TNF-Related Apoptosis-Inducing Ligand (TRAIL): A Potential Candidate for Combined Treatment of Hematological Malignancies

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Abstract: TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF gene superfamily, which induces apoptosis through engagement of death receptors. TRAIL is unusual as compared to the other cytokines of this family, as it interacts with a complex system of receptors consisting of two pro-apoptotic death receptors (TRAIL-R1 and TRAIL-R2) and three decoy receptors (TRAIL-R3, TRAIL-R4 and osteoprotegerin). Moreover, with respect to other members of the TNF superfamily, such as CD95L and TNF-α, TRAIL has generated great interest as a potential tumor-specific cancer therapeutic because as a stable soluble trimer it selectively induces apoptosis in many transformed cells but not in normal cells. Of note, TRAIL cytotoxicity is at least partially independent of the major systems involved in resistance to chemotherapy, such as p53 wild-type function and multidrug resistance (MDR) genes. Since one fundamental problem of most cancers is the development of multiple mechanisms of resistance, which progressively reduce or suppress the therapeutic efficacy of conventional chemotherapy, new therapeutic approaches that either restore the pro-apoptotic activity of chemotherapeutic drugs or by-pass the mechanisms of resistance are highly desirable. This review will focus on the potential of TRAIL for its application in the therapy of hematological malignancies, used either alone or in combination with chemotherapy. The scenario emerging from the literature is that the treatment and management of hematological malignancies will require the rational combination of TRAIL plus conventional or new drugs in a regimen that would optimize the anti-neoplastic activity in malignant cells resistant to chemotherapy through restoration of the pro-apoptotic activity of TRAIL.

Key Words: TRAIL, leukemia, multiple myeloma, tumor chemoresistance, signal transduction, NF-kB, apoptosis, caspases.

INTRODUCTION

The recently discovered ligand TRAIL, also known as Apo-2 ligand (L) [1, 2], is a 40 kDa protein that is structurally related to the TNF family of cytokines. TRAIL is expressed as a type-II transmembrane protein; however, its extracellular domain can be proteolytically cleaved from the cell surface [3]. TRAIL interacts with four high-affinity transmembrane and one soluble receptors belonging to the apoptosis-inducing TNF-receptor (R) family. Two of the receptors that bind TRAIL contain cytoplasmic "death domains" and signal apoptosis: TRAIL-R1 and TRAIL-R2. The other three receptors appear to act as "decoys". TRAIL-R3 and TRAIL-R4 have homology to the extracellular domains of TRAIL-R1 and TRAIL-R2. TRAIL-R4 has a truncated non-functional cytoplasmic death domain, while TRAIL-R3 lacks a cytosolic region and is anchored to the plasma membrane through a glycophospholipid moiety. Both receptors are therefore incapable of transmitting an apoptosis signal [4, 5]. The soluble TNF-R family member osteoprotegerin (OPG) was initially discovered to bind the TNF superfamily member RANKL, but later it was found to bind TRAIL. However, a biological connection between OPG and TRAIL remains to be firmly established [5]. Examination of cancer cell lines and tumors failed to provide any correlations between the expression of decoy receptors and TRAIL resistance. However, almost all of these studies relied on detecting mRNA rather than looking for cell surface expression of the proteins. It is possible that even detection of receptors by immunoblot may be misleading since the decoy receptors may localize within the cell rather than on the cell surface [4, 5]. Therefore, it is not fully clear how widespread the decoy receptor surface expression is in tumor or normal cells, or how these receptors modulate TRAIL signaling.

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PROMISING ANTI-TUMOR PROPERTIES OF TRAIL

One fundamental problem of most cancers, including hematological malignancies, is the development of multiple mechanisms of resistance, which progressively reduce or suppress the therapeutic efficacy of conventional radio/chemotherapy. Most chemotherapeutic drugs can induce tumor cell death by apoptosis [6]. The main so-called "intrinsic" pathway of apoptosis induction triggered by chemotherapeutic drugs involves activation of procaspase 9 in the cytosol after release from the mitochondrial intermembrane space. At least in some cell types, anticancer drugs also upregulate the expression of death receptors and sensitize tumor cells to their cognate ligands, which activate the "extrinsic" pathway of apoptosis. The extrinsic pathway involves death receptor engagement, death-inducing signaling complex (DISC) formation, proteolytic activation of the

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apical caspases-8 and -10, and consequently, activation of effector caspases-3, -6, and -7. In certain types of cells, effector caspase activation requires amplification of DISC signals by engagement of the intrinsic pathway, which is preferentially activated by DNA-damaging agents such as chemo- and radiotherapeutic agents. A critical step in the intrinsic pathway is the activation (e.g., through p53-dependent gene transcription, or by phosphorylation) and translocation of the Bcl-2 family of proteins that regulate cell sensitivity mainly at the mitochondrial level [6].

Like other members of the TNF ligand family, TRAIL induces apoptosis in a variety of cell lines and primary tumor cells *in vitro*, including several tumor cell lines resistant to chemotherapeutic agents or ionizing radiation, due to mutations in the p53 tumor suppressor gene [5]. In particular, it has been shown that TRAIL is effective in inducing apoptosis in cells derived from cancers of the colon, lung, breast, prostate, pancreas, kidney, skin, central nervous system and thyroid, as well as from leukemia and multiple myeloma (this latter issue will be dealt in detail later) [5]. However, while the therapeutic potential of CD95 (Fas) ligand (L) and TNF-α is limited by their acute toxic effects on normal tissues, TRAIL preferentially induces apoptosis in tumor cells, while sparing most normal cells, suggesting that it may prove to be a powerful cancer therapeutic. Although it is not conclusively established whether TRAIL causes liver toxicity in humans [6, 7], preclinical studies are very promising. Recombinant human TRAIL protein systemically injected in mice and non-human primates promotes potent apoptosis-inducing activity against tumor cells without significant cytotoxicity on normal organs or tissues [8-11]. Moreover, monoclonal antibodies that functionally engage TRAIL-R1 or TRAIL-R2, therefore displaying agonistic activity, also exhibit apoptosis-mediated anti-tumor activity both *in vitro* and in xenograft (mouse) models, without hepatocyte cytotoxicity [12].

It has also underlined the role of TRAIL as mediator of anti-cancer activity of the immune system. Although it has been demonstrated that TRAIL is not found on the surface of resting peripheral blood T (PBT) cells, its expression is dramatically induced when PBT cells are stimulated with anti-CD3 antibodies in the presence of interferons (IFNs) [13]. Of note, these stimulated PBT cells exhibit enhanced cytotoxicity against tumor cells that is TRAIL-dependent. The anti-tumor effects of IFN-α/γ on multiple myeloma is also associated with rapid upregulation of TRAIL, and is blocked by a dominant negative form of TRAIL-R5 [14, 15]. The relevance of TRAIL in these processes is strengthened by data on graft versus-tumor activity in mouse models: in particular, alloreactive T cells can express TRAIL, and TRAIL deficiency is associated with a significantly lower graft-versus-tumor effect, but with no important differences in graft-versus-host disease, a major complication of allogeneic hematopoietic cell transplantation [16]. The seminal study by Schmaltz *et al*. underlines the role of T lymphocytes expressing TRAIL as pivotal effectors in graft-versus-tumor activity [16]. Therefore, strategies aimed at increasing TRAIL expression and/or activity of donor T cells could enhance the anti-tumor effect of allogeneic hematopoietic cell transplantation. More generally, in addition/alternative to the use of recombinant TRAIL, the positive modulation of TRAIL on effector cells can be exploited as boost of immune surveillance toward malignancies [17].

TRAIL AND HEMATOLOGIC MALIGNANCIES

The cytotoxic activity of TRAIL has been evaluated in different types of hematological malignancies by different groups of investigators, including us (Table **1**).

Acute and Chronic Leukemias

In general, the activity of TRAIL used as a single agent in primary acute leukemias is modest. In particular, Clodi *et al*. [18] demonstrated that TRAIL has a modest activity as it killed less than 30% of cells in a limited subset of primary precursor B-cell acute lymphoblastic leukaemias (ALL) within 18 hours compared with killing 75% of Jurkat T lymphoblastoid cell line. Apparently, the sensitivity to TRAIL did not correlate with the pattern of expression of TRAIL receptors, cellular FLICE-inhibitory protein (c-FLIP) [19] or multidrug resistance (MDR) [18**,** 20-22]. Regarding acute myeloid leukemia (AML), only 2/19 AML isolates showed a >10% increase in apoptotic cells following ex-vivo TRAIL treatment [23]. On the other hand, it has been reported that recombinant TRAIL significantly reduced the *in vitro* growth of AML progenitors [24]. Moreover, recombinant TRAIL significantly reduced the number of myeloid colonies (CFU-GM) and clusters from patients with myelodysplastic syndromes (MDS) and chronic myeloid leukemia (CML) [24-26], suggesting that TRAIL has cytotoxic activity on the growth of at least a subset of patients affected by MDS and CML.

The activity of TRAIL has been investigated also on low-grade chronic lymphocytic leukemias (CLL) [27, 28]. B-CLL represents quintessential example of human malignancies that are caused primarily by defects in the normal pathways for apoptosis. These contribute to chemoresistance, rendering tumor cells less sensitive to the cytotoxic actions of currently available anticancer drugs, and can also promote resistance to cellular immune responses. Olsson *et al*. [27] found TRAIL-R1 and -R2 death receptor, and TRAIL-R3 and -R4 decoy receptor mRNA expression in most of the 57 B-CLL patients studied. Despite TRAIL death receptor expression, B-CLL cells were relatively resistant to induction of apoptosis by recombinant human TRAIL. Western blot analysis showed higher constitutive expression of c-FLIP in B-CLL as compared to normal tonsillar B cells. Consistently, MacFarlane [28] demonstrated that resistance to TRAIL was upstream of caspase-8 activation. In fact, in TRAIL-treated B-CLL cells, both caspase-8 and c-FLIP were cleaved to form two stable intermediates of approximately 43 kDa, which remained associated with the DISC, and caspase-8 was not further processed to its active heterotetramer. Taken together, these findings suggest the involvement of FLIP in the resistence of B-CLL cells to TRAIL-induced apoptosis and highlight the possibility of sensitizing B-CLL cells to TRAIL by modulation of c-FLIP levels.

Multiple Myeloma

A number of studies from several groups of investigators allowed to clearly establish that multiple myeloma (MM) is

Table 1. Trail and Hematological Malignancies

Pathology	Cell Types	Effects of TRAIL (+/- Drugs)	References
ALL	Primary bone marrow (BM) cells	Induction of apoptosis	Clodi *et al.* [18]
AML	Primary BM and peripheral blood (PB) cells	Induction of apoptosis in 2/19 patients; Fludarabine, Ara-C, Daunorubicin sensitize cells to TRAIL-induced apoptosis.	Jones *et al* . [23]
AML, CML, MDS	Primary BM cells	Reduction of number of colonies and clusters.	Plasilova *et al.* [24]
MDS	Primary BM cells	Induction of apoptosis in liquid BM cell cultures; decrease number of colonies in LTMC from advanced MDS and increase number of colonies in less advanced MDS.	Zang *et al.* [25]
CML, ALL	Primary BM and PB cells and cell lines	Induction of apoptosis in 6/10 primary ALL cultures, in 5/7 AML lines and in 2/5 CML-BC lines.	Uno *et al.* [26]
B-CLL	Primary B cells from PB	Resistance to TRAIL-induced apoptosis; Actinomycin D sensitizes B cells to TRAIL-induced apoptosis.	Olsson *et al.* [27]; MacFarlane. [28]
MM	Cell lines; primary flow sorted CD38 ^{bright}/CD45^{-} myeloma cells from BM	Induction of apoptosis; synergy between Arsenic trioxide and TRAIL in inducing apoptosis.	Gazitt *et al.* [29, 30, 33]; Liu *et al.* [31, 32]
MM	Cell lines	Synergy between Adriamycin and TRAIL in inducing apoptosis.	Jazirehi *et al.* [34]
MM	Chemotherapy and Dexametasone resistant or sensitive cell lines, flow sorted CD38 ^{bright}/CD45^{-} myeloma cells from BM	Induction of apoptosis in cell lines and in primary tumour cells (irrespective of refractoriness to Dexametasone); synergy between Doxorubicin and TRAIL in inducing apoptosis; NF-kB inhibitors (PS341 and SN50) sensitizes MM cells to TRAIL-induced apoptosis.	Mitsiades *et al.* [35]
MM	Cell lines, flow sorted CD38+/138+ myeloma cells from BM	Induction of apoptosis; reduction of CD138+ myeloma cells in 31% of patients; sinergy between immunomodulatory–analog of Thalidomide ImiD1and TRAIL in inducing apoptosis.	Lincz *et al.* [36] Mitsiades *et al.* [38] Mitsiades *et al.* [71]
AML	Cell line (HL-60) and CD34+-derived monocytic cells	Induction of apoptosis and induction of monocytic maturation.	Secchiero *et al.* [90]
AML	Cell lines	Increase of TRAIL-induced apoptosis in cells pre-treated with Etoposide, Ara-C, Doxorubicin followed by TRAIL treatment.	Wen *et al.* [86]
AML, CML	Cell lines, freshly isolated AML blasts (CD34+) from PB	Resistance to TRAIL-induced apoptosis in freshly isolated AML cells; Triterpenoids (CDDO, CDDO-m) sensitise 2/4 leukemia lines to TRAIL-induced apoptosis.	Suh *et al.* [87]
AML, CML	Cell lines	Resistance to TRAIL-induced apoptosis in Bcr-Abl positive leukemic cells; STI-571 sensitizes cells to TRAIL-induced-apoptosis.	Nimmanapalli *et al.* [88]

the most susceptible hematological malignancy to recombinant TRAIL [29-39] used alone as well as in combination therapy (as discussed in detail later). Concerning TRAIL used as single agent, Gazitt *et al*. demonstrated for the first time in 1999 that TRAIL induces substantial apoptosis in freshly isolated, flow-sorted MM cells obtained from different MM patients, while it is not cytotoxic on purified CD34+ hematopoietic stem cells [29, 30]. Other studies demonstrated that TRAIL effectively kills MM cells *in vitro* irrespective of refractoriness to dexamethasone and chemotherapy [35, 36]. A rapid cleavage of caspases-8, -9, -3, and -6 was observed upon TRAIL treatment in sensitive MM cells. These phenomena were not observed or were significantly delayed in TRAIL-resistant MM cells, suggesting that resistance to TRAIL-apoptosis may arise from inhibition at the level of apical caspase-8 activation. Higher levels of expression for various apoptosis inhibitors, including c-FLIP, were indeed present in TRAIL-resistant MM cells [36, 37].

In MM cell lines, TRAIL is a potent inducer of apoptosis independent of Bcl-2 [31]. It is also of particular interest that overexpression of p53, by using adenovirus-p53 (Ad-p53), induce an increase in the expression of TRAIL-R1/-R2 in a subset of Ad-p53-sensitive myeloma cell lines [31]. The relevance of the p53 status for the response to TRAIL-induced apoptosis, was further outlined from the same group, by using myeloma cell lines with wild-type p53, and with null or dysfunctional expression of p53, caused by single nucleotide substitutions leading to non-conservative amino acid changes [32, 33]. In particular, treatment of these cell lines with arsenic trioxide induced apoptosis in two different

modes depending on p53 status: *i*) in cells with mutated/null p53, arsenic trioxide induced G(2)/M arrest, activation of caspase-8 and -3, and rapid and extensive apoptosis, concomitant with induction of TRAIL; *ii*) in cells expressing wild-type p53, arsenic trioxide induced G(1) arrest and delayed apoptosis with activation of caspase-9 and -3, while TRAIL was not induced. Of note, in both cell types arsenic trioxide upregulated TRAIL-R1 and -R2, and synergizes with exogenous recombinant TRAIL in the induction of apoptosis, suggesting the combined used of these two agents in clinical setting in myeloma patients [32, 33].

An important aspect in considering TRAIL as a potential therapeutic candidate molecule is its potential cytotoxicity on normal bone marrow. Although several studies have demonstrated that TRAIL is not cytotoxic on normal clonogenic hematopoietic progenitors [5, 25, 30], different groups of investigators, including us, have demonstrated that TRAIL selectively affects erythroid development by specifically targeting immature erythroblasts, characterized by a low-intermediate expression of surface glycophorin A [40-42]. In this respect, we have demonstrated that TRAIL negatively affects erythropoiesis by a twofold mechanism: induction of apoptosis [41] and anti-differentiative activity [42]. The latter effect is mediated by the ability of TRAIL to activate the ERK pathway in immature erythroid cells, which selectively express TRAIL-R2. It is noteworthy that an upregulation of surface TRAIL in malignant plasma cells has also been described in cells obtained from patients affected by MM and it might account for the high frequency of anemia and other complications observed in these patients [43]. Therefore, although TRAIL is a very promising agent for the treatment of MM, these findings add a cautionary note to the potential treatment of MM patients bearing anemia with recombinant TRAIL.

DISSECTING TRAIL SIGNALING: TOOL TO IMPROVE THE TRAIL-BASED ANTI-TUMOR THERAPY

Recent advancement in our understanding the events that occur downstream to the engagement of the TRAIL receptors (Fig. **1**) clarify TRAIL proper function as pro-apoptotic drug and give us a tool to better design and assay TRAIL-based combination therapies. Of note, it was originally described that both TRAIL-R1 and -R2 receptors, when engaged by TRAIL, activated caspase-8 mainly and independently of each other [44-46]; subsequent study demonstrated that also caspase-10 is activated by TRAIL irrespectively of caspase-8 and that also caspase-10, as caspase-8, is an important apical caspase involved in apoptosis of malignant cells [47-52, reviewed in 53, 54]. In fact, defects in apoptosis pathways (such as caspase-8 or caspase-10 mutations [47-52]) make important contributions to chemoresistance, suggesting a need to restore apoptosis sensitivity or to identify alternative pathways for apoptosis induction. In this

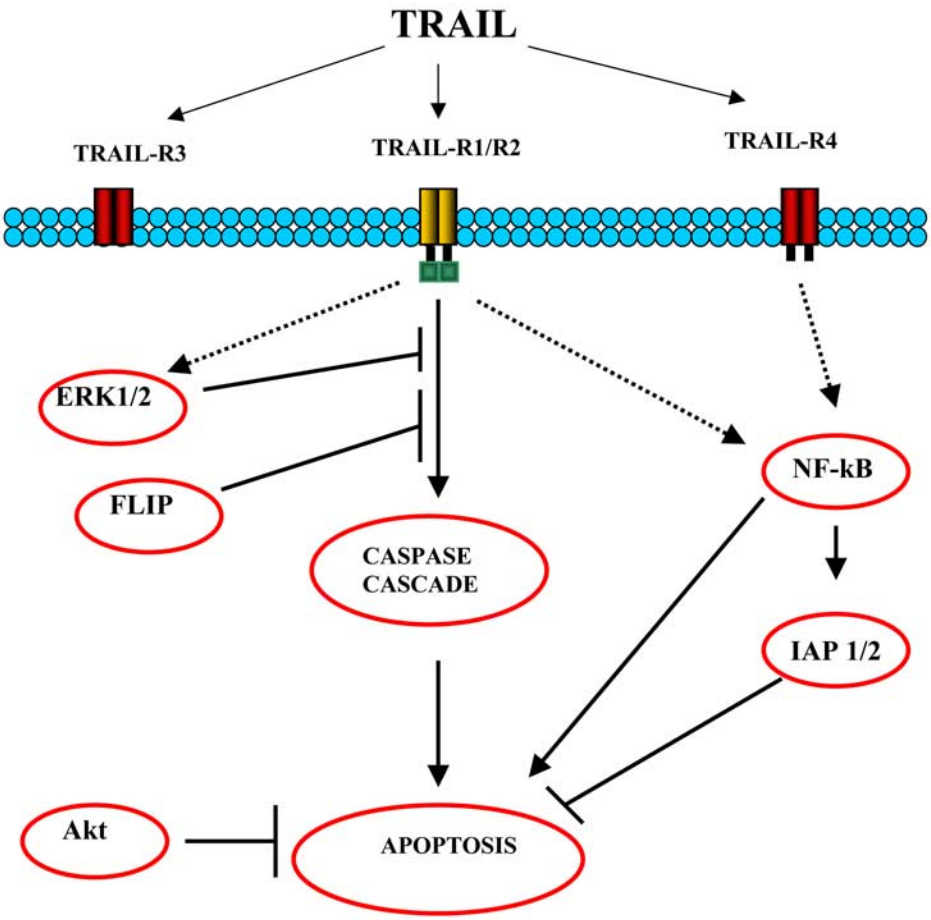

Fig. (1). A depiction of the major pathways that have been implicated in the modulation of TRAIL-induced apoptosis.

regard others and we have demonstrated a role of nitric oxide and related compounds in the cytotoxic effect of TRAIL [55, 56]. Thus, pharmacological activation of nitric-oxide synthase can synergize with TRAIL and/or overcome drug resistance in hematological malignancies [55, 56]. These data underscores the importance of discovering new pathways that are involved in mediating TRAIL activity.

TRAIL and NF-Kb/Akt Pathways

One of the most recent advances in dissecting TRAIL signaling is represented by the discovery of the role of NF-kB pathway in drug-dependent sensitivity and survival of malignant cells [57, 58]. NF-kB is potently and rapidly activated after TNF-α binding to TNF-receptor (R)-1, generating a pro-survival signal that must be overcome in many cell lines to unable TNF-α to induce apoptosis [58]. NF-kB has been reported to induce expression of c-FLIP, Bcl-Xl and inhibitors of apoptosis (IAPs), which are considered to be responsible for its ability to protect cells from death [59-64]. While TRAIL can also activate NF-kB, this stimulation is significantly attenuated and delayed as compared to that of TNF-α. Nevertheless, in cancer cell lines with high constitutive NF-kB activity, specific downregulation of NF-kB significantly sensitized the cells to TRAIL [65-70]. In this respect, it has been demonstrated that insulin-like growth factor-1 (IGF-1) stimulates sustained activation of the transcription factor NF-kB and of the serine/threonine kinase Akt in MM cells, and decreases TRAIL-sensitivity of these cells [38]. Consistently with the potential role of these pathways in counteracting apoptosis induction by TRAIL (Fig. (**1**)), as well as by chemotherapy, the NF-kB inhibitor SN50, as well as the Akt inhibitor IL-6-Hydroxy-methyl-chiro-inositol 2-(R)-2-O-methyl-3-O-octadecylcarbo-nate, abrogated the protection against TRAIL and/or chemotherapy induced-apoptosis by IGF-1 [38, 39]. Interestingly, thalidomide, an established anti-MM agent, inhibits NF-kB activity as part of its diverse actions, and potentate the activity of TRAIL on MM [39, 71]. Taken together these finding define important roles of NF-kB/Akt pathways in modulating tumor cell responsiveness to TRAIL and provide the framework for targeting NF-kB/Akt activities in novel TRAIL-based combination therapy (i.e., with thalidomide) to improve the outcome in MM.

Although in some contexts NF-kB can moderate sensitivity to TRAIL, more recent studies have outlined the complexity of the interplay between NF-kB and TRAIL/TRAIL-R system [70, 72-75]. In fact, it has been shown that the transcription of TRAIL itself as well as of TRAIL-R1, TRAIL-R2 [72-75] and TRAIL-R3 [70] is upregulated by NF-kB family members (Fig. (**2**)). It has been demonstrated that etoposide and doxorubicin induce the NF-kB-dependent expression of both pro- and anti-apoptotic proteins including TRAIL and its death receptor, TRAIL-R2, and IAPs. Inhibition of NF-kB activation in response to genotoxic agents resulted in loss of cell surface expression of TRAIL and TRAIL-R2, aggressive growth and chemotherapy resistance of tumors in nude mice. In the same study, it was shown that also the sensitivity of normal lung and breast epithelium to undergo genotoxin-induced apoptosis correlates strongly with cell surface expression of TRAIL [72]. Moreover, NF-kB has been shown to be essential for upregulating TRAIL mRNA and protein expression in T cells by T cell receptor mimetics, such as phytohemagglutunin and anti-CD3/CD28 antibody [75].

Thus, NF-kB family is involved in mediating both pro-survival and pro-apoptotic activity (Fig. (**1**)). Recent studies have began to address the role of single members of the NF-kB family in mediating TRAIL cytotoxicity [73, 74], demonstrating that p65/RelA subunit acts as a survival factor by inhibiting caspase-8 and TRAIL-R1 and TRAIL-R2 expression and enhancing expression of prosurvival c-IAP1 and c-IAP2 after TRAIL treatment [73]. By comparison, overexpression of p75/c-Rel subunit of NF-kB enhances TRAIL-R1, TRAIL-R2, and Bcl-Xs and inhibits c-IAP1, c-IAP2, and survivin after TRAIL treatment [74]. Therefore, the dual function of NF-kB, as an inhibitor or activator of apoptosis, depends on the relative levels of RelA and c-Rel subunits (Fig. (**2**)).

Besides the NF-kB transcription factors, TRAIL expression is also regulated by Akt activity, which on one hand blocks TRAIL cytotoxicity in leukemic cells [76], as described before, and, on the other hand, downregulates TRAIL expression through phosphorylation and inhibition of FOXO family of forkhead transcription factors [58, 77] (Fig. (**2**)). Recently, Ghaffari *et al*. [78] reported that hematopoietic cytokines such as IL-3 and erythropoietin in normal cells, as well as Bcr-Abl oncoprotein in transformed CML cells, inhibit transcription of TRAIL. Using small interfering RNAs, it was shown that the inhibition of TRAIL function is sufficient to partially rescue cytokine-deprived cells from apoptosis [77]. Based on these findings, it has been suggested that the Bcr-Abl-induced decrease of TRAIL expression in hematopoietic cells may be involved in the tumorigenicity of CML, by increasing survival of the tumor cells [79].

TRAIL and Mitogen Activated Protein Kinase (MAPK) Pathway

MAPK form a large family of serine-threonine protein kinases conserved through evolution [80, 81]. In mammalian cells, three major MAPK cascades have been identified: extracellular signal regulated kinases (ERK), c-Jun amino-terminal kinases (JNK) or stress-activated protein kinases (SAPK), and p38 MAP kinase (p38). In particular, the classical MAP kinases (ERK1 and ERK2) are activated by a variety of cell growth and differentiation stimuli and play a central role in survival and mitogenic signaling. A number of studies performed in different cell models, including Hodgkin disease, have underlined the importance of the ERK pathway in counteracting the cytotoxic activity of death inducing ligands, and in particular of TRAIL [82-85]**,** thus favoring the survival of malignant cells. In particular, Zheng *et al*. reported that the active phosphorylated form of MAPK/ERK is aberrantly expressed in cultured and primary Hodgkin disease cells [82]. Inhibition of the upstream MAPK kinase (also called MEK) by the small molecule UO126 inhibited the phosphorylation of ERK and demonstrated a dose- and time-dependent anti-proliferative activity in Hodgkin disease cell lines. Furthermore, UO126 potentiated the activity of TRAIL and chemotherapy-induced cell death. Consistently with these findings, Tran *et al*. demonstrated that a number of cell lines insensitive to death

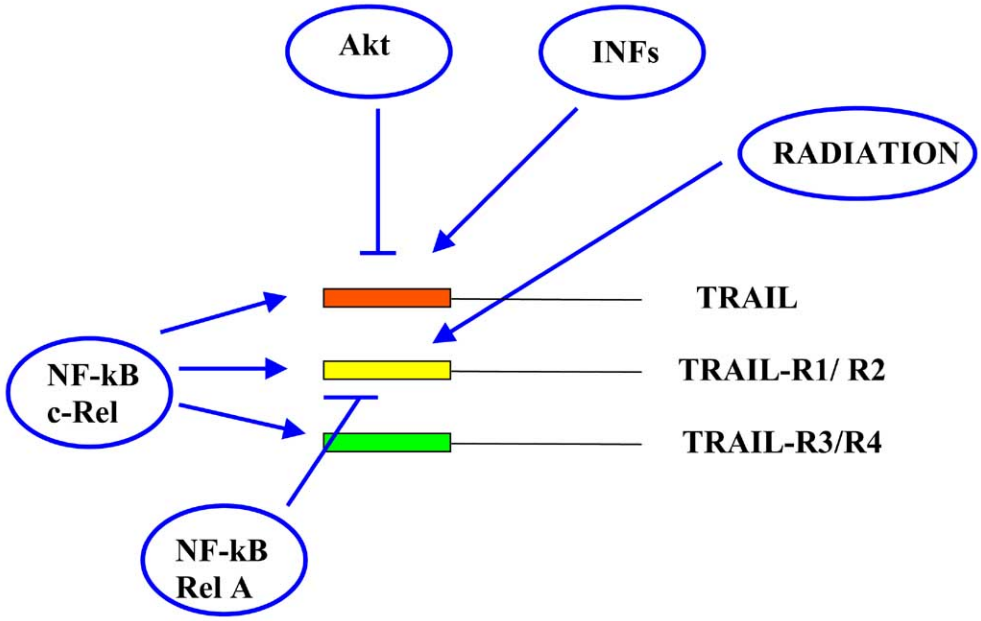

Fig. (2). Pathways that modulate the expression of TRAIL and TRAIL receptors.

inducing ligand-mediated apoptosis are able to redirect the proapoptotic signaling to an anti-apoptotic ERK1/2 signal [83]. In fact, activation of TRAIL-R rapidly induced subsequent ERK1/2 activation, which prevented caspase-8 activation. These findings indicate that ERK1/2 has a dominant protecting effect over apoptotic signaling from the death receptors (Fig. (**1**)). Consistently with a widespread role of the ERK pathway in hematological malignancies, it has also been shown that 14 out of 18 blast samples taken from individuals affected by AML show high levels of ERK1/2 [84]. The downmodulation of ERK activation in the 14 cases of AML with 20-40 μM of PD98059, a selective inhibitor of MEK1 phosphorylation, induced a significant decrease of blast cell proliferation compared with untreated controls. In contrast, the proliferation of blast cells that expressed low or undetectable levels of ERK activity was not inhibited. The primary effect of ERK downmodulation was a cell cycle arrest followed by the apoptosis of a significant percentage of the leukemic blasts [84].

Interestingly, we have also demonstrated that TRAIL itself activates the ERK pathway in primary normal erythroid progenitors [42], suggesting that the ability to activate the ERK pathway is a common feature of the TRAIL/TRAIL-R system. Thus, the ability of TRAIL to activate the pro-survival ERK pathway in malignant cells may be regarded as a feedback mechanism to temper the pro-apoptotic activity of TRAIL.

TRAIL IN COMBINATION THERAPY IN HEMATOLOGICAL MALIGNANCIES

The aforementioned signaling pathways are often modified by chemotherapeutic compounds and by ionizing radiations, among other stimuli [85]. As consequence, while the potential therapeutic of TRAIL as a single agent in hematological malignancies is limited perhaps to a subset of MM patients, several studies have shown that the association of conventional chemotherapy and/or ionizing radiations with TRAIL display additive or super-additive effects with respect to chemotherapy/ionizing radiations or TRAIL used alone (Table **1**).

It is known that various chemotherapeutic drugs have been shown to sensitize tumor cells to members of the TNF-family. However, it is not completely understood whether sensitization by drugs and sensitivity to drugs are related or distinct events. In this respect, Jazirehi *et al*. showed that adriamycin-resistant tumor cells could be sensitized by adriamycin to TRAIL-mediated apoptosis, by upregulating procaspase-9 [34]. Wen *et al*. demonstrated that sequential treatment of HL-60 cells with etoposide, Ara-C, or doxorubicin followed by TRAIL induced significantly more apoptosis than treatment with TRAIL, etoposide, doxorubicin, or cytosine arabinoside (Ara-C) alone, or cotreatment with TRAIL and the antileukemic drugs, or treatment with the reverse sequence of TRAIL followed by one of the antileukemic drugs. This because treatment with etoposide, Ara-C, or doxorubicin up-regulates TRAIL-R2 levels, in a p53-independent manner, and thus sensitizes human acute leukemia cells to TRAIL-induced apoptosis [86]. Jones *et al*. [23] demonstrated a positive action of TRAIL in combination with several drugs on cells isolated from patients with AML. In particular, incubation with TRAIL combined with fludarabine, Ara-C or daunorubicin resulted in additive or super-additive apoptosis induction in approximately half of the isolates. The ability of TRAIL and daunorubicin to induce super-additive apoptosis correlated with the ability of these agents to activate caspase-8. Thus, co-administration of TRAIL with conventional cytotoxic drugs may be of therapeutic value also in some patients with AML [23]. This favorable effect of TRAIL in combination with chemotherapeutic drugs toward AML cells is strengthened by the findings of Suh *et al*. on triterpenoids, a class of naturally occurring and synthetic compounds with demonstrated antitumor activity, including 2-cyano-3, 12-dioxoolean-1, 9-dien-28-oic acid (CDDO) and its methyl ester (CDDO-m).

CDDO or CDDO-m induced substantial increases in cell death in 5 out of 10 samples of primary AML blasts, and sensitized AML blasts to TRAIL cytotoxicity [87]. Other reports highlight the potentiation of TRAIL-induced apoptosis in Bcr-Abl positive human acute leukemia cells co-treated with STI-571 [88] and in primary effusion lymphoma in combination with azidothymidine [89] thanks to a positive effect on the apoptotic machinery as well as inhibition of antiapoptotic pathways. Moreover, the therapeutic potential of TRAIL in hematological malignancies is likely to be more complex than inducing apoptosis. In fact, we have recently demonstrated that TRAIL promotes monocytic maturation of human HL-60 leukemia cells [90]. The ability of TRAIL to induce monocytic maturation was uncoupled to its ability to induce apoptosis and, therefore, it may be of clinical relevance, especially if associated to other inducers of differentiation, such as all-*trans*-retinoic acid.

Combination of TRAIL and ionizing radiation has been shown to induce additive or synergistic apoptotic effects and eradication of clonogenic tumor cells thereby increasing the therapeutic efficacy of ionizing radiation [91-94]. The major goal of modern radiation in oncology is the achievement of a maximal tumor control with minimal normal tissue damage. However, normal tissue tolerance may preclude the application of tumoricidal radiation doses. In order to overcome this limitation, attempts to minimize the required radiation dose by reducing the number of malignant clonogenic cells are promising. For this purpose, TRAIL is a very good candidate, since it triggers apoptosis even in cells not undergoing apoptosis in response to radiation. Our and other groups have demonstrated that additive or synergistic apoptotic effects of ionizing radiation+TRAIL can be explained, al least in part, by the increase in expression of TRAIL-R1 and -R2, induced by radiation, thus sensitizing target cells to the effect of TRAIL [92, 93].

CONCLUSIONS

TRAIL protein offers great promise as a cancer therapeutic in hematological malignancies, but also in solid tumors, given that TRAIL has shown cytotoxic activity also in tumor xenograft models. The progress made in understanding the signaling of the TRAIL pathway is pivotal for further development of TRAIL as an effective anti-neoplastic drug alone or, more feasibly, in combination with either other anti-neoplastic drugs or with radiation therapy.

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