Distinct function of monoclonal antibody to TRAIL-R2 as potentiator or inhibitor of the ligand TRAIL-induced apoptosis

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Abstract Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) specifically induces apoptosis in tumor cells but may be toxic to human hepatocytes. Although hepatocytes are susceptible to apoptotic signals mediated by TRAIL-receptor 2 (TRAIL-R2), we previously reported that some anti-TRAIL-R2 monoclonal antibodies (mAbs) produce little hepatocyte toxicity. Those mAbs neutralized the cytotoxic activity of TRAIL by inhibiting receptor–ligand binding. The hepatocyte-toxic mAbs did not compete with TRAIL for binding to TRAIL-R2, and potentiated ligand activity in both cancer cells and hepatocytes. A neutralizing antibody to TRAIL inhibited hepatocyte death by anti-TRAIL-R2 mAbs, suggesting that the toxicity may reflect their ability to potentiate membrane-bound TRAIL on hepatocytes.

Keywords: Tumor necrosis factor-related apoptosis-inducing ligand; Tumor necrosis factor-related apoptosis-inducing ligand-receptor 2; Apoptosis; KM mice™; Monoclonal antibody

1. Introduction

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of TNF superfamily and activates two distinct receptors [1]; TRAIL-R1 (DR4) and TRAIL-R2 (DR5) contain cytoplasmic death domains that can mediate apoptosis. Soluble TRAIL (sTRAIL) potently induces apoptosis in various cancer cell lines, but spares most normal cell types in vitro [2,3]. Furthermore, TRAIL exhibits anti-tumor activity in mice xenograft models of human cancers although in non-human primates its administration produces little toxicity [2–4]. However, sTRAIL has been reported to induce hepatocyte toxicity, which has raised concern about its safety as a cancer therapy [5]. In mice, membrane-bound TRAIL transduced by adenoviral systems produced hepatitis [6], indicating that overexpression of membrane-bound TRAIL can mediate apoptosis in normal murine liver cells. In addition, high-level TRAIL expression is effective in killing human hepatocytes in vitro [7]. Although considerable controversy surrounds the potential hepatocyte toxicity of sTRAIL [8], these observations present the possibility that TRAIL may trigger the apoptosis of normal human liver cells in vivo.

To examine the role of TRAIL receptors (TRAIL-Rs) in signaling tumor apoptosis and hepatocyte toxicity, we generated fully human monoclonal antibodies (mAbs) specific to TRAIL-R1 and TRAIL-R2 using KM mice™ [9]. Antibodies to TRAIL-R1 and TRAIL-R2 induced apoptosis in several human cancer cell types, indicating that the mAbs mimic the action of the natural ligand. We also reported that both TRAIL-R1 and TRAIL-R2 mediated death signals in human hepatocytes. All of the mAbs to TRAIL-R1 induced significant hepatocyte death, whereas the mAbs to TRAIL-R2 could be separated into two groups, one with and the other without severe hepatocyte toxicity [9].

We here report that certain mAbs to TRAIL-R2, which exhibit little toxicity towards hepatocytes, are efficient inhibitors of TRAIL-induced apoptosis, whereas other anti-TRAIL-R2 mAbs, which induce hepatocyte death, enhance the activity of sTRAIL in both cancer cells and human hepatocytes. Furthermore, hepatocyte toxicity by this latter group of mAbs is partially inhibited by a neutralizing antibody to TRAIL.

2. Materials and methods

2.1. Cell lines

Colo205 (colorectal adenocarcinoma) and HepG2 (hepatoma) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Colo205 was maintained in RPMI1640 supplemented with 10% fetal bovine serum and streptomycin. HepG2 was maintained in minimum essential medium supplemented with 10% fetal bovine serum, 1.0 mM sodium pyruvate and 0.1 mM non-essential amino acids.

2.2. Reagents

The sTRAIL was purchased form R&D systems (Minneapolis, MN). The preparation is supplied as non-tagged protein but it affects hepatocyte viability such as his-tagged one (unpublished data), which may be due to inappropriate Zn content. Anti-TRAIL neutralizing mAb, RIK-2, was purchased from eBioscience (San Diego, CA). Anti-Fas agonistic mAb, CH-11, was purchased from MBL (Nagoya, Japan). Fully human mAbs to TRAIL-R2 were generated using KM mice™ as previously described [9].

2.3. Surface plasmon resonance analysis

Binding experiments were performed at 25 °C using a Biacore 3000 (Biacore K.K., Tokyo, Japan). Anti-TRAIL-R2 mAbs were coupled to CM5 sensor chip (Biacore K.K.) surface at a level of about 6000 resonance units using the amine coupling kit (Biacore K.K.). Injection of 60 μl of recombinant sTRAIL-R2 proteins fused to human IgG Fc portion at 9 μg/ml into the flow cells was followed by 60 μl of sTRAIL at 1 μg/ml, both at a flow rate of 20 μl/min.

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Abbreviations: TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; sTRAIL, soluble TRAIL; TRAIL-R, TRAIL receptor; TRAIL-R2, TRAIL receptor 2; mAb, monoclonal antibody

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2.4. Cytotoxicity assay

Cancer cells were cultured in 96-well plates (1×10^4 cells/well) and exposed to the indicated concentrations of mAbs to TRAIL-R2. For combined analysis with sTRAIL, subsequently sTRAIL was added and incubated for 48 h at 37 °C. Cell viability was determined using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI).

2.5. Human hepatocytes

Human hepatocytes have been described previously [9]. Hepatocytes were cultured in CM5300 (CEDRA, Austin, TX) containing mAbs to TRAIL-R2 in the presence of anti-human IgG (10 μg/ml) with or without RIK-2 (1 μg/ml), for 8 h. For combined analysis with sTRAIL or CH-11, hepatocytes were incubated in CM5300 containing the indicated concentrations of sTRAIL or CH-11 and mAbs to TRAIL-R2 in the absence of goat anti-human IgG for 24 h. Hepatocyte viability was determined using the CytoTox 96 Non-Radioactive Cytotoxic Assay (Promega).

2.6. Statistics

All data are expressed as mean ± S.D. Differences between experimental and control groups were determined using the Mann–Whitney *U* test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Anti-TRAIL-R2 mAbs differ in the inhibition of receptor–ligand binding

We investigated the binding characteristics of TRAIL-R2 to anti-TRAIL-R2 mAbs and sTRAIL by surface plasmon resonance analysis. sTRAIL did not bind to E11 and L30-bound TRAIL-R2 (Fig. 1), indicating that E11 and L30 compete with sTRAIL for binding to TRAIL-R2. By contrast, sTRAIL was able to bind to H48 and F4-bound TRAIL-R2 (Fig. 1), suggesting that TRAIL-R2 binding region of H48 and F4 is different from that of sTRAIL. This result demonstrates that mAbs to TRAIL-R2 are different in the blocking of TRAIL-R2–sTRAIL interaction.

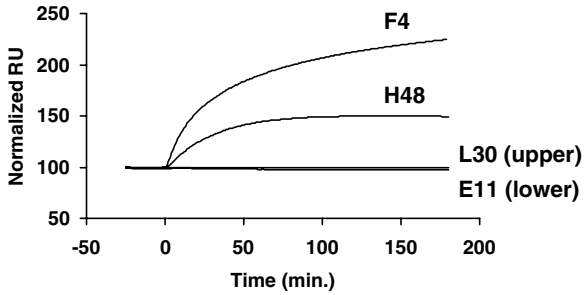

Fig. 1. Binding activity of mAbs to TRAIL-R2 against TRAIL-R2 with TRAIL. Anti-TRAIL-R2 mAbs, E11, L30, H48 and F4 were coupled to CM5 sensor chip surface. Recombinant sTRAIL-R2 proteins were injected into the flow cells, and the amount of TRAIL-R2 that bound to immobilized mAbs to TRAIL-R2 was normalized as 100 RU. sTRAIL was injected at time 0.

3.2. Cytotoxic activity of sTRAIL is modulated by mAbs to TRAIL-R2 in cancer cells

We treated Colo205 cells with the mAbs to TRAIL-R2 and sTRAIL to investigate the effect of the mAbs to TRAIL-R2 on TRAIL activity. Because all of the mAbs to TRAIL-R2 exhibited potent cytotoxic activity in Colo205 cells upon cross-linking (Fig. 2), mAb treatment was performed in the absence of anti-human IgG. The mAbs E11 and L30 blocked TRAIL-induced death of Colo205 cells in a dose-dependent manner. On the contrary, H48 and F4 dose-dependently enhanced the apoptosis-inducing activity of the ligand (Fig. 3A). We also tested the activity of mAbs co-incubated with sTRAIL in hepatoma HepG2 cells (Fig. 3B), which were relatively resistant to TRAIL (Fig. 3A, B), and obtained the result similar to that in Colo205 cells. Moreover, E11 and L30 inhibited effector caspase activation by TRAIL whereas H48 and F4 augmented

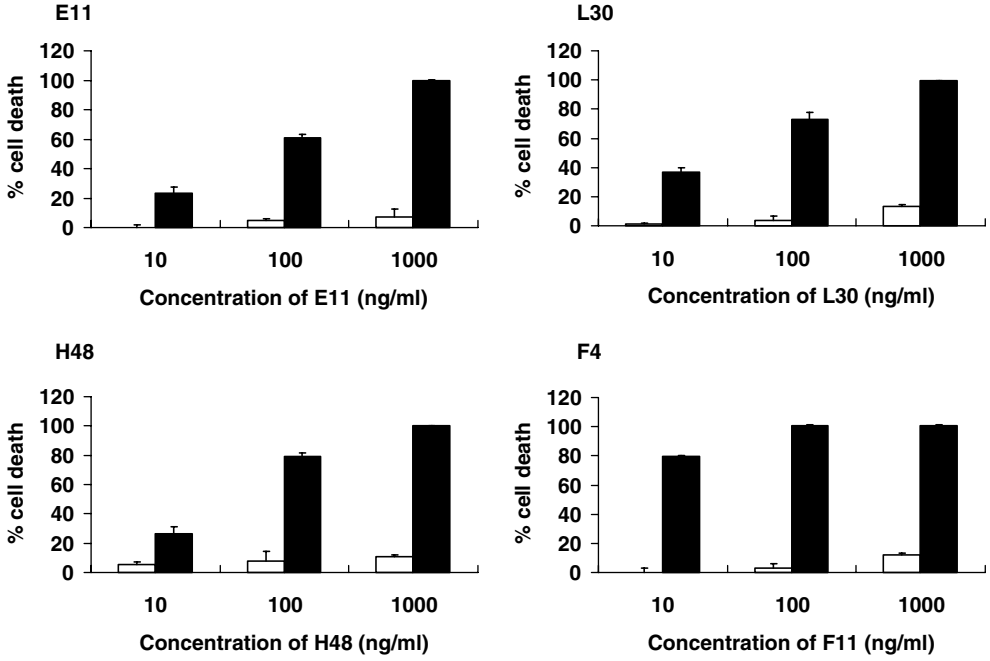

Fig. 2. Induction of cancer cell death by cross-linked mAbs to TRAIL-R2. Colo205 cells were incubated with the indicated concentrations of mAbs, E11, L30, H48 and F4 in the presence (■) or absence (□) of anti-human IgG for 48 h. Cell viability was determined as described in Section 2.