MINI-REVIEW

TRAIL Mediated Signaling in Pancreatic Cancer

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Abstract

Research over the years has progressively shown substantial broadening of the tumor necrosis factor alpha-related apoptosis-inducing ligand (TRAIL)-mediated signaling landscape. Increasingly it is being realized that pancreatic cancer is a multifaceted and genomically complex disease. Suppression of tumor suppressors, overexpression of oncogenes, epigenetic silencing, and loss of apoptosis are some of the extensively studied underlying mechanisms. Rapidly accumulating *in vitro* and *in vivo* evidence has started to shed light on the resistance mechanisms in pancreatic cancer cells. More interestingly a recent research has opened new horizons of miRNA regulation by DR5 in pancreatic cancer cells. It has been shown that DR5 interacts with the core microprocessor components Drosha and DGCR8, thus impairing processing of primary let-7. Xenografting DR5 silenced pancreatic cancer cells in SCID-mice indicated that there was notable suppression of tumor growth. There is a paradigm shift in our current understanding of TRAIL mediated signaling in pancreatic cancer cells that is now adding new layers of concepts into the existing scientific evidence. In this review we have attempted to provide an overview of recent advances in TRAIL mediated signaling in pancreatic cancer as evidenced byfindings of *in vitro* and *in vivo* analyses. Furthermore, we discuss nanotechnological advances with emphasis on PEG-TRAIL and four-arm PEG cross-linked hyaluronic acid (HA) hydrogels to improve availability of TRAIL at target sites.

Keywords: Pancreatic cancer – TRAIL – apoptosis - nanotechnology

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Introduction

Mechanistically, multifaceted mechanisms including crosstalk of signaling pathways, genetic/epigenetic mutations, inactivation of tumor suppressors and activation of oncogenes in pancreatic cancer have gained tremendous appreciation in the past decade (Diamantidis et al., 2008). In the field of signaling pathways, it is getting sequentially more understandable that pancreatic cancer cells are difficult to target because of rewiring of intracellular signaling cascades. Linear and integrated signaling cascades also impair tumor necrosis factor alpha-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in pancreatic cancer cells. TRAIL mediated intracellular signaling has emerged as a deeply studied cascade in cancer cells (Röder et al., 2011; Arlt et al., 2013). TRAIL is a ligand and gained tremendous appreciation over the years because of its ability to selectively induce apoptosis in cancer cells. TRAIL signals through its receptors DR4 and DR5. Death-receptor signaling is initiated by ligand-induced receptor trimerization that results in formation of the death-inducing signaling complex (DISC). Death receptors have death domains (DD), which recruit FADD, to the receptor complex.Through death effector domains (DED) in FADD, recruitment and activation of the apoptosis-initiating caspase-8 occurs. Caspase-8 activates its downstream effector caspase-3. It is surprising to note that intrinsic pathway is triggered via an additional amplification loop that involves the cleavage of Bid by caspase-8 to truncated Bid (tBid). tBid moves into the mitochondrion to facilitate release of cytochrome c. Cytochrome c in concert with pro-caspase-9 and apoptotic protease activating factor (APAF) forms apoptosome. Bax and Bak are in an inactive state at the outer mitochondrial membrane however upon stimulation, pro-apoptotic proteins including Puma or Bim trigger activation of Bax and Bak via dissociation from anti-apoptotic Bcl-2 family.

It has previously been convincingly revealed using pancreatic cancer cell lines differentially expressingBcl-XL that TRAIL induced apoptosis was impaired in Bcl-XL overexpressing cancer cell lines (Panc-1 and PancTuI). However, enforced expression of a pro-apoptotic protein Bax or gene silencing of Bcl-XL induced apoptosis in TRAIL resistant cancer cells (Hinz et al., 2000). *In vitro*

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assays provided evidence that in five pancreatic cancer cell lines, TRAIL, DR4 and DR5 mRNA expression were detectable. Still TRAIL did not induce apoptosis in pancreatic cancer cells primarily because of over-expression of anti-apoptotic proteins (Ozawa et al., 2001). It is noteworthy that actinomycin improved TRAIL induced apoptosis in resistant cancer cells via targeting of cFLIP (Matsuzaki et al., 2001). Another contemporary study indicated that differentially high levels of decoy receptors also inhibited an efficient TRAIL induced apoptosis (Ibrahim et al., 2001). Increasingly it is being realized that TRAIL induced pro-survival signals in pancreatic cancer cells as evidenced byactivation of protein kinase C and NF-kappaB in TRAIL treated pancreatic adenocarcinoma cell line PancTuI. Synthetic agent mediated inhibition of NF-kappaB or transient transfection with a dominant negative mutant of IkappaBalpha induced apoptosis in TRAIL treated cancer cells (Trauzold et al., 2001). NEMO-binding domain (NBD) peptide is a novel selective NF-kappaB inhibitor and results revealed that NBD treated L3.6 cell line (TRAIL resistant) displayed TRAIL induced apoptosis (Thomas et al., 2002). TRAIL induced apoptosis was notably enhanced in gemcitabine treated SW1990 cancer cells. There was a marked increase in expression of Smac/DIABLO and cytochrome C (Zhao et al., 2011).

Tissue Expression

It has previously been reported that TRAIL-R3 expression was low and TRAIL-R4 expression ranged from moderate to high levels. However weakness of the study was limited number of cases studied (Liao et al., 2001).

Molecular Basis of TRAIL Resistance

It has previously been persuasively revealed that constitutively activated NFKB, but not TRAIL induced NF-kappaB modulated apoptosis. Panc-1 cells have constitutively activated NFKB and it has been experimentally verified that introduction of IκB-α super-repressor considerably reduced NF-κB DNA-binding activity (Braeuer et al., 2006). Shikonin, a naphthoquinone derivative has been shown to repress constitutive as well as gemcitabine-induced activation of NF-κB and NF-κB-target genes. Mice xenografted with pancreatic cancer cells displayed decreased proliferation, decreased microvessel density and increased apoptosis upon treatment with Wang et al. (2014). α-Mangostin, an antioxidant derived from the *Garcinia mangostana L.* Expression of pNF-κB/p65Ser552 was also found to be inhibited in α-Mangostin treated pancreatic cancer cell lines (Hafeez et al., 2014). Wogonin, a naturally occurring flavone was noted to suppress cFLIP in Capan-1 cell line thus restoring TRAIL induced apoptosis. Moreover, DR5 was upregulated in wogonin treated Capan-1 cancer cells (Ding et al., 2012). Zyflamend is a formulation of 10 standardized herbal extracts and reported to be effective in restoration of apoptosis in cancer cells. Zyflamend triggered expression of DR5 via CCAAT/enhancer-binding protein-homologous protein (CHOP). Mechanistically it was shown that DR5 promoter region contained a CHOP binding site. Cancer cells transfected with DR5 promoter plasmids, containing either a wild-type or mutated CHOP binding site, revealed that Zyflamendupregulated expression of DR5 in cancer cells transfected with wild-type CHOP binding site containing plasmids (Kim et al., 2012a). Pro-survival signaling induced in TRAIL treated cancer cells has added another layer of information into the molecular biology of TRAIL induced transduction cascade. TRAIL-induced expression of uPA and IL-8 was noted to be severely impaired in DR4 and DR5 blocked Colo357 cells and Panc89 cells. Colo357 cells stably transfected with TRAF2, upon treatment with TRAIL induced upregulation of uPA and IL-8. Similar results were noted in Colo357 cells transfected with Bcl-xl (Zhou et al., 2013).

MUC5AC, a secretory mucin expressed frequently in cancer cells and inoculating nude mice with MUC5AC silenced SW1990 cells did not induce tumor growth. Additionally it was shown that MUC5AC silenced SW1990 cells displayed remarkably enhanced TRAIL induced apoptosis (Hoshi et al., 2013). Mesothelin affinity for MUC16 has recently been utilized for targeting of MUC16 overexpressing pancreatic cancer cells. TRAIL-based fusion protein Meso-TR3 was designed by insertion of cDNA of mesothelin to the 5'-end of a TR3 expression plasmid. Construct was expressed in mammalian 293T cells as a fusion protein. BxPC3 pancreatic cancer cells treated with Meso-TR3 displayed considerably enhanced apoptotic rate (Garg et al., 2014).

Intriguingly, Dihydroartemisinin (DHA), a semi-synthetic derivative of artemisinin exerted its effects via ROS mediated upregulation of DR5 in BxPC-3 and PANC-1 cells. DHA also substantially reduced tumor development in nude mice xenografted with BxPC-3 (Kong et al., 2012). Concordant with similar approach, another study highlighted ROS/ERK/p53-mediated upregulation of death receptors in Gamma-tocotrienol treated Panc-1 and MiaPaca-2 cells (Kannappan et al., 2010).

AEG35156/GEM 640, a second-generation antisense oligonucleotide against XIAP has shown notable efficacy against Panc-1 pancreatic carcinoma cells. TRAIL induced apoptosis was substantially enhanced in Panc-1 cells treated with AEG35156 (LaCasse et al., 2006). Subsequent study has shown that gene silencing strategy against XIAP also improved TRAIL mediated apoptosis. Mechanistically it was reported that targeted inhibition of XIAP in Bcl-2 overexpressing cancer cells potentiated TRAIL induced apoptosis. Xenografting PancTu1 cells transduced with XIAP shRNA in nude mice induced regression of tumor in combination with TRAIL (Vogler et al., 2008).

Gimatecan, a potent lipophilic camptothecin has earlier been tested for efficacy in combination with CpG-oligodeoxynucleotides in pancreatic cancer xenograft (Petrangolini et al., 2008).

Benzyl isothiocyanate (BITC) is reported to induce apoptosis in pancreatic cancer cells. *In vitro* studies revealed that it triggered intrinsic pathway via activation of Bid. BITC also potentiated TRAIL induced apoptosis

via inhibition of Rac1 in pancreatic ductal carcinoma cells CFPAC-1 (Basu and Haldar, 2009).

Confluence of information suggests that gene silencing of c-FLIP(L) and c-FLIP(S) restored apoptosis in pancreatic cancer cells. More interestingly cells reconstructed with c-FLIP induced TRAIL resistant phenotype. It is relevant to mention that 5-fluorouracil (5-FU), cisplatin or gemcitabine induced apoptosis via inhibition of c-FLIP and enforced expression of c-FLIP impaired drug induced efficacy (Haag et al., 2011). Triptolide, a diterpene triepoxide improved TRAIL mediated apoptosis in pancreatic cancer cells via upregulation of DR5 and downregulation of c-FLIP (Chen et al., 2014).

There is a direct piece of evidence suggesting that Bcl-xl knockdown BxPC-3 cells transduced with empty vector demonstrated notably enhanced apoptotic rate following GSK-3i or TRAIL exposure. However, Bcl-xl knockdown cells reconstituted with Bcl-xl, indicated partial reversal of GSK-3 inhibitor and TRAIL-induced apoptosis. Mechanistically it was reported that GSK-3i treatment resulted in an increased SIRT1 and HDAC3 loading at BCL2L1 promoter that consequently resulted in formation of repressive chromatin (Zhang et al., 2014). Embelin, isolated from the fruit of *Embelia ribes* has been shown to inhibit growth of AsPC-1 xenografts in Balb C nude mice. EMT was also notably repressed in xenografted nude mice. Tissue analysis of xenografted mice revealed that Embelin triggered upregulation of DR4 and DR5 Huang et al, 2014. Garlic oil has been shown to exert its inhibitory effects on proliferation of AsPC-1, PANC-1, and Mia PaCa-2 cells Lan et al, 2013.

Regulation of miRNA by DR5 in Pancreatic Cancer Cells

Recently emerging hints have started to scratch the surface of new role of death receptor as a negative regulator of miRNA. It has lately been shown that DR5 is located in nucleus of pancreatic cancer cells. It has also been experimentally verified that Drosha and DGCR8 are associated with DR5 in cancer cells as evidenced by immunoprecipitation of nuclear DR5. Sequential methodologies including gene silencing of DR5 indicated that DR5 silenced pancreatic cancer cells did not display an increase in the levels of pri-let-7. However, processing activity of Drosha towards pri-let-7 was substantially enhanced underscoring the fact that DR5 interaction with Drosha considerably impaired its activity (Haselmann et al., 2014).

Preclinical Studies

It has lately been shown in an orthotopic model of a sensitive tumor that there was a considerably reduced primary and metastatic tumor burden in TRAIL treated mice. More importantly it was revealed that site of tumor engraftment did not alter the inherent sensitivity of patient xenografts to TRAIL (Sharma et al., 2014).

Pancreatic cancer cells frequently express EMMPRIN, a membrane-bound glycoprotein. Experimentally verified data revealed that EMMPRIN regulated expression of Matrix metalloproteinases, thus inducing degradation of extracellular matrix components. SCID mice xenografted with MIA PaCa-2 and PANC-1 cancer cells were treated with anti-EMMPRIN antibody and results revealed that it exerted its inhibitory effects on cell proliferation and endothelial cell densities. More interestingly, anti-EMMPRIN antibody and monomeric monoclonal antibody TRA-8 were used in combination in xenografted mice and considerably inhibited tumor growth in mice (Kim et al., 2012b). Monoclonal antibodies against DR4 (HGS-ETR1, mapatumumab) or DR5 (HGS-ETR2, lexatumumab) were found to be effective when used as combination therapy in MiaPaCa2 cells. Mapatumumab efficiently induced regression of tumor growth in xenografted mice (Stadel et al., 2010). Oral administration of phenethyl isothiocyanate inhibited tumor growth of MIAPaca2 cancer cells in xenografted mice (Stan et al., 2013).

GANT-61 (Gli transcription factor inhibitor) has been shown to exert its inhibitory effects on tumor growth via upregulation of DR4 and DR5 as evidenced by tumor tissues derived from NOD/SCID IL2Rγ null mice (Fu et al., 2013).

It is now well known that epithelial cell adhesion molecule (EpCAM) is frequently overexpressed by pancreatic cancer cells. XenograftingEpCAM-positive BxPc-3 cancer cells in immunodeficient mice induced tumor growth, both in absence of TRAIL transfected lymphocytes. However, EpCAM-positive BxPc-3 cancer cells did not establish tumor in TRAIL transfected lymphocytes bearing mice. More importantly, intraperitoneally injected EpCAMxCD3 Bispecific antibody substantially inhibited tumor formation in xenografted mice (Groth et al., 2012). It has recently been shown that a multipronged approach, consisting of atorvastatin, celecoxib and tipifarnib considerably reduced growth of the tumors in SCID mice xenografted with pancreatic cancer cells (Ding et al., 2014). PARP inhibitor (Olaparib) and the pan-Bcl-2 inhibitor (Obatoclax) represented anti-tumor activity in xenografted mice (Chen et al., 2014). Triptolide, isolated from *Tripteryglum wilfordii* was noted to significantly reduce tumor growth *in mice* (Liu and Cui, 2013).

Clinical Trials

Tigatuzumab, a humanized version of the agonistic murine monoclonal antibody TRA-8 has been tested for efficacy in chemotherapy-naive patients. Phase 2 trial evaluated the efficacy of combinatorial approach consisting of tigatuzumab and gemcitabine in 62 chemotherapy-naive patients and results revealed that it was well tolerated (Forero-Torres et al., 2013).

The Role of Nanotechnology on Antitumor Activity of TRAIL

To overcome the resistance of many cancer cells to TRAIL-induced apoptosis, wide ranging natural and synthetic agents have shown potential. Matsuzaki et al. (2001) showed that the combination of TRAIL and

actinomycin D prompted apoptosis in different human pancreatic cancer cell lines, including TRAIL-resistant cells. Likewise, Bai et al. (2005) studied a TRAIL-based triple chemotherapy to overcome the chemoresistance in pancreatic cancer cells. The combination of geldanamycin, PS-341, and TRAIL dramatically enhances TRAIL cytotoxic effects and could be a novel therapeutic strategy for pancreatic cancer.

Nanotechnology approaches have been widely used in medical research, medical imaging, and clinical applications. In an attempt to gain a greater and specific antitumor activity of TRAIL, the research on nanocarriers encapsulating TRAIL (Lim et al., 2011) or TRAIL-encoding plasmid (Mangipudi et al., 2009; Miao et al., 2013) gained a special attention over the last decade. The delivery of TRAIL and classical antitumor drugs using different kinds of nanotechnological approaches have also been investigated as a promising strategy to achieve a greater and more effective killing activity against cancer cells (Guo et al., 2011; 2012; Lee et al., 2011a; 2011b).

As mentioned, researchers are constantly looking for new strategies to gain a more efficient cancer treatment by using specific ligands with selective affinity to cancer cells. Different strategies including encapsulating TRAIL alone or in combination with other antitumor drugs have been studied to kill tumor cells in different kinds of cancer. Intratumoral injections of PEG-TRAIL (50μg) in mice xenografted with Mia Paca-2 cancer cells exerted inhibitory effects on tumor formation (Byeon et al., 2013). It is interesting to note that PEGylated TRAIL has further been tested using a specific delivery system consisting of four-arm PEG cross-linked hyaluronic acid (HA) hydrogels. The results revealed that antitumor activity of TRAIL in mice xenografted with Mia Paca-2 was remarkably higher because of stability of PEGylated TRAIL in HA hydrogels (Byeon et al., 2014).

Bae et al. (2012) designed human serum albumin (HSA) nanoparticles with the surface modified by TRAIL and transferrin, and containing doxorubicin, which were demonstrated to have a significant *in vitro* killing activity against different cancer cell lines. Among these cells, the authors studied the cytotoxic and apoptotic activities of the nanoparticles on CAPAN-1, a metastatic pancreatic cancer cell line. These nanoparticles were also tested in HCT 116-xenografted nu/nu mice and localization of HSA-NPs was noted in tumors.

Therefore, in view of the need for more efficacious treatments, together with the numerous advantages reported regarding the use of nanomaterials in clinical applications, the delivery of TRAIL-using different nanotechnological approaches to treat pancreatic cancer demands more investigation.

Concluding Remarks

The potential of TRAIL to selectively induce apoptosis in cancer cells is clear and already proven in many studies. Likewise, nano-based drug delivery systems are emerging and promising technologies that have the ability to specifically deliver chemotherapeutics to cancer cells. Numerous studies have demonstrated that drug-loaded nanomaterials are more active to kill cancer cells than free antitumor drugs, which might result in better outcomes of clinical treatment protocols. Therefore, TRAIL encapsulation in nanodevices is particularly encouraging in an attempt to achieve an increased antitumor activity.

However, despite the merits that make TRAIL a promising apoptotic agent, it has drawbacks such as rapid clearance from the systemic circulation and short biological half-life. To overcome these problems, the nanoencapsulation of TRAIL has become a promising strategy to achieve a greater apoptotic activity. Furthermore, combination of nano-based drug delivery devices containing TRAIL and other chemotherapeutic agent is more likely to reach enhanced antitumor activity.

Altogether, the growing research on nanotechnology and the need for innovative formulations that can enhance the biological performance of biomolecules with known antitumor activity, highlight TRAIL-nanovectors as potential new approaches to treat many kinds of tumors, including the pancreatic cancer that have a high lethality level. In conclusion, it can be stated that TRAIL delivery needs further improvement using nanotechnological strategies.

References

Arlt A, Müerköster SS, Schäfer H (2013). Targeting apoptosis pathways in pancreatic cancer. *Cancer Lett*, **332**, 346-58.

Bae S, Ma K, Kim TH, et al (2012). Doxorubicin-loaded human serum albumin nanoparticles surface-modified with TNF-related apoptosis-inducing ligand and transferrin for targeting multiple tumor types. *Biomaterials*, **33**, 1536-46.

Bai J, Sui J, Demirjian A, et al (2005). 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*, **65**, 2344-52.

Basu A, Haldar S (2009). Anti-proliferative and proapoptotic effects of benzyl isothiocyanate on human pancreatic cancer cells is linked to death receptor activation and RasGAP/Rac1 down-modulation. *Int J Oncol*, **35**, 593-9.

Braeuer SJ, Büneker C, Mohr A, Zwacka RM (2006). Constitutively activated nuclear factor-kappaB, but not induced NF-kappaB, leads to TRAIL resistance by up-regulation of X-linked inhibitor of apoptosis protein in human cancer cells. *Mol Cancer Res*, **4**, 715-28.

Byeon HJ, Choi SH, Choi JS, et al (2013). Apoptotic activity and antitumor efficacy of PEGylated TNF-related apoptosis-inducing ligand (TRAIL) in a Mia Paca-2 cell-xenografted mouse model. *Biomed Pharmacother*, **68**, 65-9.

Byeon HJ, Choi SH, Choi JS, et al (2014). Four-arm PEG cross-linked hyaluronic acid hydrogels containing PEGylated apoptotic TRAIL protein for treating pancreatic cancer. *ActaBiomater*, **10**, 142-50.

Chen S, Wang G, Niu X, et al (2014). Combination of AZD2281 (Olaparib) and GX15-070 (Obatoclax) results in synergistic antitumor activities in preclinical models of pancreatic cancer. *Cancer Lett*. Feb, **348**, 20-8.

Chen Z, Sangwan V, Banerjee S, et al (2014). Triptolide sensitizes pancreatic cancer cells to TRAIL-induced activation of the death receptor pathway. *Cancer Lett*, **348**, 156-66.

Diamantidis M, Tsapournas G, Kountouras J, Zavos C (2008). New aspects of regulatory signaling pathways and novel therapies in pancreatic cancer. *Curr Mol Med*, **8**, 12-37.

Ding J, Polier G, Köhler R, et al (2012). Wogonin and related

natural flavones overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein resistance of tumors by down-regulation of c-FLIP protein and up-regulation of TRAIL receptor 2 expression. *J Biol Chem*, **287**, 641-9.

Ding N, Cui XX, Gao Z, et al (2014). A triple combination of atorvastatin, celecoxib and tipifarnib strongly inhibits pancreatic cancer cells and xenograft pancreatic tumors. *Int J Oncol*, **44**, 2139-45.

Forero-Torres A, Infante JR, Waterhouse D, et al (2013). Phase 2, multicenter, open-label study of tigatuzumab (CS-1008), a humanized monoclonal antibody targeting death receptor 5, in combination with gemcitabine in chemotherapy-naive patients with unresectable or metastatic pancreatic cancer. *Cancer Med*, **2**, 925-32.

Fu J, Rodova M, Roy SK, et al (2013). GANT-61 inhibits pancreatic cancer stem cell growth *in vitro* and in NOD/SCID/IL2R gamma null mice xenograft. *Cancer Lett*, **330**, 22-32.

Garg G, Gibbs J, Belt B, et al (2014). Novel treatment option for MUC16-positive malignancies with the targeted TRAIL-based fusion protein Meso-TR3. *BMC Cancer*, **14**, 35.

Groth A, Salnikov AV, Ottinger S, et al (2012). New gene-immunotherapy combining TRAIL-lymphocytes and EpCAMxCD3 Bispecific antibody for tumor targeting. *Clin Cancer Res*, **18**, 1028-38.

Guo L, Fan L, Ren J, et al (2011). A novel combination of TRAIL and doxorubicin enhances antitumor effect based on passive tumor-targeting of liposomes. *Nanotechnology*, **22**, 265105.

Guo L, Fan L, Ren J, et al (2012). Combination of TRAIL and actinomycin D liposomes enhances antitumor effect in non-small cell lung cancer. *Int J Nanomedicine*, **7**, 1449-60.

Haag C, Stadel D, Zhou S, et al (2011). Identification of c-FLIP(L) and c-FLIP(S) as critical regulators of death receptor-induced apoptosis in pancreatic cancer cells. *Gut*, **60**, 225-37.

Hafeez BB, Mustafa A, Fischer JW, et al (2014). α-Mangostin: a Dietary antioxidant derived from the pericarp of *Garcinia mangostana L.* inhibits pancreatic tumor growth in xenograft mouse model. *Antioxid Redox Signal*, doi:10.1089/ars.2013.5212..

Haselmann V, Kurz A, Bertsch U, et al (2014). Nuclear death receptor TRAIL-R2 inhibits maturation of Let-7 and promotes proliferation of pancreatic and other tumor cells. *Gastroenterology*, **146**, 278-90.

Hinz S, Trauzold A, Boenicke L, et al (2000). Bcl-XL protects pancreatic adenocarcinoma cells against CD95- and TRAIL-receptor-mediated apoptosis. *Oncogene*, **19**, 5477-86.

Hoshi H, Sawada T, Uchida M, et al (2013). MUC5AC protects pancreatic cancer cells from TRAIL-induced death pathways. *Int J Oncol*, **42**, 887-93.

Huang M, Tang SN, Upadhyay G, et al (2014). Embelin suppresses growth of human pancreatic cancer xenografts, and pancreatic cancer cells isolated from KrasG12D mice by inhibiting akt and sonic hedgehog pathways. *PLoS One*, **2**, 92161.

Ibrahim SM, Ringel J, Schmidt C, et al (2001). Pancreatic adenocarcinoma cell lines show variable susceptibility to TRAIL-mediated cell death. *Pancreas*, **23**, 72-9.

Kannappan R, Ravindran J, Prasad S, et al (2010). Gamma-tocotrienol promotes TRAIL-induced apoptosis through reactive oxygen species/extracellular signal-regulated kinase/p53-mediated upregulation of death receptors. *Mol Cancer Ther*, **9**, 2196-207.

Kim H, Zhai G, Samuel SL, et al (2012). Dual combination therapy targeting DR5 and EMMPRIN in pancreatic adenocarcinoma. *Mol Cancer Ther*, **11**, 405-15.

Kim JH, Park B, Gupta SC, et al (2012). Zyflamend sensitizes tumor cells to TRAIL-induced apoptosis through up-regulation of death receptors and down-regulation of survival proteins: role of ROS-dependent CCAAT/enhancer-binding protein-homologous protein pathway. *Antioxid Redox Signal*, **16**, 413-27.

Kong R, Jia G, Cheng ZX, et al (2012). Dihydroartemisinin enhances Apo2L/TRAIL-mediated apoptosis in pancreatic cancer cells via ROS-mediated up-regulation of death receptor 5. *PLoS One*, **7**, 37222.

LaCasse EC, Cherton-Horvat GG, Hewitt KE, et al (2006). Preclinical characterization of AEG35156/GEM 640, a second-generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. *Clin Cancer Res*, **12**, 5231-41.

Lan X, Sun H, Liu J, et al (2013). Effects of garlic oil on pancreatic cancer cells. *Asian Pac J Cancer Prev*, **14**, 5905-10.

Lee AL, Dhillon SH, Wang Y, et al (2011). Synergistic anti-cancer effects via co-delivery of TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) and doxorubicin using micellar nanoparticles. *MolBiosyst*, **7**, 1512-22.

Lee AL, Wang Y, Pervaiz S, Fan W, Yang YY (2011). Synergistic anticancer effects achieved by co-delivery of TRAIL and paclitaxel using cationic polymeric micelles. *MacromolBiosci*, **11**, 296-307.

Liao Q, Friess H, Kleeff J, Büchler MW (2001). Differential expression of TRAIL-R3 and TRAIL-R4 in human pancreatic cancer. *Anticancer Res*, **21**, 3153-9.

Lim SM, Kim TH, Jiang HH, et al (2011). Improved biological half-life and anti-tumor activity of TNF-related apoptosis-inducing ligand (TRAIL) using PEG-exposed nanoparticles. *Biomaterials*, **32**, 3538-46.

Liu Q, Chen W, Jiao Y, et al (2014). Pulsatilla saponin A, an active molecule from Pulsatilla chinensis, induces cancer cell death and inhibits tumor growth in mouse xenograft models. *J Surg Res*, **188**, 387-95.

Liu Y, Cui YF (2013). Synergism of cytotoxicity effects of triptolide and artesunate combination treatment in pancreatic cancer cell lines. *Asian Pac J Cancer Prev*, 14, 5243-8.

Mangipudi SS, Canine BF, Wang Y, Hatefi A (2009). Development of a genetically engineered biomimetic vector for targeted gene transfer to breast cancer cells. *Mol Pharm*, **6**, 1100-9.

Matsuzaki H, Schmied BM, Ulrich A, et al (2001). Combination of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and actinomycin D induces apoptosis even in TRAIL-resistant human pancreatic cancer cells. *Clin Cancer Res*, **7**, 407-14.

Miao L, Zhang K, Qiao C, et al (2013). Antitumor effect of human TRAIL on adenoid cystic carcinoma using magnetic nanoparticle–mediated gene expression. *Nanomedicine*, **9**, 141-50.

Ozawa F, Friess H, Kleeff J, et al (2001). Effects and expression of TRAIL and its apoptosis-promoting receptors in human pancreatic cancer. *Cancer Lett*, **163**, 71-81.

Petrangolini G, Tortoreto M, Perego P, et al (2008). Combination of metronomic gimatecan and CpG-oligodeoxynucleotides against an orthotopic pancreatic cancer xenograft. *Cancer BiolTher*, **7**, 596-601.

Röder C, Trauzold A, Kalthoff H (2011). Impact of death receptor signaling on the malignancy of pancreatic ductal adenocarcinoma. *Eur J Cell Biol*, **90**, 450-5.

Sharma R, Buitrago S, Pitoniak R, et al (2014). Influence of the implantation site on the sensitivity of patient pancreatic tumor xenografts to Apo2L/TRAIL therapy. *Pancreas*, **43**, 298-305.

Stadel D, Mohr A, Ref C, et al (2010). TRAIL-induced apoptosis is preferentially mediated via TRAIL receptor 1 in pancreatic carcinoma cells and profoundly enhanced by XIAP inhibitors. *Clin Cancer Res*, **16**, 5734-49.

Stan SD, Singh SV, Whitcomb DC, Brand RE (2013). Phenethyl isothiocyanate inhibits proliferation and induces apoptosis in pancreatic cancer cells in vitro and in a MIAPaca2 xenograft animal model. *Nutr Cancer*, **66**, 747-55.

Thomas RP, Farrow BJ, Kim S, et al (2002). Selective targeting of the nuclear factor-kappaB pathway enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated pancreatic cancer cell death. *Surgery*, **132**, 127-34.

Trauzold A, Wermann H, Arlt A, et al (2001). CD95 and TRAIL receptor-mediated activation of protein kinase C and NF-kappaB contributes to apoptosis resistance in ductal pancreatic adenocarcinoma cells. *Oncogene*, **20**, 4258-69.

Vogler M, Walczak H, Stadel D, et al (2008). 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*, **68**, 7956-65.

Wang Y, Zhou Y, Jia G, et al (2014). Shikonin suppresses tumor growth and synergizes with gemcitabine in a pancreatic cancer xenograft model: involvement of NF-κB signaling pathway. *Biochem Pharmacol*, **88**, 322-33.

Zhang JS, Herreros-Vilanueva M, Koenig A, et al (2014). Differential activity of GSK-3 isoforms regulates NF-κB andTRAIL- or TNFα induced apoptosis in pancreatic cancer cells. *Cell Death Dis*, **5**, 1142.

Zhao B, Li L, Cui K, et al (2011). Mechanisms of TRAIL and gemcitabine induction of pancreatic cancer cell apoptosis. *Asian Pac J Cancer Prev*, **12**, 2675-8.

Zhou DH, Yang LN, Roder C, Kalthoff H, Trauzold A (2013). TRAIL-induced expression of uPA and IL-8 strongly enhanced by overexpression of TRAF2 and Bcl-xl in pancreatic ductal adenocarcinoma cells. *Hepatobiliary Pancreat Dis Int*, **12**, 94-8.