A novel receptor for Apo2L/TRAIL contains a truncated death domain

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Apo2 ligand (Apo2L [1], also called TRAIL for tumor necrosis factor (TNF)-related apoptosis-inducing ligand [2]) belongs to the TNF family and activates apoptosis in tumor cells. Three closely related receptors bind Apo2L: DR4 and DR5, which contain cytoplasmic death domains and signal apoptosis, and DcR1, a decoy receptor that lacks a cytoplasmic tail and inhibits Apo2L function [3–5]. By cross-hybridization with DcR1, we have identified a fourth Apo2L receptor, which contains a cytoplasmic region with a truncated death domain. We subsequently named this protein decoy receptor 2 (DcR2). The DcR2 gene mapped to human chromosome 8p21, as did the genes encoding DR4, DR5 and DcR1. A single *DcR2* mRNA transcript showed a unique expression pattern in human tissues and was particularly abundant in fetal liver and adult testis. Upon overexpression, DcR2 did not activate apoptosis or nuclear factor-κB; however, it substantially reduced cellular sensitivity to Apo2L-induced apoptosis. These results suggest that DcR2 functions as an inhibitory Apo2L receptor.

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Results and discussion

We screened a human fetal lung complementary DNA (cDNA) library by hybridization to a probe based on the extracellular domain (ECD) of DcR1 [4]. We identified two virtually identical cDNA clones encoding a previously undescribed receptor, which we designated DcR2. The clones predict a 386-residue polypeptide precursor (Figure 1a). The clones have a single base difference in codon 310, encoding either a serine residue (TCG) or a leucine residue (TTG). Hydropathy analysis (data not shown) suggests that DcR2 has a type I transmembrane protein topology (Figure 1a). The ECD contains two cysteine-rich regions (Figure 1b), and shows remarkably greater identity to the ECDs of DR4 (55%), DR5 (56%) or DcR1 (67%) than to those of tumor necrosis factor receptor-1 (TNFR1) (26%), Fas/Apo1 (CD95) (27%) [6] or Apo3/DR3 (19%) [7–10] (Figure 1b). The intracellular domain (ICD) also shows greater homology to DR4 (60%) or DR5 (49%) than to TNFR1 (18%), CD95 (14%) or Apo3/DR3 (10%) (Figure 1c). This region contains an apparently truncated 'death domain', which is about one-third of the length of the death domains found in other TNFR superfamily members and in associated adaptor proteins [6]. Notably, five out of six death-domain residues that are essential for signaling by TNFR1 [11], including the position that corresponds to the CD95-inactivating mouse *lpr* mutation [6], are identical or similar in DR4 and DR5, but are absent in DcR2 (Figure 1c). PCR analysis of several cDNA libraries with primers that flank the truncated DcR2 death domain revealed a single product, which matched the corresponding region of our two clones in size and in sequence (data not shown), suggesting that there is one predominant splice form of DcR2.

We investigated the chromosomal location of the human DcR2 gene and its close relatives by radiation-hybrid analysis (Research Genetics) with PCR primers based on the unique 3′ untranslated region of each receptor. *DcR2* was linked to marker SHGC33989 (LOD = 7.2); *DR4* to D8S2127 (LOD = 13.0); *DR5* to DS481 (LOD = 11.1); and *DcR1* to WI6536 (LOD > 3.0). Each of the first three markers is linked to D8S2055, which maps physically to chromosome 8p21, and WI6536 is linked to D8S298, which maps also to 8p21.

Next, we analyzed the expression of *DcR2* mRNA in human tissues (Figure 2). We detected only one mRNA transcript of approximately 4 kilobases (kb), consistent with a single splice form of DcR2. The transcript was expressed in fetal kidney, lung and particularly liver, and in many adult tissues, especially testis and kidney. This expression pattern differs from that of DR4, DR5 or DcR1 [3–5]: DR4 is particularly abundant in peripheral blood leukocytes (PBL) and spleen, DR5 in ovary, liver and lung, and DcR1 in PBL, spleen, lung and placenta.

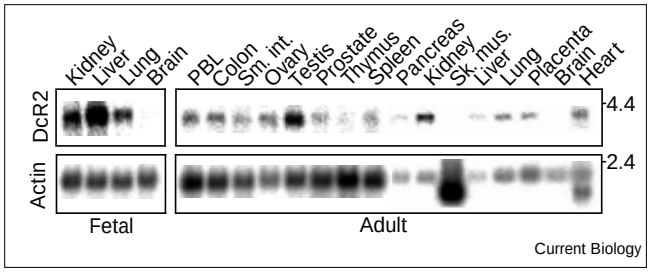
To investigate whether DcR2 binds to Apo2L, we generated IgG fusion proteins (immunoadhesins) based on the ECDs of DcR2 and other receptors. Immunoadhesins

Figure 1

(a) Nucleotide and predicted amino-acid sequence of human DcR2 (GenBank accession number 147230). Indicated are the putative signal peptide as determined by amino-terminal sequence analysis of DcR2–IgG (yellow), the transmembrane domain (magenta) and potential N-linked glycosylation sites (red). Serine 310 of clone 35663 is replaced by a leucine in clone 35664. (b) Extracellular sequence alignment. Highlighted are conserved amino acids (blue), and the cysteines of each cysteine-rich domain, CRD (red). (c) Cytoplasmic sequence alignment. Conserved amino acids are in blue. Red indicates positions that are important for death signaling by TNFR1 [11] and CD95 [6]. DD, death domain. (d) Domain organization of Apo2L receptors. S, signal sequence; CRD1, CRD2, cysteine-rich domains; TM, transmembrane domain; TD, truncated death domain; DD, death domain. In DcR1, 1–5 are pseudorepeats of 15 amino acids. The topology of DR4 is based on [3].

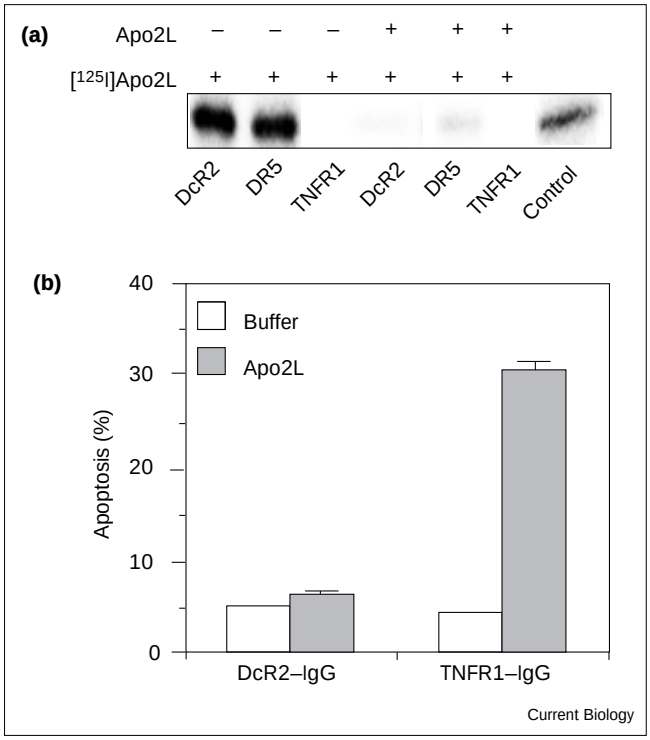
based on DcR2 or DR5, but not those based on TNFR1, co-precipitated radiolabeled Apo2L; this co-precipitation was blocked by excess unlabeled Apo2L (Figure 3a). Biacore analysis demonstrated that the DcR2 immunoadhesin bound to Apo2L, but not to TNF, lymphotoxin α or CD95 ligand (data not shown). Furthermore, DcR2, but not TNFR1, immunoadhesin blocked the ability of Apo2L to induce apoptosis in human HeLa cells (Figure 3b). These results indicate that DcR2 is a specific receptor for Apo2L.

Figure 2



Expression of *DcR2* mRNA in human tissues. Poly(A)$^+$ RNA blots derived from human fetal and adult tissues (Clontech) were hybridized to a 200 bp ^{32}P-labeled DNA probe based on the 3′ untranslated region of DcR2. PBL, peripheral blood leukocytes; Sm. int., small intestine; Sk. mus., skeletal muscle.

Figure 3



Interaction of DcR2 with Apo2L. (a) An immunoadhesin containing the ECD of DcR2 (amino acids 1–212) fused to the hinge and Fc regions of human IgG1 was generated as described [14]. DcR2, DR5 [4] or TNFR1 [15] immunoadhesin (2.5 μ g) was incubated with ^{125}I-labeled soluble Apo2L [1] (1 ng, specific activity 10.7 μ Ci μ g^{-1}) with or without 1 μ g unlabeled Apo2L (1 h at 24°C). Complexes were precipitated by protein A–Sepharose and resolved by electrophoresis under reducing conditions. In the right-hand lane (control), ^{125}I-Apo2L was loaded directly. (b) Human HeLa S3 cells were incubated with buffer or Apo2L (125 ng ml^{-1}) in the presence of DcR2 or TNFR1 immunoadhesin (10 μ g ml^{-1}) for 5 h, and analyzed for apoptosis by annexin V binding as described [7]. The data are means ± s.e.m. of triplicate determinations.

Overexpression of death-domain-containing receptors such as DR4 or DR5 leads to activation of apoptosis and of nuclear factor-κB (NF-κB) [3–5]. HeLa cells transfected with either DcR2 clone showed no increase in cell death, whereas cells transfected with DR4 or DR5 showed higher levels of apoptosis (Figure 4a). Similarly, transfection of human 293 cells with DcR2 did not increase NF-κB activity, whereas DR4 or DR5 caused NF-κB activation (Figure 4b). These results suggest that the truncated death domain of DcR2 is not functional.

We tested the effect of DcR2 transfection on the induction of apoptosis by Apo2L. DcR2-transfected cells were substantially less sensitive than control vector-transfected cells to Apo2L-induced apoptosis (Figure 4c), but showed little difference in sensitivity to induction of apoptosis through CD95 (Figure 4d). These data support the conclusion that DcR2 does not signal cell death and demonstrate that high levels of DcR2 can specifically inhibit the cytotoxic action of Apo2L. Deletion of the ICD of DcR2 did not affect its activity (Figure 4c), indicating that this region is not critical for the inhibition of Apo2L.

Although there is evidence that Apo2L may be involved in apoptosis of activated peripheral T cells [12], the biological role of this cytokine is not fully understood. The existence of four receptors for Apo2L indicates unusually complex regulation. The close linkage of the genes for the four Apo2L receptors on human chromosome 8p21 suggests that they arise from recent gene duplication events. To date, no other TNFR family members have been mapped to chromosome 8p. It is notable that 8p21 is a frequent site of chromosomal abberation in a variety of human cancers [13]. DcR2 has a unique mRNA expression pattern compared with those of other Apo2L receptors, suggesting that it has a distinct role. Our initial characterization of DcR2 indicates that its truncated death domain is not capable of signaling. Ectopic DcR2 expression provides protection against Apo2L-induced apoptosis independently of the ICD of DcR2, suggesting that this receptor can act as a decoy that sequesters the ligand from its death-signaling receptors. Hence, DcR2 may fulfil an inhibitory role similar to that of DcR1, perhaps in tissues that express high levels of DcR2, such as fetal liver or adult testis. Nonetheless, because the cytoplasmic sequence of DcR2 is distinct from that of DR4 or DR5, it is conceivable that DcR2 may have some signaling function of its own.

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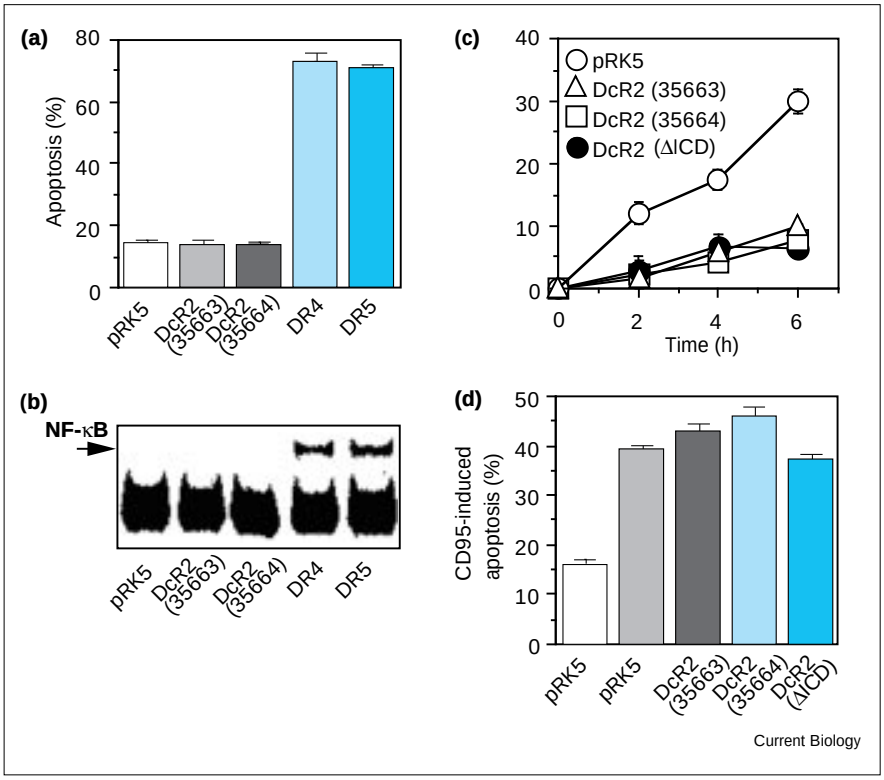
Figure 4



DcR2 inhibits Apo2L activity. (a) HeLa S3 cells (10^6 per assay) were transfected by electroporation with pRK5, or with pRK5 encoding DcR2 (clone 35663 or 35664), or DR4 or DR5 (16 μg), along with pRK5 encoding CD4 (4 μg) as a transfection marker. The level of apoptosis in CD4-expressing cells was assessed 24 h later, by analysis of annexin V binding [7]. (b) Human 293 cells (5×10^6 per assay) were transfected by calcium-phosphate precipitation with 20 μg pRK5, or pRK5 encoding either DcR2 clone, or DR4 or DR5. The cells were analyzed 24 h later for activation of NF-κB (arrow) by electrophoretic mobility shift assay [7]. (c) Human 293 cells (10^6 per assay) were transfected with 4 μg pRK5, or pRK5 encoding either DcR2 clone, or a DcR2 mutant in which the intracellular domain (amino acids 236–386) was deleted (ΔICD), along with 1 μg pRK5 encoding green fluorescent protein (GFP). After 24 h, the cells were treated with Apo2L (1 μg ml^{-1}) for the indicated time periods, stained with Hoechst 33342 dye (10 μg ml^{-1}), and double-positive cells were scored for apoptotic morphology. (d) HeLa S3 cells were transfected with the indicated plasmids. After 15 h, cycloheximide was added (10 μg ml^{-1}), and 1 h later, buffer (white bar) or agonist anti-CD95 antibody CH-11 (1 μg ml^{-1}; shaded bars) was added for 5 h and apoptosis was analyzed as in (a). The data in (a,c,d) are means ± s.e.m. of triplicate determinations.

References

1. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A: Induction of apoptosis by Apo-2 Ligand, a new member of the tumor necrosis factor receptor family. *J Biol Chem* 1996, 271:12697-12690.
2. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, *et al.*: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 1995, 3:673-682.
3. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, *et al.*: The receptor for the cytotoxic ligand TRAIL. *Science* 1997, 276:111-113.
4. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, *et al.*: Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 1997, 277:818-821.
5. Pan G, Dixit VM: An antagonist decoy receptor and a new death domain-containing receptor for TRAIL. *Science* 1997, 277:815-818.
6. Nagata S: Apoptosis by death factor. *Cell* 1997, 88:355-365.
7. Marsters S, Sheridan J, Donahue C, Pitti R, Gray C, Goddard A, *et al.*: Apo3, a new member of the tumor necrosis factor receptor family, contains a new death domain and activates apoptosis and NF-κB. *Curr Biol* 1996, 6:1669-1676.
8. Chinnaiyan AM, O'Rourke K, Yu GL, Lyons RH, Garg M, Duan DR, *et al.*: Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science* 1996, 274:990-992.
9. Kitson J, Raven T, Jiang YP, Goeddel DV, Giles KM, Pun KT, *et al.*: A death-domain-containing receptor that mediates apoptosis. *Nature* 1996, 384:372-375.
10. Bodmer JL, Burns K, Schneider P, Hofmann K, Steiner V, Thome M, *et al.*: TRAMP, a novel apoptosis-mediating receptor with sequence homology to tumor necrosis receptor 1 and Fas (Apo-1/CD95). *Immunity* 1997, 6:79-88.
11. Tartaglia LA, Ayers TM, Wong GHW, Goeddel DV: A novel domain within the 55 kd TNF receptor signals cell death. *Cell* 1993, 74:845-853.
12. Marsters S, Pitti R, Donahue C, Ruppert S, Bauer K, Ashkenazi A: Activation of apoptosis by Apo-2 ligand is independent of FADD but blocked by CrmA. *Curr Biol* 1996, 6:750-752.
13. Mitelman F, Mertens F, Johansson B: A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nature Genet* 1997, 15:417-474.
14. Ashkenazi A and Chamow SM: Immunoadhesins: An alternative to human monoclonal antibodies. *Methods: a Companion to Methods in Enzymology* 1995, 8:104-115.
15. Ashkenazi A, Marsters SA, Capon DJ, Chamow SM, Figari IS, Pennica D, *et al.*: Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin. *Proc Natl Acad Sci USA* 1991, 88:10535-10539.