



H-cont H-TRAIL-R4



supplementary Figure 1



supplementary Figure 2

supplementary figure legends

Material and Methods

Reagents:

For Western blotting experiments, anti-TRAIL-R1, TRAIL-R2 and TRAIL-R4 antibodies were obtained from Milipore (Molsheim, France). Anti-TRAIL-R1, and TRAIL-R2 antibodies used for flow cytometry or immunoprecipitation were from DIACLONE (Besançon, France). Oligonucleotide primers and Pfu polymerase were purchased from Eurogentech (Angers, France) and Sigma-Aldrich (Lyon, France) respectively. Remaining reagents are described in the material and method section of this manuscript.

Construction of chimeric TRAIL receptor expressing retroviral vectors

Vectors were constructed using standard cloning procedures. TRAIL-R2 and TRAIL-R4 intracellular domains (icd) were obtained by polymerase chain reaction from pCRIII vectors encoding full length TRAIL-R2 and TRAIL-R4 as described earlier [1], with the following primer pairs : TRAIL-R2 forward primer (5'- <u>GTC GAC</u> TGT TCT CTC TCA GGC ATC-3') ; reverse primer (5'- <u>CTC GAG</u> CGG CCG CCA GTG TGA TGG-3') and TRAIL-R4 forward primer (5'- <u>GTC GAC</u> TAT CAC TAC CTT ATC ATC -3') ; reverse primer (5'- <u>CTC GAG</u> TCA CAG GCA GGA CGT AGC -3') containing a SalI and a XhoI site, respectively. The resulting amplified fragments were subcloned into pCR-Blunt (Invitrogen, Cergy Pontoise, France) and checked by sequencing. TRAIL-R2-icd and TRAIL-R4-icd were subcloned between the SalI and XhoI sites of pCRIII vectors encoding the extracellular domains (ecd) of TRAIL-R1 (aa 1-239, PS688), TRAIL-R2 (aa 1-212, PS664) or TRAIL-R4 (aa1-211, PS690), kindly provided by Dr Pascal

Schneider (Lausanne, Switzerland). Resulting TRAIL-R1ecd-TRAIL-R2icd, TRAIL-R4ecd-TRAIL-R2icd and TRAIL-R2ecd-TRAIL-R4icd DNA fragments were subcloned into a pMSCV-Puro retroviral vector between HindIII and XhoI, generating pMSCV-Puro-TRAIL-R1ecd-TRAIL-R2icd (OM043), pMSCV-Puro-TRAIL-R4ecd-TRAIL-R2icd (OM051) and pMSCV-Puro-TRAIL-R2ecd-TRAIL-R4icd (OM050).

Legends :

Supplementary Figure 1 : (A) The proliferative index of H-Ctl and H-TRAIL-R4 cells was measured in the presence or in the absence of 10 μ g recombinant Fc-TRAIL-R2, as described in the manuscript figure 1E. Fc-TRAIL-R2 was added to the culture daily for 4 days. (B) Representative picture of nude mice xenografted with HeLa control (H-Ctl on the left flank) and HeLa expressing TRAIL-R4 (H-TRAIL-R4 on the right flank) and the corresponding tumors harvested from mice pictured.

Supplementary Figure 2: (A) Schematic representation of TRAIL receptor chimeric constructs (OM043, OM050 and OM051). (B) Receptor expression in HeLa cells was analyzed by flow cytometry after infection of TRAIL receptor fusion constructs (OM043, OM050 and OM051) or the corresponding empty vector (OM181). (C) Biochemical analysis of TRAIL chimeric receptors. Immunoprecipitations were performed using a control antibody (lanes 1, 4, 7, 10, 13 and 16) or antibodies targeting TRAIL-R1 (lanes 2, 3, 5 and 6), TRAIL-R2 (lanes 8, 9, 11 and 12) and TRAIL-R4 (lanes 14, 15, 17 and 18). Antibodies were applied either after lysis (1, 3, 4, 6, 7, 9, 10, 12, 13,

15, 16 and 18), or on intact cells (2, 5, 8, 11, 14 and 17) for 1 h on ice. The latter samples were subsequently subjected to lysis in NP40 lysis buffer and samples were precleared on sepharose 6B beads before immunoprecipitation using agarose protein G beads. Samples were then washed extensively and subjected to western blot using anti-TRAIL-R1, -R2 and -R4 antibodies. Note that TRAIL-R4's extracellular domain is not recognized by the anti-TRAIL-R4 antibody from Chemicon. *ns stands for non specific. (**D**) AKT activation was analyzed by western blot as described in the text.

1. Merino D, Lalaoui N, Morizot A, Schneider P, Solary E, et al. (2006) Differential inhibition of TRAIL-mediated DR5-DISC formation by decoy receptors 1 and 2. Mol Cell Biol 26: 7046-7055.