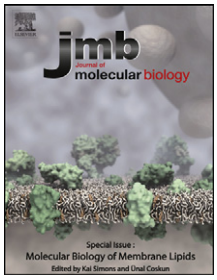

Death Receptor 5 Networks Require Membrane Cholesterol for Proper Structure and Function

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Abstract

Death receptor 5 (DR5) is an apoptosis-inducing member of the tumor necrosis factor receptor superfamily, whose activity has been linked to membrane cholesterol content. Upon ligand binding, DR5 forms large clusters within the plasma membrane that have often been assumed to be manifestations of receptor co-localization in cholesterol-rich membrane domains. However, we have recently shown that DR5 clusters are more than just randomly aggregated receptors. Instead, these are highly structured networks held together by receptor dimers. These dimers are stabilized by specific transmembrane helix–helix interactions, including a disulfide bond in the long isoform of the receptor. The complex relationships among DR5 network formation, transmembrane helix dimerization, membrane cholesterol, and receptor activity has not been established. It is unknown whether the membrane itself plays an active role in driving DR5 transmembrane helix interactions or in the formation of the networks. We show that cholesterol depletion in cells does not inhibit the formation of DR5 networks. However, the networks that form in cholesterol-depleted cells fail to induce caspase cleavage. These results suggest a potential structural difference between active and inactive networks. As evidence, we show that cholesterol is necessary for the covalent dimerization of DR5 transmembrane domains. Molecular simulations and experiments in synthetic vesicles on the DR5 transmembrane dimer suggest that dimerization is facilitated by increased helicity in a thicker bilayer.

Introduction

Death receptor 5 (DR5) is an apoptosis-inducing member of the tumor necrosis factor (TNF) receptor (TNFR) superfamily that is activated by TNF-related apoptosis-inducing ligand (TRAIL) or other agonists [1–5]. The TRAIL–DR5 signaling pathway is of great therapeutic interest, as exogenous TRAIL prevents tumor growth and, in contrast to other apoptosis-inducing ligands such as FasL, does not exhibit systemic cytotoxicity [6]. Thus, DR5 is widely regarded as a potential target in the treatment of cancer [7–10], with a number of its ligands, including recombinant human TRAIL and antibody agonists (as we will study here), currently in clinical trials [11–16] (see also clinicaltrials.gov). However, TRAIL resistance has proven to be a challenging barrier to the success of DR5-based cancer therapies [17,18]. Thus, continued efforts to understand the complex events involved in DR5 signal propagation, including the initiating events at the membrane, will be useful for understanding the therapeutics currently in clinical trials and for discovering novel DR5-targeted therapeutics.

TRAIL- and agonist-induced apoptosis are initiated by the formation of large (~300–500 nm diameter) DR5 receptor networks in the plasma membrane [19–22]. We and others have shown that these networks are held together by dimeric receptor junctions [21,23–26]. Ligand-induced receptor dimerization is consistent with crystallographic and functional evidence, showing that

both intracellular protein domains and downstream proteins form stable and functional dimeric complexes [25,27–31]. We also showed that the dimeric junctions of DR5 networks are stabilized through interactions between amino acids in the transmembrane (TM) helices, which form tight dimeric bundles in the membrane [21,32]. In the long isoform of DR5, a disulfide bond forms between TM helices upon activation. Although the short isoform lacks the disulfide bond, both the long and short isoforms contain the well-established GxxxG TM helix interaction motif, which allows for tight packing at the interface of the two monomeric helices [33,34].

We have also recently suggested that the receptor dimer of TNFR1 (a structurally homologous member of the same superfamily as DR5) undergoes a conformational transition upon activation [26]. This is a very different model than what has previously been proposed for TNFR activation. In earlier models, activation was thought to involve receptor trimerization and a stoichiometric change in the cytosolic domain that required no conformational changes (i.e., receptor backbone rearrangements) [35,36]. Our molecular calculations and electron paramagnetic resonance experiments on the TM domains, on the other hand, support a model in which signal initiation is a consequence of the mechanical strain placed on the receptors as the network grows. More specifically, we have suggested that the extracellular domain of the receptor dimer pivots outwards upon network formation and that as a consequence, the TM dimer opens like scissors (closed in the inactive state, open in the active state) [26,32]. This model of activation has been supported by several recent studies on TNFR1 [37–39].

Given that ligand-induced dimeric interactions of DR5 occur in part via TM residues, it stands to reason that the membrane itself may play an active role in driving the dimeric interactions that promote network formation. Cholesterol-rich microdomains in the plasma membrane play an active role in signal transduction in a number of pathways [40–42] including several that involve members of the TNFR superfamily. Ligand-bound TNFR1 localizes to cholesterol-rich domains and recruits downstream signaling proteins [43,44]. The death receptor Fas has been shown to localize to cholesterol-rich domains in a ligand-dependent manner [45], although others have observed ligand-independent cholesterol co-localization [46]. In DR5, Song *et al.* showed that caspase-8 cleavage in TRAIL-sensitive cells could be inhibited by treatment with methyl-β-cyclodextrin (MβCD), which strips membrane cholesterol [47]. Multiple other studies have shown that DR5 function correlates with its migration into cholesterol-rich domains [47–51]. However, it is unknown whether this localization is associated with receptor oligomeric structure, that is, DR5 dimerization and network formation.

Here, we find that the extent of agonist-induced DR5 clustering is not diminished by cholesterol depletion. However, cholesterol depletion greatly reduced the ability of DR5 to initiate caspase-dependent apoptosis. We attribute this to the formation of non-functional ligand–receptor networks that differ from functional networks by a reduction in the population of constituent disulfide-linked DR5 dimers. Collectively, these results offer the first evidence that membrane heterogeneity plays a central role in dictating the structural details and functional activity of DR5 networks. These results further support a model in which DR5 networks have a specific structure and that cholesterol-rich membrane domains do not simply corral high local concentrations of receptors but play an essential role in driving ligand–receptor network architecture.

Results

DR5 signal transduction in response to agonistic antibody is cholesterol dependent

It was previously shown that the TRAIL activation of DR5 induces cholesterol-dependent caspase-8 activation and concomitant migration of DR5 to cholesterol-rich, detergent-resistant membrane (DRM) fractions [47]. Here, we use the agonistic antibody, mAb631, in lieu of TRAIL to trigger DR5 signaling; thus, we first determined that DR5 activated by mAB631 recapitulates the same cholesterol-dependent behavior as when it is activated by TRAIL. We first depleted the membrane cholesterol with MβCD, then treated the cells with agonistic antibody, and measured caspase-8 activation. Jurkat cells with membrane cholesterol (i.e., not treated with MβCD) efficiently activate caspase-8 upon the addition of DR5 agonist (Fig. 1a, compare gray and black distributions). Pretreatment with MβCD results in a reduced ability of these cells to activate caspase-8 (Fig. 1b, compare gray and black distributions). We then showed that activation by agonistic antibody causes DR5 to relocate from high-density fractions to cholesterol-rich fractions with lower density (Supplementary Fig. 1). These results show that cholesterol-dependent DR5 behavior is consistent when activated by either mAB631 or TRAIL.

Ligand–receptor networks form in the absence and presence of membrane cholesterol

DR5 agonistic antibody is known to induce DR5 network formation [21], and we have confirmed above that it drives the co-localization of the receptor to cholesterol-rich membrane fractions. However, there is no clear evidence that cholesterol-rich domains induce DR5 network formation or vice versa. Ligand–receptor networks are routinely identified using confocal fluorescence microscopy, as in