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Mini-review

# Engineering death receptor ligands for cancer therapy

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ABSTRACT

CD95, TNFR1, TRAILR1 and TRAILR2 belong to a subgroup of TNF receptors which is characterized by a conserved cell death-inducing protein domain that connects these receptors to the apoptotic machinery of the cell. Activation of death receptors in malignant cells attracts increasing attention as a principle to fight cancer. Besides agonistic antibodies the major way to stimulate death receptors is the use of their naturally occurring "death ligands" CD95L, TNF and TRAIL. However, dependent from the concept followed to develop a death ligand-based therapy various limiting aspects have to be taken into consideration on the way to a "bedside" usable drug. Problems arise in particular from the cell associated transmembrane nature of the death ligands, the poor serum half life of the soluble fragments derived from the transmembrane ligands, the ubiquitous expression of the death receptors and the existence of additional non-death receptors of the death ligands. Here, we summarize strategies how these limitations can be overcome by genetic engineering.


## 1. Introduction

The ligands and receptors of the tumor necrosis factor (TNF) family fulfill a variety of functions in the immune system, but have also been implicated in developmental processes and in the control of tissue homeostasis. With respect to the activation of intracellular signaling pathways three subgroups of TNF receptors can be defined ([1] and Fig. 1). Firstly, TNF receptors that stimulate intracellular signaling pathways by recruitment of members of the TNF-receptor associated factor (TRAF) family of adapter proteins. Secondly, TNF receptors that have a conserved protein protein interaction domain in their cytoplasmic tail, called the death domain, which enables signaling by homotypic interaction with death domain containing-adapter proteins. As most of these receptors can trigger apoptosis by virtue of their death domain, they have been named death receptors. Especially, CD95 (Fas), tumor necrosis factor (TNF) receptor-1 (TNFR1), TNF-related apoptosis inducing ligand (TRAIL) receptor-1 (TRAILR1; DR4) and TRAILR2 (DR5) have been identified as potent inducers of apoptosis in a variety of cell types in vitro and in vivo. The ligands of these death receptors, CD95L, TRAIL and TNF, are accordingly often designated as death ligands. Thirdly, soluble and membrane-bound decoy receptors which compete with receptors of the two other subgroups for ligand binding.

There is evidence that transformed cells are particularly sensitive for death receptor-induced apoptosis. So, there are currently considerable efforts to exploit death ligands in tumor therapy [2]. With respect to the development of a death ligand-based therapeutic concept, two principle strategies can be pursued. First, treatment with recombinant ligand proteins and secondly gene therapy with cells engineered to express death ligands. Dependent from the concrete strategy adopted to create a death ligand-based therapy, one or more of the following aspects has to be taken into consideration on the way to the bedside.

- The ligands of the TNF family, including all death ligands, are initially expressed as transmembrane proteins. From these transmembrane molecules soluble

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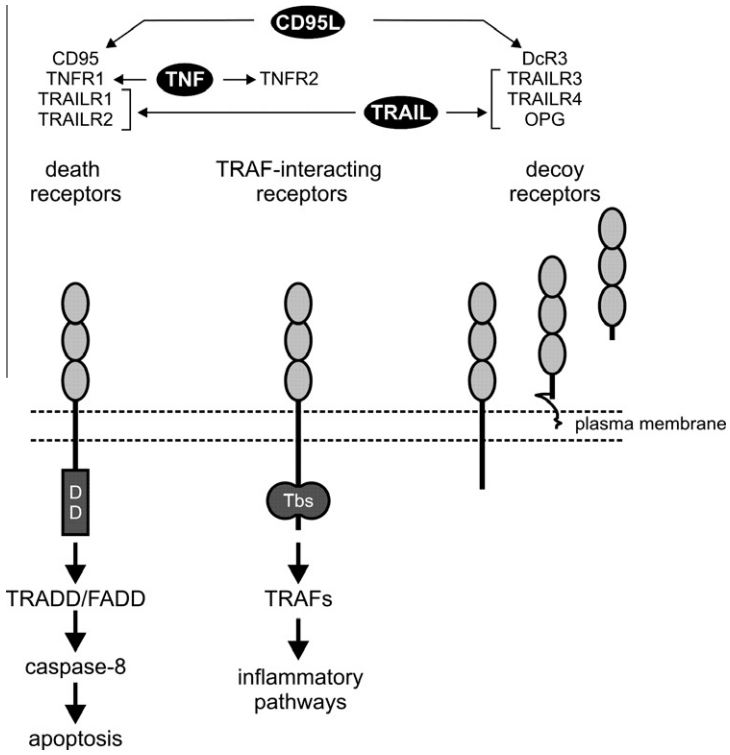


**Fig. 1.** The members of the TNF receptor superfamily can be subdivided in death receptors, TRAF interacting receptors and decoy receptors. The death ligands CD95L, TRAIL and TNF do not only interact with their corresponding death receptors CD95, TRAILR1 and TRAILR2 and TNFR1, but also with receptors of the other subgroups (CD95L: DcR3; TRAIL: TRAILR3, TRAILR4, OPG; TNF: TNFR2).

trimeric ligands can be derived naturally by proteolytic processing or alternative splicing and/or experimentally by genetic engineering (Fig. 2). However, TNF receptors differ in their capability to become activated by soluble variants of their corresponding ligand (Fig. 3). For example, CD95 is not or poorly activated by soluble CD95L, but readily signals in response to membrane CD95L whereas TNFR1 signaling is robustly triggered by soluble and membrane TNF [2].

- Soluble variants of ligands of the TNF family can be instable. So, for TNF and Glucocorticoid-induced TNF-related ligand (GITRL) protomer dissociation-related inactivation of the soluble molecules has been observed at low concentrations [3–6].
- All three death ligands interact with more than one receptor, TNF with TNFR1 and TNFR2, TRAIL with TRAILR1, TRAILR2, TRAILR3, TRAILR4 and osteoprotegerin (OPG) and CD95L with CD95 and decoy receptor-3 (DcR3) [1,2]. Not all of these receptors are involved in the anti-tumoral effect of their corresponding death ligand and may even antagonize the aimed therapeutic effect or cause unwanted side effects.
- Death receptors are broadly expressed also on non-transformed cells. Despite the higher apoptosis sensi-

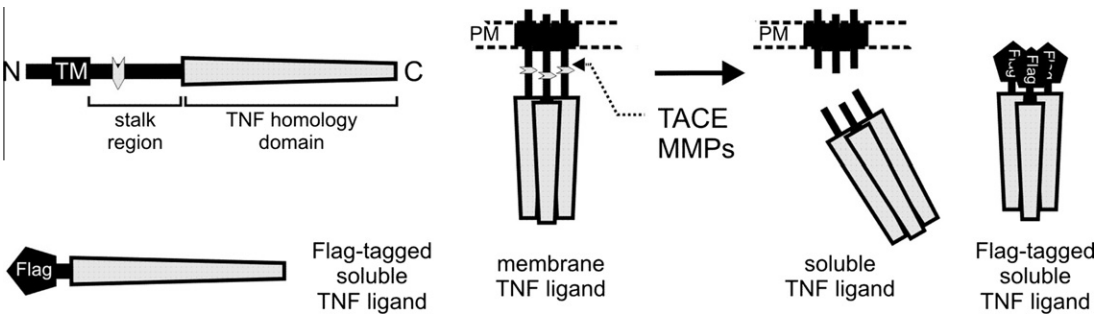


**Fig. 2.** Death ligands, as other members of the TNF ligand family, are initially expressed as type II membrane proteins. Soluble ligands can be released from these membrane-bound molecules by metallopeptidases, such as a disintegrin and metalloprotease-10 (ADAM10) and ADAM17 (=TACE).