Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand Pathway and Its Therapeutic Implications

Elisabeth G.E. de Vries, Jouirik A. Gietema, and Steven de Jong

Background

Apoptosis can be executed through a mitochondria-dependent (intrinsic) and a death receptor–dependent, mitochondria-independent (extrinsic) pathway. The intrinsic pathway is initiated by, for example, chemotherapy and/or radiotherapy, and the extrinsic pathway is initiated by ligand-induced activation of death receptors on the cell membrane. One of the reasons for chemotherapy resistance is the failure of tumor cells to go into apoptosis due to defects in the intrinsic apoptotic pathway (e.g., changes in p53). Circumvention of chemotherapy resistance via apoptosis induction by death ligands belonging to the tumor necrosis factor family of cytokines, including tumor necrosis factor, Fas ligand, and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), exhibits cytotoxicity in tumor cell lines. The risk of side effects precludes the systemic use of recombinant forms of tumor necrosis factor and Fas ligand, but this is not the case with recombinant human TRAIL (rhTRAIL). Therefore, exploitation of the TRAIL-mediated apoptosis pathway for translation to the clinic is of interest.

TRAIL signaling pathway. TRAIL, is a type II membrane protein and its COOH terminus can be processed proteolytically to form a soluble ligand. Both membrane-expressed TRAIL and soluble TRAIL can induce apoptosis in a wide variety of tumor cell lines, whereas normal cells are relatively resistant. TRAIL binds to five different receptors. Following binding to its death receptors, DR4 and DR5, TRAIL can induce apoptosis. The membrane-bound decoy receptors DcR1 and DcR2, having no or a truncated intracellular death domain, cannot induce apoptosis. Osteoprotegerin is a soluble receptor inhibiting TRAIL. In the mouse, only a single receptor resembling human DR4 and DR5 exists, along with two DcRs that are only distantly related by sequence to human DcR1 and DcR2, and one close homologue of osteoprotegerin. Human and murine TRAIL proteins share only 65% sequence identity. This difference underscores the difficulty to predict side effects of rhTRAIL in humans based on studies in mice. In contrast, human and cynomolgus monkey TRAIL are 98% homologous, and the receptor ectodomain sequence identity is 91% for DR4, 88% for DR5, 84% for DcR2, and 99% for osteoprotegerin, whereas cynomolgus DcR1 seems to be a pseudogene. Therefore, testing TRAIL in monkeys potentially better predicts its toxicity.

TRAIL binding to DR4 and DR5 at the cell surface leads to formation of the death-inducing signaling complex (Fig. 1). This complex consists of the trimerized receptor, the adaptor protein Fas-associated death domain, and caspase-8. On death-inducing signaling complex formation, caspase-8 is activated, which in type 1 cells leads to activation of caspase-3 and subsequently apoptosis. In type II cells, TRAIL also triggers the mitochondrial pathway. Active caspase-8 causes cleavage of BID into tBID, which translocates to the mitochondria leading to release of cytochrome c and Smac/DIABLO. Cytochrome c together with Apaf-1 can activate caspase-9, which then activates caspase-3 (Fig. 1).

Recent Advances

Function of TRAIL in physiology and pathophysiology. Studies with a neutralizing rat anti-mouse TRAIL antibody showed a role for TRAIL in immune surveillance against tumor development in mice (4, 5). In mice, this antibody promoted tumor outgrowth of TRAIL-sensitive tumors (5). TRAIL gene knockout mice show an increased susceptibility to tumor initiation and metastases and autoimmune diseases (6).

However, recent evidence indicates that in several tumor cells, including human colon cancer cells, TRAIL can mediate tumor cell proliferation, which may have a dominant effect over the apoptotic signal (7). Moreover, a role for DR5 as a candidate tumor suppressor was established. In human colon cancer cell lines, silencing of DR5 in vivo led to accelerated growth of bioluminescent tumor xenografts and conferred resistance of the tumor cells to the chemotherapeutic agent 5-fluorouracil (8).

Chronic TRAIL blockade in mice exacerbates collagen-induced arthritis and experimental autoimmune encephalomyelitis. TRAIL deficiency also increases the susceptibility of mice to autoimmune arthritis and diabetes (9). The Fas-Fas ligand pathway has been studied extensively in humans in relation to systemic lupus erythematosus. Soluble TRAIL concentrations in the serum, however, are also increased in these patients. This seems to be disease specific, as levels in patients with rheumatoid arthritis and Wegener’s granulomatosis were not elevated, suggesting a role for TRAIL in systemic lupus erythematosus pathophysiology (10). TRAIL also plays a role in infections. Dysregulated immune and coagulation systems are held responsible for the septic syndrome. However, additional pathophysiologic mechanisms, such as uncontrolled...
apoptosis induced by death receptor ligands, might well play a role. Endotoxin administration to volunteers caused not only an increase in tumor necrosis factor but also a 10-fold increase in plasma soluble TRAIL 2.5 hours after administration (11). In addition, virally infected cells are more susceptible for TRAIL-induced apoptosis (12). This illustrates that TRAIL does play a role in normal physiology and diseases and illustrates that the human body can tolerate certain TRAIL levels.

Osteoprotegerin is a key regulator of osteoclastogenesis, but recently also osteoprotegerin involvement in the control of endothelial cell survival through inhibition of TRAIL activity was suggested. Endothelial osteoprotegerin expression was associated with high tumor grade and certain histologic types in 400 breast tumors. This suggests a potential biological role for osteoprotegerin in development and/or maintenance of tumor vasculature (13).

Preclinical in vivo antitumor efficacy and toxicity of rhTRAIL and TRAIL receptor antibodies. In animals, rhTRAIL reduced growth of human tumor xenografts as well as xenotransplants and potentiated chemotherapy and radiotherapy effect. Furthermore, rhTRAIL administration in nonhuman primates showed no serious toxicity towards normal tissue. The rhTRAIL half-life in chimpanzees, however, was only 25 minutes (3). Most chimpanzee and human tissues express TRAIL receptors, whereas a few differences were observed between human and chimpanzee tissues. Lack of liver toxicity in chimpanzees after rhTRAIL administration despite DR4 and DR5 expression was reassuring for rhTRAIL application in humans (14). Moreover, no TRAIL hepatotoxicity was observed in human hepatocytes within the chimeric liver of severe combined immunodeficient mice (15). Several agonistic antibodies against the human receptors DR4 and DR5 have been developed. These antibodies, just like rhTRAIL, showed antitumor activity alone in several preclinical tumor models and both potentiated the effect of chemotherapy (16–19). There were no signs of serious side effects in animals following DR4 and DR5 antibody administration. However, one has to take in account that these animals have been exposed to antibodies specific for human DR4 and DR5.

Translational advances. In a few studies, the prognostic significance of TRAIL and TRAIL receptor expression in tumors has been determined. No differences in TRAIL mRNA expression levels were observed between stage I/II and stage III/IV ovarian cancer, but high TRAIL expression was associated with longer survival (20). DR4 was an independent prognostic factor for disease-free survival in 129 patients with stage II/III colon cancer (21). In non–small cell lung cancer, DR5-positive staining was associated with increased risk of death (22). Similarly, high DR5 expression in breast cancer strongly correlated with decreased survival using tissue microarrays containing specimens from 655 breast cancer patients (23).

Clinical studies. Clinical studies with compounds directed at TRAIL receptors are done as phase I study with rhTRAIL.
(Genentech, South San Francisco, CA) and with one agonistic TRAIL-DR4 antibody and two agonistic TRAIL-DR5 antibodies (all Human Genome Sciences, Rockville, MD). For the clinical study with rhTRAIL, no data are yet available. The TRAIL-DR4 antibody was well tolerated in phase I studies. The maximum tolerated dose was not yet reached with data up to 10 mg/kg per cycle. The mean terminal elimination half-life was 16 days. Stable diseases occurred in heavily pretreated/refractory patients (24, 25). Phase II studies in 38 non–small cell lung cancer patients showed that 10 mg/kg doses i.v. per 21 days was well tolerated and gave stable disease in 29% (26). In 38 colon cancer patients, stable disease was observed in 31.6% (27). In a phase II study with 40 non-Hodgkin’s lymphoma patients, one complete and two partial tumor responses, all in follicular lymphoma patients, occurred (28). This study proves that the TRAIL-DR4 antibody has antitumor activity on its own. Based on preclinical data showing potentiation of chemotherapy effect, studies are ongoing in which TRAIL-DR4 antibody is added to gemcitabine/cisplatin or paclitaxel/carboplatin combination regimens (29, 30). In these studies, no pharmacokinetic interaction is observed and the optimal dose is not yet known.

Several studies have been done with TRAIL-DR5 antibodies (i.e., two phase I studies with ETR2 and a phase I with TR2J), but no data of the TR2J study are available yet. With the ETR2, one ongoing study in 28 patients noticed minimal toxicity at doses of 10 mg/kg every 21 days (31). Eight patients experienced a stable disease after two cycles. In the second study, ETR2 was well tolerated at doses of 10 mg/kg every 2 weeks (32).

Early results from the clinical studies show that, perhaps in contrast to the expectations, these drugs can be administered safely at relevant doses to patients. As rhTRAIL and agonistic DR4 and DR5 antibodies differ in pharmacokinetic behavior and target DR4 and DR5 versus DR4 or DR5, it is too early to decide which of these TRAIL pathway targeted drugs is to be preferred in the clinic.

The effect of recombinant osteoprotegerin was studied in patients with juvenile Paget’s disease (33). This disease is characterized by an accelerated bone turnover due to inactivating mutations in the gene encoding osteoprotegerin. Recombinant osteoprotegerin increased radical bone mass. It is unknown whether this might potentiate tumor development.

### Clinical Translational Implications

Interestingly, premalignant lesions of the cervix and colon adenomas also overexpress DR4 and DR5 (34, 35). In addition, in vitro exposure of these premalignant tissues to TRAIL can induce apoptosis. This opens opportunities for secondary prevention when the TRAIL receptor targeted compounds can be administered safely.

There is ongoing research directed at the development of more specific and more potent TRAIL analogues (36, 37).
Initially, TRAIL receptor targeted drugs were especially considered as a relevant adjunct to chemotherapy. However, with the availability of numerous smart drugs, targeting relevant players along the TRAIL signaling pathway combinations of several of these drugs, also based on additive effects in preclinical models, seems of major interest. Interesting combinations (Fig. 2) are envisioned with the following:

1. Proteasome inhibitors as the proteasome is responsible for the degradation of the vast majority of intracellular proteins, including proapoptotic proteins, and thus affects multiple signaling pathways within cells (38).
2. Histone deacetylase inhibitors as they induce differentiation and/or apoptosis in a variety of cell types by activating transcription of target genes. Histone deacetylase inhibitors synergize with TRAIL by, for example, inducing TRAIL-DR4 and TRAIL-DR5 through nuclear factor-κB activation and some proapoptotic members of the Bcl-2 family and engaging the mitochondrial pathway (39).

Inhibitors of antiapoptotic Bcl-2 family members (Bcl-2 and Bcl-XL) or inhibitors of apoptosis protein that thus have the capacity to remove roadblocks on the TRAIL signaling pathway (Fig. 2).

Given the large number of proteins that play a role in TRAIL-induced apoptosis, it can be envisioned that tumor profiling of the relevant genes will be major importance for patient tailored therapy with these drugs.

References

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