Review

Targeting Apoptosis Signaling in Pancreatic Cancer

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Abstract: The ability to escape apoptosis or programmed cell death is a hallmark of human cancers, for example pancreatic cancer. This can promote tumorigenesis, since too little cell death by apoptosis disturbs tissue homeostasis. Additionally, defective apoptosis signaling is the underlying cause of failure to respond to current treatment approaches, since therapy-mediated antitumor activity requires the intactness of apoptosis signaling pathways in cancer cells. Thus, the elucidation of defects in the regulation of apoptosis in pancreatic carcinoma can result in the identification of novel targets for therapeutic interference and for exploitation for cancer drug discovery.

Keywords: apoptosis; pancreatic cancer; TRAIL; IAPs; mitochondria

1. Introduction

Tissue homeostasis critically depends on a subtle balance between cell growth and cell death and is typically disturbed in human cancers [1]. This implies that a reduced rate of programmed cell death (apoptosis) and/or an increase in cell proliferation can lead to tumor formation [1]. Apoptosis is a highly conserved form of cell death that already occurs in lower organisms [2]. Apoptosis exerts vital functions in many physiological processes and is found to be deregulated in a large variety of human diseases [1]. For example, a hallmark of human cancers including pancreatic carcinoma is the inability of cells to undergo apoptosis in response to an apoptotic stimulus [3,4]. Deregulated apoptosis programs are often also the underlying cause of primary or acquired resistance of pancreatic carcinoma to current therapies, including chemo-, radio- or immunotherapy, since these treatment strategies primarily act by triggering the intrinsic cell death program in target cancer cells [5]. The elucidation of
apoptosis pathways and their deregulation in pancreatic cancer therefore harbors a great potential for the development of novel experimental cancer therapeutics that are targeted to the underlying molecular mechanisms of treatment resistance. Such an approach is expected to have the ability to bypass classical drug resistance mechanisms.

2. Pancreatic Cancer

Pancreatic cancer represents currently one of the leading causes of cancer deaths in the Western world with a very poor prognosis despite intensive protocols [6,7]. This poor outcome is, at least in part, due to the resistance of pancreatic cancer to the currently available treatment options and still constitutes one of the most challenging problems in oncology [8]. Evasion of apoptosis substantially contributes to this treatment failure, since cytotoxic cancer therapies depend on the induction of apoptosis in cancer cells in order to be effective [5]. Thus, strategies that target defective apoptosis programs may open new perspectives to improve the prognosis of pancreatic cancer patients.

3. Apoptosis Signaling Pathways

A large variety of stimuli that originate both from the exterior of the cell, for example death receptors, chemotherapeutic agents or therapeutic antibodies, or alternatively from the inside of a cell, such as reactive oxygen species or metabolic end products, can initiate apoptosis [1]. In case of caspase-dependent apoptosis, the activation of apoptosis signaling pathways results in the activation of caspases [9]. Caspases are a family of cysteine proteases that act as death effector molecules in many forms of cell death [9]. Caspases are synthesized as inactive proenzymes and cleavage or dimerization is required for their activation [9].

Caspases can become activated by two principal pathways, i.e., the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway [10]. In the death receptor pathway, the engagement of death receptors by their corresponding ligands, for example agonistic TRAIL receptors, leads to the activation of the initiator caspase-8 [11]. Once activated caspase-8 either directly cleaves effector caspases such as caspase-3 or, alternatively, initiates the mitochondrial pathway by cleaving Bid. The processed form of Bid, i.e., tBid, in turn translocates to mitochondrial membranes, where it initiates the release of mitochondrial intermembrane space proteins such as cytochrome c into the cytosol [12,13]. In the cytosol, cytochrome c forms a multimeric complex together with Apaf-1 and caspase-9 termed the apoptosome complex, which is responsible for caspase-3 activation [13].

It is important to note that there are also additional forms of cell death beyond apoptosis [14]. These non-apoptotic cell death modes include e.g. necrosis, autophagy, lysosomal cell death or mitotic catastrophe [14]. There is mounting evidence that the type of cell death is highly dependent on the cellular context and that different forms of cell death may occur in parallel in a given system.

4. Targeting Apoptosis for the Treatment of Pancreatic Cancer

4.1. Death Receptor Signaling as Therapeutic Target in Pancreatic Cancer

Death receptors are part of the tumor necrosis factor (TNF) receptor gene superfamily of transmembrane receptors that are characterized by an intracellular domain called the
“death domain” [11]. This domain transmits the death signal from the surface of a cell to intracellular signaling pathways and serves as a platform for the recruitment of signaling molecules that transmit the death signal such as the adaptor protein FADD and caspase-8 [11]. Binding of death receptor ligands such as TRAIL to their corresponding receptors leads to the recruitment of FADD and caspase-8 to the activated death receptor. This results in the formation of the death-inducing signaling complex (DISC) at the plasma membrane, i.e., which in turn leads to caspase-8 activation [11].

The concept is to target death receptors for cancer therapy, since death receptors can directly engage the cell death program of cancer cells. To this end, TRAIL presents the most promising clinical candidate, since it preferentially triggers apoptosis in cancer over non-malignant cells [11]. The presence of TRAIL receptors exposed on the surface of cell membranes offers the possibility of inducing apoptosis in cancer cells by the exogenous administration of compounds capable of directly engaging two agonistic TRAIL receptors, i.e., TRAIL-R1 and TRAIL-R2 [11]. By comparison, two antagonistic decoy receptors, TRAIL-R3 and TRAIL-R4, bind TRAIL, but do not signal cell death, and osteoprotegerin acts as a soluble decoy receptor [11]. At least one of the agonistic TRAIL receptors has been reported to be expressed in human pancreatic carcinoma tissue, while conflicting reports have been made on expression of TRAIL decoy receptors [15-18]. Although the majority of pancreatic carcinoma cell lines were found to express the essential signaling molecules of the TRAIL pathway [19-21], most of them turned out to be refractory to TRAIL [21,22]. However, sensitivity towards TRAIL could be restored by various combination therapies, e.g. using chemotherapeutics (camptothecin, cisplatin, celecoxib) that downregulate c-FLIP expression [23]. Also in a xenografts mouse model of pancreatic adenocarcinoma, TRAIL together with gemcitabine showed a greater anti-tumor effect than either monotherapy used alone [24]. Along this line, anti-TRAIL receptor 2 antibody combined with CPT-11 caused inhibition of tumor growth in an orthotopic model of pancreatic cancer [25]. Also, pharmacologic or genetic inhibition of CDK4 enhanced the TRAIL sensitivity [26]. Moreover, the estradiol metabolite 2-Methoxyestradiol promoted TRAIL-induced apoptosis by upregulating TRAIL receptors via generation of oxidative stress and JNK activation [27]. Combination therapy with the proteasome inhibitor bortezomib similarly enhanced TRAIL-induced apoptosis via upregulation of TRAIL-R1/TRAIL-R2, downregulation of c-FLIP, and increase in Bak [28]. These various combination strategies indicate that the antitumor activity of TRAIL can substantially be augmented by the addition of other cytotoxic principles.

Expression levels of O-glycosyltransferases may serve as biomarkers to predict TRAIL sensitivity, as they are overexpressed in several cancers and regulate TRAIL-induced apoptosis via modulation of TRAIL-R1 or -R2 [29].

Various agents have been developed in recent years to target the TRAIL system for clinical application [30]. For example, soluble TRAIL and agonistic antibodies directed against the TRAIL receptors TRAIL-R1 and -R2 were developed [31,32]. In pancreatic carcinoma, Apomab (agonistic antibody against TRAIL-R2) was shown to be effective in pancreatic cancer as a single agent and in combination with chemotherapy [33]. At present, TRAIL-R1 monoclonal antibodies are being tested in early clinical trials alone or in combination with chemotherapeutic drugs [34].

In addition to the induction of apoptosis, TRAIL may also exert non-apoptotic functions, including activation of survival cascades such as NF-κB, PI3K/Akt or Ras/Raf/ERK pathways [35]. For example, TRAIL was shown to enhance metastasis formation in an orthotopic mouse model of human
pancreatic carcinoma [36]. Thus, clinical protocols with TRAIL have to take also those activities into consideration.

4.2. “Inhibitor of Apoptosis” (IAP) Proteins as Therapeutic Targets in Pancreatic Cancer

“Inhibitor of Apoptosis” (IAP) proteins comprise a family of endogenous caspase inhibitors with eight human analogues, i.e., XIAP, cIAP1, cIAP2, survivin, livin (ML-IAP), NAIP, Bruce (apollon) and ILP-2 [37]. All IAP proteins harbor at least one baculovirus IAP repeat (BIR) domain, which presents the interaction domain with caspases. Among the IAP family proteins, XIAP has shown the most potent anti-apoptotic effects by inhibiting active caspase-3 and -7 and by preventing caspase-9 activation [37,38]. The IAP family protein survivin not only regulates apoptosis, but also controls mitotic events [39]. IAP proteins are negatively controlled at several levels, e.g. by mitochondrial (Smac/DIABLO) or nuclear proteins (i.e., XIAP-associated factor 1 (XAF-1)) [37]. IAP proteins are also targets of caspase-mediated cleavage as well as ubiquitination-mediated proteasomal degradation via auto- and heteroubiquitination through the RING domain of IAP proteins [37].

In pancreatic cancer, high expression levels of several of the IAP proteins have been reported in comparison to non-malignant pancreatic ductal cells or pancreatic tissue, including XIAP, cIAP2, survivin and livin [40-43]. In addition, low expression levels of XAF1 were recently shown to correlate with shorter survival times in pancreatic cancer [44]. In multivariate analysis, XAF1 expression turned out as an independent prognostic indicator of the survival of these patients [44].

In order to target the expression and/or function of IAP proteins in pancreatic cancer, a number of approaches were developed. So far, most of these strategies are directed against XIAP, since XIAP possesses the most potent anti-apoptotic properties among the IAP proteins [38], for example, RNA interference (RNAi) or antisense oligonucleotides. To this end, RNAi-mediated downregulation of XIAP was reported to increase apoptosis of pancreatic carcinoma cells following treatment with death receptor ligands, such as TRAIL or agonistic anti-CD95 antibodies, as well as after γ-irradiation and also suppressed colony formation [21,45]. Additionally, the combination of XIAP inhibition and TRAIL even overcame Bcl-2-conferred resistance [46]. This involved a switch of type II cells, which require the mitochondrial contribution to TRAIL-induced apoptosis, to type I cells in which TRAIL triggers apoptosis irrespective of Bcl-2 overexpression [46]. Also, inhibition of XIAP cooperated with TRAIL to trigger regression of established pancreatic carcinoma in a tumor regression model in xenograft-bearing mice [46]. Similarly, loss of XIAP protein upon administration of XIAP antisense oligonucleotides increased TRAIL-mediated apoptosis in a pancreatic carcinoma cell line [47]. Downregulation of XIAP or cIAP2 also increased the response to anticancer drugs including doxorubicin, paclitaxel, gemcitabine and cisplatin, at least in some pancreatic cancer cell lines [41,42]. Loss of XIAP protein upon administration of XIAP antisense oligonucleotides correlated with increased sensitization to TRAIL-mediated apoptosis in a pancreatic carcinoma cell line [47]. XIAP antisense oligonucleotides against XIAP are currently under evaluation in early clinical trials [48].

The binding groove of the BIR3 domain of XIAP, which binds Smac, has served as a target for the design of compounds that inhibit XIAP [49]. In pancreatic cancer, Smac peptides were reported to sensitize pancreatic cancer cells to both death-receptor- or anticancer drug-induced apoptosis [50]. To facilitate intracellular delivery, a carrier was coupled to Smac peptides, for example the protein
transduction motif of the HIV Tat protein [50]. Smac mimetics were also found in another study to potentiate the chemotherapy response [51]. In addition, small molecule XIAP inhibitors synergized with TRAIL to induce apoptosis both in vitro and in vivo, causing regression of pancreatic carcinoma [43]. Also, XIAP inhibitors potentiated radiosensitivity of pancreatic carcinoma cells by enhancing caspase cleavage and subsequently apoptosis in response to γ-irradiation [45].

In addition to synthetic small molecule inhibitors, Embelin, a natural compound from the Japanese Ardisia herb, was identified as a XIAP inhibitor [52]. In pancreatic carcinoma cell lines, Embelin enhanced TRAIL-induced apoptosis [53].

Besides the BIR2 domain of XIAP, small molecule compounds were also designed against the BIR2 domain of the protein. To this end, the screening of a polyphenylurea library resulted in the identification of several non-peptidic compounds with potent binding to the BIR2 domain of XIAP [54,55]. In pancreatic carcinoma cells, these polyphenylurea compounds induced apoptosis as single agents and also increased the induction of apoptosis following treatment with gemcitabine, TRAIL or irradiation [40].

4.3. Mitochondria as Therapeutic Target in Pancreatic Cancer

The Bcl-2 family of proteins comprises both anti-apoptotic members, e.g. Bcl-2, Bcl-X\textsubscript{L}, Mcl-1, and pro-apoptotic proteins including Bax, Bak, Bad and BH3 domain only-proteins [12]. Imbalances in the ratio of anti-apoptotic versus pro-apoptotic Bcl-2 proteins with a relative increase in the anti-apoptotic molecules have been reported for several human cancers. How BH3-only proteins initiate the activation of Bax and Bak has not exactly been identified and there are currently two alternative working models. In the direct activation model [56], BH3-only proteins that act as direct activators, i.e., Bim and cleaved Bid (tBid), bind to Bax and Bak to trigger their activation, while BH3-only proteins that act as sensitizers, e.g. Bad, bind to the pro-survival Bcl-2 proteins. According to the indirect activation model, BH3-only proteins activate Bax and Bak indirectly by engaging anti-apoptotic Bcl-2 proteins, thereby freeing up Bax and Bak [57,58]. Furthermore, Bak activation requires inactivation of both, Bcl-X\textsubscript{L} and Mcl-1 [59].

Various strategies have been developed over the last years to antagonize anti-apoptotic Bcl-2-related proteins in human cancers. For example, targeting of the protein-protein interaction site between anti-apoptotic Bcl-2 proteins and the multimeric pro-apoptotic Bcl-2 proteins Bax or Bak yielded small molecule antagonists that bind to the surface groove of Bcl-2, Bcl-X\textsubscript{L} and Bcl-w in a similar manner as the BH3 domain of Bax or Bak [60]. ABT-737 represents the prototypic compound of this class of inhibitors that has been extensively characterized in preclinical models [61]. ABT-737 was shown to directly trigger apoptosis in susceptible cell lines, e.g. chronic lymphocytic leukemia cells, or to sensitize cancer cells for apoptosis [60]. Recently, ABT-737 and TRAIL were found to synergize in the induction of cell death in pancreatic cancer cells by stimulating the intrinsic and extrinsic apoptotic pathways, respectively [62]. Obatoclax, another BH3 mimic, antagonizes Bcl-2, Bcl-X\textsubscript{L}, Bcl-w as well as Mcl-1 [63]. In pancreatic cancer Obatoclax has shown to potentiate TRAIL-triggered apoptosis [64]. ABT-263, an oral analogue with improved pharmacokinetic properties, is currently evaluated in early clinical trials in small-cell lung cancer and B-cell malignancies [65]. TW-37 presents another small-molecule inhibitor of Bcl-2, which was shown to inhibit cell growth and invasion and increased apoptosis in pancreatic cancer [66]. Another approach to target anti-apoptotic
Bcl-2 proteins is the use of antisense oligonucleotides. For example, Bcl-XL antisense oligonucleotides enhanced gemcitabine- or irradiation-induced cytotoxicity in pancreatic cancer cells [67,68].

5. Conclusions

Pancreatic cancer harbors multiple defects in apoptosis signaling pathways that contribute to tumorigenesis and favors treatment resistance, including high levels of anti-apoptotic proteins and/or reduced expression or function of pro-apoptotic proteins. Several components of apoptosis signaling pathways may be exploited as targets for the development of experimental cancer therapies, for example the TRAIL system, IAP proteins or anti-apoptotic Bcl-2 proteins. The transfer of this knowledge on apoptosis signaling into the design of experimental clinical trials may offer novel perspectives to improve the prognosis of pancreatic cancer patients.

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References

1. Lockshin, R.A.; Zakeri, Z. Cell death in health and disease. J. Cell Mol. Med. 2007, 11, 1214-1224.
2. Taylor, R.C.; Cullen, S.P.; Martin, S.J. Apoptosis: Controlled demolition at the cellular level. Nat. Rev. Mol. Cell Biol. 2008, 9, 231-241.
3. Fulda, S. Tumor resistance to apoptosis. Int. J. Cancer 2009, 124, 511-515.
4. Fulda, S. Apoptosis pathways and their therapeutic exploitation in pancreatic cancer. J. Cell Mol. Med. 2009, 13, 1221-1227.
5. Fulda, S.; Debatin, K.M. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene 2006, 25, 4798-4811.
6. Li, D.; Xie, K.; Wolff, R.; Abbruzzese, J.L. Pancreatic cancer. Lancet 2004, 363, 1049-1057.
7. Maitra, A.; Hruban, R.H. Pancreatic cancer. Annu Rev Pathol 2008, 3, 157-188.
8. Schneider, G.; Siveke, J.T.; Eckel, F.; Schmid, R.M. Pancreatic cancer: Basic and clinical aspects. Gastroenterology 2005, 128, 1606-1625.
9. Logue, S.E.; Martin, S.J. Caspase activation cascades in apoptosis. Biochem. Soc. Trans. 2008, 36, 1-9.
10. Hengartner, M.O. The biochemistry of apoptosis. Nature 2000, 407, 770-776.
11. Ashkenazi, A. Targeting the extrinsic apoptosis pathway in cancer. Cytokine Growth Factor Rev. 2008, 19, 325-331.
12. Adams, J.M.; Cory, S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007, 26, 1324-1337.
13. Kroemer, G.; Galluzzi, L.; Brenner, C. Mitochondrial membrane permeabilization in cell death. Physiol. Rev. 2007, 87, 99-163.
14. Okada, H.; Mak, T.W. Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat. Rev. Cancer* 2004, 4, 592-603.
15. Ozawa, F.; Friess, H.; Kleepf, J.; Xu, Z.W.; Zimmermann, A.; Sheikh, M.S.; Buchler, M.W. Effects and expression of TRAIL and its apoptosis-promoting receptors in human pancreatic cancer. *Cancer Lett.* 2001, 163, 71-81.
16. Satoh, K.; Kaneko, K.; Hirota, M.; Masamune, A.; Satoh, A.; Shimosegawa, T. Tumor necrosis factor-related apoptosis-inducing ligand and its receptor expression and the pathway of apoptosis in human pancreatic cancer. *Pancreas* 2001, 23, 251-258.
17. Sanlioglu, A.D.; Dirice, E.; Elpek, O.; Korcum, A.F.; Balci, M.K.; Omer, A.; Griffith, T.S.; Sanlioglu, S. High levels of endogenous tumor necrosis factor-related apoptosis-inducing ligand expression correlate with increased cell death in human pancreas. *Pancreas* 2008, 36, 385-393.
18. Liao, Q.; Friess, H.; Kleepf, J.; Buchler, M.W. Differential expression of TRAIL-R3 and TRAIL-R4 in human pancreatic cancer. *Anticancer Res.* 2001, 21, 3153-3159.
19. Hinz, S.; Trauzold, A.; Boenicke, L.; Sandberg, C.; Beckmann, S.; Bayer, E.; Walczak, H.; Kalthoff, H.; Ungefroren, H. Bcl-XL protects pancreatic adenocarcinoma cells against CD95- and TRAIL-receptor-mediated apoptosis. *Oncogene* 2000, 19, 5477-5486.
20. Trauzold, A.; Schmiedel, S.; Roder, C.; Tams, C.; Christgen, M.; Oestern, S.; Arlt, A.; Westphal, S.; Kapischke, M.; Ungefroren, H.; Kalthoff, H. Multiple and synergic deregulations of apoptosis-controlling genes in pancreatic carcinoma cells. *Br. J. Cancer* 2003, 89, 1714-1721.
21. Vogler, M.; Durr, K.; Jovanovic, M.; Debatin, K.M.; Fulda, S. Regulation of TRAIL-induced apoptosis by XIAP in pancreatic carcinoma cells. *Oncogene* 2007, 26, 248-257.
22. Bai, J.; Sui, J.; Demirjian, A.; Vollmer, C.M., Jr.; Marasco, W.; Callery, M.P. Predominant Bcl-XL knockdown disables antiapoptotic mechanisms: Tumor necrosis factor-related apoptosis-inducing ligand-based triple chemotherapy overcomes chemoresistance in pancreatic cancer cells *in vitro*. *Cancer Res.* 2005, 65, 2344-2352.
23. Wang, P.; Zhang, J.; Bellail, A.; Jiang, W.; Hugh, J.; Kneteman, N.M.; Hao, C. Inhibition of RIP and c-FLIP enhances TRAIL-induced apoptosis in pancreatic cancer cells. *Cell. Signal.* 2007, 19, 2237-2246.
24. Hylander, B.L.; Pitoniak, R.; Penetrante, R.B.; Gibbs, J.F.; Oktay, D.; Cheng, J.; Repasky, E.A. The anti-tumor effect of Apo2L/TRAIL on patient pancreatic adenocarcinomas grown as xenografts in SCID mice. *J. Transl. Med.* 2005, 3, 22.
25. DeRosier, L.C.; Buchsbaum, D.J.; Oliver, P.G.; Huang, Z.Q.; Sellers, J.C.; Grizzle, W.E.; Wang, W.; Zhou, T.; Zinn, K.R.; Long, J.W.; Vickers, S.M. Combination treatment with TRA-8 anti death receptor 5 antibody and CPT-11 induces tumor regression in an orthotopic model of pancreatic cancer. *Clin. Cancer Res.* 2007, 13, 5535s-5543s.
26. Retzer-Lidl, M.; Schmid, R.M.; Schneider, G. Inhibition of CDK4 impairs proliferation of pancreatic cancer cells and sensitizes towards TRAIL-induced apoptosis via downregulation of survivin. *Int. J. Cancer* 2007, 121, 66-75.
27. Basu, A.; Castle, V.P.; Bouziane, M.; Bhalla, K.; Haldar, S. Crosstalk between extrinsic and intrinsic cell death pathways in pancreatic cancer: Synergistic action of estrogen metabolite and ligands of death receptor family. *Cancer Res.* 2006, 66, 4309-4318.
28. Koschny, R.; Ganten, T.M.; Sykora, J.; Haas, T.L.; Sprick, M.R.; Kolb, A.; Stremmel, W.; Walczak, H. TRAIL/bortezomib cotreatment is potentially hepatotoxic but induces cancer-specific apoptosis within a therapeutic window. *Hepatology* **2007**, *45*, 649-658.

29. Wagner, K.W.; Punnoose, E.A.; Januario, T.; Lawrence, D.A.; Pitti, R.M.; Lancaster, K.; Lee, D.; von Goetz, M.; Yee, S.F.; Totpal, K.; Huw, L.; Katta, V.; Cavet, G.; Hymowitz, S.G.; Amler, L.; Ashkenazi, A. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat. Med.* **2007**, *13*, 1070-1077.

30. Ashkenazi, A.; Herbst, R.S. To kill a tumor cell: The potential of proapoptotic receptor agonists. *J. Clin. Invest.* **2008**, *118*, 1979-1990.

31. Ashkenazi, A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. *Nat. Rev. Drug Discov.* **2008**, *7*, 1001-1012.

32. Humphreys, R.C.; Halpern, W. Trail receptors: Targets for cancer therapy. *Adv. Exp. Med. Biol.* **2008**, *615*, 127-158.

33. Adams, C.; Totpal, K.; Lawrence, D.; Marsters, S.; Pitti, R.; Yee, S.; Ross, S.; Deforge, L.; Koeppen, H.; Sagolla, M.; Compaan, D.; Lowman, H.; Hymowitz, S.; Ashkenazi, A. Structural and functional analysis of the interaction between the agonistic monoclonal antibody Apomab and the proapoptotic receptor DR5. *Cell Death Differ.* **2008**, *15*, 751-761.

34. Mom, C.H.; Verweij, J.; Oldenhuis, C.N.; Gietema, J.A.; Fox, N.L.; Miceli, R.; Eskens, F.A.; Loos, W.J.; de Vries, E.G.; Sleijfer, S. Mapatumumab, a fully human agonistic monoclonal antibody that targets TRAIL-R1, in combination with gemcitabine and cisplatin: A phase I study. *Clin. Cancer Res.* **2009**, *15*, 5584-5590.

35. Falschlehner, C.; Emmerich, C.H.; Gerlach, B.; Walczak, H. TRAIL signalling: Decisions between life and death. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 1462-1475.

36. Trauzold, A.; Siegmund, D.; Schniewind, B.; Sipos, B.; Egberts, J.; Zorenkov, D.; Emme, D.; Roder, C.; Kalthoff, H.; Wajant, H. TRAIL promotes metastasis of human pancreatic ductal adenocarcinoma. *Oncogene* **2006**, *25*, 7434-7439.

37. LaCasse, E.C.; Mahoney, D.J.; Cheung, H.H.; Plenchette, S.; Baird, S.; Korneluk, R.G. IAP-targeted therapies for cancer. *Oncogene* **2008**, *27*, 6252-6275.

38. Eckelman, B.P.; Salvesen, G.S.; Scott, F.L. Human inhibitor of apoptosis proteins: Why XIAP is the black sheep of the family. *EMBO Rep.* **2006**, *7*, 988-994.

39. Altieri, D.C. New wirings in the survivin networks. *Oncogene* **2008**, *27*, 6276-6284.

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

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

42. Lopes, R.B.; Gangeswaran, R.; McNeish, I.A.; Wang, Y.; Lemoine, N.R. Expression of the IAP protein family is dysregulated in pancreatic cancer cells and is important for resistance to chemotherapy. *Int. J. Cancer* **2007**, *120*, 2344-2352.
43. Vogler, M.; Walczak, H.; Stadel, D.; Haas, T.L.; Genze, F.; Jovanovic, M.; Bhanot, U.; Hasel, C.; Moller, P.; Gschwend, J.E.; Simmet, T.; Debatin, K.M.; Fulda, S. Small molecule XIAP inhibitors enhance TRAIL-induced apoptosis and antitumor activity in preclinical models of pancreatic carcinoma. *Cancer Res.* 2009, 69, 2425-2434.

44. Huang, J.; Yao, W.Y.; Zhu, Q.; Tu, S.P.; Yuan, F.; Wang, H.F.; Zhang, Y.P.; Yuan, Y.Z. XAF1 as a prognostic biomarker and therapeutic target in pancreatic cancer. *Cancer Sci* 2010, 101, 559-567.

45. Giagkousiklidis, S.; Vellanki, S.H.; Debatin, K.M.; Fulda, S. Sensitization of pancreatic carcinoma cells for gamma-irradiation-induced apoptosis by XIAP inhibition. *Oncogene* 2007, 26, 7006-7016.

46. Vogler, M.; Walczak, H.; Stadel, D.; Haas, T.L.; Genze, F.; Jovanovic, M.; Gschwend, J.E.; Simmet, T.; Debatin, K.M.; Fulda, S. Targeting XIAP bypasses Bcl-2-mediated resistance to TRAIL and cooperates with TRAIL to suppress pancreatic cancer growth *in vitro* and *in vivo*. *Cancer Res.* 2008, 68, 7956-7965.

47. LaCasse, E.C.; Cherton-Horvat, G.G.; Hewitt, K.E.; Jerome, L.J.; Morris, S.J.; Kandimalla, E.R.; Yu, D.; Wang, H.; Wang, W.; Zhang, R.; Agrawal, S.; Gillard, J.W.; Durkin, J.P. Preclinical characterization of AEG35156/GEM 640, a second-generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. *Clin. Cancer Res.* 2006, 12, 5231-5241.

48. LaCasse, E.C.; Kandimalla, E.R.; Winocour, P.; Sullivan, T.; Agrawal, S.; Gillard, J.W.; Durkin, J. Application of XIAP antisense to cancer and other proliferative disorders: Development of AEG35156/ GEM640. *Ann. N. Y. Acad. Sci.* 2005, 1058, 215-234.

49. Shiozaki, E.N.; Shi, Y. Caspases, IAPs and Smac/DIABLO: Mechanisms from structural biology. *Trends Biochem. Sci.* 2004, 29, 486-494.

50. Fulda, S.; Wick, W.; Weller, M.; Debatin, K.M. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma *in vivo*. *Nat. Med.* 2002, 8, 808-815.

51. Dineen, S.P.; Roland, C.L.; Greer, R.; Carbon, J.G.; Toombs, J.E.; Gupta, P.; Bardeesy, N.; Sun, H.; Williams, N.; Minna, J.D.; Brekken, R.A. Smac mimetic increases chemotherapy response and improves survival in mice with pancreatic cancer. *Cancer Res.* 2010, 70, 2852-2861.

52. Nikolovska-Coleska, Z.; Xu, L.; Hu, Z.; Tomita, Y.; Li, P.; Roller, P.P.; Wang, R.; Fang, X.; Guo, R.; Zhang, M.; Lippman, M.E.; Yang, D.; Wang, S. Discovery of embelin as a cell-permeable, small-molecular weight inhibitor of XIAP through structure-based computational screening of a traditional herbal medicine three-dimensional structure database. *J. Med. Chem.* 2004, 47, 2430-2440.

53. Mori, T.; Doi, R.; Kida, A.; Nagai, K.; Kami, K.; Ito, D.; Toyoda, E.; Kawaguchi, Y.; Uemoto, S. Effect of the XIAP inhibitor Embelin on TRAIL-induced apoptosis of pancreatic cancer cells. *J. Surg. Res.* 2007, 142, 281-286.

54. Schimmer, A.D.; Welsh, K.; Pinilla, C.; Wang, Z.; Krajewska, M.; Bonneau, M.J.; Pedersen, I.M.; Kitada, S.; Scott, F.L.; Bailly-Maitre, B.; Glnsly, G.; Scudiero, D.; Sausville, E.; Salvesen, G.; Nefzi, A.; Ostresh, J.M.; Houghten, R.A.; Reed, J.C. Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. *Cancer Cell* 2004, 5, 25-35.
55. Wang, Z.; Cuddy, M.; Samuel, T.; Welsh, K.; Schimmer, A.; Hanaei, F.; Houghten, R.; Pinilla, C.; Reed, J.C. Cellular, biochemical, and genetic analysis of mechanism of small molecule IAP inhibitors. *J. Biol. Chem.* 2004, 279, 48168-48176.

56. Letai, A.; Bassik, M.C.; Walensky, L.D.; Sorcinelli, M.D.; Weiler, S.; Korsmeyer, S.J. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* 2002, 2, 183-192.

57. Chen, L.; Willis, S.N.; Wei, A.; Smith, B.J.; Fletcher, J.I.; Hinds, M.G.; Colman, P.M.; Day, C.L.; Adams, J.M.; Huang, D.C. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol. Cell* 2005, 17, 393-403.

58. Willis, S.N.; Fletcher, J.I.; Kaufmann, T.; van Delft, M.F.; Chen, L.; Czabotar, P.E.; Ierino, H.; Lee, E.F.; Fairlie, W.D.; Bouillet, P.; Strasser, A.; Kluck, R.M.; Adams, J.M.; Huang, D.C. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* 2007, 315, 856-859.

59. Willis, S.N.; Chen, L.; Dewson, G.; Wei, A.; Naik, E.; Fletcher, J.I.; Adams, J.M.; Huang, D.C. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. *Genes Dev.* 2005, 19, 1294-1305.

60. Oltersdorf, T.; Elmore, S.W.; Shoemaker, A.R.; Armstrong, R.C.; Augeri, D.J.; Belli, B.A.; Bruncko, M.; Deckwerth, T.L.; Dinges, J.; Hajduk, P.J.; Joseph, M.K.; Kitada, S.; Korsmeyer, S.J.; Kunzer, A.R.; Letai, A.; Li, C.; Mitten, M.J.; Nettesheim, D.G.; Ng, S.; Nimmer, P.M.; O’Connor, J.M.; Oleksijew, A.; Petros, A.M.; Reed, J.C.; Shen, W.; Tahir, S.K.; Thompson, C.B.; Tomaselli, K.J.; Wang, B.; Wendt, M.D.; Zhang, H.; Fesik, S.W.; Rosenberg, S.H. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 2005, 435, 677-681.

61. Zhang, L.; Ming, L.; Yu, J. BH3 mimetics to improve cancer therapy: mechanisms and examples. *Drug Resist. Updat.* 2007, 10, 207-217.

62. Huang, S.; Sinicrope, F.A. BH3 mimetic ABT-737 potentiates TRAIL-mediated apoptotic signaling by unsequestering Bim and Bak in human pancreatic cancer cells. *Cancer Res.* 2008, 68, 2944-2951.

63. Nguyen, M.; Marcellus, R.C.; Roulston, A.; Watson, M.; Serfass, L.; Murthy Madiraju, S.R.; Goulet, D.; Viallet, J.; Belec, L.; Billot, X.; Acoca, S.; Purisima, E.; Wiegmans, A.; Cluse, L.; Johnstone, R.W.; Beauparlant, P.; Shore, G.C. Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. *Proc. Natl. Acad. Sci. USA* 2007, 104, 19512-19517.

64. Huang, S.; Okumura, K.; Sinicrope, F.A. BH3 mimetic obatoclax enhances TRAIL-mediated apoptosis in human pancreatic cancer cells. *Clin. Cancer Res.* 2009, 15, 150-159.

65. Tse, C.; Shoemaker, A.R.; Adickes, J.; Anderson, M.G.; Chen, J.; Jin, S.; Johnson, E.F.; Marsh, K.C.; Mitten, M.J.; Nimmer, P.; Roberts, L.; Tahir, S.K.; Xiao, Y.; Yang, X.; Zhang, H.; Fesik, S.; Rosenberg, S.H.; Elmore, S.W. ABT-263: A potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* 2008, 68, 3421-3428.

66. Wang, Z.; Song, W.; Aboukameel, A.; Mohammad, M.; Wang, G.; Banerjee, S.; Kong, D.; Wang, S.; Sarkar, F.H.; Mohammad, R.M. TW-37, a small-molecule inhibitor of Bcl-2, inhibits cell growth and invasion in pancreatic cancer. *Int. J. Cancer* 2008, 123, 958-966.
67. Xu, Z.; Friess, H.; Solioz, M.; Aebi, S.; Korc, M.; Kleeff, J.; Buchler, M.W. Bcl-x(L) antisense oligonucleotides induce apoptosis and increase sensitivity of pancreatic cancer cells to gemcitabine. *Int. J. Cancer* **2001**, *94*, 268-274.

68. Masui, T.; Hosotani, R.; Ito, D.; Kami, K.; Koizumi, M.; Mori, T.; Toyoda, E.; Nakajima, S.; Miyamoto, Y.; Fujimoto, K.; Doi, R. Bcl-XL antisense oligonucleotides coupled with antennapedia enhances radiation-induced apoptosis in pancreatic cancer. *Surgery* **2006**, *140*, 149-160.

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