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Apoptosis: Targets in Pancreatic Cancer

Sabine Westphal and Holger Kalthoff*

Address: Molecular Oncology, Clinic for General and Thoracic Surgery, University of Kiel, Arnold-Heller-Str. 7, 24105 Kiel, Germany

Email: Sabine Westphal - sabinewe@yahoo.de; Holger Kalthoff* - hkalthoff@email.uni-kiel.de

* Corresponding author

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Abstract

Pancreatic adenocarcinoma is characterized by poor prognosis, because of late diagnosis and lack of response to chemo- and/or radiation therapies. Resistance to apoptosis mainly causes this insensitivity to conventional therapies. Apoptosis or programmed cell death is a central regulator of tissue homeostasis. Certain genetic disturbances of apoptotic signaling pathways have been found in carcinomas leading to tumor development and progression. In the past few years, the knowledge about the complex pathways of apoptosis has strongly increased and new therapeutic approaches based on this knowledge are being developed. This review will focus on the role of apoptotic proteins contributing to pancreatic cancer development and progression and will demonstrate possible targets to influence this deadly disease.

Review

Pancreatic cancer is one of the most malignant tumors with a very poor prognosis. Although pancreatic cancer has an incidence of about 10 cases/100,000 persons it is still the fourth male and fifth female leading cause of cancer-related death in the Western world [1]. Most of the newly diagnosed patients present with an already unresectable tumor stage. The 5-year survival rate of patients with pancreatic cancer receiving surgery and chemotherapy ranges from 1%–2% [2]. One of the reasons for this low survival rate is the insensitivity of pancreatic cancer to most oncologic therapies like chemotherapy, radiotherapy and immunotherapy [3–10]. Tumor development and progression as well as resistance to most oncologic therapies result mainly from lacking response to apoptotic stimuli.

Apoptosis or programmed cell death is a central regulator of tissue homeostasis [reviewed in [11]]. Multicellular organisms eliminate redundant, damaged or infected cells by apoptosis. Because chemotherapy and radiotherapy act primarily by inducing apoptosis, defects in the apoptotic pathway can cause cancer cell resistance [12,13]. Tumor cells utilize multiple pathways to down-modulate apoptosis [14].

Apoptosis mediated by death receptors belonging to the tumor-necrosis factor (TNF) receptor superfamily is the best-studied pathway in cells (Figure 1) [15,16]. Members of the TNF receptor family, TNF, Fas (Apo-1, CD95) and TRAIL (TNF-related apoptosis-inducing ligand)-R [16] share a common internal domain, the so-called death domain [17]. These receptors are activated by their natural ligands TNFα, FasL, and TRAIL, respectively. The interaction between receptor and ligand causes trimerization of receptor followed by recruitment of FADD (Fas-associated death domain protein) and procaspase-8 to the death domain forming the DISC (death-inducing signaling complex) [18]. At the DISC, cleavage of procaspase-8 yields the active form of this protease [18]. In type I cells, the amount of activated caspase-8 is sufficient to initiate apoptosis via direct activation of the central effector caspase,

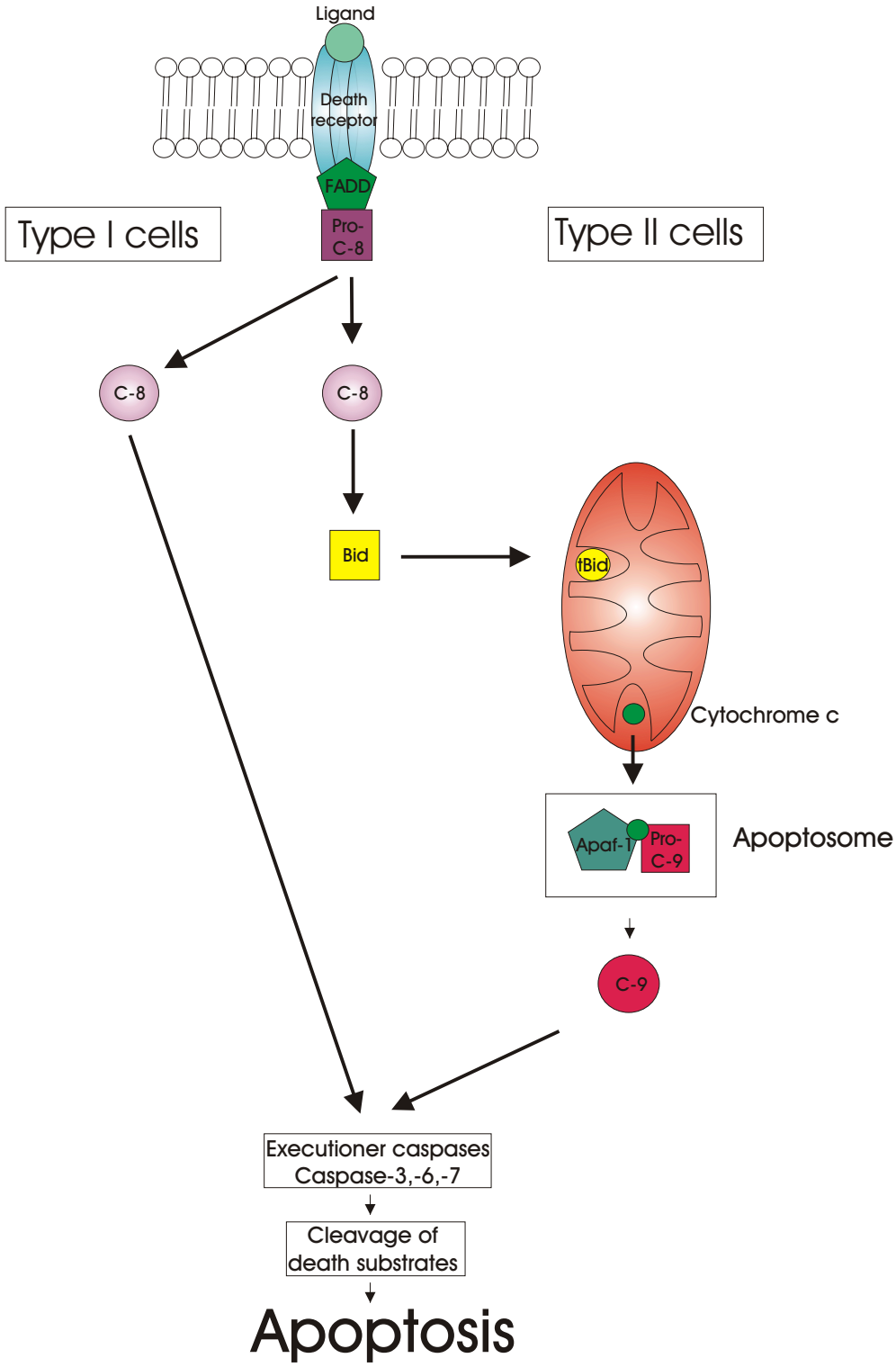

Figure 1

Apoptosis mediated by death receptors of the TNF family in type I and type II cells. Apoptosis can be initiated by two alternative pathways: in type I cells the amount of initiator caspases is sufficent to induce executioner caspases directly or in type II cells the enhancing effect of mitochondria is necessary. Active executioner caspases cleave the death substrates, which results in apoptosis.

caspase-3. In type II cells, the signal enhancing-effect of mitochondria is needed to induce apoptosis [19]. The Bcl-2 family member BID mediates activation of mitochondria in response to death receptor activation. BID is cleaved by active caspase-8 producing tBID, which is translocated to the mitochondria [19]. tBID becomes integrated into the mitochondria membrane and induces release of cytochrome c and other apoptogenic factors from the intermembranous space of mitochondria [20,21]. In the cytoplasm, cytochrome c forms a complex with Apaf-1 (apoptotic protease activating factor-1), ATP and procaspase-9 termed the apoptosome. Like caspase-8, caspase-9 can be considered an initiator caspase, which is activated by cleavage at the apoptosome and activates in turn executioner caspases, mainly caspase-3, -6 and -7 [22]. Cleavage of death substrates, DNA fragmentation, and cleavage of cytoskeletal proteins finally lead to cell death [22].

This complex pathway is controlled and influenced by a variety of different pro- and anti-apoptotic factors. The balance of these effectors is important to ensure tissue homeostasis. Activation or downregulation of pro- and anti-apoptotic genes influence cancer cell viability, cancer cell sensitivity to chemotherapy and radiotherapy, and tumor development and progression (Figure 2).

This review will focus on the knowledge about deregulation of apoptotic proteins and pathways in pancreatic cancer and possible therapeutic approaches based on these findings.

Death receptors

As described above, apoptosis is mediated mainly by members of the TNF death receptor superfamily proteins including Fas (Apo-1, CD95) and TRAIL-R and their natural ligands. Deregulation of these pathways may contribute to abnormal tumor growth [23,24].

The Fas-FasL system is believed to represent one of the main apoptotic cell death-signaling pathway [25]. Fas or FasL over- and under-expression has been shown in a variety of human carcinomas including lung [26], renal [27] and colon cancer [28]. Findings concerning Fas receptor expression in pancreatic cancer are contradictorily. It has been demonstrated recently that Fas mRNA was increased in pancreatic carcinomas [29]. Contrarily, an in vivo study revealed that both membranous Fas and cytoplasmic Fas receptors could not be detected invasive ductal-type pancreatic adenocarcinomas [30]. Together, these data suggest that tumor cells can evade apoptosis by downregulation of the Fas receptor.

In contrast to FasL, TRAIL is not toxic to normal cells [31]. Unlike other tumor cells, TRAIL receptors are highly expressed at the surface of pancreatic carcinoma cells [32]. Additionally, it has been demonstrated that repeated treatment of different pancreatic cell lines with TRAIL caused sustained and profound cell death [33]. These results show that the TRAIL system in principal is functional in pancreatic cancer but is blocked at some stage downstream in the apoptotic pathway among which is the expression of non-functional receptors, so-called decoy receptors. Approaches to initiate apoptosis by application of TRAIL to cancer cells were successful in mammary carcinomas, intracranial gliomas and colon carcinomas [34]. However, the fact that under certain conditions this therapy induces apoptosis also in normal healthy hepatocytes causing hepatic necrosis [35] dampened the optimistic expectations.

FAP-1

FAP-1 (Fas-associated phosphatase-1) is a non-receptor protein tyrosine phosphatase. It has been demonstrated that FAP-1 can block the function of Fas by interaction with its carboxy-terminal three amino acids [10,36,37]. Cell lines resistant to Fas-mediated apoptosis strongly overexpressed FAP-1 and it also was highly expressed in tumor cells in pancreatic carcinoma tissues [37]. In contrast, Capan-1, a pancreatic cancer cell line highly sensitive to Fas-mediated apoptosis, totally lacks FAP-1 expression [37]. Stable transfection of Capan-1 cells with a FAP-1 cDNA strongly decreases the sensitivity to Fas-mediated apoptosis [37]. Inhibition of Golgi anterograde transport by Brefeldin A suggested that FAP-1 could prevent translocation of Fas from intracellular stores to the cell surface [37] resulting in insufficient receptor density at the cell surface. Modulation of FAP-1 expression or suppression of its enzymatic activity may be the basis of a novel therapy for pancreatic cancer.

The role of mitochondria

In type II cells, the apoptosis-enhancing effect of mitochondria is necessary to induce the full apoptotic phenotype. It has been demonstrated that pancreatic cancer cell lines are type II cells [19,32,38]. The Bcl-2 family members play the major role in this pathway.

Bcl-2 family

At the center of the cell's decision to live or to die in response to an apoptotic signal is the Bcl-2 family of apoptotic regulators [39]. The Bcl-2 family is the best-characterized group of apoptosis-mediating factors and can be divided into two main groups according to their functional properties; anti-apoptotic proteins like Bcl-x_L and Bcl-2 and pro-apoptotic proteins, such as Bax, Bak and Bad. Bcl-2 proteins interact with other molecules through an α-helical domain termed BH-3 domain. This interaction is believed to be important for regulation of apoptosis [40].

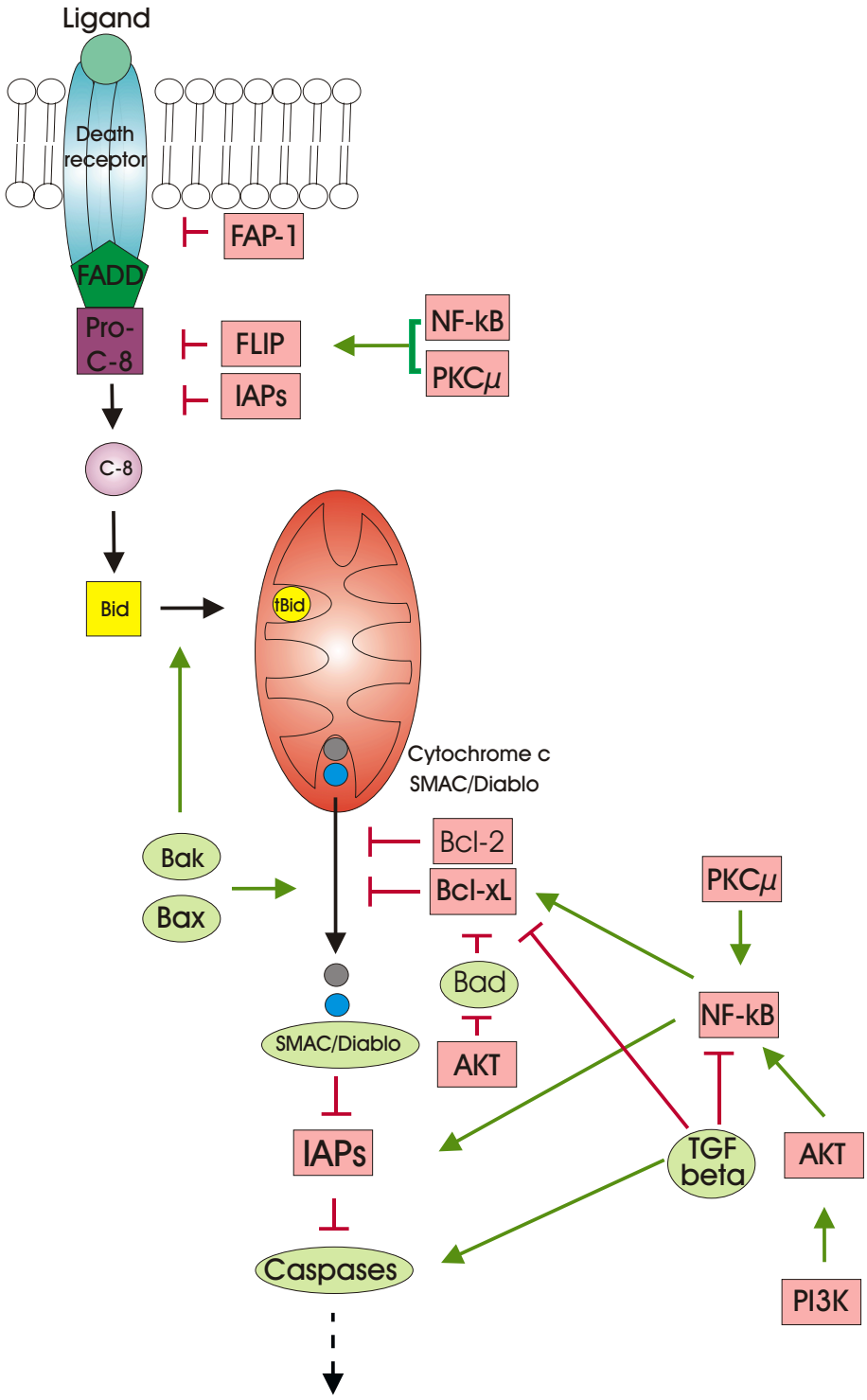

Figure 2
Influences of pro-and anti-apoptotic effectors on death receptor-mediated apoptosis. Apoptosis is controlled by several pro-(green) and anti-(red) apoptotic proteins. The balance of these proteins are important to ensure tissue homeostasis.

Bcl-2 is located at the cytoplasmic face of the mitochondrial outer membrane, at the ER-membrane and the nuclear envelope and may register damage of these compartments and affect their behavior [41]. Bcl-2 directly or indirectly prevents the release of cytochrome c from mitochondria in a variety of tissues [41]. High expression of Bcl-2 is found in various human tumors [42]. Interestingly, it has been shown that Bcl-2 expression is normal or even decreased in pancreatic cancer cells [43,44]. Preclinic animal and clinical phase II studies using Bcl-2 antisense constructs, such as G3139, in combination with different chemotherapeutics show significant regression of different cancers [45,46]. However, it is uncertain if this therapeutic approach will be successful in pancreatic cancer showing normal or decreased Bcl-2 expression levels.

Bcl-x exists in two distinct isoforms in humans. Bcl-x_L, the longer form, functions in an anti-apoptotic manner. Bcl-x_S, the shorter form, in contrast, functions as an apoptosis promoter. Like Bcl-2, Bcl-x_L also prevents cytochrome c release from mitochondria [reviewed in [47]]. Different studies showed that most likely every cell type is protected by at least one member of anti-apoptotic Bcl-2-like proteins. In pancreatic carcinoma cells Bcl-x_L plays the most important role in protecting from Fas and TRAIL-mediated apoptosis [32]. Furthermore, Bcl-x_L is believed to bind to Apaf-1 and may therefore inhibit the association of Apaf-1 with procaspase-9 and thereby prevent caspase-9 activation [48]. Unlike Bcl-2, Bcl-x_L is constitutively overexpressed in pancreatic cancer cell lines highly resistant to Fas and TRAIL-mediated apoptosis [32]. Therefore Bcl-x_L may be an ideal target for pancreatic cancer therapy. Overexpression of Bcl-x_L in cell lines with low Bcl-x_L expression like Colo357, showed complete suppression of apoptosis. On the other hand, inhibition of Bcl-x_L function by overexpression of Bax or administration of antisense oligonucleotides against Bcl-x_L mRNA resulted in sensitization of cells expressing high levels of Bcl-x_L like Panc-1 or Panc-TuI [32]. Additionally, another study revealed that Bcl-x_L antisense oligonucleotide inhibited pancreatic cancer cell growth and caused apoptosis by reducing Bcl-x_L protein levels in different pancreatic cancer cell lines [49]. Bcl-x_L antisense nucleotides also increased the sensitivity to chemotherapeutics like gemcitabine. This was confirmed by another study [50]. Activation of Bcl-2 family member Bcl-x_L after repeated exposure to the chemotherapeutic drugs 5-FU and gemcitabine contribute to chemo-resistance of pancreatic cancer cells [50]. Correlation of the molecular data with clinical patient parameters revealed that patients whose tumors exhibited no, faint, or weak Bcl-x_L expression lived significantly longer after tumor resection than patients whose tumors exhibited moderate Bcl-x_L mRNA expression [51].

Bax is a pro-apoptotic member of the Bcl-2 family that resides in the cytosol and translocates to mitochondria upon induction of apoptosis [52]. It has been demonstrated that ectopically expressed Bax in human embryonic kidney cells (293T cells) induced cytochrome c release and caspase activation [53]. However, overexpression of Bax did not influence the apoptosis rate or expression of Bcl-2 and Bcl-x_L in human pancreatic cancer cells transduced with a retroviral expression vector [54]. But Bax significantly increased the sensitivity to chemotherapeutic drugs like gemcitabine and 5-Fu when transduced to pancreatic cancer cell line ASPC-1 cells [54]. Although Bax failed to substantially increase susceptibility of the pancreatic cell line Colo357 towards gemcitabine *in vitro*, *in vivo* data from a SCID mouse model suggest that pancreatic tumors overexpressing Bax caused higher sensibility to gemcitabine and therefore stronger tumor regression [B. Schniewind, unpublished data]. That indicates that overexpression of Bax may have therapeutic application in enhancing the efficacy of chemotherapy in pancreatic cancer. A recent study revealed that a binary adenoviral vector system (Ad/GT-Bax + Ad/hTERT-GV16) using the human telomerase reverse transcriptase (hTERT) promoter to induce Bax gene expression was able to induce apoptosis in pancreatic cancer cell lines [55]. Transduced cells not only showed Bax overexpression but also an increased level of caspase-3. Another study revealed that increased levels of Bax and reduced expression of Bcl-2 are involved in the growth-inhibitory effect of cisplatin in pancreatic cancer cells. [56].

Another member of pro-apoptotic proteins of Bcl-2 family is Bak. In pancreatic cancer, Bak expression and apoptosis occur in regions of chronic inflammation surrounding the cancer cells but not in the tumor cells themselves [57]. This may facilitate tumor growth and spread. Recent studies have demonstrated that Bad, a typical pro-apoptotic protein of Bcl-2 family, binds with its BH3 domain to both Bcl-2 and Bcl-x_L but mediates its pro-apoptotic functions through inhibition of Bcl-x_L, but not Bcl-2 [58].

To restore the function of downregulated pro-apoptotic Bcl-2 family members, there are many efforts to develop peptides and non-peptide agents that mimic the function of these proteins [59].

The key enzymes for arachidonic acid metabolism, lipoxygenases (LOXs) and cyclooxygenases (COXs) influence the development and progression of several human cancers including pancreatic cancer [60]. It has been shown that both COXs and LOXs are upregulated in human pancreatic cancer tissues. Inhibitor studies revealed that blocking these enzymes inhibit pancreatic cancer growth and induce apoptosis. Further investigations pointed out that blockade of LOXs induce apoptosis through

cytochrome c release, caspase-9 activation and changes in the levels of Bcl-2 family proteins [61].

In summary, the deregulation of Bcl-2 family both the pro- and the anti-apoptotic proteins play a crucial role in development, growth and expansion of pancreatic cancer. Several approaches based on these findings have been demonstrated to be a tool potentially influencing pancreatic cancer also in a clinical setting.

Caspases and caspase inhibitors

Caspases

The central component of apoptosis is a proteolytic system involving a family of cysteine proteases termed caspases. All caspases are expressed as proenzymes. Activation involves proteolytic processing [62]. Caspases initiate and execute cell death by inactivating anti-apoptotic proteins, shutting down DNA replication and repair [63], reorganization of the cytoskeleton [64] and disruption of the nuclear lamina [65].

Caspases are highly regulated by a variety of different inhibitors and activators. The induced proximity or oligomerization model explains that caspases exist as inactive monomers. The effectors of caspases bring the caspases in close proximity, allowing for their intermolecular autoproteolytic activation [66]. For example, activation of procaspase-8 requires association with its cofactor FADD (Fas-associated protein with death domain) [67] and procaspase-9 must interact with APAF-1 to become functional [68].

Important caspase-8 inhibitors represent the FADD-like ICE inhibitory proteins (FLIPs) [69]. Two forms, $FLIP_L$ (long form) and $FLIP_S$ (short form) have been characterized [70]. $FLIP_L$ is a structural homologue of caspase-8 but it lacks amino acid residues that are critical for caspase activity [70–72]. $FLIP_L$ competes with procaspase-8 for binding to FADD at the DISC, thus preventing caspase activation [66]. Very interestingly, it has been shown that $FLIP_L$ is overexpressed both in pancreatic cancer cell lines resistant to Fas mediated apoptosis and pancreatic tumors [73]. This may promote progression of malignant pancreatic tumors.

Recent studies suggest that FLIP is not simply an inhibitor of death-receptor-induced apoptosis but it is involved in activation of NF-κB by recruiting adaptor proteins like TRAF [74]. NFκ-B is known to regulate several genes that mediate tumorigenesis and metastasis [reviewed in [75]]. This may also contribute to tumor growth. Downregulation of FLIP in prostate cancer cell line resulted in sensitization to Fas-mediated apoptosis [76]. Recently, it has been demonstrated that natural and synthetic ligands of the transcription factor PPARγ (peroxysome proliferator-activated receptor γ) sensitize tumor cells to apoptosis by decreasing the level of FLIP [77]. Equivalent data concerning pancreatic cancer are eagerly awaited. It has been shown that caspase expression is more or less normal in pancreatic carcinoma. In contrast, effectors blocking caspase activation or function like FLIPs are deregulated in pancreatic cancer leading to resistance to death receptor mediated apoptosis.

A new therapeutic approach uses somatostatin receptor subtype 2 (sst2) to activate caspase-3 and therefore to induce apoptosis in pancreatic cancer [78]. Likewise, sst2 treatment of human cancer cell lines with Diospyrin, a bisnaphthoquinonoid natural product, resulted in induction of apoptosis mediated via activation of caspase-3 and caspase-8 [79].

Irofulven (MGI 114, 6-hydroxymethylacylfulvene) induces apoptosis via caspase activation in pancreatic carcinoma cells [80].

IAP family

The inhibitor of apoptosis (IAP) family including cIAPs, XIAP and survivin can block apoptosis through interaction with members of the caspase family. IAPs are characterized by a domain termed the baculoviral IAP repeat (BIR) necessary for caspase interaction [81,82]. At least one BIR domain is necessary for suppression of apoptosis [83]. Another structural feature of IAPs is the presence of a carboxy terminal RING zinc finger domain [83]. But it seems that this domain is not always strictly required for inhibition of apoptosis [84–87]. Overexpression of XIAP, cIAP1, cIAP2 and survivin has been demonstrated to suppress apoptosis [reviewed in [83]]. The cellular function of the IAP family members cIAP1, cIAP2 and XIAP is largely unclear. These proteins seem to be involved in pathogenesis of small-cell lung cancer [88], non-small cell lung cancer [89], myeloid leukemia cells [90] and prostrate cancer [91]. Concerning pancreatic cancer, there are only few data on the involvement of cIAP1, cIAP2 and XIAP in the pathogenesis of this type of cancer. Our own results indicate increased levels of cIAP1, cIAP2 and XIAP in pancreatic cancer cell lines highly resistant to Fas- and TRAIL-induced apoptosis (A. Trauzold, unpublished data). Therefore, changes of expression of cIAP1, cIAP2 and XIAP may also have a role in the pathogenesis also in pancreatic cancer.

Survivin

Survivin has been identified as a new member of the inhibitor of apoptosis (IAP) family. Survivin is characterized by a unique structure that discriminates it from all other members of the IAP family. It contains only a single BIR repeat and lacks a carboxy terminal RING finger domain. Survivin is expressed in the G_2/M phase of the cell cycle in

a cycle-regulated manner [92]. It directly binds to and inhibits both caspase-3 and caspase-7 activity leading to arrest of apoptosis [93].

Survivin expression is not detectable in differentiated normal adult cells of any organ [94] but it is highly expressed in a wide range of cancer tissues [95] including neuroblastoma [96], colorectal [97] and stomach [98] carcinoma. It has been demonstrated recently that survivin is also frequently expressed in malignant pancreatic ductal tumors [99]. Because survivin is a potent caspase-inhibitor, its overexpression in cancer cells is implicated in the resistance to different apoptotic stimuli including chemotherapy. Molecular manipulation of survivin expression may enhance chemotherapy and radiation therapy not only in pancreatic cancer.

SMAC/DIABLO

IAPs are inhibited by a protein named SMAC/DIABLO (Second mitochondria-derived activator of caspase/direct IAP binding protein with low pI) [100,101]. SMAC/DIABLO is synthesized as a precursor protein and is imported into mitochondria [100]. Upon cellular stress, SMAC/DIABLO is released into the cytosol. In the cytosol it promotes cell death by preventing IAP inhibition of caspases [102]. Like cytochrome c, release of SMAC/DIABLO from the mitochondria is inhibitable by Bcl-2 [100]. SMAC/DIABLO could have a therapeutic application in enhancing the effect of chemotherapeutics by binding IAPs that are overexpressed in a variety of carcinoma cells including pancreatic cancer. Recently it has been shown that SMAC/DIABLO fusion peptide was able to enhance apoptosis induced by diverse anticancer agents including paclitaxel, etoposide and others in MCF-7 breast cancer cells [103].

Growth Factors

Growth factor independence is a hallmark of tumors and is known to give cells a selective growth advantage [104]. Mutations of tumor suppressor genes and oncogenes lead to a loss of regulation in the expression and/or activation of both growth factors and their receptors [105]. Human pancreatic cancer cells are reported to produce multiple growth factors including insulin-like growth factor (IGF) and the receptors IGF-1 receptor (IGF-1R) and IGF-2 receptor, epidermal growth factor (EGF), EGF receptor (EGFR) and ErbB2, transforming growth factor-α (TGF-α), and transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factors (VEGF) and their receptors [106–115].

Insulin-like growth factor (IGF)

Insulin-like growth factors (IGFs), including IGF-1 and IGF-2, are mitogenic peptides involved in the regulation of cell proliferation, differentiation and apoptosis [116,117]. Exogenous IGF-1 increased cell proliferation and activated MAPK and AKT signaling pathways in pancreatic cell lines [118,119]. IGF-2 might function in the switch from the quiescent to the proliferative state [120]. In multiple myeloma cells IGF-1 stimulates sustained activation of NF-κB and AKT and upregulates a series of anti-apoptotic proteins including FLIP, survivin, cIAP-2 and XIAP [121].

IGF-1R shows the highest binding affinity for IGF-1, although IGF-2 can also bind and activate the receptor [117]. IGF-1R as well as IGF-1 and IGF-2 have been found to be upregulated in pancreatic cancer [122]. This leads to down-modulation of apoptosis and survival of cancer cells through the PI3K/AKT pathway.

Epidermal growth factor (EGF)

Epidermal growth factor (EGF) stimulates proliferation and differentiation in a wide range of cell types through its corresponding receptor, EGFR. The EGFR gene is overexpressed in the majority of pancreatic ductal adenocarcinomas and human pancreatic cancer cell lines [123]. EGFR binds EGF and TGF-α with high affinity, and both ligands are overexpressed in pancreatic cancer [123]. Additionally, the overexpression of both the EGF receptor and its ligand in human pancreatic cancer is associated with enhanced tumor aggressiveness and shorter survival following tumor resection [124].

Treatment of pancreatic cancer cell lines with Erbitux (IMC-C225) anti-EGFR antibody in combination with gemcitabine and radiation resulted in complete regression or growth inhibition of cancer cells in a preclinical setting [125]. This combinatory treatment shows potential clinical application in the treatment of pancreatic cancer in humans.

Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)

Vascular endothelial growth factor (VEGF) is a potent and specific angiogenic factor. VEGF is known to be a key player for tumor growth [126,127]. Fibroblast growth factors (FGFs) are mitogenic polypeptides that signal via FGF receptors (FGFRs). Both certain FGFs and VEGF and their receptors are overexpressed in cancer cells including pancreatic cancer cells [111,112]. A soluble receptor for VEGF (sVEGF) functions as a dominant-negative inhibitor of receptor suppresses tumor angiogenesis and enhances apoptosis of cancer cells [128]. This effect was increased by combined use of sVEGF and a soluble FGF receptor (sFGFR1) in lung and pancreatic cancer cell lines [129].

Transforming growth factor alpha (TGF-α)

TGF-α is structurally and functionally related to members of the EGF family [130]. Numerous human solid tumors express high levels of TGF-α [131]. Recent work has

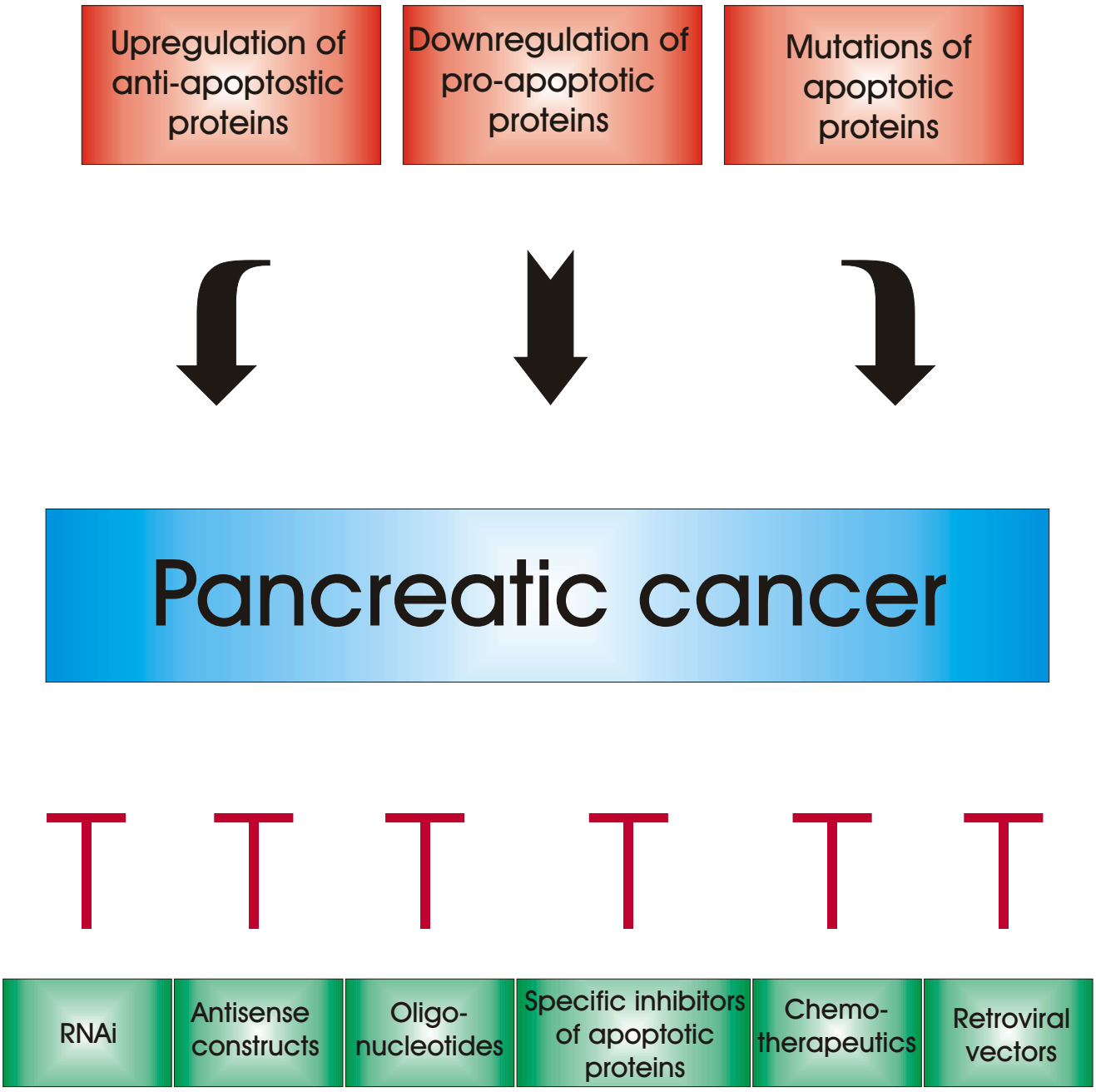

Figure 3
Effects causing pancreatic cancer and possible therapeutic approaches. Multiple changes of apoptotic proteins contribute to pancreatic cancer development and progression. But many therapeutic approaches are developed to restore normal sensitivity to apoptotic stimuli and therefore to repress pancreatic cancer.

shown that overexpression of TGF-α in liver has a dramatic protective effect on Fas-mediated apoptosis in TGF-α transformed mice. Mice with TGF-α overexpression in the pancreas develop ductal pancreatic cancer [132]. Interestingly, expression of Bcl-x_L, an anti-apoptotic protein, was greatly increased in these transgenic mice and may partic-

ipate in protective role of TGF-α [133]. This may contribute to apoptosis resistance in pancreatic cancer cells.

Transforming growth factor beta (TGF-β)

TGF-β is a multifunctional cytokine regulating a broad spectrum of cellular activity including cell-cycle control, differentiation, and apoptosis [reviewed in [134]]. TGF-β is a factor mediating growth inhibition and induction of apoptosis [134,135]. TGF-β is involved in many events of apoptotic pathways. It cooperates with Fas [136] and TGF-α [137], down-regulates the anti-apoptotic proteins Bcl-x_L and Bcl-2 and activates caspase-1, -3, -8 and -9 [138,139]. Another mechanism of TGF-β-induced apoptosis has been proposed following the finding that TGF-β down-regulates NF-κB activity by the induction of the expression of IκB α, a specific inhibitor of NF-κB [140]. TGF-β initiates its cellular response by activation of specific downstream intracellular effectors termed SMAD proteins [reviewed in [141]] Escape from TGF-β/SMAD-induced apoptosis is frequently observed in tumors. Mutations and changes of expression levels of TGF-β and SMAD proteins could be observed also in pancreatic cancer tissues [142,143]. For example, SMAD 4, a tumor suppressor in the TGF-β signaling pathway is genetically inactivated in about 55% of all pancreatic adenocarcinomas. Patients with pancreatic adenocarcinomas with SMAD4 expression live significantly longer [144].

Other factors and signaling pathways

Nuclear factor of κB (NF-κB)

Nuclear factor κB (NFκB) is a sequence-specific transcription factor that is involved in many cellular activities including the inflammatory and immune response. NF-κB also inhibits apoptosis by activation of several anti-apoptotic proteins [[145], reviewed in [146]] like cIAPs, FLIPs and the Bcl-2 family member Bcl-x_L [147]. Normally, NF-κB remains as a heterodimeric complex consisting of p50, p65, and is kept by IκBα in an inactive state in the cytoplasm. A variety of stimuli like cytokines, pathogens, carcinogens or stress can lead to degradation of IκBα and p50–p65 heterodimer is translocated to the nucleus, binds the DNA at the promoter region and activates anti-apoptotic genes [148]. It has been demonstrated recently that NF-κB contributes to apoptosis resistance in different cancers including pancreatic cancer [38,148]. This suggests that NF-κB is an ideal target for chemotherapeutic therapies of cancer. Inhibition of NF-κB by Gliotoxin, MG132 or Sulfasalazine [149] sensitizes pancreatic cancer cells to apoptosis induced by etoposide (VP16) or doxorubicin. Furthermore, inhibition of NF-κB by food-derived polyphenols like quercitin decreased pancreatic cancer growth and prevented metastasis [150]. Quercitin decreased primary tumor growth, increased apoptosis by mitochondrial depolarization, cytochrome c release, caspase 3 activation, and inhibition of NF-κB activation.

PKCμ

Protein kinases control and modulate a variety of cellular activities. Members of the protein kinase C (PKC) family have been shown to be involved in cell proliferation and differentiation [151]. Screening of expression pattern of PKC isoforms pointed out that the expression of PKCμ correlates with the resistance to Fas-mediated apoptosis in different pancreatic cancer cell lines [40]. Cell lines highly resistant to Fas-mediated apoptosis show high PKCμ expression levels [40]. It has been demonstrated that PKCμ regulates apoptosis in several cancer cells including pancreatic carcinoma cells [152,153]. Overexpression of PKCμ reduces the sensitivity of the cells to apoptosis induced by TNF [153]. This effect correlates with an enhanced expression of anti-apoptotic proteins like cIAP2, TRAF1, cFLIP and survivin as well as increased expression level of NF-κB dependent genes [152–154].

Survival signaling through PI3K/AKT

Survival signals like growth factors, cytokines and hormones activate phosphatidylinositol 3-kinase (PI3K) [155]. Subsequently, PI3K activates AKT/PKB [156] that interferes with the apoptotic machinery. Activated AKT/PKB mediate cell survival via the regulation of numerous apoptotic relevant proteins such as the Bcl-2 family members BAD and Bcl-x_L and the transcription factor NF-κB [157,158]. Survival signaling by AKT is counteracted by PTEN that antagonizes the action of PI3K. PI3K and AKT are overexpressed in a variety of cancers [159,160]. In addition, PTEN is frequently deleted in advanced tumors [161,162]. These alterations lead to a 'constitutively active' survival-signaling pathway that enhances the insensitivity of tumor cells to apoptosis induction. Additionally, EGFR directly influences PI3K [163]. Because PI3K mediates survival signals, EGFR overexpression leads to a decrease in the apoptotic response and therefore a stronger survival of cancer cells. Recent work has shown that the IGF-1R suppresses apoptosis by signaling through PI3K and AKT [120]. Activated AKT in turn phosphorylates the intracellular transducer, Bad, which modulates the activity of the apoptosis suppressors Bcl-2 and Bcl-x_L [164]. Additionally, AKT can directly phosphorylate caspase-9, which leads to inactivation of caspase-9 [165]. Strategies are pursued that aim to block the enzymatic activity of PI3K and PKB/AKT, in order to prevent inactivation of pro-apoptotic Bad. Wortmannin was shown to be a potent inhibitor of PI3K [160]. In several cancers including non-small-cell lung cancer [160] and pancreatic cancer [166], treatment of cells with wortmannin leads to inhibition of proliferation and increased apoptosis. Additionally, wortmannin enhanced gemcitabine-induced apoptosis in human pancreatic cells in vitro [167] and in vivo [168]. In contrast, studies investigating different pancreatic cancer cell lines pointed out that the PI3K/AKT pathway is not involved in gemcitabine-resistance [A. Arlt, unpublished data]. Nei-

ther did the basal AKT-activity correlate with the sensitivity towards gemcitabine treatment, nor did the inhibition of PI3K/AKT alter gemcitabine-induced apoptosis.

p53

The p53 pathway represents the molecular connection between the cell cycle and apoptosis. The p53 gene encodes a 53-kDa nuclear phosphoprotein. p53 inhibits cell growth through activation of cell cycle arrest and apoptosis [169]. The p53 gene is mutated in over 50% of human cancers. Pancreatic cell lines showed mutations in p53 at frequencies of 95% [170]. This enhances tumor development and progression. Currently, many therapeutic approaches were attempted to normalize p53 function in a variety of cancers including pancreatic cancer. Transfer of wild-type p53 induces apoptosis and produces significant growth suppression of pancreatic cancer in vitro and in vivo [170–172].

Conclusion

In the past, many efforts were made to cure patients from pancreatic cancer. Changes of expression and mutations of apoptotic proteins are common in pancreatic cancer cells and contribute to tumor development and progression. Consequently, pancreatic cancer has developed multiple resistance mechanisms to apoptosis. Many efforts to restore apoptosis and thereby reducing tumor growth were made with considerable success at least under preclinical conditions (Figure 3). Future therapies have to translate this knowledge into the clinic. They need to combine various therapeutic strategies and have to modulate selectively the sensitivity of pancreatic cancer cells to apoptosis without affecting normal cells.

Authors' contribution

SW conducted literature review and wrote this article. HK supported this work by critical reading the manuscript and supervised the final editing. All authors read and approved the final manuscript.

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