

TRUNDD, a new member of the TRAIL receptor family that antagonizes TRAIL signalling

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Received 12 January 1998

Abstract TRAIL/Apo-2L induces rapid apoptosis of a variety of tumor cell lines. A family of tumor necrosis factor receptor-related molecules have been identified as receptors for TRAIL. Herein, we report the identification of another member of the TRAIL receptor family, TRUNDD (TRAIL receptor with a truncated death domain). The TRUNDD transcript was detected in multiple human tissues. TRUNDD is highly homologous to all known TRAIL receptors and has an extracellular TRAIL-binding domain but lacks a functional intracellular death domain and does not induce apoptosis. Consistent with an inhibitory role, ectopic expression of TRUNDD attenuated TRAIL-induced apoptosis in mammalian cells.

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Key words: Tumor necrosis factor receptor; TRAIL receptor; Apoptosis; Death domain; Decoy receptor

1. Introduction

Apoptosis, or programmed cell death, is an intrinsic cell suicide mechanism of fundamental importance to development and homeostasis in all multicellular organisms [1]. Members of the tumor necrosis factor (TNF) ligand family, TNF α and FasL are well-studied physiologic activators of apoptosis and play an important role in inflammatory and immune responses [2,3]. Recently, an additional cytotoxic ligand termed TRAIL/Apo-2L was characterized [4,5]. TRAIL is expressed in a variety of human tissues and induces apoptosis in many transformed cell lines. Three receptors for TRAIL have been identified (Fig. 4) [6–12]. DR4 and DR5 are signalling receptors and fully capable of engaging the death pathway. Previously described death receptors, including TNFR1, Fas and DR3, contain a cytoplasmic death domain that binds to the death domain-containing adapter molecule FADD [13]. In turn, FADD recruits and activates the apical death protease designated caspase-8. This initiates a proteolytic cascade resulting in cleavage of death substrates and eventual demise of the cell [14]. Surprisingly, DR4 (TRAIL-R1) and DR5 (TRAIL-R2), despite containing a death domain, induce caspase-dependent apoptosis independent of FADD, suggesting the existence of another death domain-containing adapter molecule [6–8,12]. The third receptor, TRID (DcR1/TRAIL-R3/LIT), is a non-signalling decoy receptor that is GPI (glycosyl-phosphatidylinositol)-linked to the cell surface and antagonizes TRAIL-induced apoptosis [7,8,11]. DR4 and DR5 are expressed in many human tissues and cancer cell lines.

Remarkably, the decoy receptor (TRID) is differentially expressed: easily detectable in normal human tissues, but not in most tumor cell lines, consistent with a protective role in normal tissues. In keeping with this hypothesis, overexpression of TRID protects mammalian cells from TRAIL-induced apoptosis. Collectively, the data suggest that TRID/DcR1 may play a protective role that allows normal tissues to withstand the potential cytotoxic effect of TRAIL.

Here we report the identification and characterization of another TRAIL receptor, designated TRUNDD (see below). TRUNDD is highly related to existing members of the TRAIL receptor family and binds TRAIL. Unlike DR4 and DR5, TRUNDD lacks a functional death domain. Ectopic expression of TRUNDD attenuates TRAIL-induced apoptosis, in keeping with a role as a dominant negative receptor.

2. Materials and methods

2.1. Construction of mammalian expression vectors

To facilitate detection, TRUNDD (amino acids 56–386) was cloned into pCMV1FLAG (IBI Kodak) as an in-frame fusion to the signal sequence and FLAG-epitope tag encoded by the vector. The cDNA encoding the extracellular domain of TRUNDD (amino acids 56–210) was obtained by PCR and subcloned into a modified pCMV1FLAG vector that allowed for in-frame fusion with the Fc portion of human IgG. DR4-Fc, TNFR1-Fc, Fc, soluble TRAIL and TNF α expression constructs have been described previously [6].

2.2. Northern blot analysis

Human multiple tissue Northern blots (Clontech) were probed with an internal fragment of TRUNDD cDNA according to the manufacturer's instructions.

2.3. Preparation of proteins and in vitro binding assays

Receptor-Fc fusions and soluble ligands were prepared and in vitro binding performed as previously described [6,7].

2.4. Cell death blocking assays

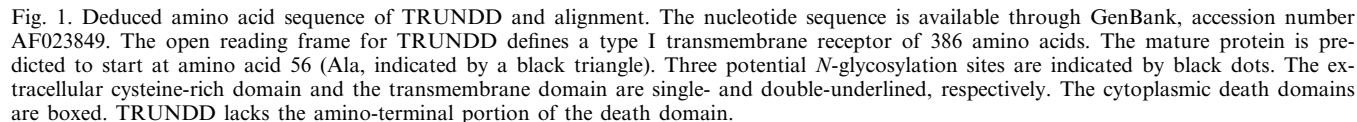
Blocking assays using receptor-Fc fusions were carried out as described [6,7].

3. Results

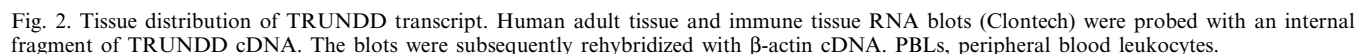
3.1. Molecular cloning and identification of TRUNDD as a novel TNF receptor

Homology searching of EST (expressed sequence tag) databases with the DR5 sequence and subsequent library screening revealed a novel cDNA clone that was identified in four different cDNA libraries. Its open reading frame encoded a protein of 386 amino acids. Protein sequence comparison analysis revealed the molecule to be a member of the TNF receptor family possessing significant homology to DR4, DR5 and TRID/DcR1 (60–70% amino acid identity) (Fig. 1). A long putative signal sequence was present at the

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Since the entire sequence of TRUNDD, especially the ex-



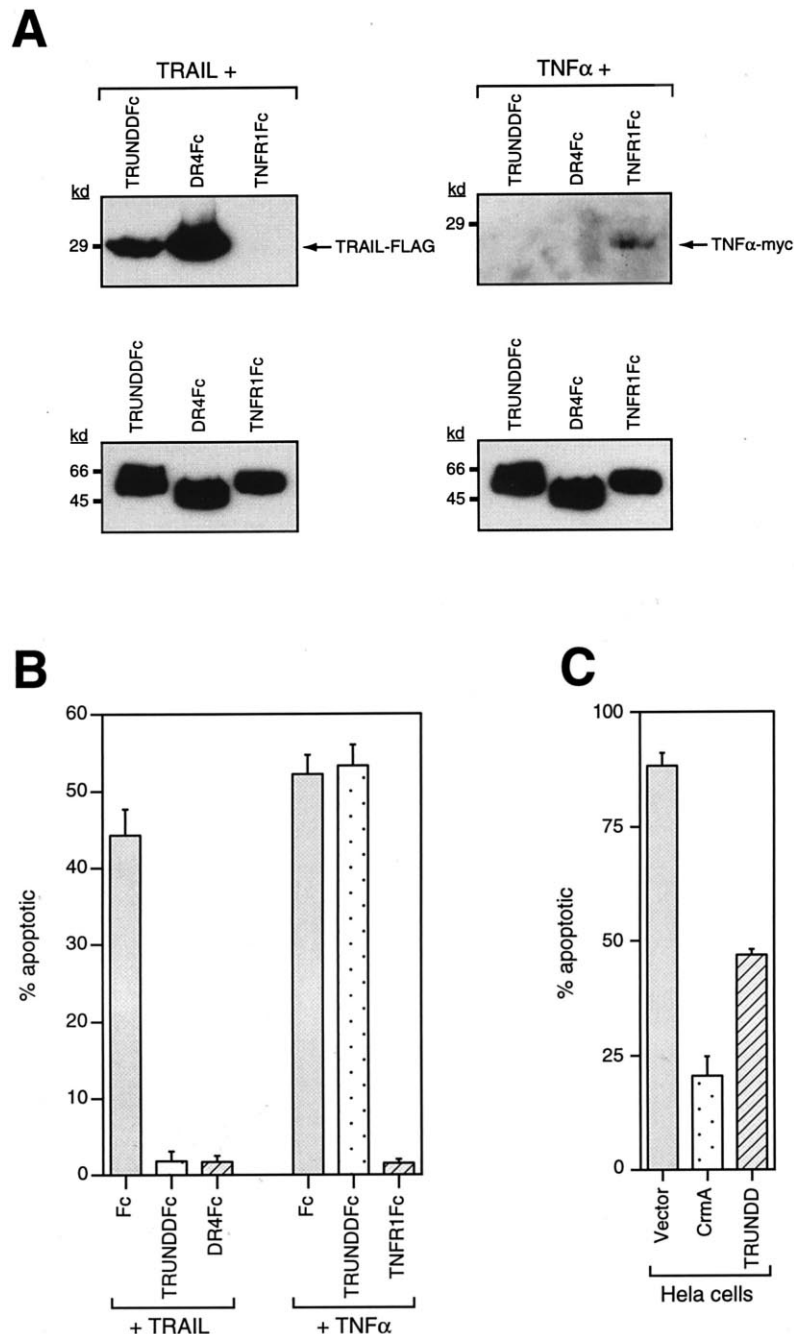


Fig. 3. The extracellular domain (ECD) of TRUNDD binds TRAIL and blocks TRAIL-induced apoptosis. A: The Fc-ECDs of TRUNDD, DR4, TNFR1 and corresponding ligands were used in binding assays as described elsewhere [6,7]. The respective Fc fusions were precipitated with protein G-Sepharose and coprecipitated soluble ligands detected by immunoblotting with antibody to FLAG or Myc epitopes. Bottom panel shows the amount of input Fc fusions present in the binding assays. B: MCF7 cells were treated with soluble TRAIL (200 ng/ml, left) or soluble TNF α (40 ng/ml, right) in the presence of equal amounts of Fc fusions or control Fc as indicated. Cells were stained with DAPI and examined as described [6]. The data (mean \pm S.D.) shown are the percentage of apoptotic nuclei among total nuclei counted ($n=3$). C: TRUNDD protects cells from TRAIL-induced apoptosis. HeLa cells were transfected with TRUNDD, CrmA expression construct or vector alone together with a β -galactosidase reporter construct. Twenty-four hours later, TRAIL was added as indicated. Four hours later, cells were fixed and stained with 5-bromo-4-chloro-3-indoxyl- β -galactopyranoside and examined microscopically as described [6].

tracellular cysteine-rich domains, was highly homologous to that of other TRAIL receptors, we asked if TRAIL could indeed bind TRUNDD. The extracellular domain of TRUNDD was expressed as a secreted chimera fused to the Fc portion of human IgG in 293 cells. Conditioned medium from transfected cells was mixed with bacterially expressed soluble His-FLAG-tagged TRAIL. The resulting complex

was precipitated with protein G-Sepharose and bound TRAIL detected by Western blotting with anti-FLAG antibody. Like DR4, DR5 and TRID, TRUNDD bound TRAIL (Fig. 3A). Corroborating this ability to bind TRAIL was the finding that TRUNDD-Fc, like DR4-Fc, could efficiently block TRAIL-induced apoptosis (Fig. 3B).

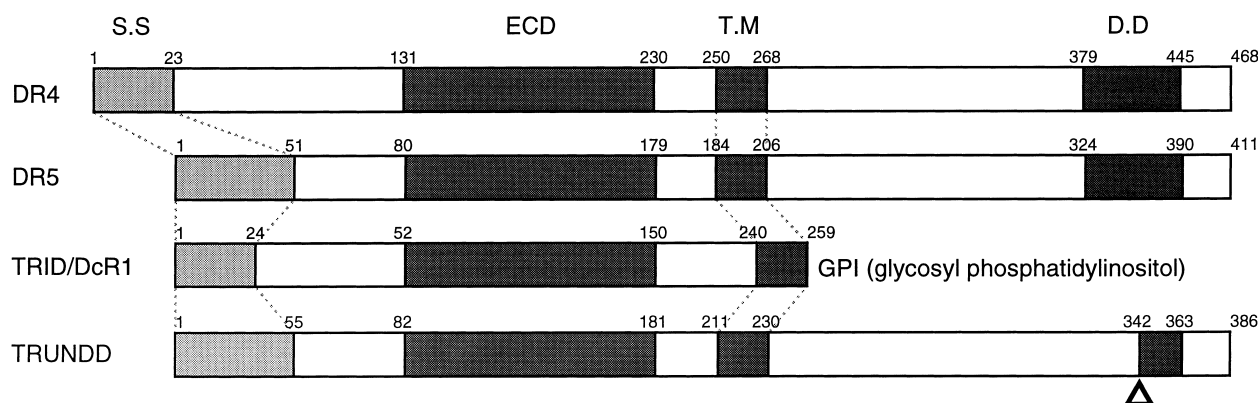


Fig. 4. Members of the TRAIL receptor family. Salient features of the receptor sequences are shown. Locations of the various domains are indicated by numbers. TRID is a GPI-linked cell surface molecule. TRUNDD contains a partial death domain and the truncated segment is indicated by an open triangle. S.S., signal sequence; ECD, extracellular domain; T.M., transmembrane domain; D.D., death domain.

3.4. TRUNDD antagonizes TRAIL signalling in mammalian cells

In keeping with TRUNDD possessing a truncated non-functional death domain (Fig. 1), its overexpression did not induce cell death in mammalian cells (not shown), rather, as might be expected, it could act as a dominant negative receptor antagonizing TRAIL-induced apoptosis (Fig. 3C). Therefore, ectopic expression of TRUNDD, like that of the decoy receptor TRID substantially attenuated TRAIL-induced cell death, suggesting that TRUNDD may function to antagonize TRAIL signalling.

4. Discussion

TRAIL is a newly identified cytotoxic ligand that belongs to the TNF family and rapidly induces apoptosis in a wide variety of tumor cell lines. Unlike Fas ligand, which is predominantly expressed in activated T cells, natural killer cells and at sites of 'immune privilege' including the cornea and testis [17], TRAIL transcripts are constitutively expressed and detectable in a variety of human tissues. Thus, TRAIL must not be injurious to most normal tissues in vivo. Recently, a family of TRAIL receptors have been identified (Fig. 4) [6–12]. Like other known death receptors (including TNFR1, Fas and DR3/Wsl/Apo3/TRAMP/LARD), DR4 and DR5 contain a cytoplasmic death domain that is capable of engaging the cell death pathway [13,18–22]. Paradoxically, the signalling TRAIL receptors DR4 and DR5 are present in both normal tissues and cancer cell lines [6–8], raising the question of why transformed cells are sensitive but normal tissues resistant to TRAIL-induced cell death. Identification of the third member of the TRAIL receptor family, TRID/DcR1, provided a potential solution to this conundrum. TRID/DcR1 has an extracellular TRAIL-binding domain but lacks a cytoplasmic domain. It is expressed in normal tissues, but importantly, not in most cancer lines. TRID acts as an antagonistic decoy receptor since its ectopic expression protects mammalian cells from TRAIL-induced apoptosis and its endogenous expression largely correlates with resistance to TRAIL. Therefore, TRID/DcR1 may in part explain the non-lethality of constitutively expressed TRAIL. It is not known for certain how TRID functions to antagonize TRAIL-induced apoptosis. It is possible that TRID simply binds and sequesters TRAIL

from the signalling receptors DR4 and DR5 or that TRID may oligomerize with these receptors and interfere with their capacity to signal. TRUNDD, the fourth TRAIL receptor may also function as a dominant negative by using a different mechanism. Unlike TRID, TRUNDD contains a cytoplasmic domain with a non-functional death domain. Consistent with this, TRUNDD does not induce apoptosis, but instead, protects mammalian cells from TRAIL-induced death, suggesting that it like TRID may serve as a dominant negative receptor capable of attenuating TRAIL signalling in tissues where it is highly expressed such as adult lung and fetal liver. However, at this early stage we cannot rule out the possibility that TRUNDD may engage other cell signalling pathways. Regardless, these findings indicate that TRAIL signalling in vivo is controlled by a complex receptor mechanism that exists to balance agonist and antagonist functions.

Acknowledgements: We like to thank members of the Dixit lab for helpful discussion, B. Schumann for secretarial help and I. Jones for assistance in preparing the figures. G. Pan holds a Special Fellow Award from the Leukemia Society of America. Supported by NIH Grants ES08111 and DAMD17-96-1-6085.

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