. (2000) The pattern of CPP32/caspase-3 expression reflects the biological behavior of the human pancreatic duct cell tumors. Pancreas 21, 532 – 537.

37 Roy, S., Bayly, C. I., Gareau, Y., Houtzager, V. M., Kargman, S., Keen, S. L., C., Rowland, K., Seiden, I. M., Thornberry, N. A. and Nicholson, D. W. (2001) Maintenance of caspase-3 proenzyme dormancy by an intrinsic "safety catch" regulatory tripeptide. Proc. Natl. Acad. Sci. USA 98, 6132 – 6137.

38 Putt, K. S., Chen, G. W., Pearson, J. M., Sandhorst, J. S., Hoagland, M. S., Kwon, J-T., Hwang, S-K., Jin, H., Churchwell, M. I., Cho, M-H., Doerge, D. R., Helferich, W. G. and Hergenrother, P. J. (2006) Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy. Nat. Chem. Biol. 2, 543 – 550.

39 Hruban, R. H., van Mansfeld, A. D., M., Offerhaus, G. J., van Weering, D. H., Allison, D. C., Goodman, S. N., Kensler, T. W., Bose, K. K., Cameron J. L. and Bos J. L. (1993) K-*ras* oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization, Am. J. Pathol. 143, 545 – 554.

40 Graff, J. R., Konicek, B. W., McNulty, A. M., Wang, Z., Houck, K., Allen, S., Paul, J. D., Hbaiu, A., Goode, R. G., Sandusky, G. E., Vessella, R. L. and Neubauer, B. L. (2000) Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing $p27^{KIP1}$ expression. J. Biol. Chem. 275, 24500 – 24505.

41 Jones, R. G., Parsons, M., Bonnard, M., Chan, V. S., Yeh, W. C., Woodgett, J. R. and Ohashi, P. S. (2000) Protein kinase B regulates T lymphocyte survival, nuclear factor κB activation and Bcl-x_L levels *in vivo.* J. Exp. Med. 191, 1721 – 1734.

42 Gilmore, A. P., Valentjin, A. J., Wang, P., Ranger, A. M., Bundred, M., O'Hare, M. J., Wakeling, A., Korsmeyer, S. J. and Streuli, C. H. (2002) Activation of BAD by therapeutic inhibition of epidermal growth factor receptor and transactivation by insulin-like growth factor receptor. J. Biol. Chem. 277, 27643 – 27650.

43 Kermer, P., Ankerhold, R., Klocker, N., Krajewski, S., Reed, J. C. and Bahr, M. (2000) Caspase-9: Involvement in secondary death of axotomized rat retinal ganglion cells *in vivo.* Brain Res. Mol. Brain Res. 85, 144 – 150.

44 Ng, S. S., W., Tsao, M. S., Chow, S. and Hedley, D. W. (2000) Inhibition of phophatidylinositide 3-kinase enhances gemcitabine-induced apoptosis in human pancreatic cancer cells. Cancer Res. 60, 5451 – 5455.
45 Ng, S. S., Tsao, M. S., Nicklee, T. and Hedley, D. W. (2001) Wortmannin inhibits Pkb/Akt phosphorylation and promotes gemcitabine antitumor activity in orthotopic human pancreatic cancer xenografts in immunodeficient mice. Clin. Cancer Res. 7, 3269 – 3275.
46 Baker, C. H., Trevino, J. G., Summy, J. M., Zhang, F., Caron, A., Nesbit, M., Gallick, G. E. and Fidler, I. J. (2006) Inhibition of PDGFR phosphorylation and Src and Akt activity by GN963 leads to therapy of human pancreatic cancer growing orthotopically in nude mice. Int. J. Oncol. 29, 125 – 138.
47 Korc, M., Chandraseker, B., Yamanaka, Y., Friess, H., Büchler, M., and Berger, H. G. (1992) Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the level of epidermal growth factor and transforming growth factor alpha. J. Clin. Invest. 90, 1352 – 1360.
48 Salabat, M. R., Ding, X. Z., Flesche, J. B., Ujiki, M. B., Robin, T. P., Talamonti, M. S., Bell, R. H. and Adrian, T. E. (2006) On the mechanisms of 12-otetradecanoylphorbol-13-acetate-induced growth arrest in pancreatic cancer cells. Pancreas 33,148 – 155.
49 Miura JWiener, H. HWright LSaba, S. GYim DFein APKC regulates a farnesyl-electrostatic switch on K-Ras that promotes its association with Bcl-XL on mitochondria and induces apoptosis. Mol. Cell 21, 481 – 493.
50 Moore, M. J., Goldstein, D., Hamm, J., Kotecha, J., Gallinger S., Au, H. J., Nomikos, D., Ding, K., Ptaszynski, M. and Parulekar, W. (2005) Erlotinib improves survival when added to gemcitabine in patients with advanced pancreatic cancer. A phase III trial of the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG). J. Clin. Oncology 23 (Suppl.), 1.
51 Tang, P. A., Tsao, M. S. and Moore, M. J. (2006) A review of erlotinib and its clinical use. Expert. Opin. Pharmacother. 7, 177 – 193.
52 Kwak, E. L., Jankowski, J., Thayer, S. P., Lauwers, G. Y., Brannigan, B. W., Harris, P. L., Okimoto, R. A., Haserlat, S. M., Driscoll, D. R., Ferry, D., Muir, B., Settleman, J., Fuchs, C. S. et al. (2006) Epidermal growth factor receptor kinase domain mutations in esophageal and pancreatic adenocarcinomas. Clin. Cancer Res. 12, 4283 – 4287.
53 Bianco, C., Giovannetti, E., Ciardiello, F., Mey V., Nannizzi S., Tortora G., Troiani T., Pasqualetti, F., Eckhardt, G., de Liguoro, M., Ricciardi, S., Del Tacca, M. et al. (2006) Synergistic antitumor activity of ZD6474, an inhibitor of vascular endothelial growth factor receptor and epidermal growth factor receptor signaling, with gemcitabine and ionizing radiation against pancreatic cancer. Clin. Cancer Res. 12, 7099 – 7107.
54 Xiong, H. Q., Rosenberg, A., LoBuglio, A., Schmidt, W., Wolff, R. A., Deutsch J., Needle M. and Abbruzzese, J. L. (2004) Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: A multicenter Phase II trial. J. Clin. Oncol. 22, 2610 – 2616.
55 Graeven, U., Kremer, B., Sudhoff, T., Killing, B., Rojo, F., Weber, D., Tillner, J., Unal, C. and Schmiegel, W. (2006) Phase I study of the humanized anti-EGFR monoclonal antibody matuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. Br. J. Cancer 94, 1293 – 1299.
56 Messersmith, W. A., Hidalgo, M., Carducci, M. and Eckhardt, S. G. (2006) Novel targets in solid tumors: MEK inhibitors. Clin. Adv. Hematol. Oncol. 4, 831 – 836.
57 Lev-Ari, S., Starr, A., Vexler, A., Karaush, V., Loew, V., Greif, J., Fenig, E., Aderka, D. and Ben-Yosef, R. (2006) Inhibition of pancreatic and lung adenocarcinoma cell survival by curcumin is associated with increased apoptosis, down-regulation of COX-2 and EGFR and inhibition of Erk1/2 activity. Anticancer Res. 26, 4423 – 4430.
58 Safran, H., Iannitti, D., Ramanathan, R., Schwartz J. D., Steinhoff M., Nauman C., Hesketh P., Rathore R., Wolff R., Tantravahi U., Hughes, T. M., Maia C. et al. (2004) Herceptin and gemcitabine for metastatic pancreatic cancers that overexpress Her-2/neu. Cancer Invest. 22, 706 – 712.
59 Karin, M. and Lin, A. (2002) NF-κB at the crossroads of life and death. Nat. Immunol. 3, 221 – 22.
60 Arlt, A., Vorndamm, J., Breitenbroich, M., Folsch, U. R., Kalthoff, H., Schmidt, W. E. and Schäfer, H. (2001) Inhibition of NF-κB sensitizes human pancreatic carcinoma cells to apoptosis induced by etoposide (VP16) or doxorubicin. Oncogene 20, 859 – 868.
61 Li, Y., Ahmed, F., Ali, S., Philip, P. A., Kucuk, O. and Sarkar, F. H. (2005) Inactivation of nuclear factor κB by soy isoflavone genestein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells. Cancer Res. 65, 6934 – 6942.
62 Takahashi, H., Funahashi, H., Sawai, H., Matsuo, Y., Yamamoto, M., Okada, Y., Takeyama, H. and Manabe, T. (2007) Stem cell factor/c-kit receptor signaling enhances the proliferation and invasion of colorectal cancer cells through the PI3K/Akt pathway. Dig. Dis. Sci. [Epub ahead of print].
63 Chen J., Ouyang, Z. G., Zhang, S. H. and Zhen, Y. S. (2007) Down-regulation of the nuclear factor-kappaB by lidamycin in association with inducing apoptosis in human pancreatic cancer cells and inhibiting xenograft growth. Oncol. Rep. 17, 1445 – 1451.
64 Kunnumakkara, A. B., Guha, S., Krishnan, S., Diagaradjane, P., Gelovani J. and Aggarwal, B. B. (2007) Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. Cancer Res. 67, 3853 – 3861.
65 Wang, Z., Banerjee, S., Li, Y., Rahman, K. M., Zhang, Y. and Sarkar, F. H. (2006) Down-regulation of notch-1 inhibits invasion by inactivation of nuclear factor-kappaB., vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells. Cancer Res. 66, 2778 – 2784.
66 Wang, Z., Zhang, Y., Banerjee, S., Li, Y. and Sarkar, F. H. (2006) Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. Cancer 106, 2503 – 2513.
67 Garcea, G., Manson, M. M., Neal, C. P., Pattenden, C. J., Sutton, C. D., Dennison, A. R. and Berry DP. (2007) Glycogen synthase kinase-3 beta; a new target in pancreatic cancer? Curr. Cancer Drug Targets 7, 209 – 215.
68 Mann, A. P., Verma, A., Sethi, G., Manavathi, B., Wang, H., Fok, J. Y., Kunnumakkara, A. B., Kumar, R., Aggarwal, B. B. and Mehta, K. (2006) Overexpression of tissue transglutaminase leads to constitutive activation of nuclear factor-{kappa}B in cancer cells: Delineation of a novel pathway. Cancer Res. 66, 8788 – 8795.
69 Saidi, R. F., Williams, F., Ng, J., Danquah, G., Mittal, V. K., ReMine, S. G. and Jacobs, M. J. (2006) Interferon receptors and the caspase cascade regulate the antitumor effects of interferons on human pancreatic cancer cell lines. Am. J. Surg. 191, 358 – 363.
70 Morton J. P., Mongeau, M. E., Klimstra, D. S., Morris J. P., Lee, Y. C., Kawaguchi Y., Wright, C. V., Hebrok M. and Lewis, B. C. (2007) Sonic hedgehog acts at multiple stages during pancreatic tumorigenesis. Proc. Natl. Acad. Sci. USA 104, 5103 – 5108.
71 Ghaneh, P., Greenhalf, W., Humphreys, M., Wilson, D., Zumstein, L., Lemoine, N. R. and Neoptolemos, J. P. (2001) Adenovirus-mediated transfer of p53 and p16 (INK4a) results in pancreatic cancer regression in vitro and in vivo. Gene Ther. 8, 199 – 208.
72 Ding, X. Z., Kuszynski, C. A., El-Metwally, T. H. and Adrian, T. E. (1999) Lipoxygenase inhibitors induce apoptosis, morphological changes and carbonic anhydrase expression in

pancreatic cancer cells. Biochem. Biophys. Res. Commun. 266, 392 – 399.
73 Ding, X. Z., Tong, W. G. and Adrian, T. E. (2000) Blockade of cyclooxygenase-2 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Anticancer Res. 20, 2625 – 2631.
74 Hennig, R., Ding, X. Z., Tong, W. G., Schneider, M. B., Standop, J., Friess, H., Buchler, M. W., Pour, P. M. and Adrian, T. E. (2002) 5-Lipoxygenase and leukotriene B(4) receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. Am. J. Pathol. 161, 421 – 428.
75 Hennig R., Grippo P., Ding, X. Z., Rao, S. M., Buchler, M. W., Friess H., Talamonti, M. S., Bell, R. H. and Adrian, T. E. (2005) 5-Lipoxygenase, a marker for early pancreatic intraepithelial neoplastic lesions. Cancer Res. 65, 6011 – 6016.
76 Ding, X. Z., Tong, W. G. and Adrian, T. E. (2001) 12-lipoxygenase metabolite 12(S)-HETE stimulates human pancreatic cancer cell proliferation via protein tyrosine phosphorylation and ERK activation. Int. J. Cancer 94, 630 – 636.
77 Ding, X. Z., Tong, W. G. and Adrian, T. E. (2003) Multiple signal pathways are involved in the mitogenic effect of 5(S)-HETE in human pancreatic cancer. Oncology 65, 285 – 294.
78 Tong, W. G., Ding, X. Z. and Adrian, T. E. (2002) Leukotriene B4 receptor antagonist LY293111 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Clin. Cancer Res. 8, 3232 – 3242.
79 Hennig, R., Ventura, J., Segersvard, R., Ward, E., Ding, X. Z., Rao, S. M., Jovanovic, B. D., Iwamura, T., Talamonti, M. S., Bell, R. H. and Adrian, T. E. (2005) LY293111 improves efficacy of gemcitabine therapy on pancreatic cancer in a fluorescent orthotopic model in athymic mice. Neoplasia 7, 417 – 425.
80 Ding, X. Z. and Adrian, T. E. (2002) Resveratrol inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Pancreas 25, e71-e76.
81 Ujiki, M. B., Ding, X. Z., Salabat, M. R., Bentrem, D. J., Golkar, L., Milam, B., Talamonti, M. S., Bell, R. H., Iwamura, T. and Adrian, T. E. (2006) Apigenin inhibits pancreatic cancer cell proliferation through G2/M cell cycle arrest. Mol. Cancer 5, 76.
82 Park, Y. J., Wen, J., Bang, S., Park, S. W. and Song, S. Y. (2005) [6]-Gingerol induces cell cycle arrest and cell death of mutant p53-expressing pancreatic cancer cells. Yonsei Med. J. 47, 688 – 697.
83 Dong, M. L., Ding, X. Z., Collin, P. and Adrian, T. E. (2003) A commercial sea cucumber lipid extract, Frondanol-A5 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Pancreas 27, 378.
84 Ujiki, M. B., Milam B., Ding, X. Z., Roginsky, A. B., Salabat, M. R., Talamonti, M. S., Bell, R. H., Gu W., Silverman, R. B. and Adrian, T. E. (2006) A novel peptide sansalvamide analogue inhibits pancreatic cancer cell growth through G0/G1 cell-cycle arrest. Biochem. Biophys. Res. Commun. 340, 1224 – 1228.