Mini-review
Targeting apoptosis pathways in pancreatic cancer
Alexander Arlt, Susanne Sebens Müerköster, Heiner Schäfer

Laboratory of Molecular Gastroenterology and Hepatology, Dept. of Internal Medicine 1, UKSH-Campus Kiel, Schittenhelmstr. 12, 24105 Kiel, Germany

ARTICLE INFO
Keywords:
Programmed cell death
Anti-cancer therapy
Chemoresistance

ABSTRACT
Pancreatic cancer – here in particular pancreatic ductal adenocarcinoma (PDAC) – is still a highly therapy refractory disease. Amongst the mechanisms by which PDAC cells could escape any non-surgical therapy, anti-apoptotic protection seems to be the most relevant one. PDAC cells have acquired resistance to apoptotic stimuli such as death ligands (FasL, TRAIL) or anti-cancer drugs (gemcitabine) by a great number of molecular alterations either disrupting an apoptosis inducing signal or counteracting the execution of apoptosis. Thus, PDAC cells exhibit alterations in the EGFR/MAPK/Ras/raf1-, PI3K/Akt-, TRAIL/TRAF2-, or IKK/NF-κB pathway accompanied by deregulations in the expression of apoptosis regulators such as cIAP, Bcl2, XIAP or survivin. Along with protection against apoptosis, PDAC cells also overexpress histone deacetylases (HDACs) giving rise to epigenetic patterns of chemoresistance and to acetylation of other regulatory proteins, as well. With respect to the multitude of anti-apoptotic pathways, a great number of molecular targets might be of high potential in novel therapy strategies. Thus, natural compounds as well as novel synthetic drugs are considered to be used in single or combined therapy of PDAC. A number of proteasome and HDAC inhibitors or selective inhibitors of IKK, EGFR, Akt and mTOR have been widely explored in preclinical settings and clinical studies. Even though these early studies encouraged an application in a clinical setting, most of the trials have been rather disappointing yet. Thus, new molecular targets and novel concepts of combination therapies need to get access into clinical trials – either in neoadjuvant/adjuvant or in palliative treatments.

1. Introduction
Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive of all human malignancies exhibiting an overall 5-year survival rate of less than 2% and ranking 4th among cancer-related death in the western world [1]. PDAC is characterized by a rapid disease progression and absence of specific symptoms, largely precluding an early diagnosis and curative treatment [2,3]. In most cases, PDAC is already locally advanced at time of diagnosis exhibiting encasement or occlusion of adjacent vascular structures.

Surgical resection represents the only curative treatment, but due to late diagnosis most patients present in an advanced stage and only a minority (10–20%) of them is amenable to surgical intervention [4–6]. Owing to the high recurrence rate, surgical PDAC patients require adjuvant chemotherapy with or without radiotherapy providing a 5-year survival rate of 15–25% [7]. A recent study reported that postoperative gemcitabine treatment significantly delayed the development of recurrent disease after complete resection of pancreatic cancer compared with
observation alone [8]. Given the mostly inoperable state of PDAC, the vast majority of patients rely on palliative treatment using conventional chemotherapy. Despite providing some improvements in life quality, the current standard of care treatment with gemcitabine or 5-fluorouracil (5-FU) result in a median survival of just a few months [9,10]. This strong limitation of conventional treatment is mainly due to the profound resistance of PDAC cells towards anticancer drugs emerging from the efficient protection against chemotherapeutic drugs by an altered balance of pro- and anti-apoptotic proteins which results in a markedly reduced apoptotic responsiveness [11,12]. Since the majority of established strategies in cancer therapy depends on the elimination of tumor cells by apoptosis the capability of tumor cells to escape the execution of cell death is the major obstacle [13,14].

As outlined in more detail elsewhere in this special issue, apoptosis or programmed cell death is a central regulator of normal tissue homeostasis and there are two alternative intracellular routes to initiate apoptosis – the intrinsic or mitochondrial pathway and the extrinsic or death-receptor pathway. Physiologically, apoptosis is essential for the elimination of redundant, damaged and infected cells. In particular, apoptosis represents a fundamental anti-neoplastic mechanism preventing tumorigenesis of normal cells [14]. Almost all neoplastic changes during the transformation of a normal cell to a cancer cell, like DNA-damage, oncogene activation or cell cycle deregulation, are potent inducers of the programmed cell death pathway. Therefore, overcoming the apoptotic failsafe is observed in many cancers as discussed recently [15] and elsewhere in this special issue. Mostly, these mechanisms of anti-apoptotic protection are commonly found in cancer (see separate reviews in this special issue), but some are more typically for PDAC (as outlined below). Thus, in accordance with the multi-step theory of carcinogenesis, the natural history of PDAC seems to gradually evolve through precursor lesions, the so-called pancreatic intraepithelial neoplasia (PanIN) 1–3 to invasive PDAC [16]. In PanIN 1 and 2 lesions, no apoptotic cells could be detected [17], arguing for the contribution of anti-apoptotic mechanisms quite early in the carcinogenesis of PDAC ensuring the survival of premalignant cells under oncogenic stress conditions.

Like other cancer cells, PDAC cells have evolved resistance mechanisms especially acting on the death receptor level and resulting in an impaired initiation of apoptosis by TNFα, FasL and TRAIL. [18]. In addition, PDAC cells gain protection against the mitochondrial pathway of apoptosis by overexpression of Bcl-family proteins (Bfl1, BCL-XL, MCL-1) [18] or by blocking activation of caspases – e.g. by the overexpression of caspase inhibitors (cIAP, XIAP1, survivin), the epigenetic downregulation of procaspase gene expression or the direct caspase inhibition by cysteine nitrosylation [19,20].

---

**Fig. 1.** Scheme of anti-apoptotic pathways and involved molecular targets. Depicted are the anti-apoptotic pathways and molecular targets (in blue) and the corresponding inhibiting compounds (in red) as further outlined in this review. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Table 1
Overview of molecular targets and their inhibitors used in preclinical and clinical studies. References are indicated by numbers in square brackets.

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>Targeting drug</th>
<th>Application in clinical or preclinical study</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR4/DR5</td>
<td>Agonistic antibodies: Mapatumumab, Conatumumab/AMG 655 in combination with anti-cancer drugs [27]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Bcl-2 family</td>
<td>Inhibitors: Obatoclax [28] or ABT-737 [29] in combination with anti-cancer drugs</td>
<td>Preclinical</td>
</tr>
<tr>
<td>XIAP</td>
<td>Smac mimetic peptides [30,31], XIAP inhibitors: Embelin [32], AEG35156 [33]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Survivin</td>
<td>Resveratrol [34], BIPCD [35]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>cFLIP</td>
<td>Lupeol [36]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>cIAP</td>
<td>HGSI1029 [37]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>NF-κB</td>
<td>IKK inhibitors CHS-828, PS-1145, BAY 11-7082 [64,65] thymoquinone (TQ), sulforaphane (SFN), dihydroartemisinin (DHA) or 3,3-diindolylmethane (I3C)/DIM [61,73,74]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>MEK</td>
<td>AZD6244 [123]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>mTOR</td>
<td>CCI-779 [125], everolimus, [126], ridaforolimus [127]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>PI3K</td>
<td>NVP-BEZ235 [124], GDC-0980, CAL-120 [128]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Akt</td>
<td>MK-2206129]</td>
<td>Preclinical</td>
</tr>
<tr>
<td>HDAC classes I, II, IV</td>
<td>SAHA/Vorinostat [132–135]</td>
<td>Preclinical, phase I study</td>
</tr>
<tr>
<td>HDAC class I</td>
<td>Bortezomib/Velcade [140,144]</td>
<td>FDA approved for certain malignancies</td>
</tr>
<tr>
<td>20S proteasome</td>
<td>PR171, NPI0052 and CEP18770 [145]</td>
<td>Phase II study, preclinical</td>
</tr>
</tbody>
</table>

A better understanding of these mechanisms has opened access to novel molecular targets that might be of high potential to overcome therapy-resistance of PDAC. Thus, novel strategies have been developed or will be so in near future aiming at the commitment of PDAC cells to undergo apoptosis efficiently in response to quite novel drugs – either administered alone or in combination with conventional anti-cancer drugs. Below, the most promising molecular targets in apoptosis pathways are featured in detail: TRAIL/TRAIL-R, Bcl/Mcl, cIAP/XIAP/Smac, cFLIP/sur-lateral targets in apoptosis pathways are featured in conventional anti-cancer drugs. Below, the most promising molecular targets in apoptosis pathways are featured in detail: TRAIL/TRAIL-R, Bcl/Mcl, cIAP/XIAP/Smac, cFLIP/survivin, NF-κB, EGFR, Ras/Raf/MAPK, PI3K/Akt/mTOR, HDACs, the proteasome and miRNAs (Fig. 1). Novel compounds affecting these pathways are listed in Table 1.

2. Death receptor pathways (TRAIL/CD95L/TNFα)

Tumor Necrosis Factor (TNF) alpha and its family members of so-called death ligands CD95L and Tumor Necrosis Factor-related apoptosis-inducing ligand (TRAIL) all possess great potential in the induction of apoptosis in cancer cells in vitro [21]. However, systemic administration of TNFα and CD95L are characterized by severe adverse effects, thus their applicability as tumor cell specific agents in anti-cancer therapy is questionable. In contrast, TRAIL is a rather promising anti-cancer cytokine as it has been shown to exert great selectivity for various cancer cells. Even though TRAIL initiates apoptosis after binding to TRAIL receptors (TRAILR1/DR4, TRAILR2/DR5) via the extrinsic pathway, it recruits the mitochondrial pathway for the manifestation of the apoptotic response.

Numerous studies have explored the cell killing effect of TRAIL also in PDAC cells under in vitro and in vivo conditions. However, it was found that the majority of PDAC cells are resistant to TRAIL-mediated apoptosis mainly because of the constitutive upregulation of anti-apoptotic proteins of both the extrinsic pathway – such as FLIP and TRAF2 – as well as of the mitochondrial pathway – such as Bcl-XL, Mcl1 and XIAP, [22,23]. Moreover, PDAC cells respond to TRAIL with activation of non-apoptotic, pro-inflammatory signaling pathways resulting in the activation of NF-κB, PKC and ERK1/ERK2 [24,25]. Accordingly, apoptosis resistant PDAC cells respond to TRAIL with the expression of pro-inflammatory and invasion-promoting proteins and show invasive growth in vitro as well as in vivo in an orthotopic SCID-mouse model of pancreatic cancer [26]. Therefore, the therapeutic use of TRAIL or of DR4/DR5 agonistic antibodies (i.e. Mapatumumab, Conatumumab/AMG 655) [27] in the treatment of PDAC requires combination with sensitizing drugs, as it also has been shown for different other malignancies.

To overcome resistance to TRAIL-mediated apoptosis, particularly constituents of the NF-κB pathway (see below) as well as anti-apoptotic mediators of the mitochondrial pathway are chosen as potential molecular targets and subject of numerous cell-culture and mouse-model based studies. A quite promising strategy to trigger TRAIL-induced apoptosis is the use of Bcl-2 inhibitors such as Obatoclax (BH3 mimetic and pan-Bcl-2 inhibitor) [28] or ABT-737 (selectively inhibiting Bcl-2 and Bcl-xL, but not Mcl-1) [29]. Efficient sensitization to TRAIL has also been achieved through targeting XIAP by small molecule antagonists (Smac mimetic peptides [30,31]), inhibitors of XIAP (Embelin [32], AEG35156 antisense oligonucleotide [33]), the disruption of survivin expression (i.e. by the natural polyphenole resveratrol [34] or the CDK4 inhibitor BIPCD [35]) or the suppression of cFLIP by the dietary triterpene lupeol [36]. A recent study revealed a strong apoptosis induction in various TRAIL-resistant PDAC cell lines by combining Mapatumumab and the IAP inhibitor HGSI1029 [37].

In a number of preclinical studies, inhibitors of NF-κB such as PS-1154 (see below) [24], PKC (G66983) [25], of Hsp70 (tripolide) [38], or of the green tea constituent epigallocatechin-3-gallate (EGCG) [39] have been successfully tested. Another recent study underwent restoration of TRAIL-induced apoptosis in PDAC cells by adenovirus mediated re-expression of Human somatostatin receptor gene
2 [40]. Quite recently, overcoming resistance to TRAIL has been also tried by administration of HDAC inhibitors exerting different class specificity (see below) [41–43] which modulate the expression of DR5, cFLIP and other anti-apoptotic genes in a HDAC class specific fashion.

3. NF-κB pathway

The transcription factor NF-κB is involved in diverse cellular activities including immune response and inflammation. NF-κB binds DNA as hetero- and homodimeric protein complex composed of the Rel family members RelA (p65), RelB and c-Rel, of NF-κB1 (p50/p105) and NF-κB2 (p52/p100). Most abundant are the heterodimer RelA/NF-κB1 (p65/p50) and the homodimer NF-κB1/NF-κB1 (p50/p50), the former being transcriptionally active in many cell types. p65/p50 is usually kept in an inactive form in the cytoplasm by stable association with inhibitor proteins such as inhibitor κB-α (IκBα) or inhibitor κB-β (IκBβ). The IκBs are phosphorylated by a multiprotein kinase complex, the IκB kinase (IKK) complex. This complex contains the two catalytical kinas IκKα and IκKβ and the regulatory component IκKγ/NEMO. Activation of the IKK complex causes phosphorylation of the IκBs followed by their polyubiquitination and subsequent proteasomal degradation. This leads to the liberation and nuclear translocation of p65/p50 and subsequent target gene activation [44–47].

While there are some reports indicating a rather apoptosis-promoting role for NF-κB [48–51], the majority of studies demonstrate NF-κB as a potent apoptosis suppressor. In fact, many tumor cells exhibiting chemoresistance are characterized by constitutive activation of NF-κB. Aetologically, constitutive activation of NF-κB in tumors can occur due to various conditions and factors. First of all, chronic infections being major risk factors for various types of cancer can induce permanent NF-κB activation [52]. Furthermore, pro-inflammatory cytokines such as interleukin (IL)-1β [53] and IL-1α [54] either released by immune cells or by other adjacent stromal cells [55] might lead to constitutive activation of NF-κB. Since some of these “inducer” cytokines are NF-κB target genes at the same time, an autocrine or paracrine amplification loop emerges leading to the constitutive cytokine-driven NF-κB activation, e.g. in PDAC cells [53], which is further sustained by certain amplifications in the ubiquitin–proteasome pathway [56]. Point mutation of the K-Ras oncogene, which is a common and early event in PDAC carcinogenesis, might also result in an enduring activation of NF-κB [57]. In addition, overexpression and activation of the epidermal growth factor (EGF) receptor might contribute to tumor progression and an invasive phenotype of PDAC by permanent activation of NF-κB [58]. In addition, altered PI3K-AKT- and Notch1-pathways were shown to contribute to NF-κB activity, as well, and more recently downregulation of the stress response gene IEX-1 has been shown to cause an enhanced NF-κB activity and to correlate with a worse prognosis in PDAC [59,60].

Although the crucial anti-apoptotic role of NF-κB is widely proved, the exact mechanisms by which apoptosis prevention occurs is only now beginning to emerge. Thus, activation of NF-κB leads either to the upregulation of anti-apoptotic genes or to the down-regulation of apoptotic genes. A prevalent mechanism by which activated NF-κB induces chemoresistance in PDAC is the increased expression of cellular inhibitors of apoptosis (cIAP1, cIAP2, TRAF1, TRAF2, survivin) [22,61] or the increased expression of the prosurvival bcl-2 homologue Bfl-1/A1 or of Bcl-x(L) [61,62]. Another mechanism by which activated NF-κB mediates resistance to chemotherapeutic drugs in PDAC cells represents the direct inactivation of caspases by nitric oxide (NO) [20].

Since chemoresistance of many tumors depends on the constitutive activation of NF-κB, a multitude of strategies has been developed and verified to prevent the activation or transcriptional activity of NF-κB and thereby to enhance chemosensitivity. The most advanced strategies which rely on an interference with NF-κB activation have already proved to be feasible and, most notably, to overcome chemoresistance in PDAC [46,63]. One effective and selective approach for blocking NF-κB activation might be given by inhibition of the IKKs. So far, three main groups of agents exist that specifically inhibit IKK activity: immunomodulatory drugs (e.g. thalidomide and its derivatives, flavonoids and cyclopentenone prostaglandins), the non-steroidal anti-inflammatory drugs (NSAID) including aspirin and salicylates, sulindac and its analogues, sulfasalazine and its metabolites and newly developed selective IKK inhibitors (e.g. CHS-828, PS-1145, BAY 11-7082) [64,65].

Pre-treatment of chemoresistant PDAC cells with sulfasalazine clearly increases the apoptotic response towards cytostatic drugs such as etoposide, doxorubicin, gemcitabine or 5-FU [53,66–68]. Moreover, combined treatment of severe combined immunodeficiency (SCID) mice bearing human pancreatic tumor xenografts with sulfasalazine and either etoposide or gemcitabine significantly reduces tumor outgrowth [67]. Likewise, thalidomide has already been applied in preclinical as well as in clinical studies [69], yet the therapeutic potential in the treatment of patients with chemoresistant solid tumors awaits to be proven. A recent paper raised some concern for possible side effects of long term IKK inhibition [70]. The authors elegantly showed that IKK inhibition leads to an enhanced IL-1β expression after bacterial infection or endotoxin challenge. Since IL-1β is a central mediator of solid tumor chemoresistance [53,71,72] IKK-inhibition might under certain circumstances have tumorpromoting side effects.

Of high potential in NF-κB inhibition are proteasome inhibitors (e.g. Bortezomib, Mg132) that interfere with the degradation of IκBα during NF-κB activation. This group of highly effective pro-apoptotic agents is discussed below.

Novel concepts of NF-κB inhibition that have been recently presented include administration of natural compounds like thymoquinone (TQ), sulforaphane (SFN), dihydroartemisinin (DHA) or 3,3-diindolylmethane (I3C/ DIM). These herbal compounds block both constitutive and anti-cancer drug induced NF-κB activity and have been successfully tested in preclinical experiments for sensitization of PDAC cells against chemotherapy [61,73,74]. Other reports showed that NF-κB inhibition by curcumin
or the enediyne antibiotic lidamycin markedly inhibited growth of human pancreatic cancer xenografts in athymic mice and also suppressed angiogenesis [75–77]. Moreover, the inhibitory effects of genistein and curcumin on NF-κB activation appear to be mediated in part by the down-regulation of Notch-1 [78].

Finally, blockade of NF-κB expression can be effectively achieved by delivery of p65-specific small interfering RNA (siRNA) to PDAC cells in vivo indicating that inhibition of NF-κB activity by siRNA may have therapeutic potential [79]. Despite these promising findings, this technology requires significant improvement with respect to efficacy of delivery, duration of action and improved specificity and safety, before clinical application can be considered.

4. EGFR pathway

Many of the features of the malignant phenotype of PDAC, such as increased proliferation, angiogenesis, and evasion of apoptosis, are associated with dysregulation of certain growth factors and their receptors. The epidermal growth factor receptor (EGFR) family and their ligands have been reported to be frequently overexpressed in PDAC [80]. After ligand binding, receptors of the EGFR family undergo homo- or heterodimerization with other members of the EGFR family. Subsequently, auto- and transphosphorylation of tyrosine residues leads to the association with adaptor and signaling molecules and activation of a network of pathways including the Ras/Raf/ MAPK, Rac/JNK/p38 MAPK and phosphatidylinositol 3’-kinase/AKT cascades. Thus, targeting of EGFR with monoclonal antibodies, with tyrosine kinase inhibitors or by using selective antagonists and inhibitors targeting the downstream network of pathways are becoming valuable therapeutic tools.

Since the phosphorylated tyrosines of the EGFR (also known as HER-1 or ErbB1) serve as binding and activating sites for a number of signal transducers and adaptor molecules, there is a wide array of downstream signaling pathways. The two main signaling routes are the Ras/Raf/MAPK [81] and the PI3K/Akt pathway [82] which will be discussed in detail below. In normal cells, the activity of the EGFR is tightly regulated but inappropriate or constitutive activation can occur as a result of EGFR mutation, overexpression, structural rearrangements and/or relief of its normal auto-inhibitory and regulatory constraints [83]. In the transformed cell, dysregulated EGFR activation can promote a range of oncogenic activities such as proliferation, migration, stromal invasion, tumor neo-vascularization and resistance to pro-apoptotic signals [83]. The clinical relevance have been shown by several reports demonstrating overexpression or inappropriate activation in various solid tumors including PDAC [80] and a correlation of EGFR activity with the prognosis of the patient [84].

Two distinct therapeutic approaches targeting the EGFR system – monoclonal antibodies (i.e. cetuximab/erbitux) and small-molecule tyrosine kinase inhibitors (TKIs) – are currently employed in the treatment of PDAC. Besides promising preclinical trials either as monotherapy, in combination with other cytotoxic agents or in combination with radiotherapy, most of the EGFR based approaches failed to improve the outcome of PDAC patients, including strategies combining erbitux with gemcitabine [85,86].

Up to now, the only EGFR targeting drug with a clinical benefit is erlotinib (Tarceva®, OSI 774). Erlotinib is a tyrosine kinase inhibitor that inhibits ErbB-1 phosphorylation. Preclinical studies and clinical trials have demonstrated that gemcitabine-induced apoptosis is augmented in vitro and in vivo [80,87]. In contrast to nearly all clinical applications of other molecular targets in PDAC, one Phase III trial of erlotinib with gemcitabine was able to show at least a small gain in the survival in patients with advanced PDAC [88]. However, objective response rates were not significantly different between the arms, although more patients on erlotinib had disease stabilization. Accordingly, erlotinib has got FDA approval and access in clinical application but the therapeutic benefit for patients with advanced PDAC remained poor. Of note, the likelihood of a better clinical response to erlotinib/gemcitabine treatment might be greater in PDAC patients developing rash during therapy [89].

5. Ras/Raf/MAPK pathway

One of the central downstream pathways of the EGFR system is the Ras/Raf/MAPK pathway. H-Ras, K-Ras and N-Ras are GTPases which act as central molecular switches that control the activity of many signaling pathways and activating mutations in K-Ras are frequently found (up to 90%) in PDAC and its precursors lesions [90]. These mutations, invariably found at codons 12, 13 or 61, prevent efficient GTP hydrolysis, rendering K-Ras in an active, GTP-bound state. The observed high frequency of K-Ras mutations and their proposed central role in pancreatic diseases [91] suggested that K-Ras would be a good molecular target in PDAC therapy. Since farnesyltransferase inhibitors (FTI), the most common used drugs to target Ras GTPases, do not completely inhibit the prenylation of K-Ras, some reports indicated that farnesyltransferase and geranylgeranyltransferase inhibitors (GTI) have to be combined to achieve anti-tumor responses [92]. Nevertheless neither FTI nor a combined approach with FTI and GTI showed a significant efficacy in clinical trials [93]. Besides activating mutations of the Ras oncogene which are frequently found in PDAC and are supposed to be critical initiators of pancreatic carcinogenesis [90], the Raf–MAPK signaling pathway can also be activated by perturbation of components upstream of Ras.

As discussed above aberrant overexpression or mutational activation of the EGFR system can cause hyperactivation of Ras leading to upregulated MAPK signaling [81]. In addition to the downstream effects on apoptotic signaling pathways by the Ras–Raf–MAPK pathway (see below), Ras signaling itself leads to upregulation of the expression of ligands of the EGFR, in particularly the transforming growth factor alpha (TGFα). The resulting autocrine loop of TGFα and other EGFR ligands (e.g., heparin-binding EGF, amphiregulin) has been observed in a wide variety of cancers and neutralization or blockade of these ligands are able to block oncogenic Ras transformation in some models [94–97].
Even though the Raf/MAPK cascade is the best characterized pathway downstream of Ras there are others as well, including PI3K/Akt, the Raf small GTPase, Tiam1 and phospholipase C controlled oncogenic signals [94]. Since there is evidence that Raf/MAPK and PI3/Akt pathways are more important in PDAC these pathways are discussed in this review.

Ras activates the family of Raf kinases (c-Raf-1, A-Raf and B-Raf) by a complex sequence of events involving phosphorylation, protein–protein and protein–lipid interactions. Even though the structures of the three Raf proteins are similar, they differ considerably in their modes of regulation, tissue distributions and abilities to activate MEK [81,94]. However, it is still unclear which of the Raf isoforms is required to activate the MAPK pathway and the role of the family members is not sufficiently characterized in PDAC [91,98–102].

Once activated Raf kinases phosphorylate and activate MEK1/2 (MAPKKK) which in turn phosphorylate and activate the ERK1 and ERK2 MAPKs [81,94]. ERK1/2 activation leads to further branching of the signal pathway by regulation of the activities of an ever growing roster of substrates [103,104]. Recent reports indicated that subcellular localization of ERK1/2 can determine the effect on cell death [105] but the target genes crucial for the control of apoptosis in PDAC remain elusive. Besides the strong evidence that the Raf/MAPK pathway is critical for growth control in PDAC downstream or independently of mutated Ras the multi-kinase inhibitor sorafenib is the only drug targeting this pathway which has been established in early clinical trials [106]. Furthermore Raf mutations are not commonly seen in pancreatic cancers [107] and upregulated MAPK activation is only rarely found in most PDAC cell lines or patient tumors [108] indicating that other pathway downstream of Ras are important in PDAC. Thus, other downstream targets of Ras are of great interest, such as the serine/threonine kinase STK33 or the non-canonical IκB kinase TBK1 which are essential for the viability of mutant K-Ras dependent cells but not of cells being independent of mutant K-Ras [109,110]. Accordingly, the inhibition of these kinases would provide an efficient and tumor (if K-Ras dependent) selective strategy in cancer therapy.

6. PI3K/Akt and mTOR pathway

The other central downstream target of Ras is the PI3K/Akt pathway. Besides Ras, survival signals like growth factors, cytokines and hormones recruit this pathway for inducing proliferation and anti-apoptotic signaling [82]. By activating the phosphoinositide-dependent kinase-1 (PDK1) and recruiting the serine/threonine kinase Akt to the plasma membrane, PI3K leads to the PDK1 dependent phosphorylation of Thr308 of Akt [111]. This phosphorylation is sufficient for Akt activation, but for a maximal Akt activity an additional phosphorylation of the Ser473 residue is required. Besides the growth promoting potential of Akt, its anti-apoptotic properties are closely linked to the resistance of cancer cells to a broad spectrum of apoptotic stimuli. The mechanisms by which Akt promotes cell survival include phosphorylation and thereby inactivation of pro-apoptotic proteins such as BAD, caspase-9 and members of the Forkhead family of transcription factors [111] and reviewed elsewhere in this special issue. In addition to this direct inhibition of pro-apoptotic mediators, Akt seems to be involved in the activation of anti-apoptotic pathways such as the NF-κB signaling pathway (see above). Recent reports have demonstrated that the PI3K/Akt pathway provides chemoresistance, at least in part, by the activation of NF-κB [18,66].

Alterations in the activity of the PI3K/AKT pathway are quite common in PDAC. Up to 60% of PDAC tissues and most PDAC cell lines exhibit increased AKT activity [18,112,113]. Even if there is evidence for functional crosstalks between other potential molecular targets in PDAC – i.e. the NF-κB [114,115] and c-myc [116] pathways – there is at least some controversy of the relevance of Akt in mediating chemoresistance through these interactions. Furthermore mutations and epigenetic downregulation of the most important negative regulator of Akt/PI3K signaling, the tumor suppressor PTEN (phosphatase and tensin homologue), have been described in PDAC and are regarded as crucial for tumor development and chemoresistance [117,118]. One of the downstream targets with clinical implications (see below) is mTOR. This serine/threonine protein kinase of the PI3K superfamily provides tumor cells with a growth advantage by promoting protein synthesis [111] and is supposed to have a role in self-renewal of tumor stem cells in PDAC [119].

Contrary to the multitude of data on the role of the Akt signaling pathway for chemoresistance in solid cancers and i.e. in PDAC only limited clinical applications were tested so far. Two of these drugs supposed to target the Akt pathway are the natural compounds curcumin [76] and resveratrol [120]. Even though these drugs showed some promising effects in preclinical and clinical applications in PDAC there is some controversy of the signaling pathways or molecular mechanisms by which the anti-tumor effects are achieved [76,120]. In addition the HIV protease inhibitor nelfinavir radiosensitize tumors with activated PI3-kinase/Akt pathway in preclinical settings and showed some effects in a phase I clinical trial [121]. Interestingly metformin, a drug established in the treatment of diabetes for decades, decreases growth of PDAC cell lines and could be a potential candidate in novel treatment strategies for PDAC patients [122]. Another efficient suppression of pancreatic cancer cell growth in vivo is the combination of the mTOR inhibitor rapamycin and the MEK inhibitor AZD6244 (ARRY-142886) as reported recently [123]. Similarly, the novel PI3K/mTOR inhibitor NVP-BEZ235 proved to be efficient in PDAC inhibition in preclinical settings [124].

Besides these natural compounds and pharmaceutical drugs several more specific PI3K, Akt and mTOR inhibitors are currently tested in clinical trials for nearly all types of cancer [111]. The use of mTOR inhibitors, either alone or in combination with other anti-cancer agents, has the potential to provide anti-cancer activity in numerous tumor types including pancreatic cancer: CCI-779 (Torisel, Wyeth Pharmaceuticals) [125], everolimus (RAD001, Afinitor, Novartis Pharmaceuticals) [126], and ridaforolimus (AP23573; formerly deforolimus, ARIAD Pharmaceuticals)
translation of the expression of HDAC1-3 is in close association with the malignant phenotype of cancer cells. Under these conditions, histone proteins become less acetylated thus interacting more tightly with DNA and forming condensed and inactive DNA (heterochromatin). Thus, in addition to CpG hypermethylation of DNA, gene silencing by histone deacetylation affects the expression of tumor suppressor genes in tumor cells.

In man, four classes of HDACs are known which are characterized by their substrate specificity, cellular localization, Zn$^{2+}$ dependence and similarity to yeast homologues. Class I, II and IV enzymes consist of four, six and one HDACs, respectively, sharing Zn$^{2+}$ dependence, whereas class III consists of seven functionally distinct and Zn$^{2+}$-independent Sirtuins [130]. HDACs exhibit some redundant but also some class specific activities as well as tissue specific expression. For a long time, particular attention was given to class I HDACs (HDAC1-3, HDAC8) which are strongly overexpressed in tumors [131].

Most of the meanwhile more than 15 HDIs tested in preclinical and early clinical studies in cancer therapy efficiently – but not always selectively – suppress class I HDACs and are supposed to exert the strongest tumor killing activities. This is much more the case if HDIs are administered together with other cancer therapies. A great number of phase I and II studies already explored the effects of the hydroxamat SAHA/Vorinostat (inhibiting class I, II and IV HDACs) which is approved by the FDA for treatment of cutaneous T-cell lymphoma (CTCL) in the therapy of other hematological malignancies and advanced solid tumors [132–135], but providing only little to no clinical benefit. In PDAC it was shown that the combined upregulation of the expression of HDAC1-3 is in close association with an elevated NF-κB activity [136]. Preclinical studies revealed that blockade of class I HDACs in PDAC cells by the rather class specific HDIs trichostatin (TSA) and SAHA or the more class I selective agent valproic acid (VPA) strongly suppressed constitutive NF-κB activity which is a major determinant of malignant transformation and drug resistance in PDAC (see above). The class II enzyme HDAC6 has been shown to play a role in aggresome formation which accumulates during proteasome inhibition (see below), and the disruption of aggresomes by HDAC6 inhibition in PDAC cells augments proteasome inhibitor (PI) induced apoptosis [137]. Thus, HDAC6 might be an interesting target, as well, either by the panspecific HDIs or by the HDAC6 selective agent tubacin [138]. More recently, HDAC2 was identified as crucial mediator of drug resistance in PDAC, as well. It was shown that HDAC2 inhibition by VPA augments etoposide induced expression of the BH3-only protein NOXA which in turn downregulates the anti-apoptotic protein Mcl-1 [139]. Moreover, VPA has been reported to trigger TRAIL-induced apoptosis by downregulation of cFLIP expression [42,43].

Accordingly, the targeting of class I HDACs (particularly HDAC1 and HDAC2) as well as of HDAC6 might be promising strategies in the treatment of PDAC patients: (1) to restore tumor suppressor gene expression by blocking the silencing effect of HDAC1 or HDAC2 mediated histone acetylation, (2) to decrease constitutive NF-κB activation and (3) to disrupt aggresome formation in the presence of PI. Such selective HDAC1 and 2 or HDAC6 specific therapies would be highly desirable because panspecific HDAC inhibitors (i.e. SAHA) would broadly de-repress gene expression along with rather pro-tumorigenic as well as general cytotoxic alterations in non-malignant cells. Thus, a great number of novel HDIs have been established including the class I HDIs depsipeptide, entinostat and MGCD0103 [118]. Yet, extended clinical trials of these agents in the treatment of solid tumors are underway.

8. Proteasome inhibitors

Polyubiquitination and subsequent proteasomal degradation of regulatory proteins through the ubiquitin–proteasome system (UPS) play a crucial role in numerous signal transduction pathways, including those controlling the cell cycle, apoptosis or cell migration. Amongst the anti-apoptotic pathways under the direct control of the UPS, NF-κB activation via IKK/IKBz is archetypical (see above), and other pathways interconnected with NF-κB act via the UPS, too. Thus, the UPS plays a pivotal role in tumorigenesis and the interference with the UPS is a major goal in pursuing novel concepts in the treatment of cancer.

Of particular relevance has become the inhibition of the proteasome, an organelle like high molecular weight protein complex which executes the signal induced degradation of ubiquitinated target proteins such as I kBz. In the past decade a plenty of preclinical and clinical studies have shown the high potential of proteasome inhibitors for efficient treatment of cancer, including PDAC. At first, less specific compounds such as the tripeptide aldehydes Mg132 and ALLN or the fungal antibiotic gliotoxin, and the more specific microbial metabolites lactacytin and epoxomicin have been used as proteasome inhibitors, but many other inhibitory effects by these drugs on other proteases limit their application in the clinic [140,141]. Nevertheless,
using these compounds, PDAC cells could be broadly sensitized to apoptotic stimuli such as TRAIL, Fasl, anti-cancer drugs like gemcitabine, etoposide or irradiation. To a significant extent, these pro-apoptotic effects could be attributed to the blockade of NF-κB activation by proteasome inhibition [24,56,66,68,142,143].

In 2003, a synthetic and reversible proteasome inhibitor – PS341/Bortezomib (Decade™) – has been FDA approved for cancer treatment. Bortezomib is highly selective for proteasome inhibition and was the first proteasome inhibitor used in a number of clinical trials yielding encouraging results – particularly with multiple myeloma and melanoma patients. However, in the treatment of other malignancies the results were rather poor. Thus, in the most cases of cancer therapy, combinations of Bortezomib with conventional anti-cancer drugs have to be considered [140,144]. Besides Bortezomib, novel compounds exerting irreversible and more pathway specific proteasome inhibition have been introduced. Amongst these, PR171, NPI0052 and CEP18770 are subject of clinical trials to determine their efficacy in a broad spectrum of solid tumors [145].

9. MicroRNA based strategies

Another important achievement in apoptosis related therapy might come from the recognition of microRNAs (miRNAs) as potential targets and measures to interfere with apoptotic signaling in tumor cells [146]. MiRNAs are small non-coding RNA molecules consisting of 17–25 nucleotides that specifically target about 30% of cellular mRNAs. MiRNAs modulate the expression of their target genes either through cytoplasmatic degradation of the corresponding mRNA or through translational inhibition.

Recent work revealed that tumors – including PDAC – exhibit characteristically altered patterns of miRNA expression that determine the expression profiles of certain target genes [146–148]. These tumor and tumor stage specific alterations result from aberrant transcriptional as well as epigenetic control of miRNAs which are either downregulated, e.g. Let-7 controlling K-Ras/MAPK [149] or miR34a involved in p53 dependent pathways [150] or upregulated, e.g. miR21 and other miRNAs affecting pro-apoptotic signaling [151]. Consequently, in tumor cells the balance of pro- against anti-apoptotic mediators shifts towards anti-apoptotic protection and this could be related to specific changes in miRNA expression. In this way, specific expression patterns of miRNAs offer clues to pancreatic tumorigenesis as well as to the prediction of therapy responsiveness and even to the selection of the most suitable therapeutic strategy.

Thus, restoration of downregulated miRNAs, e.g. those of the p53 pathway, could be one measure to turn tumor cells more susceptible to apoptosis. This can be achieved either by HDIs in order to release epigenetic silencing of endogenous miRNA expression (see above). Another strategy would be the application of lentiviral miRNA expression vectors, e.g. for restoration of let-7 [149] or of miR34a [152], leading to enhanced sensitivity of PDAC cells to chemotherapy. In turn, the targeting of upregulated miRNAs or their precursors might be achieved by synthetic, chemically-modified antisense oligonucleotides including Locked Nucleic Acid oligonucleotides or cholesterol conjugates of 20-O-methyl, 20-O-methoxyethyl. Given a deepened understanding of the regulatory functions of miRNAs in pancreatic cancer development and a better interpretation of miRNA expression signatures, miRNAs can serve as valuable diagnostic or predictive markers and therapeutic targets for PDAC treatment.

10. Outlook and perspectives

Even though the above mentioned therapeutic approaches targeting apoptosis pathways are promising, their beneficial effects in clinical application still remain to be proven. Up to now, many novel strategies produced impressive effects in cell culture experiments and to some extent in animal studies, but failed to provide a therapeutic benefit for PDAC patients. One explanation for the failure of these strategies in the clinic is that tumor cells do not exist and expand as an isolated cell entity but rather in a continuous and reciprocal communication with non-neoplastic cells of the adjacent microenvironement – and this holds particularly true for the stroma-rich PDAC. Recent studies provided compelling evidence that the sensitivity of tumor cells towards anti-cancer treatment is influenced by the tumor microenvironment [153]. Thus, current research in translational oncology strongly aims to identify molecular targets affecting tumor stroma interactions and to overcome stroma-mediated chemoresistance.

Regardless of these efforts in therapy, the major goal and challenge to combat PDAC is the availability of improved diagnostic markers, identifying the disease in a pre-metastatic stage and making a curative treatment accessible to more patients. Given an earlier diagnosis, surgical interventions together with adjuvant radio/chemotherapy or neoadjuvant strategies [154] are certainly the most promising options. Considering the currently 5-year survival rate of not greater than 20% of surgically treated PDAC patients, the urgent need for an individualized and more effective adjuvant therapy is evident. This will probably be the major application area of novel drugs and small molecules targeting the apoptotic pathways described above. In particular, patients subject of Whipple or pylorus preserving duodenopancreatomy (PPDP) surgery will be eligible for individualized treatments. This would implicate the identification of molecular markers and signatures from the resected tumor material, thereby defining those signaling pathways that account for the anti-apoptotic protection of the tumor. In this way, potential targets for the most effective pro-apoptotic therapy together with conventional anti-cancer drugs could be established for each patient, giving rise of the potentially most effective adjuvant treatment. First trials on such kind of individualized therapy are currently underway.

As long as an earlier diagnosis is still not available, one has to deal with a majority of PDAC patients that do not have curative options by surgery and adjuvant/neoadjuvant radio-/chemotherapy. Here, the currently available strategies of palliative treatment improve the outcome of PDAC patients rather marginally in terms of a longer
progress free disease and survival time, even though some amendments have been achieved in terms of life quality. Besides the aggressive tumor spread which occurs quite early in PDAC tumorigenesis along with regional metastases as well as metastases in other organs, tumor cachexia is the greatest limitation in curative cancer therapy. In many cases, not the expanding tumor and/or metastases themselves lead to the rapid decease of patients suffering from advanced PDAC, but rather the patient’s vanishing nutritive status and weight loss. Of note, signaling pathways accounting for anti-apoptotic protection – e.g. NF-kB or the UPS – are also associated with tumor cachexia and should be taken into account for an application here [155]. Thus, it seems to be worth getting detailed insight into the molecular mechanisms of tumor cachexia thereby allowing a better management of this major clinical threat. If this will be possible in the future, more time is afforded to the patients who then longer can get improved anti-cancer therapies by the combined use of new compounds affecting apoptosis pathways and conventional drugs. Under such circumstances the duration of novel therapies might be sufficient as to effectively eliminate the tumor and to extend the survival of PDAC patients.

Conflict of interest

None declared.

Acknowledgement

The funding by the German Research Society (DFG) to H.S. (SCHA677/7-3) and S.S.M (SE1381/2-1) is greatly acknowledged.

References


