HHS Public Access

Author manuscript

J Cell Biochem. Author manuscript; available in PMC 2017 December 11.

Published in final edited form as:

J Cell Biochem. 2016 February ; 117(2): 279–288. doi:10.1002/jcb.25284.

Control of Apoptosis in Treatment and Biology of Pancreatic Cancer

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Abstract

Pancreatic cancer is estimated to be the 12th most common cancer in the United States in 2014 and yet this malignancy is the fourth leading cause of cancer-related death in the United States. Late detection and resistance to therapy are the major causes for its dismal prognosis. Apoptosis is an actively orchestrated cell death mechanism that serves to maintain tissue homoeostasis. Cancer develops from normal cells by accruing significant changes through one or more mechanisms, leading to DNA damage and mutations, which in a normal cell would induce this programmed cell death pathway. As a result, evasion of apoptosis is one of the hallmarks of cancer cells. PDAC is notoriously resistant to apoptosis, thereby explaining its aggressive nature and resistance to conventional treatment modalities. The current review is focus on understanding different intrinsic and extrinsic pathways in pancreatic cancer that may affect apoptosis in this disease.

Keywords

pancreatic cancer; apoptosis; signalling; therapy

Pancreatic ductal adenocarninoma (PDAC) is estimated to be the 12th most common cancer in the United States in 2014 and yet this malignancy is the fourth leading cause of cancer-related death in the United States. There were an estimated 46,420 new cases of pancreatic cancer and 39,590 deaths in the United States in 2014. The 5-year survival rate (2004–2010) is estimated at 6.7% which is not a substantial change from 6.2% in 2002 [Siegel et al., 2014]. Despite years of extensive research all over the world, the prognosis of pancreatic ductal adenocarcinoma (PDAC) is still dismal.

Apoptosis is an actively orchestrated cell death mechanism that serves to maintain tissue homoeostasis. Cancer develops from normal cells by accruing significant changes through one or more mechanisms, leading to DNA damage and mutations, which in a normal cell would induce this programmed cell death pathway. This would mean that most cancers, have mechanisms in place to evade apoptosis [Lowe et al., 2004; Arlt et al., 2013]. In fact, evasion of apoptosis is one of the hallmark characteristics of all cancers. In PDAC, this starts early, as the precursor lesions also lack apoptotic cells [Arlt et al., 2013; Hamacher et al., 2008]. In

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Conflict of interest: University of Minnesota has filed a patent for Minnelide, which has been licensed to Minneamrita Therapeutics, LLC. AKS is the co-founder and the Chief Scientific Officer of this company.

most treatment-responsive malignancies, the response to chemotherapy and radiotherapy is through the induction of apoptosis in the cancer cells [Schulze–Bergkamen and Krammer, 2004]. PDAC is notoriously resistant to apoptosis, thereby explaining its aggressive nature and resistance to conventional treatment modalities [Arlt et al., 2013].

ROLE OF APOPTOSIS IN BIOLOGY OF PDAC

Pancreatic ductal adenocarcinoma (PDAC) has a well worked out multi-step carcinogenesis theory. The cancer evolves through precursor lesions, known as pancreatic intraepithelial neoplasia (PanIN 1–3) to invasive ductal cancer [Hamacher et al., 2008]. PanIN 1 and 2 are hyperplastic lesions and PanIN 3 is carcinoma in situ [Ghaneh et al., 2007]. PanINs originate from the small pancreatic ducts, but the cell of origin of these PanINs or PDAC, whether ductal, acinar, or progenitor cell, still remains elusive [Reichert and Rustgi, 2011]. Gene expression from genetically engineered mouse models using mutant K-Ras supports the notion that acinar cells undergo acinar to ductal metaplasia (ADM), which functions as a precursor to PanINs [Means et al., 2005; Zhu et al., 2007; Houbracken et al., 2011]. However it is also possible that a progenitor population exists in the adult pancreas that forms PanINs with the mutant K-Ras without undergoing ADM [Rovira et al., 2010]. Different step-wise molecular alterations bring about the development of PDAC from PanINs. A gain of function mutation in K-Ras, found as early as PanIN 1, is probably the earliest event in the pathogenesis of PDAC. Other hits in the genome need to be sequentially accrued for the evolution of invasive cancer from the hyperplastic PanIN1. These include telomere shortening (PanIN1), loss of function of the tumor suppressor p16INK4a (PanIN2) and loss of function of tumor suppressors SMAD4 and TP53 (PanIN 3) [Bardeesy and DePinho, 2002; Ghaneh et al., 2007; Zhu et al., 2007]. These subcellular changes lead to upregulation of multiple prosurvival mechanisms and downregulation of apoptotic machinery as described below:

GROWTH FACTOR SIGNALING

Epidermal growth factor receptor (EGFR) family is particularly overexpressed in PDAC. EGFR is an oncogene, overexpression leads to increased cell proliferation, survival, and decreased apoptosis. These receptors are usually activated by EGF (epidermal growth factor) and TGF-α (transforming growth factor-α) in PDAC [Yarden and Sliwkowski, 2001]. On activation, they dimerize, autophosphorylating their tyrosine residues leading to activation of various intracellular pathways, namely Ras/Raf/MAPK, PI3K/AKT, and the JAK/STAT pathways [Ghaneh et al., 2007; Wong and Lemoine, 2009; Arlt et al., 2013]. These downstream changes lead to increased cell proliferation, tumor growth, and decreased apoptosis.

Ras/Raf/MAPK PATHWAY

Ras is one of the central downstream target of the EGFR family and KRAS is the most commonly mutated gene in PDAC and PanINs [Deramaudt and Rustgi, 2005]. Mutated Ras functions as an oncogene, causing continued cell proliferation, and resistance to apoptosis. Though the main downstream target of Ras is Raf leading to downstream activation of

MEK1/2 and hence ERK1/2 MAPK, it also activates PI3K/Akt and PLCγ pathways [Arlt et al., 2013].

PI3k/Akt/mTOR PATHWAY

This cell survival pathway functions downstream of the EGFR or Ras protein as mentioned above. It may also be activated by other survival signals, like cytokines and hormones [Cohenuram and Saif, 2007]. It has a role to play in cell proliferation and survival and makes the cancer resistant to apoptosis [West et al., 2002]. While Akt2 gene amplification is reported in 11–20% of PDAC, upregulation of Akt pathway is present in up to 60% of PDAC [Cheng et al., 1996; Ruggeri et al., 1996; Altomare et al., 2002; Schlieman et al., 2003]. Akt once activated, acts on mTOR (mammalian target of rapamycin), c-myc, and NF-κB as its downstream targets [Hamacher et al., 2008; Wong and Lemoine, 2009]. For PDAC, this pathway is overactivated further because of PTEN (phosphatase and tensin homolog). PTEN is a tumor suppressor that functions as a negative regulator of PI3K/Akt pathway and is mutated or down regulated in 60% of PDAC [Asano et al., 2004; Xu et al., 2010].

NF-κB PATHWAY

NF-κB pathway includes dimers made of RELA, p50, and c-REL but the most common is the RELA-p50 dimer. This pathway is proinflammatory, primarily activated in response to cytokines like IL-1 and TNF α. The constitutive activation of this pathways is present in PDAC [Li et al., 2004]. Inhibition of this pathway leads to induction of apoptosis in PDAC and increases chemosensitivity [Li et al., 2004].

EMBRYONIC PATHWAYS

- *Sonic Hedgehog*:—Three hedgehog proteins belong to this pathway, Sonic Hedgehog (SHH), Desert Hedgehog (DHH), and Indian Hedgehog (IHH). Among these, SHH, a ligand not expressed on pancreatic precursor cells or the mature pancreas, is expressed in about 70% of pancreatic cancers [Thayer et al., 2003; van den Brink, 2007]. SHH enhances proliferation of ductal epithelium and enhances K-RAS induced tumorigenesis by multiple pathways. It has been found to increase the expression of Cyclin D1 and decrease p21 in the ductal epithelium, thereby enhancing proliferation. At the same time, it makes the cells resistant to apoptosis by increasing the expression of anti-apoptotic molecules Bcl-xl and Bcl-2 [Morton et al., 2007]. SHH is found to be upregulated in pancreatic cancer stem cells [Li et al., 2009]. Recently, it has also been found that SHH leads to activation of pancreatic stellate cells and increases EMT markers like vimentin, desmin, and α-smooth muscle actin, hence causing desmoplasia [Bailey et al., 2008].
- *WNT pathway*:—WNT/β-Catenin pathway has been realized to be important in the pathogenesis of PDAC. In the presence of WNT signal, cytoplasmic degradation of β-Catenin is inhibited, leading to increased transcription of WNT dependent genes, such as cyclin D1, c-myc, VEGF, MMP-7, and others [Freese et al., 2010]. In PDAC, this pathway has been shown to have roles in angiogenesis, resistance to apoptosis, cell cycle and maintenance of cancer stem

cells [Cui et al., 2012]. Mechanistically, WNT activation, leads to increased cell proliferation by increasing the levels of cyclin D1 and c-myc. c-myc in turn further increases the expression of cyclin D1, cyclin A, CDK4, CDK2, and inhibits the cell cycle checkpoint protein p21 [Qi et al., 2007]. In addition, WNT activation inhibits apoptosis of these hyperproliferative cancer cells by increasing the expression of IAP (Inhibitor of Apoptosis Protein) and Survivin (BIRC5) [Guo et al., 2009]. Another anti-apoptotic mechanism involves WNT target MMP7, which inhibits extrinsic apoptosis by decreasing the expression of Fas receptor on the cancer cells [Almendro et al., 2009].

REGULATION OF APOPTOSIS IN PDAC

A cell can undergo apoptosis through three major pathways-Intrinsic mitochondrial, Extrinsic, or Intrinsic ER stress dependent. Cancer cells learn to evade all of them in their journey of oncogenic transformation by acquiring resistance mechanisms.

INTRINSIC MITOCHONDRIAL PATHWAY

This pathway may be activated in response to a number of intracellular derangements-DNA damage, oxidative stress, hypoxia, high intracellular calcium, to name a few. The primary organelle orchestrating this pathway is the mitochondria, which maintains a fine balance between the two types of Bcl-2 proteins, pro-apoptotic (Bax, Bad, Bim, Bak, Bid, Bik) and anti-apoptotic (Bcl-2, Bcl-X_L, Mcl-1). Whenever the intracellular factors favoring apoptosis accumulate, the pro-apoptotic Bcl-2 proteins overwhelm the anti-apoptotic proteins, leading to increased permeability of the mitochondrial membrane, which causes the release of cytochrome-c and other factors like, AIF (Apoptosis Inducing Factor), Smac (Second Mitochondria-derived Activator of Caspase), DIABLO (Direct IAP Binding protein with Low pI), HtrA2 (Omi/High temperature requirement protein A) into the cytoplasm, initiating apoptotic cascade. Once in the cytoplasm, cytochrome-c binds to Apaf-1 (Apoptotic Protease Activating Factor-1) to form the multi-protein complex known as apoptosome. This complex cleaves pro-caspase 9 into its active enzyme, caspase-9. Caspase 9 furthers the cascade by cleaving procaspases 3 and 7 into active caspases 3 and 7 which are responsible for the morphological and biochemical changes we call apoptosis. Caspases are cysteine proteases, with a unique property of cleaving after aspartic acid residues. Caspase 2, 8, 9, 10, and 12 are the initiator caspases while Caspase 3, 6, and 7 are the effector caspases. The other released proteins, Smac, Diablo, and Omi/HtrA2 promote apoptosis by binding to the IAPs [Wong, 2011]. IAP family includes proteins having a BIR (Baculovirus IAP Repeat) domain which inhibits apoptosis by binding to caspases 3 and 9, thereby hindering their activation. IAP family includes proteins like C-IAP1 (BIRC2), C-IAP2 (BIRC3), X-IAP (BIRC4), Survivin (BIRC5) and many others.

PDAC CELLS ARE RESISTANT TO INTRINSIC APOPTOSIS

- *Anti-apoptotic/pro-apoptotic imbalance*: Unlike other cancers, anti-apoptotic protein Bcl-2 is not frequently overexpressed in pancreatic cancer. In contrast, an imbalance between anti-apoptotic Bcl-X_L, and pro-apoptotic Bax was found in the TGF-α murine model of pancreatic cancer, which closely simulates human cancer. In this model, the Bcl-X_L activity was shown to be dependent upon

activation of STAT3 and NF-κB. Hence the anti-apoptotic proteins have a role to play in precancerous lesions as well as in invasive pancreatic cancer [Greten et al., 2002].

- *Inhibitors of Apoptosis Proteins (IAPs)*: IAPs have been found to be overexpressed in preinvasive and invasive pancreatic cancer. Among the many IAPs, XIAP, cIAP, and Survivin are particularly upregulated in PDAC and precancerous lesions. Survivin, a recent addition to the IAP family, is absent from adult pancreas (normal, chronic pancreatitis) but is highly expressed in cancerous tissues. There is more frequent Survivin expression in cancerous lesions compared to preinvasive lesions [Satoh et al., 2001]. Interestingly, cIAP1 and cIAP2 overexpression have been found to correlate with chemoresistance in pancreatic cancer cells [Lopes et al., 2007]. NF–κB confers resistance to apoptosis in PDAC making them chemo and radio-resistant; one of the mechanisms is upregulation of the IAPs, especially the cIAPs and XIAP [Karin et al., 2002; Lin et al., 2010].

EXTRINSIC DEATH RECEPTOR PATHWAY

This pathway is activated in a cell in response to extracellular stimuli-binding of death ligands, TNF (Tumor Necrosis Factor), FasL, TRAIL (TNF-related apoptosis inducing ligand) to death receptors on the cell surface. These death receptors belong to the TNF Receptor family, including TNFR1, FAS (APO-1/CD95) and TRAIL (APO-2) receptor, the common thread being the intracellular domain these receptors share, known as the “death” domain. When the death ligands bind their respective receptors, adapter proteins like TRADD (TNF Receptor Associated Death Domain) and FADD (Fas Associated Death Domain) and procaspases 8, 10 are recruited to the death domain, thereby forming DISC (Death Inducing Signaling Complex). Formation of the DISC cleaves procaspase 8 into the active capsase 8, which proceeds to cleave the executioner caspases leading to apoptosis.

Despite the different mechanisms of initiation, there is a lot of cross talk among the intrinsic and extrinsic pathways. Cells are divided into two types, type I are only dependent on caspase 8 (extrinsic pathway) to cause apoptosis by itself while in type II, caspase 8 increases the permeability of the mitochondrial membrane, which then causes apoptosis. PDAC are type II cells [Hamacher et al., 2008]. Fas dependent apoptosis in such cells, hence is dependent on the cellular mitochondrial activity [Walczak and Krammer, 2000].

PDAC CELLS ARE RESISTANT TO EXTRINSIC APOPTOSIS

- *FAP-1*:—PDAC seems resistant to the death receptor pathway, despite the presence of death receptors at the cell surface and intact downstream pathways. One of the culprits implicated is FAP-1 (Fas-associated phosphatase-1), which is overexpressed in pancreatic cancer. FAP-1 decreases the Fas-FasL interaction by inhibiting Fas translocation towards the membrane, thereby effectively downregulating membrane receptor activity [Ungefroren et al., 2001].
- *Decoy receptors*:—Decoy receptors are identical to the death receptors, but lack the “death” domain. The most common decoy receptor overexpressed in PDAC

is DcR3 (Decoy Receptor 3) which leads to decreased binding of the death ligands (FasL) to death receptors (Fas) with functional downstream activity, thereby inhibiting apoptosis [Elnemr et al., 2001].

- *c-FLIP$_L$*:—This molecule is structurally identical to caspase-8, but lacks the amino acids essential for caspase activity. Interestingly, c-FLIP$_L$ is overexpressed in pancreatic cancer, competing with procaspase-8 for participating in DISC formation [Elnemr et al., 2001]. Similar to DcR3, its overexpression leads to decreased apoptosis by decreasing death receptor signaling.
- *TRAF2*:—A death receptor adaptor, upregulated in PDAC, links NF–κB with extrinsic apoptosis. TRAF2 belongs to the TNF receptor associated factor (TRAF) protein family. TRAF2 functions to inhibit TNF receptor associated death signal and instead activates NF-κB signaling. Thus upregulated TRAF2 inhibits death receptor pathway induced apoptosis and makes cancer cells more invasive by increasing NF–κB and its downstream targets IL-8 and MMP-9 [Trauzold et al., 2005].

INTRINSIC ENDOPLASMIC RETICULUM PATHWAY

This mode of apoptosis is believed to be activated when ER is injured by cellular stresses like hypoxia, free radicals, and glucose deprivation. It functions independent of the mitochondria. The initiator is thought to be caspase 12, activated in response to severe ER injury [Wong, 2011]. ER is the organelle responsible for protein folding and assembly and allows only correctly folded proteins to exit into the cytosol. It is particularly sensitive to intracellular perturbations, like DNA damage, oxidative stress, decreased ER calcium levels- all these interfere with protein folding in the ER and lead to “ER stress”. The cell deals with this stress by upregulating UPR (Unfolded Protein Response) signaling in the ER. This signaling has an important role in tumorigenesis, as cancer cells are under “physiological ER stress” because of the high replicative rate. It has been shown that UPR plays a survival role in a number of types of cancer [Healy et al., 2009; Li and Lee, 2006]. Our group, interestingly, found GRP78 (Glucose Regulated protein 78kDa), an integral survival protein in the UPR response, to be upregulated in pancreatic cancer cell lines as well as human tumor samples. GRP78 is required for activation of ER stress sensors. Most of the GRP78 resides within the ER lumen, but a minor fraction functions as a transmembrane protein, which plays a role in apoptosis. It has been found that GRP78 can bind and inhibit caspase-7 and pro-apoptotic proteins like BIK and BAX.

ROLE OF APOPTOSIS IN TREATMENT OF PANCREATIC CANCER

The currently available or investigational chemotherapies exert their anticancer effects via apoptosis. These drugs can be classified into two broad classes: Broad spectrum agents that affect multiple pathways and targeted therapies with well-defined targets.

BROAD SPECTRUM ANTICANCER AGENTS

Antimetabolites—5-FU, a nucleoside analogue, was the standard therapy for advanced pancreatic cancer (Table I) prior to the FDA approval of gemcitabine. Gemcitabine is

another nucleoside analogue which is the current standard of care. After phosphorylation, gemcitabine, gets incorporated into replicating DNA resulting in premature chain termination and apoptosis [Abbruzzese, 2002]. The 1 year survival rates in a phase III trial with 126 patients were 18% for patients treated with gemcitabine vs 2% in patients treated with 5-FU [Burris et al., 1997]. However, pancreatic tumors show initial sensitivity to gemcitabine followed by the rapid development of resistance. Several genetic and epigenetic changes have been associated with this resistance that are involved in gemcitabine transport and metabolism. Alterations in hENT-1 (nucleoside transporter-1), DCK (deoxycytidine kinase), and RNR-M1 and M2 (ribonucleotide reductase), have been shown to promote gemcitabine resistance. Overexpression of prosurvival pathways like S100A4-BNIP3, NF-κB, and PI3-AKT have been implicated in gemcitabine resistance. Interestingly, all these resistance markers lead to progression towards a mesenchymal phenotype and increase in stem cell markers [Kim and Gallick, 2008].

Platinums—Oxaliplatin has been approved as a second line drug to be used in combination either with gemcitabine or FOLFIRINOX regimens. Platins interact with DNA to form DNA adducts that are primarily intrastrand crosslinks, which ultimately lead to apoptosis. However, these apoptotic signals can be attenuated and resistance to platinums is a common phenomenon in pancreatic cancer. Resistance mechanisms are due to intracellular changes that prevent platins from interacting with DNA, interfere with DNA damage signals from activating the apoptotic machinery or both. More specifically, these changes include increased DNA adduct repair by nucleotide excision repair, loss of damage recognition, activation of PI3/Akt pathway, loss of p53 function, overexpression of anti-apoptotic proteins, and decreased caspase activation [Siddik, 2003].

Taxanes—Taxanes were being used as adjunct chemotherapy in treatment of pancreatic cancer with no definitive regimen until the advent of *nab*-paclitaxel. This nanoparticle-based albumin-bound water soluble formulation of paclitaxel was approved as a first line drug for metastatic pancreatic cancer with gemcitabine after a successful phase III trial in 2013 [Von Hoff et al., 2013]. Taxanes cause mitotic arrest in cancer cells by disrupting microtubule function leading to apoptosis. However, PDAC cells exhibit resistance to taxanes by upregulating NF–κB [Dong et al., 2002], overexpressing G2/M phase transition proteins like polo like kinases and aurora kinases or altering the paclitaxel binding site of β-tubulin (Fig. 1).

Novel therapeutic agents

Minnelide: Triptolide, derived from the Chinese herb *tripterygium wilfordii*, is a potent anticancer compound but is poorly soluble in water, limiting its clinical use. Minnelide, a water soluble prodrug of triptolide has been shown to be very effective against PDAC in various preclinical models and is currently undergoing phase I trials [Chugh et al., 2012]. It acts by inhibiting multiple pathways in cancer such as Sp1 [Banerjee et al., 2013], NF-κB [Liu et al., 2014] and Hsp 70 [Phillips et al., 2007], thus activating apoptotic and autophagic cascades leading to cancer cell death [Mujumdar et al., 2010]. We also found that triptolide, decreases GRP78 in pancreatic cancer cell lines, thereby activating ER stress which further augments apoptotic response [Mujumdar et al., 2010]. Although it is effective as a

monotherapy in PDAC, triptolide sensitized resistant pancreatic cancer cell lines to cisplatin [Zhu et al., 2012] and TRAIL [Chen et al., 2014]. It induced cell death by downregulating Bcl-2, Bcl-X_L, COX2, cyclin D1, and survivin in vitro. Our group has also shown that Minnelide synergizes with paclitaxel, oxaliplatin and TRAIL in abolishing pancreatic cancer and prolonging the survival of tumor bearing mice (unpublished data).

Curcumin: Curcumin is an inhibitor of several molecules involved in proliferation of cancer cells. It also alters the expression of microRNAs in pancreatic cancer cells [Lev-Ari et al., 2007]. Phase 2 trials of curcumin alone or in combination with gemcitabine showed that curcumin might have some biological activity in PDAC [Dhillon et al., 2008].

TARGETED THERAPIES

Although laboratory results of targeted therapies have been impressive, until now only erlotinib, an EGFR tyrosine kinase inhibitor, has demonstrated marginal survival benefit in combination with gemcitabine in **phase III clinical trials**. Even though the failures of targeted therapies in the clinical setting are discouraging for PDAC, it is reasonable to surmise that major progress will evolve as the molecular biology of apoptotic pathways in pancreatic adenocarcinoma continues to be unraveled (Fig. 2).

THERAPIES DIRECTLY TARGETING APOPTOTIC PATHWAYS

Death receptoreextrinsic apoptosis pathway—Systemic administration of TNFα and CD95L is associated with severe adverse effects precluding their therapeutic use. TRAIL, on the other hand has a greater selectivity for cancer cells via binding to DR4 and DR5 receptors. Unfortunately, a majority of PDAC cells are resistant to TRAIL mediated apoptosis due to upregulated anti apoptotic pathways. TRAIL and DR4/DR5 agonistic antibodies (Conatumumab, Tigatuzumab) in PDAC cells activate prosurvival pathways like NF-κB, ERK and PKC [Trauzold et al., 2005; Khanbolooki et al., 2006], thereby causing a paradoxical increase in tumor growth and invasiveness of PDAC as shown in an orthotopic murine model of PDAC [Trauzold et al., 2001]. Resistant pancreatic cancer cells can be sensitized to death receptor mediated apoptosis by inhibiting the prosurvival pathway NF-κB or by decreasing the expression of anti-apoptotic proteins as shown by various studies [Arlt et al., 2013].

Triptolide, as shown previously by our group effectively leads to inhibition of NF-κB pathway, augments TRAIL induced lysosomal membrane permeabilization and inhibits cFLIP, XIAP, and induces cleavage of Bid to sensitize PDAC cells to TRAIL mediated apoptosis [Chen et al., 2014]. In vivo, Minnelide synergizes with TRAIL to abolish PDAC tumors in a xenograft mouse model of TRAIL resistant PDAC cells (unpublished data). Multiple other molecules targeting this apoptotic pathway are currently being developed [Werner et al., 2011].

Mitochondrial/intrinsic apoptosis pathway—The Bcl-2 family members play the major role in this pathway. Multiple therapies ranging from antisense oligonucleotides to anti Bcl-2 antibody, to ribozymes have been tested against PDAC, but none of these have proven beneficial in clinic. On the other hand, substantial efforts have been made to develop

natural or semisynthetic SMIs (small molecule inhibitors) of Bcl-2 proteins. Gossypol and its derivatives that is TW-37, AT-101 are pan Bcl-2 inhibitors that are undergoing clinical trials for various visceral and hematological malignancies. They bind to the hydrophobic groove of Bcl-2 and Bcl-xL and prevent their heterodimerization with the pro-apoptotic proteins of the same family. Other SMIs, like ABT-737 and its oral analogue ABT 269, developed by using NMR technology mimic the BH-3 domain of BAD and inhibit the anti-apoptotic Bcl-2 protein family except for Mcl-1, which is upregulated. Synergistic antitumor activity have been achieved using TRAIL and these SMIs [Wong, 2011; Masood et al., 2011; Arlt et al., 2013]. Another SMI developed via high throughput screening of natural compounds is obatoclax (indole bipyrrole), that disrupts interactions between Mcl-1 and Bak and induces Bax mediated apoptosis in solid organ malignancies. It has also been tested with TRAIL in PDAC [Wong, 2011; Masood et al., 2011].

Common apoptosis pathway—The central component for any cell undergoing apoptosis via any pathway is a proteolytic system involving caspases. Targeting these terminal components could lead to a more effective direct therapy for cancer. Several pharmacological and biological agents have been designed to synthetically activate caspases in pancreatic cancer. One example is caspase modulator I–1541, which stabilizes procaspase-3 in an “on-state” conformation that facilitates its subsequent autoactivation. It was able to induce apoptosis in multiple cell lines with defects in various apoptotic pathways upstream of caspase activation [Wolan et al., 2009]. Gambogic acid and its derivative, MX-2167, have been shown to directly activate caspase-3 and induce apoptosis in most solid organ cancers. A receptor based approach using somatostatin receptor subtype 2 was shown to activate caspase-3 and induce apoptosis in pancreatic cancer cells [Vernejoul et al., 2002]. A reovirus based therapy, reolysin, targets transformed KRas pancreatic cancer cells leading to ER stress mediated apoptosis caused by cleavage of caspase-4 [Carew et al., 2013].

IAP and survivin inhibitors—Smac/DIABLO is an intrinsic antagonist of IAPs and promotes apoptosis by antagonizing IAP-mediated caspase inhibition. Its structural analysis led to the recognition of IAP binding motif (IBM) and have helped in developing Smac mimetics/peptides and non-peptide inhibitors (SM-164) that enhance apoptosis and sensitize cells to chemotherapeutics drugs and radiation [LaCasse et al., 2008; Owens et al., 2013]. Several of these Smac mimetics are currently in phase I trials. Single stranded antisense oligonucleotides against XIAP (AEG35156), have been employed in combination with conventional chemotherapy but with little or no benefit in patients with PDAC. Others (TWX006, Xantags, and Embelin) are still being investigated [LaCasse et al., 2008; Mahadevan et al., 2013]. Survivin is one of the ubiquitous proteins found to be upregulated in human cancers and several strategies targeting survivin have been used to sensitize resistant pancreatic cancer cells to apoptosis. Antisense oligonucleotides (ISIS23722), transcriptional repressors (YM155), HSP90 antagonists (shepherdin, AICAR) and immune based anti-survivin antibodies lead to functional or chemical depletion of survivin and are being investigated in multiple solid organ cancers including pancreatic cancer [LaCasse et al., 2008; Owens et al., 2013].

SIGNAL TRANSDUCTION PATHWAYS

Epidermal growth factor receptor pathway—EGF receptor can be targeted therapeutically by monoclonal antibodies or small molecule tyrosine kinase inhibitors (TKI). Monoclonal antibodies interact with the extracellular domain of EGFR, inhibiting EGF ligand binding. The small molecule TKIs act intracellularly by interfering with the ATP binding site of the EGFR tyrosine kinase. Both the monoclonal antibodies and the small molecule TKIs efficiently block the EGFR signal transduction pathways in-vitro, inducing tumor cell cycle arrest and potentiating apoptosis. Cetuximab, matuzumab, and trastuzumab have been tested in clinical trials with mixed results [Bayraktar and Rocha-Lima, 2010]. Erlotinib, which has been FDA approved for advanced PDAC and geftinib, which is undergoing trials, are EGFR tyrosine kinase inhibitors. These TKIs also have a chemo and radiosensitizing effect as they suppress the DNA repair mechanisms induced by these conventional therapies [Bayraktar and Rocha-Lima, 2010].

Ras/Raf/MAPK pathway—Farnesyltransferase inhibitors like tipifarnib and lonafarnib that target the isoprenylation of K-Ras though very effective in in vitro studies, were not very effective in animal studies due to activation of the secondary prenylation pathway via geranylgeranyltransferase. Drugs targeting the downstream Raf kinases like sorafenib have also been tested in clinical trials but did not achieve a very meaningful response [Siu et al., 2006], which points towards other important pathways downstream to Ras in PDAC like YAP and STK33 [Scholl et al., 2009; Zhang et al., 2014]. Nevertheless, there are some exciting therapies targeting K-Ras in different ways such as SMIs that either block SOS-mediated nucleotide exchange or allosterically shift the affinity of KRAS to favor GDP over GTP [Maurer et al., 2012]. Suppression of oncogenic RAS signaling by interfering with its localization to endomembrane has also shown interesting results in human PDAC cells in vitro and in vivo [Spiegel et al., 2014].

PI3K/Akt/mTOR pathway—Despite the proven protumorigenic role of Akt signaling in PDAC only few specific inhibitors of this pathway have been tested in clinical setting. Drugs known to inhibit this pathway, albeit non-exclusively, are natural compounds (curcumin, resveratrol, and triptolide), HIV protease inhibitor nelfinavir and metformin [Arlt et al., 2013]. Several inhibitors targeting mTOR have been tested and several more are being developed against multiple cancer types including PDAC. Some of these that have been used in preclinical trials are everolimus, ridaforolimus, temsirolimus, and GDC-0980, with everolimus having completed a phase II trial with no significant anti-tumor effect [Wolpin et al., 2009].

NF-κB INHIBITORS

Constitutive and cancer induced NF-κB activation is the major factor responsible for chemoresistance in PDAC [Carbone and Melisi, 2012]. It has been proved that inhibition of NF-κB in cancer cells is possible and multiple modalities have been developed to either prevent the drug induced NF-κB activation or to suppress the constitutional NF-κB activity in pancreatic cancer cells [Carbone and Melisi, 2012]. Multiple approaches to inhibit NF-κB have been developed, namely IKK inhibitors (BAY-11-7082 BAY-11-7085, CHS828),

proteasome inhibitors (bortezomib, RP-171, carfilzomib) and DNA binding inhibitors (SN-50). Other drug classes that exhibit a strong NF-κB inhibition are NSAIDs (aspirin, indomethacin, COX-2 inhibitors), naturally occurring compounds (triptolide, curcumin, resveratrol, epigallocatechin gallate (EGCG), and luteolin) which block this pathway at various steps [Lin et al., 2010]. As NF-κB is required for various cellular and physiological responses like immune system, persistent suppression is undesirable. Intermittent inhibition of NF-κB to overcome the chemoresistance in PDAC is one way to ensure a desirable antitumor effect.

HDAC INHIBITORS

Histone acteylases (HATs) and deacetylases (HDACs) are enzymes that affect the expression of multiple genes via altering various histone and non-histone proteins and chromatin modifications [Bernstein et al., 2007]. These enzymes regulate multiple cellular functions including proliferation and apoptosis by maintaining a fine balance between acetylated and deacetylated chromatin [Marks and Xu, 2009]. HDAC inhibition leads to a reversal of epigenetic changes associated with PDAC and induces cellular differentiation and apoptosis. HDAC inhibitors can be classified into six groups but all of them have a characteristic zinc binding moiety that inhibits HDACs.

HDAC inhibitors sensitize pancreatic cancer cells to both extrinsic and intrinsic apoptotic pathways by downregulating their inhibitors (c-FLIP, XIAP, Bcl-2, Mcl-1) and upregulating death receptors (DR4, DR5) [Koutsounas et al., 2013; Feng et al., 2014]. Multiple studies have shown the synergistic anti-tumor effects of HDAC inhibitors with TRAIL, ABT-737, and other conventional chemotherapies [Koutsounas et al., 2013; Feng et al., 2014]. In addition to caspase dependent apoptosis HDAC inhibitors also induce caspase independent apoptosis by DNA fragmentation through the increased nuclear translocation of Bax-AIF-serine protease complex [Garcia–Morales et al., 2005].

HDAC inhibitors in combination therapy have been shown to synergize with multiple conventional anticancer agents such as gemcitabine, topoisomerase inhibitors, and proteasome inhibitors.

IMMUNOTHERAPY

Immune therapies hold promise as an alternative treatment modality with significantly less toxicity. There are four main categories of immunotherapy: checkpoint inhibitors, vaccines, monoclonal antibodies, and cytokines. They work either by activating T cells or inducing humoral immune response to tumor specific antigens resulting in cell mediated or antibody dependent cellular cytotoxicity in cancer cells [Laheru and Jaffee, 2005]. Checkpoint inhibitors block the T-cell inhibitory signals to enhance pre-existing anti-cancer immune responses. Agents targeting these molecules like CTLA-4 (ipilimumab: phase I), programmed cell death-1 (PD-1) (pembrolizumab: phase IB, nivolumab: phase I/II) are being tested in combination to further enhance the inherent anti-cancer immune response [Arslan and Yalcin, 2014].

Multiple types of vaccines have been tested in PDAC namely whole cell recombinant vaccine, dendritic cell vaccine, DNA, and peptide vaccines. The most studied and clinically tested vaccines in PDAC are whole cell vaccines, namely Algenpantucel-L based on allogeneic pancreatic cancer cells expressing αGal epitope and GVAX based on irradiated tumor cells expressing murine GM-CSF are undergoing phase III and II trials respectively [Gunturu et al., 2013]. Other vaccines based on telomerase (GV1001), K-Ras, mesothelin (CRS-207), heat shock protein (HSPPC-96) and antigen-pulsed dendritic cells +/− lymphokine activated killer therapy are also undergoing clinical trials.

CONCLUSION

There is abundant evidence suggesting that dysregulation of the apoptotic pathway plays a major role in pancreatic cancer carcinogenesis and that targeting apoptosis for treatment of this deadly cancer is feasible. Many such agents are undergoing either clinical or preclinical testing alone or in combination with conventional anticancer drugs. However, most of these agents have failed to translate into meaningful clinical benefit for patients suffering from pancreatic cancer. There are a multitude of reasons that could explain failure of these earlier therapies-lack of suitable preclinical models mimicking human disease, incomplete understanding of PDAC pathobiogenesis and unclear role of tumor microenvironment to name a few. However, with evolving research and better collaboration, we hope pancreatic cancer will no longer remain a death sentence.

Acknowledgments

Grant sponsor: NIH; Grant numbers: R01-CA170946, CA124723; Grant sponsor: NIH; Grant number: R01-CA184274.

This study was funded by NIH grants R01-CA170946 and CA124723 (to AKS); NIH grant R01-CA184274 (to SB); Katherine and Robert Goodale foundation support (to AKS) and Minneamrita Therapeutics LLC (to AKS).

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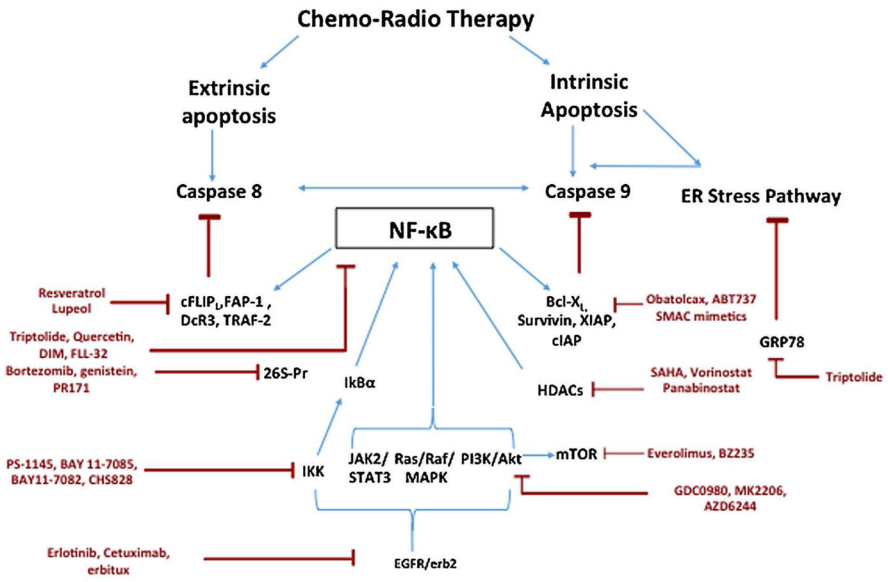

Fig. 1.
Mechanisms contributing to apoptosis evasion in pancreatic cancer.

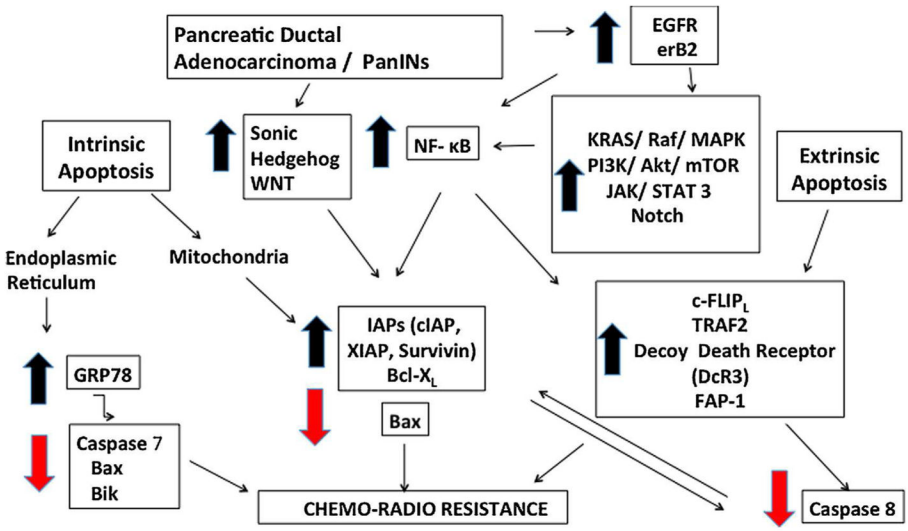

Fig. 2.
Novel targeted therapies in pancreatic cancer.

TABLE I

Newer Therapies for Pancreatic Cancer and Their Molecular Targets

Treatment target	Drug description	Mechanism of action
1 Fas-FasL interaction	Synthetic phosphodiester oligodeoxynucleotide	Modulate Fas-FasL expression
	Antibodies and antisense oligonucleotide	Decrease FasL
2 Death receptors	TRAIL (TNF related apoptosis inducing ligand) and its structural modifications	Bind and activate DR4 and DR5 receptors
	RO5458640 (humanized monoclonal antibody)	Inhibit binding of TWEAK (TNF-like weak inducer of apoptosis) ligand to its receptor (Fn14)
	Mapatumumab, Conatumumab/AMG 655	Agonistic DR4/DR5 antibody
3 Bcl-2 family of proteins	Antisense oligonucleotides	Inhibit overexpression of Bcl-2 and $Bcl_{XL.}$
	Small molecule inhibitors (Gossypol-AT101, TW-37, PPARγ-inactive TZD derivatives, Apogossypolone, ABT 737, Obatoclax, ABT 199)	Inhibit the interaction of various anti-apoptotic Bcl-2 members to pro-apoptotic members increasing the activity of latter. BH3 mimetics
4 Inhibitor of apoptosis proteins (IAPs)	SMAC mimetics (JP 1201, TL32711 (Birinapant), LCL161, SM-164,)	Inhibit IAPs after binding to their BIR3 domain
	Antisense oligonucleotides (AEG 35156, Embelin, Xantag)	Functional depletion of XIAP
5 Survivin	YM 155 (sepantronium bromide)	Transcriptional repressor
	Antisense oligonucleotides (ISIS23722, EZO-3042), siRNAs	Functional depletion of survivin
	Hsp90 inhibitors (shepherdin, AICAR)	Interfere with survivin folding and stability
6 Caspases	Gambogic acid and its derivatives (MX-2167)	Directly activate caspase-3
	Reolysin (human reovirus)	Target transformed KRAS cancer cells and activate caspase-4
7 EGFR/Ras/Raf/MAPK	EGFR inhibitors Tyrosine kinase inhibitors (Erlotinib, Geftinib)	Interfere with ATP binding site of EGFR tyrosine kinase
	Monoclonal antibodies (Cetuximab, Matuzumab, Trastuzumab)	Inhibit EGF ligand binding to EGFR
	Farnesyl transferase inhibitors (Tipifarnib, Lonafarnib)	Inhibit isoprenylation of KRAS
8 PI3K/Akt/mTOR	Natural compounds (curcumin, triptolide, resveratrol)	Possible transcriptional inhibition.
	HIV protease inhibitors (Nelfinavir), Metformin	Inhibit activation of Akt
	mTOR inhibitors (Everolimus, Ridaforolimus, Temsirolimus)	Bind to FKBP12 and inhibit mTOR
9 NF-κB	Natural compounds (triptolide, luteolin, curcumin, EGCG)	Possible transcriptional inhibition
	IKK inhibitors (BAY-11–7082, BAY-11–7085)	Prevent degradation of IκB
	Proteasome inhibitors (bortezomib)	Prevent degradation of IκBα by inhibiting 20S proteasome.
	DNA binding inhibitor (SN-50)	Inhibit NF-κB nuclear translocation
10 HDAC	Class I inhibitors (Depsipeptide)	Bind to zinc containing catalytic domain of HDACs
	Class I and II inhibitors (Vorinostat, panabinostat, abexinostat, valproic acid)	