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INVITED REVIEW

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CASPASE-8 REGULATION OF TRAIL-MEDIATED CELL DEATH

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Research on TNF-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors has advanced tremendously over the past 17 years. Initial observations of TRAIL and TRAIL receptor-mediated tumor cell toxicity led to enthusiasm of exploiting this selective, malignant cell killing for cancer therapy. Further examination revealed aberrant TRAIL signaling in some cancer cells leading to protection from TRAIL-mediated cell death. Mechanisms of TRAIL resistance often involve decreased expression or activity of initiator caspase-8, crucial for complete TRAIL signal transduction. Caspase-8 mutations, epigenetic silencing, decrease in stability, and incomplete activation have been reported. This article reviews the discovery of TRAIL and TRAIL receptors and subsequent studies that reveal how expression and function of caspase-8 are central to TRAIL-mediated cell death. This article is part of a Special Issue entitled "Apoptosis: Four Decades Later".

***Key Words*: TRAIL, caspase-8, cell death, cancer, epigenetic silencing, ubiquitination.**

INTRODUCTION

The discovery of TNF-related apoptosis-inducing ligand (TRAIL) in 1995 was the beginning of exciting research that is now approaching two decades [1]. TRAIL was identified as a member of the TNF family [2]. By 1998, five TRAIL receptors had been discovered and characterized. TRAIL-receptor 1 (DR4) and TRAIL-receptor 2 (DR5) are pro-apoptotic TRAIL receptors capable of mediating cell death. TRAIL-receptor 3 (DcR1) and TRAIL-receptor 4 (DcR2) are decoy TRAIL receptors unable to induce cell death due to deficient intracellular signaling domain or truncated death domain, respectively [3–7]. Osteoprotegerin (OPG), identified in 1998, is a soluble TRAIL decoy receptor shown to be important for osteoclast development [8].

TRAIL expression has been found in various types of human tissues. Endogenous TRAIL has both innate and adaptive immune system functions [9]. TRAIL plays a role in immunosurveillance and has been shown to kill malignant cells as wells as bacterial and viral infected cells [9]. TRAIL's ability to cause toxicity in various cancer cells while leaving normal cells unharmed made it an exciting, potential cancer therapy. TRAIL-based therapies are currently being evaluated for clinical use.

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Abbreviations used: 5-Aza-5' – aza-deoxycytidine; Apaf-1 – apoptosis protease-activating factor-1; Bcl-2 – B-cell lymphoma 2; Bid – Bcl-2 interacting protein; CARP1 – caspase-8 and caspase-10-associated RING protein 1; CARP2 – caspase-8 and caspase-10-associated RING protein 2; CUL3 – cullin 3; DcR1 – decoy receptor 1; DcR2 – decoy receptor 2; DISC – death-inducing signaling complex; DR4 – death receptor 4; DR5 – death receptor 5; FADD – Fas-associated death domain protein; HDAC – histone deacetylase; IFNγ – interferon gamma; OPG – osteoprotegerin; PHA – phytohemagglutinin; RING – really interesting new gene; RIP1 – receptor-interacting protein 1; SAHA – suberoylanilide hydroxamic acid; tBid – truncated Bid; TRAIL – TNF-related apoptosis inducing ligand; VA – valproic acid.

Cancer cell resistance to TRAIL-mediated cell death has been noted and resistance mechanisms have been investigated extensively. Mechanisms include but are not limited to aberrant TRAIL receptor cell surface expression and presentation [10], TRAIL receptor glycosylation [11], recruitment of the functionally inactive caspase-8 homologue FLIP to the death-inducing signaling complex (DISC) [12, 13], decreased oncogene *c-myc* expression [14], and increased anti-apoptotic Bcl-2 family member Mcl-1 expression [15]. These mechanisms are of importance and have provided insight into molecular determinants of TRAIL sensitivity. However, this review will focus on caspase regulation of TRAIL-induced cell death, specifically caspase-8. Initiator caspase-10 can be recruited to the DISC but cannot functionally substitute for caspase-8 in mediating cell death [16, 17]. Caspase-8 is the focal apical caspase for the TRAIL signaling pathway and this review will highlight the significance of caspase-8 expression and activity in TRAIL-induced apoptosis [18].

CASPASE-8

Caspase-8 is a 55 kDA cysteine protease. Caspase-8 has 480 amino acids and contains two death effector domains and a catalytic protease domain [18]. Procaspase-8 is the inactive zymogen that requires activation for complete activity [18]. Caspase-8 requires multiple steps for activation that include oligomerization and proteolysis cleavage [19]. Active caspase-8 consists of a tetramer with two large and small subunits. The first cleavage of caspase-8 generates p43/41 intermediate fragments. Additional cleavage product are p26/24, p18 and p10 fragments [19]. Multiple caspase-8 isoforms have been described with caspase-8/a (55 kDa) and caspase-8/b (53 kDa) being the predominant isoforms expressed in cells and the characteristic caspase-8 doublet seen by western blotting [20]. A caspase-8 alternative splice variant, caspase-8L, has been identified and can protect against caspase-8-mediated cell death [21, 22].

TRAIL SIGNALING

TRAIL signaling leads to cell death by utilizing both extrinsic and intrinsic pathways [19, 23]. The intrinsic pathway involves mitochondrial permeabilization and release of pro-apoptotic factors that lead to caspase cascade activation. TRAIL ligation to DR4 or DR5 causes receptor clustering and trimerization. Intracellular adaptor protein FADD is recruited to the death domain of the TRAIL receptor. Initiator caspase-8 is recruited to the complex collectively known as the death-inducing signaling complex (DISC) and interacts with FADD through their death effector domains [24]. DISC activation of caspase-8 leads to caspase cascade activation where downstream effector caspase-3 becomes activated and cleaves protein targets that culminate in cell death. Additionally, caspase-8 can cleave the Bcl-2 related protein Bid. Truncated Bid (tBid) translocates into the mitochondria and causes release of cytochrome c and the formation of the Apaf-1containing apoptosome and subsequent caspase-9 activation [24]. Activated caspase-9 can then activate effector caspase-3 resulting in cell death (Fig. 1).

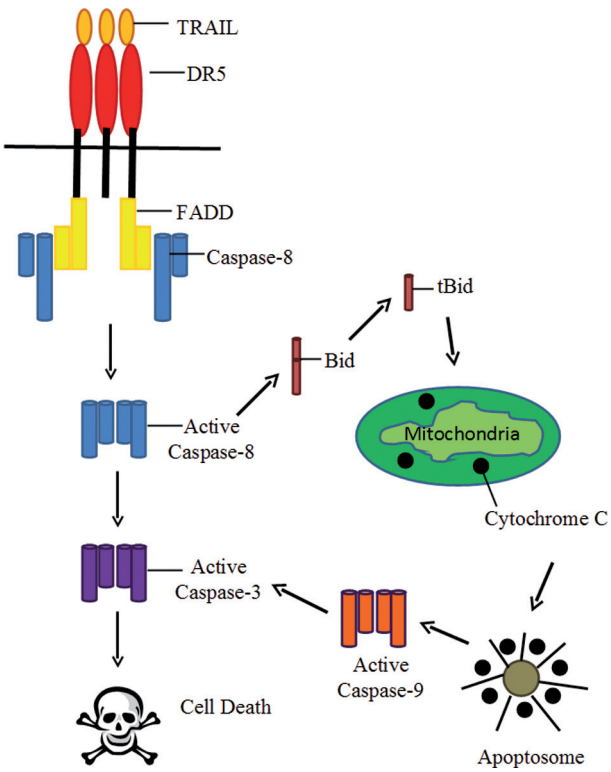

Fig. 1. Extrinsic and intrinsic TRAIL signaling pathways. TRAIL ligation to a pro-apoptotic TRAIL receptor causes receptor trimerization and activation of initiator caspase-8 and effector caspase-3, leading to cell death. Alternatively, caspase-8 can cleave Bid. Truncated Bid triggers the release of cytochrome c and formation of the apoptosome. The apoptosome activates caspase-9 resulting in activation of caspase-3 and cell death

TRAIL RESISTANCE

Caspase-8 mutations

Caspase-8 mutations in malignant cells were first noted in squamous cell carcinoma of the oral cavity. A point mutation that altered the stop codon and increased the size of the encoded protein was found in patient-derived cells but not in autologous PHA-treated blood lymphocytes [25]. These cancer cells were also less sensitive to apoptosis induced by caspase-8 over expression, suggesting that the mutation reduced caspase-8 activity. Caspase-8 mutations have also been identified in hepatocellular and gastric carcinomas with defective apoptosis activities observed in mutants [26, 27]. Five caspase-8 mutations were identified in invasive colorectal carcinomas that include three missense mutations, one frame-shift mutation, and one nonsense mutation [28]. 293T cells transfected with mutant caspase-8 expression vectors showed decreased apoptosis after DR5 overexpression. Collectively theses studies highlight that caspase-8 mutations are present in a variety of malignancies and these mutations inactivate the cysteine protease and lead to reduced spontaneous and receptor mediated-cell death.

Decreased caspase-8 expression and epigenetic silencing

The previously mentioned studies detected caspase-8 mutations in malignant cells yet these mutations were not associated with loss of caspase-8 expression. Dual observations of caspase-8 mutation and undetectable caspase-8 expression have been noted in HOC313 head and neck carcinoma cells [29]. Chemoresistance to the anti-cancer drug cisplatin was also observed in HOC313 cells. Loss of caspase-8 expression has been described in childhood neuroblastomas and medulloblastomas insensitive to TRAIL-induced cell death. Treatment with DNA methyltransferase inhibitor 5'Aza-deoxycytidine (5-Aza) increased caspase-8 mRNA and protein expression in both TRAIL-resistant neuroblastoma [30, 31] and medulloblastoma [32, 33] cancer cells, suggesting that epigenetic silencing was responsible for the downregulation of caspase-8. Increased caspase-8 expression was associated with enhanced TRAIL-mediated cell death. Further investigation of TRAIL-resistant neuroblastoma revealed that invasive neuroblastoma cells have increased TRAIL resistance and decreased caspase-8 mRNA and protein compared to non-invasive neuroblastoma cell lines [31]. Treatment of invasive neuroblastoma cells with DNA methyltransferase inhibitor Azacytidine, increased caspase-8 protein expression and increased cell death after co-treatment of Azacytidine and TRAIL. Silencing of caspase-8 expression by DNA methylation has also been noted in small cell lung carcinoma [34]. 5-Aza treatment also increased caspase-8 protein expression and improved TRAIL sensitivity.

DNA hypermethylation is not the only epigenetic mechanism found to be responsible for decreased caspase-8 expression. Histone hypoacetylation has also been found to result in caspase-8 downregulation. Medulloblastoma cell treatment with histone deacetylase (HDAC) inhibitors valproic acid (VA) and suberoylanilide hydroxamic acid (SAHA) also increased caspase-8 expression [35].

These reports underline caspase-8 epigenetic silencing by DNA methylation as a means for malignant cancer cells to decrease caspase-8 expression and avoid TRAIL-induced cell death. TRAIL resistance noted by epigenetic silencing of caspase-8, em-

phasizes the requirement of caspase-8 expression for complete TRAIL signaling. Modulation of caspase-8 has been studied to define treatments that increase caspase-8 expression and thus enhance TRAIL-mediated cell death.

Cytokine Interferon gamma (IFNγ) was previously shown to increase TRAIL sensitivity in a dose-dependent manner in Ewing sarcoma, neuroblastoma, and medulloblastoma cells [36]. Further examination revealed increased caspase-8 expression, but not effector caspase-3 expression, after treatment with IFNγ. Co-treatment with TRAIL and IFNγ resulted in markedly enhanced caspase-mediated cell death. IFNγ mediated caspase-upregulation was found to be Stat1 dependent [36]. IFNγ treatment did not change the methylation status of caspase-8 [36].

Decreased caspase-8 stability

Decreased caspase-8 stability and accelerated degradation has been reported in TRAIL resistant DLD1 colon cancer cells [37]. Restoration of caspase-8 expression in resistant DLD1 cells resulted in increased TRAIL-induced apoptosis. Caspase-8-and caspase-10-associated RING proteins, CARP1 and CARP2 mRNA were upregulated in TRAIL-resistant colon cancer cells compared to TRAIL-sensitive parental cells. CARP1 and CARP2 aid in ubiquitin-mediated proteolysis of death effector domain containing caspases [38].

Proteasome inhibitor stabilization of caspase-8 remains controversial. Caspase-8 stabilization after proteasome inhibition has been noted in some cancer cells [39]. Conversely, caspase-8 expression was unchanged in ovarian carcinoma cells after treatment with the proteasome inhibitor MG132 [40]. These differences may be cell type dependent. Additionally, autophagic lysosomal caspase-8 degradation has also been described [41].

Caspase-8 ubiquitination

Several caspase-8 post-translational modifications have been identified including caspase-8 phosphorylation and ubiquitination. Caspase-8 phosphorylation has been noted on various residues (Thr263, Tyr380, Tyr397, Tyr645) [42–47]. Caspase-8 phosphorylation is reported to inhibit caspase-8 activity. Yet, caspase-8 phosphorylation status in TRAIL-resistant cancer cells has not been thoroughly explored. In 2009, Jin and colleagues revealed that TRAIL treatment induces caspase-8 ubiquitination in TRAIL-sensitive H460 lung cancer cells [48]. The E3 ligase CUL3 was shown to interact with caspase-8 and was associated with the DISC after TRAIL treatment. Knockdown of CUL3 decreased caspase-8 ubiquitination and activity and limited TRAIL-mediated apoptosis. TRAIL treatment caused both K48 and K63 polyubiquitination chains to be added to caspase-8 [48]. Additionally, Jin et al. reported that ubiquitin binding protein p62 promoted TRAIL-induced caspase-8 aggregation. siRNA knockdown of p62 diminished TRAIL-induced caspase-8 ubiquitination and activity. No caspase-8 ubiquitination and processing was observed in two TRAIL-resistant cell lines, HeLa and ADR-RES [48]. This study highlights post-translational ubiquitination of caspase-8 for complete TRAIL sensitivity.

A recent report by Bellail and colleagues examined ubiquitin enzymes A20 and CUL3 in TRAIL-sensitive and TRAIL-resistant glioblastoma cell lines. Unlike H460 lung cancer cells, TRAIL-sensitive glioblastoma cells had no detectable caspase-8 ubiquitination or CUL3 in the DISC after TRAIL treatment [49]. Little A20 expression was detected in TRAIL-sensitive glioblastoma cells. Introduction of A20 expression plasmids reversed TRAIL sensitivity. The A20 zinc finger domain, that confers E3 ligase capability, was found to be necessary for A20-mediated TRAIL resistance [49]. Further investigation found that A20 attaches K63 polyubiquitin chains to RIP1 and these polyubiquitin chains interact with caspase-8. An *in vitro* caspase-8 cleavage assay revealed that K63 polyubiquitin chains inhibit caspase-8 processing [49]. These studies show how in two different malignant cell types, direct caspase-8 ubiquitination or association with a polyubiquitinated protein can enhance or inhibit TRAIL-mediated cell death, respectively. In TRAIL-sensitive H460 lung cancer cells, caspase-8 is ubiquitinated and undergoes aggregation required for complete caspase-8 activation and activation of downstream caspase cascade (Fig. 2). In TRAIL-resistant glioblastomas, caspase-8 interacts with A20-induced K63 polyubiquitin chains attached to RIP1 that hinder caspase-8 activation and processing (Fig. 3).

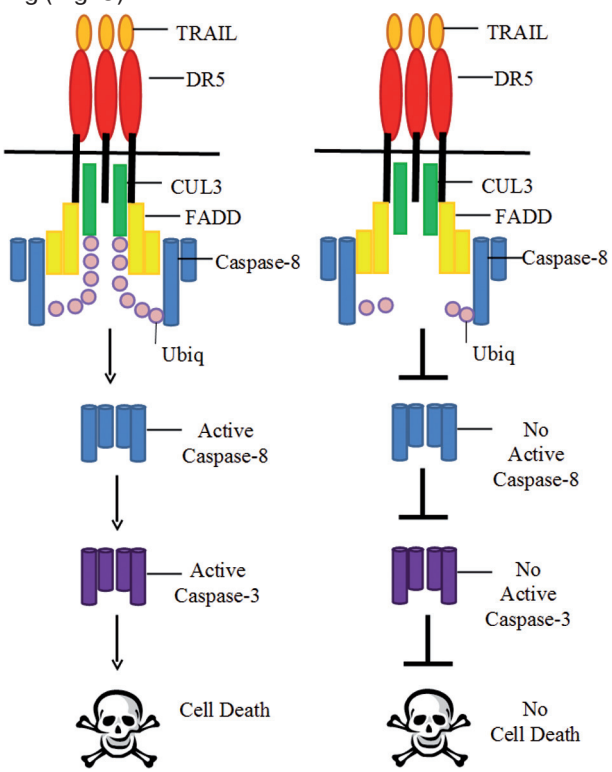

Fig. 2. Schematic of TRAIL susceptibility after caspase-8 polyubiquitination. (Left; Example TRAIL-sensitive H460 cells) TRAIL ligation to a pro-apoptotic TRAIL receptor causes E3 Ligase CUL3 to polyubiquitinate caspase-8. Ubiquitinated caspase-8 can properly aggregate and complete caspase-8 and caspase-3 activation leads to cell death. (Right; Example TRAIL-resistant HeLa cells) Caspase-8 that is not ubiquitinated cannot be fully activated which leads to failure to activate caspase-3 and cause cell death

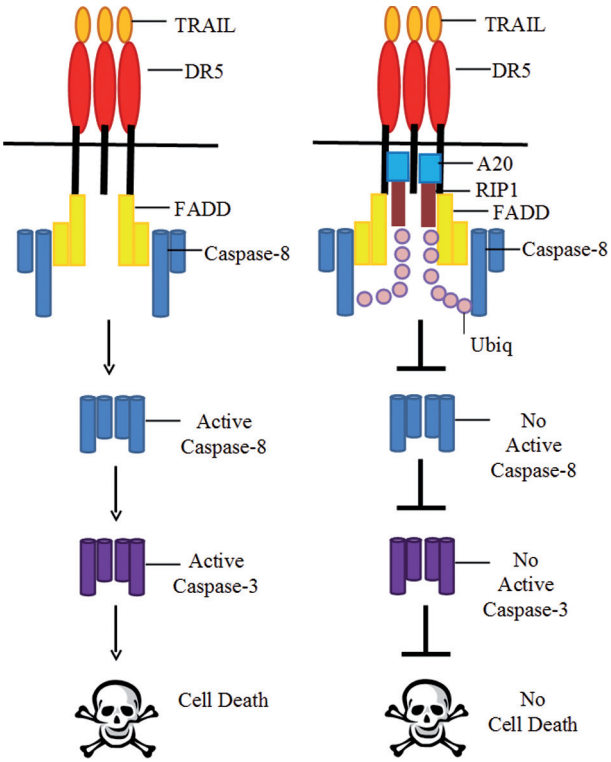

Fig. 3. Schematic of TRAIL resistance after caspase-8 association with polyubiquitinated RIP1. (Left; Example of TRAIL-sensitive glioblastoma cells LN71) TRAIL ligation causes activation of initiator caspase-8 and effector caspase-3, leading to cell death. (Right; Example of TRAIL-resistant glioblastoma cells LN443) The E3 ligase zinc finger domain of A20 polyubiquitinates RIP1. Caspase-8 interacts with polyubiquitinated RIP1. Polyubiquitinated RIP1 inhibits caspase-8 processing and subsequent signaling of apoptosis

CONCLUDING REMARKS

TRAIL susceptibility in malignant cells is the foundation for various TRAIL-based therapies currently being explored for translational use. Yet, not all cancer cells are sensitive to TRAIL-induced cell death. It is not hard to imagine that cancer cells have acquired mechanism(s) that escape TRAIL immunosurveillance and inhibit cell death. Abnormalities in expression and activation status of the upstream executioner caspase-8 are detailed in this review. Restoration of caspase-8 expression or activity sensitizes various TRAIL-resistant cancer cells. Future studies focusing on modulation of caspase-8 expression and upregulation will further contribute to the scientific knowledge of molecular determinants that confer TRAIL resistance and will likely aid in the efficacy of TRAIL-based therapies.

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