RESEARCH HIGHLIGHT

Death receptor 5 rises to the occasion

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Death receptors (DRs), mediate death and inflammation in many cell types, especially in the immune system. In a study published in *Cell Research*, Du et al. present new structures of lipid membrane-anchored DR5 ectodomains (ECDs) leading to a new model of self-associated DR5 ECDs lying horizontal to the membrane prior to ligand engagement, termed pre-ligand self-association.

The mechanisms of death receptor (DR) signaling have been extensively studied in past decades, yielding a wealth of information on how DRs transmit proinflammatory and programmed cell death (PCD) signals.[1,2] DR5 belongs to the DR subfamily of tumor necrosis factor receptor superfamily (TNFRSF), which includes FAS/CD95, TNFR1, DR3, DR4, and DR6. Each of these is a type-I transmembrane protein composed of an extracellular portion with 3 or 4 cysteine-rich domains (CRDs), a transmembrane domain and an eponymous intracellular death domain (DD).[1,2] Specific TNF-like ligands induce DRs to transduce signals for PCD mainly through apoptosis and necroptosis.[3] Nevertheless, DRs are also involved in proinflammatory responses and tissue development in the host.[4]

Early analytical biochemistry and X-ray structures showed that TNF is a homotrimeric type II membrane protein recognized as a specific ligand for TNFR1 (TNFRSF1A or CD120a) which itself can form a receptor homotrimer.[5,6] It can signal as a membrane-bound complex or in soluble form cleaved from the membrane. A simple signaling model was developed in which the trimerized TNF finds and binds available TNFR monomers that are swarming around on the plasma membrane of target cells to induce clustering of receptor chains, thereby apposing their intracellular DD and causing downstream signaling. This ligand recruitment model, though kinetically challenged, appeared convincing until the discovery of certain heterozygous dominant-negative gene mutations in autoimmune lymphoproliferative syndrome (ALPS) patients that left intact only a portion of the closely-related FAS (CD95 or APO-1) ectodomain (ECD). Curiously, these partial ECD variants lacked the FAS ligand (FASL) binding site, yet dominantly interfered with the function of the unmutated FAS chain. The presence of a non-FASL-binding FAS ECD prevents the ability of FASL trimers to recruit the wild-type FAS chains to assemble signaling proteins into the death-inducing signaling complex (DISC) or induce lymphocyte apoptosis.[7] Analysis of a large cohort of ALPS patients shows that all dominantly interfering FAS/CD95 variants share a CRD1 containing region, the most distal from the membrane, on the ECD. This region of the ECD was shown biochemically to promote ligand-independent self-association of receptor chains for both FAS and TNFR1 and was termed pre-ligand association domain (PLAD). Further studies using fluorescence resonance energy transfer demonstrate that CRDs, particularly CRD1, of FAS/CD95, TNFR1, TNFR2, and HVEM, typically self-associate through homotypic interactions.[8,9] These studies also show that PLADs are essential, in addition to the intracellular homotypic interactive DDs, for controlling signaling of FAS/CD95 and TNFR1, and other TNFRSF members. Interestingly, decoy receptor 2, a naturally-occurring paralog of DR5 protein with no DD or TRAIL-binding region, associates with DR5 in the absence of the ligand TRAIL, and inhibits TRAIL-induced DR5-dependent apoptosis.[10] Thus, evolution has used the PLAD function to exert stimulatory and inhibitory effects on DR signaling. However, the pre-ligand and post-ligand structures in the membrane were unknown until the current study.[11]

Another long-standing question regarding DRs is how the target cells avoid the random productive collision of receptor chains to spontaneously initiate signaling with lethal consequences. In T lymphocytes, the signaling components of the FAS-dependent DISC are constitutively expressed in resting or activated T cells, but there is no measurable DISC signaling without ligand stimulation. These phenomena of DR signaling control have remained a mystery in the field until now. The structures of non-overlapping interface binding between DR5 ECD chains provide new insights into structural oligomerization prior to ligand interactions and functional adaptation to the quiescent versus activation states. The current model adds a fresh perspective to the original PLAD model suggesting that a single CRD can associate with a full-length ECD to dominantly interfere with FAS signaling.[7] These observations show that other CRDs and ECD regions of DR5 are required for the pre-ligand assembly of functional trimers and even higher-order oligomeric structures.

A fascinating and non-intuitive observation in the new study is that the pre-associated DR5 ECDs lie down in the membrane to "bury" and limit exposure of TRAIL-binding sites. Functional studies measuring caspase-8 activation demonstrate that the pre-ligand-associated DR5 complexes are essentially inactive before TRAIL stimulation. This implied that the pre-ligand assembly complexes are autoinhibitory and restrain DR5 signaling. To map the functional sites and explore the underlying regulatory mechanisms of pre-assembled DR5 ECD complexes, Chou's group developed an innovative set of single-domain nanobodies, NB1–NB5 against the DR5 ECD.[11] Remarkably, the NB1 binding site on the DR5 ECD disrupts one interface between the self-associated receptor chains (interface 1), which exposes the hidden TRAIL-binding sites and promotes TRAIL-induced apoptosis signaling. On the other hand,

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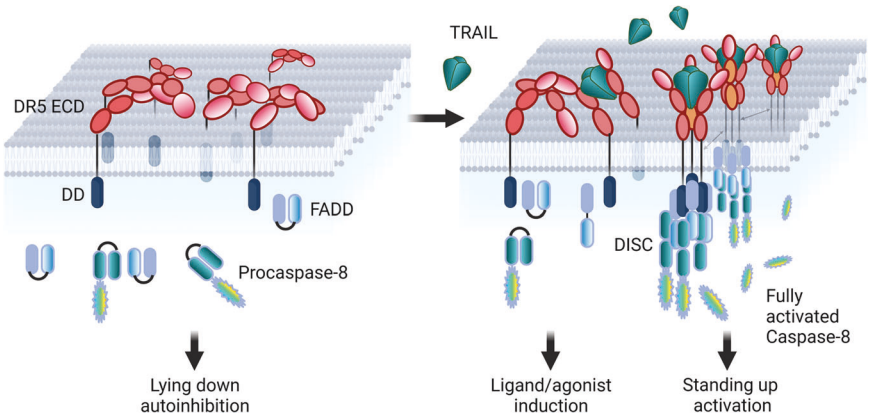

Fig. 1 Pre-ligand self-association (PLS) autoinhibits DR5 signaling. Without TRAIL, the DR5 ECD self-associate to form the "lying down" structures parallel to the surface of the lipid bilayer membrane, which buries some ligand-binding sites and prevents DR5 intracellular DD from recruiting the FAS-associated death effector domain (FADD) protein and procaspase-8 for apoptosis signaling (left). TRAIL ligand binds first to a subset of available sites of the PLS complex causing conformational changes and the exposure of more TRAIL-binding sites. Consequently, DR5 ECDs rise orthogonal to the membrane in an active TRAIL–DR5 signaling complex that clusters via interactions between the transmembrane and intracellular DDs. The active signaling complex results in the formation of DISC, caspase-8 activation and apoptosis or inflammatory signaling (right).

NB3 recognizes the main TRAIL-binding site of DR5, but, as a monomer, is incapable of signaling. The authors then link NB1 and NB3 to produce a combination nanobody ligand that significantly stimulates DR5 signaling. There is also an interface 2, formed by CRD2 and CRD3 on one monomer interacting with CRD1 and CRD2 of another, which stabilizes the pre-ligand complex and the large membrane clusters it forms. The interpretation was that alteration of the pre-associated DR5 complex by NB1 renders it more sensitive to the stimulation by NB3 which is otherwise inactive on its own. These data imply that TRAIL engages its receptor with a one-two punch. TRAIL initially touches a limited number of available ligand-binding sites on the pre-assembled complex of DR5 chains lying flaccidly in the membrane, causing sequential conformational alterations that result in the exposure of more ligand-binding sites for rearrangement to a fully activated conformation of ligand and receptor chains (Fig. 1). Therefore, the specific nanobody approach was very useful in mapping the functional sites/domains of DR5 for pre-ligand association and drawing inferences regarding signaling mechanisms.

In summary, these structures give an intriguing and detailed view of the pre-ligand assembly. The principles underlying pre-ligand association and signaling autoinhibition for DR5 are likely generalizable to other TNFRSF receptors. It would be interesting to examine similar structures of FAS and how the dominant-negative ECD mutants of FAS impair function in ALPS. These advances provide hope that the detailed understanding of this receptor family will lead to new treatments for autoimmune disorders and cancers through the controlled induction of TNFRSF receptor signaling.

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ADDITIONAL INFORMATION

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