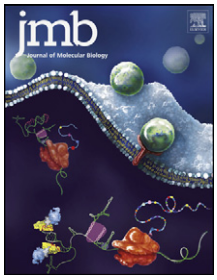

Soluble Extracellular Domain of Death Receptor 5 Inhibits TRAIL-Induced Apoptosis by Disrupting Receptor–Receptor Interactions

Nagamani Vunnam[1], Chih Hung Lo[1], Benjamin D. Grant[2], David D. Thomas[3,4] and Jonathan N. Sachs[1]

*1 - **Department of Biomedical Engineering,** University of Minnesota, Minneapolis, MN 55455, United States*
*2 - **Fluorescence Innovations Inc.,** Minneapolis, MN 55455, United States*
*3 - **Department of Biochemistry,** Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, United States*
*4 - **Photonic Pharma LLC,** Minneapolis, MN 55410, United States*

***Correspondence to Jonathan N. Sachs:**at: Department of Biomedical Engineering, University of Minnesota, Twin-cities, Minneapolis, MN 55455, United States. jnsachs@umn.edu*
http://dx.doi.org/10.1016/j.jmb.2017.08.009
Edited by James Bowie

Abstract

Dysregulation of tumor necrosis factor (TNF) receptor signaling is a key feature of various inflammatory disorders. Current treatments for TNF-related diseases function either by sequestering ligand or blocking ligand–receptor interactions, which can cause dangerous side effects by inhibiting the receptors that are not involved in the disease condition. Thus, alternate strategies that target receptor–receptor interactions are needed. We hypothesized that the soluble extracellular domain (ECD) of long isoform of death receptor 5 (DR5) could block endogenous receptor assembly, mimicking the biological effect of decoy receptors that lack the death domain to trigger apoptosis. Using live-cell fluorescence resonance energy transfer studies, we demonstrated that soluble ECD disrupts endogenous DR5–DR5 interactions. Cell viability assays were used to demonstrate the complete inhibition of TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by the ECD, although TRAIL is still able to bind to the receptor. Importantly, we used mutagenesis to prove that the inhibition of TRAIL-induced apoptosis by the ECD predominantly comes from the disruption of DR5 oligomerization and not ligand sequestration. Inhibition of death receptor activation should have important therapeutic applications in diseases such as nonalcoholic fatty liver disease. More generally, this approach should be generalized to enable the inhibition of other TNF receptor signaling mechanisms that are associated in a wide range of clinical conditions.

Introduction

Members of the tumor necrosis factor receptor (TNFR) and tumor necrosis factor (TNF) superfamilies (TNFRSF/TNFSF) play a vital role in the homeostasis of the immune system [1,2]. However, increased expression of TNFR/TNF cytokines can cause severe inflammatory reactions and tissue injury [3,4]. For example, overexpression of death receptor 5 (DR5/TNRSF10B), a member of TNFR superfamily, in hepatocytes results in nonalcoholic fatty liver disease [5,6]; likewise, accumulation of excess TNF-α in joints results in arthritis [7]. Current treatments for TNF-related ailments function either by sequestering ligand or blocking ligand–receptor interactions that activate TNFR signaling [8–16]. Unfortunately, these anti-TNF drugs cause significant side effects due to their interference with the host immune system. Furthermore, because members of the TNF family of ligands can bind to several related receptors, sequestration of ligands can cause adverse effects by inhibiting the receptors that are not involved in the disease condition [17–19]. As such, for the improved management of TNFR signaling diseases, there is a pressing need to develop receptor-specific therapies that do not interfere with ligand binding.

Recently, pre-ligand assembly of TNF receptors has been acknowledged as an essential precursor to activation [20–23]. Hence, targeting the pre-assembled TNFR structures (e.g., receptor dimers and/or

trimers) has been considered a potential therapeutic target [24,25]. DR5 is an excellent case study for this approach. This is because, unlike other members of the TNFR superfamily, the death receptors and related decoys receptors—which lack a complete functional cytoplasmic death domain and inhibit signaling—are capable of forming heterophilic complexes in a ligand-independent manner [26–29]. Originally, decoy receptors were thought to inhibit apoptosis by sequestering ligand from the death receptors [30]. However, recent studies have suggested that ligand-independent heteromeric complex formation between decoy receptors and death receptors, as opposed to ligand sequestration, may be the primary mechanism of signal inhibition [26–28]. As a consequence, mimicking the biological action of decoy receptors with soluble proteins or small peptides should, in principle, serve as a receptor-specific approach to inhibition.

Here, we show that it is possible to inhibit TNFR signaling by perturbing native, pre-assembled receptor–receptor complexes. Specifically, we targeted the pre-ligand assembly of DR5 to inhibit the apoptosis induced by TNF-related apoptosis-inducing ligand (TRAIL/TNFSF10/Apo2L). DR5 is a pro-apoptotic TNF-receptor with an N-terminal extracellular domain (ECD) made up of three cysteine-rich domains (CRDs; CRD1–3), a transmembrane domain, and a cytoplasmic death domain, which is crucial for initiation of death signaling upon binding of TRAIL. We aimed to control TRAIL-induced apoptosis by using the isolated, soluble ECD of DR5, which lacks the functional death domain. Soluble ECD, which contains both the presumed pre-ligand assembly domain (PLAD; CRD1) and ligand-binding domains (CRD2 and CRD3), could form both ECD–DR5 and ECD–TRAIL complexes (Fig. 1). We hypothesized that soluble ECD might act as a competitive inhibitor, which we anticipated in our experimental design, by masking the endogenous DR5 interaction site and making it inaccessible for its binding partner. Using a combination of biophysical, biochemical, and cell-based techniques, we show that the soluble ECD binds directly to endogenous DR5 and effectively inhibits activation. Importantly, we prove that this mode of inhibitory action of ECD is at the receptor–receptor interface and thus provides the first direct demonstration that this approach is viable even while ligand is still able to bind. This strategy may be applicable to all the members of clinically important TNFR superfamily.

Results

Biochemical characterization of soluble ECD and recombinant FLAG-tagged TRAIL proteins

We postulated that soluble ECD could inhibit TRAIL-induced apoptosis by forming both ECD–DR5 and ECD–TRAIL complexes, which lack the functional death domain complexes (Fig. 1). To test this hypothesis, we produced soluble ECD and FLAG-tagged TRAIL using the champion pET-SUMO and pT7-FLAG-1 expression systems, respectively. Purified proteins were characterized by electrophoresis under denaturing and reducing conditions. ECD appeared as a single band at 17 kDa and Flag-tagged TRAIL at 22 kDa, which are similar to their theoretical molecular weights based on the amino acid sequences (Fig. 2A). Soluble ECD was further confirmed by immunoblotting (Fig. 2B). The secondary

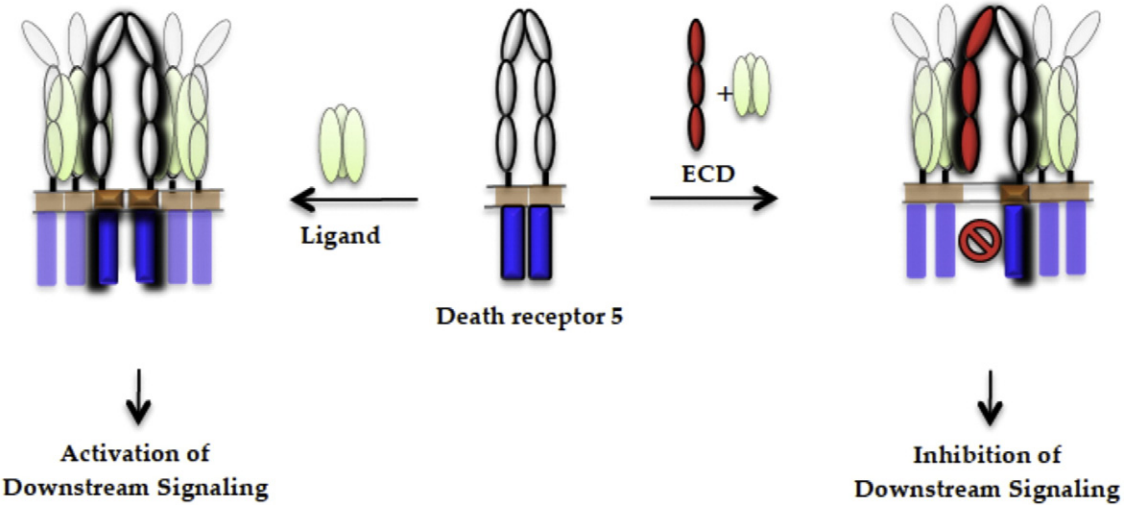

Fig. 1. Viable strategy for inhibition of DR5 signaling. Isolated ECD protein could prevent ligand-induced apoptosis by complexing with DR5 and preventing the formation of the requisite cytosolic death domain dimer.