

Chapter 2

TRAIL-R3/R4 and Inhibition of TRAIL Signalling in Cancer

Lubna Danish, Daniela Stöhr, Peter Scheurich, and Nadine Pollak

Abstract The tumour necrosis factor (TNF) ligand family member TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis predominantly in tumour cells, but not in normal tissues, representing therefore an attractive candidate for cancer therapy. The human TRAIL/TRAIL receptor system is very complex, four different membrane receptors bind the ligand. Two of these receptors, TRAIL-R1 and TRAIL-R2, transmit apoptotic but also non-apoptotic signals, whereas the other two, TRAIL-R3 and TRAIL-R4, act as inhibitors. Most tumour cells co-express several TRAIL receptors, allowing receptor interference. Several molecular mechanisms have been proposed by which TRAIL-R3 and TRAIL-R4 may counteract pro-apoptotic TRAIL signalling at the plasma membrane level, but possibly also intracellularly. A detailed understanding of the role of the individual TRAIL receptors and their interplay will be advantageous for the development of new TRAIL receptor agonists for cancer therapy. In fact, new TRAIL formulations will be needed since first clinical studies with soluble TRAIL or receptor agonistic antibodies showed only limited success. This review summarizes the complex TRAIL/TRAIL receptor system and the mechanisms by which TRAIL-R3 and TRAIL-R4 may interfere with TRAIL-mediated apoptosis induction. In addition, we discuss the prognostic and predictive value of TRAIL receptor expression in patients' tumour material.

Keywords Apoptosis • Death receptor • Decoy receptor • Membrane receptor • Signalling complex • TNFSF • TNFRSF • TRAIL • TRAIL receptor

2.1 Introduction

Human cytokines belonging to the tumour necrosis factor (TNF) ligand family and their cognate TNF receptors regulate a multitude of biological processes. Their functions are crucial in the regulation of immune responses, such as co-stimulatory

Lubna Danish and Daniela Stöhr contributed equally to this work.

L. Danish • D. Stöhr • P. Scheurich • N. Pollak (✉)
Institute of Cell Biology and Immunology, University of Stuttgart,
Allmandring 31, 70569 Stuttgart, Germany
e-mail: nadine.pollak@izi.uni-stuttgart.de

signalling or cell death induction in potentially harmful cells [1]. Accordingly, many of these cytokines and their receptors are interesting candidates as therapeutic agents or targets [2]. TNF superfamily ligands as well as their receptors are structurally related. Most ligand members are initially expressed as homotrimeric type I transmembrane proteins. In addition, soluble forms of the ligands can be generated by proteolytic cleavage or alternative splicing with significant consequences on bioactivity of these molecules, being typically higher in the membrane-presented mode [3]. Ligand bioactivity is exerted by binding and activating the corresponding partner(s) within the TNF receptor family. Typically, TNF receptors are expressed as type II transmembrane proteins and contain one to six cysteine-rich domains (CRDs) in their extracellular part. These CRDs have been demonstrated to not only harbour the ligand interaction site but additionally a homophilic interaction site called the pre-ligand binding assembly domain (PLAD).

Induction of apoptosis, a form of programmed cell death, is attributed to a subgroup within the TNF receptor members, the death receptors like TNF-R1, Fas (CD95), TRAIL-R1 and TRAIL-R2. In particular, the TRAIL system has gained enormous attraction since its apoptotic capacity is restricted to cancer cells whereas normal cells are insensitive to TRAIL [4]. Remarkably, the TRAIL receptor system is of particular complexity. Four specific membrane-expressed TRAIL receptors are capable to bind the ligand. Two of them, TRAIL-R1 and TRAIL-R2, are characterized by an intracellular death domain (DD) and are thus known as death receptors transmitting the apoptotic TRAIL-induced signal. TRAIL-R3 is membrane-anchored by a glycosylphosphatidylinositol (GPI) moiety and presumably not capable of intracellular signalling at all while TRAIL-R4 features a truncated DD with restricted signalling capacity. TRAIL-R3 and TRAIL-R4 are often referred to as decoy or regulatory receptors since they interfere with TRAIL-induced apoptosis induction. Intracellularly located TRAIL receptors have been detected more recently and proposed to be functional, representing a further level of complexity within the TRAIL signalling system. In particular, nuclear TRAIL-R2 has been proposed to enhance the malignancy of tumour cells [5].

In this chapter, we will introduce the TRAIL signalling system strongly focusing on the interference of TRAIL-R3 and TRAIL-R4 with TRAIL death receptors and the value of TRAIL receptor expression in different types of cancer as prognostic factor since this might determine the successful application of TRAIL-based therapeutics.

2.2 The TRAIL Signalling System

2.2.1 Structure of TRAIL

The ligand TRAIL, also called Apo2 ligand (Apo2L), TNF superfamily member 10 (TNFSF10), TNF ligand 2 (TL2), CD253 or TNF ligand gene 6A (TNLG6A), was first described in the late nineties by two independent groups [6, 7]. TRAIL was soon

shown to represent a potential anti-tumour agent, while physiologically playing a role in the regulation of the innate and adaptive immune responses. The gene coding for human TRAIL consists of five exons and is located on chromosome 3. The molecule TRAIL shows 23% sequence homology to the death receptor ligand member FasL and 19% identity with TNF [6]. Until now, three splice variants of the full-length protein (TRAIL α) are known. These are TRAIL β , lacking exon 3, TRAIL γ , lacking exons 2 and 3, and TRAIL δ , lacking exons 3 and 4 [8]. All these latter molecules are depleted in their receptor interaction domain and are thus thought to be unable of receptor activation. They have been rather suggested to compete with TRAIL α for translation, in this way regulating TRAIL signalling [9, 10]. TRAIL transcripts are found in many different tissues such as liver, lung, colon, brain, heart, testis and kidney, but also in different cells of the haematopoietic system where TRAIL expression is inducible [6, 11]. When expressed on the surface of human cells, TRAIL is a type II transmembrane glycoprotein with a short amino-terminal cytoplasmic and a long extracellular carboxyl-terminal domain composed of two anti-parallel β -sheets [6, 7]. Similar to other TNF superfamily members, the extracellular domains (ECD) of TRAIL protomers associate at hydrophobic interfaces to form compact homotrimers. Unique for TRAIL is a zinc-binding site at the trimer interface which is indispensable for the structure and stability of the trimer, hence also for its bioactivity [12]. Limited proteolysis of membrane-bound TRAIL by cysteine proteases has been suggested as a mechanism to generate the soluble form of the ligand [13]. However, soluble TRAIL is capable to induce apoptosis predominantly via TRAIL-R1 whereas full activation of TRAIL-R2 is achieved only by membrane-integrated TRAIL [14].

2.2.2 Structure and Function of TRAIL Receptors

As mentioned above the TRAIL/TRAIL receptor system is relatively complex, comprising altogether six receptor molecules, of which four are membrane-bound and two are soluble (Fig. 2.1). Among the membrane-bound receptors, TRAIL-R1, also called death receptor 4 (DR4) or TNFR superfamily member 10 A (TNFRSF10A), was the first to be discovered in 1997 by a group around Dixit while searching the human genome database for sequences with homology to the TNF receptor 1 [15]. A second receptor, TRAIL-R2, DR5, TNFRSF10B, Apo2, TRAIL receptor inducer of cell killing 2 (TRICK2), or KILLER was discovered independently by several groups [16–22]. The receptors are encoded by two genes located on chromosome 8p and are expressed as type I transmembrane proteins with one partial and two complete CRDs in their extracellular portion and a DD in their cytoplasmic region. TRAIL-R1 and TRAIL-R2 both exist in two splice variants including full-length TRAIL-R1 comprising 468 amino acids and its splice variant (bDR4) lacking 168 amino acids within the extracellular ligand-binding region [23] as well as long DR5 (DR5(L)) containing 440 amino acids and short DR5 (DR5(S)) lacking 29 amino acids within the ECD and the predicted transmembrane domain [17].

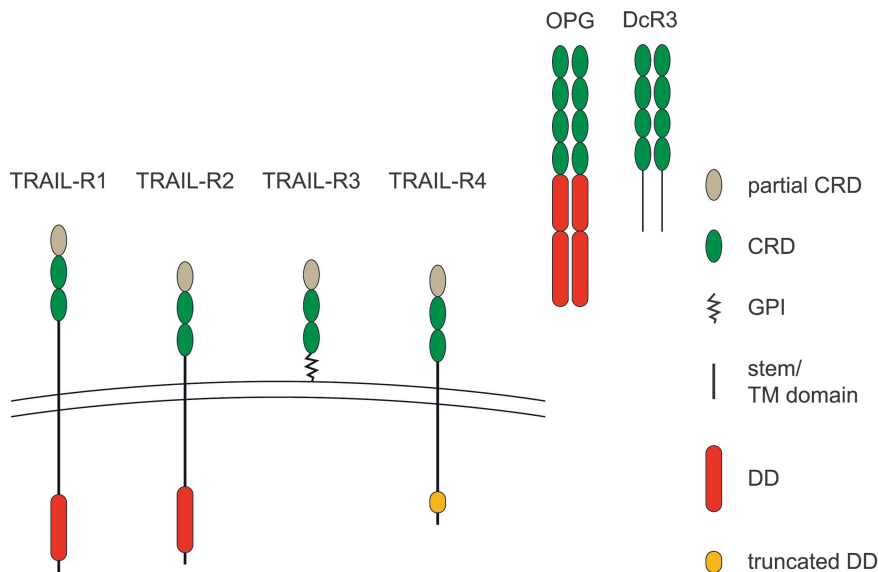


Fig. 2.1 Schematic representation of the structural features of human TRAIL receptors. The extracellular parts of TRAIL-R1 to TRAIL-R4 consist of cysteine-rich domains (CRDs, *grey and green*), a structural feature of receptors belonging to the TNF receptor superfamily. The first CRD, a partial one, mediates homophilic interactions between pre-ligand binding assembly domains (PLAD-PLAD) while the membrane proximal CRD2 and CRD3 form the ligand interaction site. The death receptors TRAIL-R1 and TRAIL-R2 are characterized by a functional death domain (DD, *red*) in their intracellular part, this domain is truncated for TRAIL-R4. TRAIL-R3 completely lacks an intracellular domain, it is linked to the membrane via a glycosylphosphatidylinositol (GPI) anchor. Splice variants for TRAIL-R1, TRAIL-R2 and TRAIL-R4 are reported, here only the longer protein variants are depicted (see text for detail). In addition to the four membrane receptors two soluble receptors have been suggested to be capable of TRAIL binding, namely OPG and DcR3. Both secreted receptors form disulfide-linked dimers. OPG consists of four CRDs and two DD-related domains while DcR3 is composed of four CRDs followed by a heparin-binding domain at its C-terminus

Interestingly, chimpanzees and humans have two death-inducing TRAIL receptors, whereas all other vertebrates solely display one. Since TRAIL-R1 shows only about 50% sequence identity with TRAIL-R2, one could speculate of different functions for these two receptor molecules. It is clear that both TRAIL death receptors can induce apoptosis in tumour cells, however, divergent reports exist regarding their contribution in cells co-expressing both receptors [24]. Moreover, recent reports show that both receptors can be also found in the cytoplasm and the nucleus of different cell lines in addition to their localization at the cell surface [25]. The mechanism of action of intracellular receptors is mainly undefined, but data suggest a proliferative role for nuclear TRAIL-R2 rather than a pro-apoptotic function [5]. Furthermore, a novel apoptosis-counteracting function for membrane-bound TRAIL-R2 in *KRAS* mutated tumour cells has been revealed. Here, TRAIL binding promotes migration and invasion via a DD-independent signalling pathway [26].

In addition to TRAIL-R1 and TRAIL-R2 two more receptors have been discovered in the late nineties referred to as TRAIL-R3, Decoy Receptor 1 (DcR1), TNFRSF10C, TRAIL Receptor without an Intracellular Domain (TRID), CD263 or Lymphocyte Inhibitor of TRAIL (LIT) [16, 18, 20, 27, 28] and TRAIL-R4, also named DcR2, TNFRSF10D, CD264, or TRAIL Receptor with a truncated Death Domain (TRUNDD) [29–31]. As already suggested by the names, these two molecules are most likely incapable of apoptotic signalling since TRAIL-R3 completely lacks any transmembrane and intracellular domain, whereas TRAIL-R4 has only a truncated DD. Similar to TRAIL-R1 and TRAIL-R2, TRAIL-R3 and TRAIL-R4 display one partial and two complete CRDs in their extracellular parts (Fig. 2.1).

TRAIL-R3 is not a transmembrane protein but is rather anchored in the cell membrane via a GPI moiety. It consists of 259 amino acids and in contrast to the other membrane-bound TRAIL receptors no splice variant has yet been identified. Signalling of TRAIL-R3 has so far not been studied intensively. However, the complete absence of a transmembrane and an intracellular domain indicates the lack of classical signalling capabilities, although signalling via its GPI anchor cannot be excluded [32].

Two splice variants have been identified for TRAIL-R4, TRAIL-R4- α and TRAIL-R4- β , with 386 and 348 amino acids, respectively, the latter lacking the first complete CRD [33]. As TRAIL-R4 contains only an incomplete DD it is suggested to be not capable to transmit an apoptotic signal upon TRAIL binding. However, it is still under debate whether TRAIL-R4 transmits non-apoptotic signals (see below). The role of TRAIL-R3 and TRAIL-R4 as inhibitory receptors rendering cells resistant against TRAIL-mediated apoptosis is discussed in more detail later in this chapter.

Besides the membrane-integrated receptors TRAIL-R1 to TRAIL-R4, two soluble members of the TNF receptor family have been reported to be capable of binding TRAIL, namely osteoprotegerin (OPG, TNFRSF11B) and decoy receptor 3 (DcR3, TNFRSF6B) (Fig. 2.1). OPG is secreted by osteoblasts and regulates osteoclast differentiation based on its ability to block receptor activator of nuclear factor “kappa-light-chain-enhancer” of activated B-cells ligand (RANKL)-stimulated osteoclast formation [34]. OPG was shown to bind to TRAIL, although with very low affinity, thereby also acting as a decoy receptor in TRAIL signalling [35]. Recent studies indicate that OPG might be involved in the pathogenesis of cardiovascular disease, in the development of both type 1 and type 2 diabetes, in endothelial cell biology and in kidney diseases, but the role of TRAIL binding in these processes is still undefined [36].

The *DcR3* gene is frequently amplified in tumour cells and the protein is overexpressed in various cancer cells [37, 38]. Ligands of DcR3 beside TRAIL also include FasL, TNF-like molecule 1A (TL1A), and LIGHT [37, 39, 40]. The interaction between DcR3 and TRAIL was demonstrated only recently in pancreatic cells, revealing a lower affinity as compared to that of LIGHT [41]. In these cells the apoptotic response to either TRAIL or FasL was enhanced when DcR3 levels were decreased [41, 42]. In summary, OPG and DcR3 might play a role in cancer progression, but whether these molecules do this by interference with the TRAIL signalling system remains to be investigated.

2.2.3 *TRAIL Receptor Signalling*

2.2.3.1 Apoptotic Signalling

The term apoptosis was introduced by Kerr and colleagues in 1972 [43] and describes a tightly regulated process of cell death with distinct morphological changes such as cell shrinkage, membrane blebbing and fragmentation of the nucleus. In contrast to unregulated cell death, necrosis, where loss of membrane integrity leads to the release of damage-associated molecular patterns (DAMPs) causing an inflammatory response, apoptosis is a rather immunological silent form of cell death since apoptotic cells become phagocytosed by immune cells to prevent activation of the immune system [44]. In the human body each day around ten billion of malignant and infected but also overaged and redundant cells die by apoptosis [45]. Interestingly, apoptosis not only occurs in the adult body but takes part also in embryogenesis, being involved for example in the development of limbs and modelling of the nervous system. Apoptosis can be triggered either by binding of death ligands like TNF, FasL or TRAIL to their cognate cell surface receptors resulting in the execution of the so-called extrinsic apoptotic pathway. Alternatively, intracellular stimuli such as DNA damage or oxidative stress induce apoptosis via activation of the intrinsic pathway of this signalling network. Depending on the cell type TRAIL-mediated apoptosis either involves solely the extrinsic pathway or includes both pathways. In type I cells, binding of TRAIL, TRAIL-fusion proteins or agonistic antibodies specific for TRAIL-R1 or TRAIL-R2, often referred to as pro-apoptotic receptor agonists (PARAs), initially triggers receptor clustering, thereby forming the death-inducing signalling complex (DISC), comprising Fas-associated protein with death domain (FADD), procaspase -8/-10 and cellular FLICE-like inhibitory protein (cFLIP). Essential for DISC formation are homophilic interactions of the DDs of the TRAIL receptors with those of FADD and, in addition, death effector domain (DED) interactions between FADD and the initiator procaspases. During DISC assembly procaspase -8/-10 oligomerizes and gets activated through proximity-induced formation of procaspase dimers and autoproteolysis, allowing the activated initiator caspases to further cleave and activate effector procaspases -3/-6/-7 to finally dismantle the cell. In type II cells the very same signalling pathway becomes activated by stimulation of TRAIL-R1 and/or TRAIL-R2, but remains at the level of only a very weak activation of initiator caspases not sufficient for strong effector caspase activation. Accordingly, these cells need amplification of the apoptotic signal by the mitochondrial intrinsic pathway to successfully trigger apoptosis. To this, receptor-activated caspase-8 cleaves truncated BH3 interacting domain death agonist (Bid), a pro-apoptotic B-cell lymphoma 2 (Bcl-2) family member, to form truncated Bid (tBid) which then translocates to the mitochondria to become an integral membrane protein triggering the polymerization of proapoptotic Bcl-2 family members Bax and/or Bak in the mitochondrial outer membrane. This leads to release of the pro-apoptotic factors cytochrome c, apoptosis inducing factor (AIF) and second mitochondria derived activator of apoptosis (Smac) into the cytoplasm. In the presence of dATP, cytochrome c induces

formation of the apoptosome, comprising also procaspase-9 and the apoptotic protease activating factor-1 (Apaf-1). Activated caspase-9 subsequently cleaves and activates effector procaspases to allow execution of the apoptotic program [46, 47].

2.2.3.2 Necroptotic Signalling

More recently it was shown that members of the TNF superfamily such as TRAIL, but also various toxic stimuli, can induce another form of programmed cell death referred to as necroptosis. In this signalling pathway TRAIL binding to TRAIL-R1/R2 leads to the recruitment of receptor-interacting kinase 1 (RIP1), as well as cellular inhibitors of apoptosis proteins, cIAP1 and cIAP2, into the signalling complex, resulting in RIP1 polyubiquitination. Polyubiquitinated RIP1 subsequently binds FADD and procaspase-8 forming a complex called ripoptosome in which procaspase-8 becomes activated and is also capable to execute the apoptotic program. However, in the case of inhibited caspase-8 activity, RIP1 can alternatively bind RIP3 to form the so-called necroptosome leading to auto-phosphorylation and activation of RIP3 and further recruitment and oligomerization of mixed lineage kinase domain-like (MLKL). Disruption of the cell membrane, causing a necrotic type of cell death is finally induced when MLKL oligomers are inserted into the membrane.

As mentioned before necrosis but also necroptosis can lead to the release of DAMPs and as a consequence to an extensive inflammatory response in the human body. Concerning tumour development these released immunostimulatory molecules can have two sides. On the one hand it was shown that production of DAMPs during necroptosis can help to eliminate cancer cells by activating natural killer (NK) cells and cytotoxic T cells, on the other hand these molecules can also have a tumour promoting function facilitating tumour angiogenesis as well as invasion and metastasis. In any case, necroptosis seems to represent a backup mechanism in the human body to eliminate infected, damaged or malignant cells which are for any reason resistant against apoptotic cell death [44, 48].

2.2.3.3 Non-cytotoxic Signalling

In TRAIL-resistant cells, ligation of TRAIL-R1 and/or TRAIL-R2 not only initiates apoptotic signals, but also stimulates cellular survival via activation of the transcription factor nuclear factor “kappa-light-chain-enhancer” of activated B-cells (NF- κ B), as well as the mitogen-activated protein kinases (MAPKs) and the phosphoinositide-3-kinase/Akt (PI3K/Akt) pathways [49]. Concerning the molecular mechanism of this pro-survival signalling, the formation of a secondary signalling complex including FADD, procaspase-8, RIP1, TNFR-associated factor 2 (TRAF2) and the inhibitor of κ B kinase (IKK γ) was proposed [50]. However, more recent publications suggest an RIP1-independent process [51] or a subdivided mechanism [52]. Beside TRAIL-R1 and TRAIL-R2, TRAIL-R4 was also suggested to be capable of

intracellular pro-survival NF- κ B and Akt signalling, as discussed further in more detail below.

Interestingly, beside the differential expression levels of intracellular proteins also the localization of TRAIL receptors at the plasma membrane was proposed to determine the formation of differential signalling complexes. It was shown that activation of aggregated TRAIL receptors in cholesterol-rich membrane microdomains (often called “lipid rafts”) leads to the formation of complexes capable of apoptotic signalling. In contrast, receptors outside of such microdomains are mainly involved in non-apoptotic signalling [53].

2.2.4 *TRAIL's Role In Vivo*

Although TRAIL is mainly known for its tumour cell killing capacity it is involved in numerous other regulatory mechanisms in the human body including the defence against viral and bacterial infections as well as haematopoiesis. To exert its function as a regulator of immune responses TRAIL is expressed in different leukocytes, both from the innate and adaptive immune systems. Most important are hereby the NK and T cells, but also B cells, dendritic cells, eosinophils, neutrophils, macrophages and monocytes which all can express TRAIL after stimulation with different activating agents like interferons (IFNs) or antigens [54]. Interestingly, TRAIL seems to be not only involved in the signalling of immune cells but even in their development [55].

NK cells play an important role in the innate immune response against intracellular pathogens but are at the same time also involved in combating tumour cells. The activation status of NK cells is dependent on different activating and inhibitory receptors as well as cytokines like IFNs and interleukins (ILs). Among these, especially IFN- γ and IFN- α are known to induce TRAIL expression [56, 57]. Interestingly, it was shown that NK cells are also involved in balancing adaptive immune responses versus autoimmunity since they have been shown to limit the autoimmune response during chronic infections by TRAIL-mediated killing of T helper cells [58, 59]. Furthermore, in hepatitis B, C and lymphocytic choriomeningitis infection, NK cells not only directly eliminate virus-infected cells but also regulate T cell responses via TRAIL release [60–62]. T cells, or more precisely T helper (Th) and cytotoxic T cells are also able to express TRAIL after stimulation of the T cell receptor together with type I IFNs [63, 64]. Similar to NK cells these are also involved in the elimination of malignant and virus infected cells in the human body. Additionally, TRAIL seems to be involved in the regulation of the Th type 1 (Th1) and Th2 responses, since Th2 cells are resistant to TRAIL-mediated apoptosis but express TRAIL after T cell receptor stimulation whereas stimulated Th1 cells are sensitive for TRAIL and up-regulate FasL. Furthermore, the inhibition of TRAIL in mice which display allergic airway diseases inhibited homing of Th2 cells to the airways [65–67].

Concerning viral and bacterial infections of the human body TRAIL seems to play the role of a double-edged sword. On the one hand TRAIL is involved directly and indirectly in the elimination of infected cells, but on the other hand some viral and bacterial species manipulate TRAIL signalling to evade from the immune response and to increase replication. A participation of TRAIL in viral defence of the human body can for example be observed during influenza infection. Hereby, infected alveolar cells are eliminated by TRAIL positive cytotoxic T cells, but TRAIL is believed also to limit the immune response reducing the chance for infection-induced immunopathology [68–70]. However, it was also described that TRAIL can be released by activated alveolar macrophages leading to damage of uninfected lung tissue [71]. Also, during hyper-inflammation TRAIL might play a conflicting role, depending on the stage of the disease. At the beginning, TRAIL appears to have mainly a protective function while eliminating activated neutrophils. Later in the disease TRAIL acts as an immune suppressor, thereby, contributing to the severe development [72, 73].

Shortly after its discovery in the late nineties, TRAIL was shown to not only kill tumour cells when applied exogenously, but proved to be effective in the human body as an endogenously expressed protein leading to the suppression of tumour growth [74]. As mentioned above, cells of the immune system, especially NK cells, T cells, macrophages and neutrophils, not only kill virus infected cells but can also identify and eliminate malignant cells *in vivo* and *in vitro* by releasing TRAIL among other cytokines when activated with IFN- γ [64, 75–79].

Interestingly, TRAIL and its receptors are expressed not only on malignant cells and cells of the immune system but also in many other tissues of the human body including testis, lung, colon, kidney and endothelium [11]. Their biological functions, however, are in most cases largely undefined. In the human testis, TRAIL for example has been shown to regulate germ cell apoptosis during the first wave of spermatogenesis [80] and it was further demonstrated that high levels of TRAIL in the seminal plasma protect spermatozoa [81]. Also, the endothelium is known to express TRAIL receptors and the medial smooth cell layer of the aorta and pulmonary arteries produce the ligand TRAIL [82, 83] which was demonstrated to promote survival as well as proliferation of endothelial cells [84]. Further, TRAIL takes part in the interplay of endothelial cells with leucocytes, modelling cell adhesion by down regulation of chemokine receptors [85]. The application of TRAIL resulted in enhanced phosphorylation of oxide synthase in endothelial cells, which was shown to increase cell migration as well as cytoskeleton reorganization and might also affect blood vessel vasodilatation and angiogenesis [86, 87].

2.3 The TRAIL System in Cancer

Cancer is a major cause of death and therefore an enormous health challenge. Worldwide cancer incidents have been increased during the last decades, mostly attributable to increased average life expectancy. On the other hand, significant

survival improvement could be achieved during the last 20 years by new medical diagnosis tools and better adjuvant therapies.

Cancer is treated typically by surgical resection of the tumour combined with adjuvant therapy. The classical approach is radiotherapy and chemotherapy, however, both are not cancer cell specific and in addition tumour cells can acquire resistance. To overcome these limitations, numerous novel therapeutic strategies have been developed and approved by the U.S. Food and Drug Administration during the last decade, such as targeted therapies and small molecule inhibitors [88]. In addition, much effort is put into the identification of predictive or prognostic tumour biomarkers, including characteristic genetic alterations or particular protein expression pattern. The expression of markers in different types and grades of cancers might be a rational basis to design an effective adjuvant treatment strategy and to improve the overall survival of patients [89]. For example, colorectal cancers are frequently mutated in *KRAS* and consequently patients will not respond to epidermal growth factor receptor (EGFR)-based therapies [90]. In fact, *KRAS* represents the first biomarker routinely used in clinical practice [89].

Tumours are frequently altered for p53 activity and are thus resistant to several classes of conventional chemotherapy. A promising therapeutic strategy is to trigger the extrinsic apoptotic pathway using PARAs as therapeutic agents, since cell death induced via this route is independent from p53. Consequently, the successful application of PARAs in tumour therapy requires plasma membrane expression of the respective death receptors in tumour cells and a functional downstream signalling network leading to the apoptotic machinery.

2.3.1 Predictive and Prognostic Significance of TRAIL Receptor Expression

Quite a few studies analysed patient-derived tumour tissues from different cancer types and grades, treated or untreated, for cellular TRAIL receptor expression using conventional immunohistochemistry. In addition, reports on TRAIL receptor gene expression and mutational analysis are found in the literature. These studies focused on the expression profile of pro-apoptotic proteins including TRAIL-R1, TRAIL-R2, caspase-8 and TRAIL itself as well as anti-apoptotic proteins such as TRAIL-R3, TRAIL-R4, c-Flip and Bcl-2 family members. In general, TRAIL receptors are frequently expressed in carcinomas from different origins as well as in the respective normal surrounding tissue [91–96]. Receptor expression was detected in various subcellular localizations, namely, the cytoplasm, the nucleus and membrane-bound. Interestingly, oncogenic mutations in the MAPK pathway, *KRAS* and/or *BRAF*, frequently found in colorectal tumours, were linked to high TRAIL-R1 and TRAIL-R2 expression [94].

Reports of genetic loss or mutation of TRAIL receptors have been also described, but were suggested to represent not a common event in cancer cells [97, 98].

Polymorphisms in the *TRAIL-R1* gene and a loss of function mutation of *TRAIL-R2* potentially increase the risk for development of head and neck cancer [99, 100]. A polymorphism in the *TRAIL-R4* gene appears to be associated with reduced breast cancer risk [101]. A recent study identified a single nucleotide polymorphism (SNP)-SNP interaction pair, located in the NF- κ B pathway genes *TRAF2* and *TRAIL-R4*, to be correlated with improved survival in breast cancer [102]. Notably, both NF- κ B and TRAIL-R4 have been rather implicated in the protection from TRAIL-induced apoptosis although constitutive activation of NF- κ B signalling is associated with tumour progression.

Both negative and positive correlations of the expression pattern with the grade of malignancy and other clinical features such as overall survival have been shown for TRAIL-R1 to TRAIL-R4. But it should be mentioned that there are also reports demonstrating no correlation of either TRAIL receptor expression with grade or survival [95, 103, 104]. High TRAIL-R1 expression is correlated with tumour grade in breast cancer patients with invasive ductal carcinoma and bladder cancer [105, 106] and with an unfavourable overall survival in adjuvant treated stage III colon cancer [93]. High TRAIL-R2 expression was shown to be associated with a higher grade of malignancy in breast, bladder, hepatocellular, renal, head and neck carcinomas and with less differentiated areas of non-small cell lung cancer of different grades [92, 106–112]. Increased TRAIL-R2 expression also matched a larger tumour size in head and neck cancer tissues [113]. Accordingly, TRAIL-R2 was found to negatively correlate with overall survival in breast, renal and lung cancers. Conversely, TRAIL-R2 expression was significantly decreased in prostate cancer of higher tumour grade [114].

Reports on TRAIL receptor expression as positive prognostic markers can also be found. High TRAIL-R1 expression was correlated with well-differentiated tumours and with better prognosis in colorectal cancer patients [91, 115]. In cervical cancer, TRAIL-R1 in the nucleus might be a predictive biomarker for radiotherapy [116]. In glioblastoma multiforme, a strong membrane staining for both TRAIL-R1 and TRAIL-R2 was correlated with better survival [117]. Since TRAIL exerts its bioactivity after binding to TRAIL receptors in the plasma membrane, loss of surface expression may explain tumour resistance to TRAIL-based therapies despite uniform cellular receptor expression. In fact, a poorer prognosis for patient survival was associated with loss of membrane staining for TRAIL-R2, but also TRAIL-R1, in early-stage colorectal carcinoma, breast cancer and pancreatic carcinoma [96, 118, 119].

Quite unexpected, recent studies revealed evidence that the subcellular localization of TRAIL receptors, in particular of TRAIL-R2, is an important determinant for the signalling outcome. Nuclear TRAIL-R2 was shown to inhibit microRNA maturation thereby enhancing malignancy of tumours, however, the functional role of cytoplasmic TRAIL receptors has not been addressed so far [5]. High cytoplasmic levels of TRAIL-R1 or TRAIL-R2 correlated with an improved recurrence-free rate for bladder cancer patients [106]. Accordingly, the differential distribution of the receptors in cellular compartments could be one reason for the inconsistent correlation of receptor expression with prognosis in cancer.

Most human cell lines co-express TRAIL-R3 and TRAIL-R4, albeit at comparably low levels. Accordingly, the expression of TRAIL-R3 and TRAIL-R4 was also evaluated for prognostic impact in several studies. TRAIL-R4 expression level is increased in prostate, pancreatic, breast, hepatocellular cancers and meningioma [107, 108, 120–122]. A strong correlation of its expression with higher tumour grades as well as poor clinical outcome and decreased survival has been demonstrated. In colorectal carcinoma, high TRAIL-R3 expression, in combination with low TRAIL-R1, is linked to poor response to 5-fluorouracil and shorter progression-free survival [123]. In head and neck cancer, TRAIL-R3 and TRAIL-R4 expressions were also significantly increased but did not correlate with tumour staging or prognosis [110, 124].

Remarkably, a positive correlation was found between the expression levels of TRAIL death receptors and inhibitory receptors. TRAIL-R2 upregulation correlates with increased TRAIL-R4, as reported for meningioma, hepatocellular, pancreatic and head and neck cancers, however, without relevance for the overall patient survival [108, 122, 124]. In contrast, high TRAIL-R2 levels along with low TRAIL-R4 expression were shown to be associated with higher grade of renal cell cancer and worse survival [109]. Similarly, high levels of TRAIL-R3 are associated with low TRAIL-R1 [123]. These data appear puzzling, more knowledge about the molecular mechanisms which control expression of the individual receptors could help to understand them better.

Interestingly, the ligand itself was highlighted as a prognostic marker. Patients newly diagnosed with acute myeloid or acute lymphoblastic leukaemia have significantly lower serum concentrations of TRAIL compared to healthy volunteers [125, 126]. Upon start of the therapy an increase of TRAIL expression resulting in higher serum concentrations was predictive of better overall patient survival [126]. Higher TRAIL expression was also associated with low tumour grade and better progression-free survival in ovarian and renal cancer patients [95, 127]. Loss of TRAIL expression in oral and cervical carcinoma is an early event and is correlated with malignant progression [113, 116]. These results indicate that downregulation of TRAIL expression enables cancerous cells to evade apoptosis suggesting that TRAIL contributes to a positive therapeutic response. On the other hand, a high level of TRAIL was associated with shorter survival for patients with renal and colorectal cancers [109, 128]. It was argued that high TRAIL expression may protect cancer cells from the immune system while promoting metastasis. TRAIL was also shown to have no prognostic value [91, 93] and there was no correlation between TRAIL expression and survival [117].

In summary, numerous studies have evaluated the prognostic relevance of the expression pattern of TRAIL and TRAIL receptors in tumour entities. The results are interesting, but also very conflicting. As most data have not been confirmed in larger validation studies, their clinical value remains limited [89]. Recently, it has become evident that the subcellular localization of TRAIL receptors may regulate specific functions and thus correlates with pro-apoptotic versus pro-survival signalling. Accordingly, it will be necessary to carefully re-evaluate the pattern of TRAIL receptor staining in primary tissue to further analyse its significance on tumour growth and response to TRAIL-based therapeutics.

2.3.2 *TRAIL Death Receptor Agonists*

Shortly after its discovery in the late nineties, TRAIL was shown to represent a promising candidate in the battle against cancer since it selectively eliminates malignant cells while sparing normal tissue [4]. Although early reports claimed a cytotoxic effect of recombinant TRAIL in hepatocytes and cells in human brain slices, this was later shown to be caused by the protein's affinity tags resulting in the formation of supramolecular aggregates [129]. However, despite that TRAIL is well tolerated in vivo and shows cytotoxicity against tumour cells in vitro, until today there is no TRAIL-based anti-tumour drug available on the market. The main reason might be that TRAIL receptor-directed clinical studies showed only moderate effects.

Among all generated TRAIL death receptor agonists investigated so far, homotrimeric recombinant TRAIL protein (dulanermin) was one of the first. In different phase I studies dulanermin proved to be well tolerated but did not reveal any significant anti-tumour activities [129]. Likely explanations for this failure are the very short half-life of only 20 min of this molecule [4, 130] but also the fact that soluble TRAIL might only fully activate TRAIL-R1, but not TRAIL-R2 [3, 14]. To address this problem oligomerized derivatives have been produced by the addition of a leucine or isoleucine zipper domain [4, 131] or a tenascin-c (TNC) domain [132]. Approaches to prolong the half-life in vivo include coupling of TRAIL with polyethylene glycol or human serum albumin [129]. An interesting approach is the use of single-chain TRAIL (scTRAIL) which, in contrast to the classical homotrimeric TRAIL, is expressed as a single protein chain consisting of the three TRAIL “monomers” covalently connected by short peptide linkers [133]. This molecule, possessing only a single N- and C-terminus, has been further fused with the Fc portion of human IgG1 to obtain a hexameric molecule with enhanced bioactivity [134].

Beside the stability and half-life also the targeting of the molecule to cancer cells is of great interest. Fusing of the abovementioned TRAIL variants to single-chain variable fragment (scFv) domains specific for different tumour-expressed antigens such as EGFR, fibroblast activation protein, CD19, CD33, or CD20 enables targeting and enhances protein stability [129, 135]. In addition, as these constructs bind to cell surface antigens thereby increasing receptor cross linking they show enhanced bioactivities. In another approach, the TRAIL death receptor agonists were not targeted to the tumour cells but to cells of the immune systems to enhance their tumour killing capacity [136–138].

Beside the large group of TRAIL fusion proteins also antibodies specific for either TRAIL-R1 or TRAIL-R2 have been developed like mapatumumab and lexatutumumab. All of them have been demonstrated to effectively kill tumour cells in vitro, but did not display compelling anti-tumour activity in clinical trials [139]. The reasons for this limited success might include insufficient receptor cross linking, i.e., the need for molecules with higher affinity/avidity, problems to reach the tumour cells at sufficient concentration and the fact that cells in a solid tumour might show a relative TRAIL resistance as indicated by data from multicellular

tumour spheroids in vitro, where downregulation of TRAIL-R1 and TRAIL-R2 has been demonstrated [140, 141]. To increase the efficacy of PARAs it appears feasible to use sensitizing drugs, like bortezomib, doxorubicin, cisplatin and 5-fluorouracil, which all have been shown to enhance TRAIL-mediated apoptosis in vitro [142].

2.3.3 The Role of TRAIL-R3 and TRAIL-R4 in Resistance to TRAIL-Mediated Apoptosis

A major hurdle in developing TRAIL-based therapies is the primary or acquired resistance to TRAIL, as it has been reported that around 50% of all cancer cell lines show resistance to TRAIL treatment [4]. Clearly, TRAIL resistance can be generated at multiple levels in the apoptotic signalling network, but the first control point exists at the membrane at the level of TRAIL receptors, e.g., by overexpression of decoy receptors or downregulation of death receptors. Initial studies have proposed that high expression of decoy receptors TRAIL-R3 and/or TRAIL-R4 in normal cells would cause protection against TRAIL-induced apoptosis [18, 30]. But later on it has been clarified that the situation is not that simple since inhibition of the decoy receptors does not necessarily result in a sensitization of normal cells to TRAIL. So, their functional role and mode of action are much more complicated than what initially was proposed. Nevertheless, a broad range of studies has shown that transformed cells can evade TRAIL-induced apoptosis by overexpression of decoy receptors [143–147].

2.3.3.1 The Decoy Model for TRAIL-R3 and TRAIL-R4

Unlike other TNF receptor family members, TRAIL-R3 completely lacks a cytoplasmic domain as it is anchored in the plasma membrane by a GPI residue [27]. In contrast, TRAIL-R4 contains a cytoplasmic region representing only one-third of a typical DD and which is therefore considered to be non-functional [29]. However, the ECDs of all four membrane-bound receptors TRAIL-R1 to TRAIL-R4 show strong homologies in their structure and all consist of three CRDs. Since most of the cells simultaneously co-express both pro- and anti-apoptotic receptors on their surface, it was initially thought that TRAIL-R3 and TRAIL-R4 compete with TRAIL-R1 and TRAIL-R2 for binding of the ligand through their ECDs [148]. Consequently they were referred to as “decoy receptors”. In fact, various initial in vitro studies have linked a high expression level of decoy receptors with TRAIL resistance and vice versa [145, 149]. Sanlioglu and co-workers found that the TRAIL sensitive breast cancer cell line MDA-MB-231 expresses less TRAIL-R4 on the cell surface as compared to the higher expression in the TRAIL resistant MCF7 cell line [144]. Further, enforced overexpression of TRAIL-R3 has been shown to inhibit TRAIL-mediated apoptosis induction in various cancer cells and this resistance could be abolished by cleavage of the GPI anchor to remove

TRAIL-R3 from the cell surface [150]. Although these and other studies linked the expression of the decoy receptors to TRAIL resistance, finally no significant correlation was found between TRAIL sensitivity and the expression levels of TRAIL-R3 and/or TRAIL-R4 on tumour cells [151–153].

Clearly, the principle of the proposed decoy mechanism is functional, i.e., TRAIL-R3 and TRAIL-R4 consume ligand without induction of a resulting pro-apoptotic signal, thereby reducing the ligand amount available for TRAIL-R1 and TRAIL-R2. But it is also obvious, that inhibition of TRAIL sensitivity by the decoy mechanism must be overcome when using extremely high concentrations of TRAIL which so far could not be demonstrated (Neumann, S., unpublished data). There exist some exceptional situations where one might expect the decoy mechanism to play a major role, for example in case of extremely high expression levels of these receptors and/or at very low ligand concentrations [154]. Alternatively, one might expect that the ligand binding affinity values of the decoy receptors should be significantly higher compared to those of the death receptors. The first reports determining binding affinities suggested that all four receptors bind to TRAIL with comparable affinities [27, 30]. However, later investigations demonstrated that the death receptors possess somewhat higher binding affinity values at physiological temperature as compared to the decoy receptors [155, 156], arguing further against the importance of the decoy mechanism under (patho)physiological conditions *in vivo*. Together, the idea that the inhibitory effects of TRAIL-R3 and TRAIL-R4 mainly depends on ligand consumption became hard to accept as a general and important mechanism.

2.3.3.2 Formation of Heteromeric Complexes Affected in Signalling

A second possibility for TRAIL-R3 and TRAIL-R4 to interfere with TRAIL-mediated apoptosis induction is the formation of heteromeric ligand/receptor complexes. As mentioned TRAIL forms homotrimers capable to bind three receptor molecules in the clefts between the individual protomers [12]. In a cell co-expressing for example equal levels of TRAIL-R1 and TRAIL-R3, four different initial complexes could be formed upon ligation: TRAIL-(R1)₃, TRAIL-(R1)₂R3, TRAIL-R1(R3)₂ and TRAIL-(R3)₃. The potential signalling capabilities of the two mixed complexes are totally unclear, but if we assume that TRAIL-R3 is incapable to signal at all, we would end up in any case with a situation of partly inhibited apoptotic signalling as compared to a cell line expressing only TRAIL-R1, but no TRAIL-R3. Additional constraints exist, however, which could regulate the efficiency of formation of the different homomeric and heteromeric complexes. First, individual receptors might be enriched in the cell membrane in different compartments, like the already mentioned cholesterol-rich microdomains. Taken as an extreme case, in our example above TRAIL-R1 and TRAIL-R3 could then be separated totally in distinct microdomains, which would not allow significant formation of heteromeric complexes upon ligand binding at all. Second, TRAIL receptors, like other members of the TNF receptor family, possess a homophilic interaction domain allowing

homomultimer formation of the membrane-expressed receptors even in the absence of ligand, the PLAD. This domain is located at the membrane distal (partial) first CRD (CRD1) of the four TRAIL receptors. Moreover, in the TRAIL receptor system, but e.g., not in the TNF receptor system, this domain allows the formation of heteromeres [154, 156–158]. The PLAD has been originally detected and described in the TNF and the Fas systems [159, 160]. The stoichiometry of PLAD-mediated multimer formation is not well defined. The group of Lenardo originally described TNF receptor and Fas homotrimers, whereas later studies using TNF receptor chimeras suggested homodimers [161]. More recently, we confirmed heteromeric TRAIL receptor interactions in the absence of ligand by acceptor photobleaching fluorescence resonance energy transfer (FRET) studies and also found predominant TRAIL receptor homodimer and heterodimer formation [154]. It cannot be excluded, however, that trimer vs. dimer formation might be regulated in a cell type-specific manner or is mainly attributable to the chemical cross linker used.

Convincing evidence exists that the PLAD not only serves to mediate multimer formation of receptors in the absence of ligand, but also after ligation. Accordingly, driven by two different interaction sites which do not sterically interfere with each other, the formation of large ligand/receptor clusters is allowed. Originally proposed in the TNF system on the basis of studies with TNF receptor mutants [161], the group of Sachs later confirmed the formation of TRAIL-R2 dimers within high molecular weight ligand/receptor networks [158]. Further studies of this group with TNF-R1 and TRAIL-R2 resulted in an activation model for these receptors. According to this model unligated receptor homodimers are in an “OFF” stage when homodimerized via PLAD interaction, but are twisted to “ON” when becoming incorporated in large ligand/receptor clusters [162, 163]. In the formation of these clusters the initial step is likely the binding of one TRAIL molecule with one of its three binding sites on the “back” of the homodimerized receptor molecules (Fig. 2.3a). Mathematical modelling studies suggest that the PLAD-PLAD interaction of the receptor dimer then opens to allow binding of the second receptor to one of the two other binding sites of the TRAIL molecule as detailed in [164]. These initial complexes TRAIL-(TRAIL-R)₂ would then further aggregate upon diffusion in the membrane.

Affinity data obtained from plasmon resonance studies of purified soluble ECDs from TRAIL receptors indicate comparable affinity values for homomeric and heteromeric PLAD-mediated interactions and were found to be in the micromolar range [165]. These low affinity values determined, however, cannot be taken for the “effective” PLAD affinities of membrane-expressed receptor molecules, because the latter are oriented and arranged in the cell membrane, whereas one binding partner in the studies by Lee et al. freely diffused in solution, whereas the other partner was immobilized (for discussion of this point, see [154]). Notably, no measurable affinity values were found for the interaction between the ECD of TRAIL-R2 and those of the two decoy receptors. It is unlikely that some molecules in this study were simply misfolded, because proper interactions in all other eight ECD combinations could be determined. These data indicate an interesting difference between

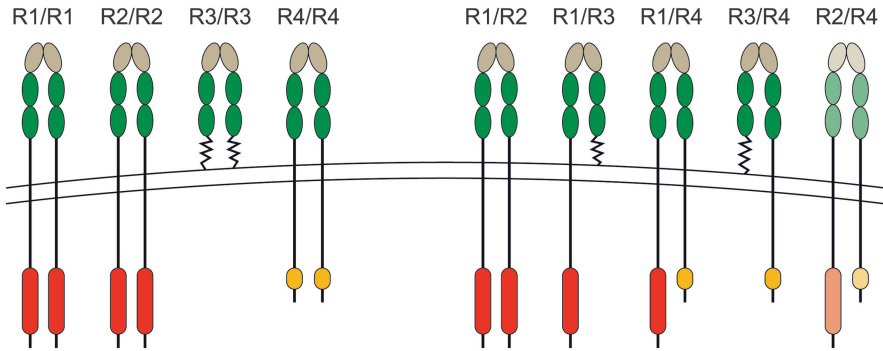


Fig. 2.2 Homotypic and heterotypic interactions between TRAIL receptor dimers. TRAIL receptors on the plasma membrane exist as pre-formed dimers in the absence of ligand, although unligated receptor trimers have also been proposed. Dimerization is mediated by the PLAD and occurs either as a homotypic (*left*) or a heterotypic (*right*) interaction between individual TRAIL receptor monomers. Note that TRAIL-R3 might be sequestered in cholesterol-rich membrane microdomains. Although heteromeric TRAIL-R3 interactions with TRAIL-R1 or TRAIL-R4 have been reported, their existence remains fairly speculative. Similarly, the heteromeric TRAIL-R2/TRAIL-R4 interaction remains inconclusive (see text for detail)

TRAIL-R1 and TRAIL-R2, the first capable to interact with all four receptors, whereas the latter can interact only with TRAIL-R1 and TRAIL-R2. However, in contrast to these data FRET and co-immunoprecipitation experiments suggested an interaction between TRAIL-R2 and TRAIL-R4 [154, 156]. Having all these facts in mind, we end up with a very complex situation: We have up to four different receptors co-expressed in a single cell, which might form up to four different homodimers and four or even five heterodimers (Fig. 2.2), which then react with the homotrimeric ligand TRAIL to finally produce large ligand/receptor clusters (Fig. 2.3).

2.3.3.3 TRAIL-R3 and TRAIL-R4 Incorporation in Ligand Receptor Clusters

Based on initial PLAD data, Clancy and colleagues introduced a new term for TRAIL-R4, “regulatory receptor” instead of “decoy receptor”. They claimed that the inhibitory action of TRAIL-R4 does not entirely depend on the consumption of TRAIL, but is rather mediated by ligand independent formation of mixed complexes with TRAIL-R2 [156]. However, there are conflicting results regarding the role of TRAIL in the formation of homomeric and heteromeric complexes, as Merino and colleagues concluded that this interaction between TRAIL-R4 and TRAIL-R2 is ligand dependent while other studies suggested a ligand independent process, mediated by the PLAD [156, 157].

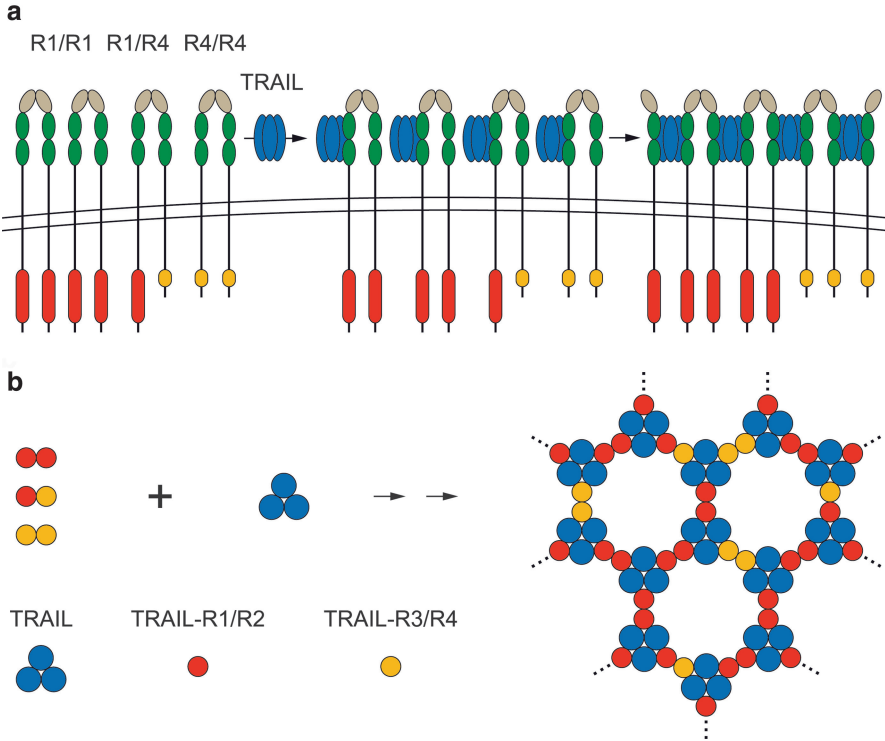


Fig. 2.3 Formation of heteromeric ligand-receptor clusters driven by PLAD-mediated receptor-receptor and ligand-receptor interaction. According to the current model, binding of TRAIL molecules (*blue*) on the “back” of pre-ligated homomeric or heteromeric receptor dimers on the cell membrane induces conformational changes of the receptors, results in opening of the PLAD-PLAD interaction and subsequent binding of a second TRAIL receptor dimer (a). Formation of large ligand-receptor clusters referred to as signalling protein oligomeric transduction structures (SPOTS) are shown in hexagonal arrangement. TRAIL-R3- and TRAIL-R4-mediated interference with death receptor signalling is based on the reduction of signalling-competent receptor complexes (b)

In a more recent work our group investigated the effects of TRAIL-R4 on TRAIL-R1 signalling in detail, aided by mathematical modelling [154]. As expected, the experimental data demonstrate that TRAIL-R4 effectively inhibited TRAIL-R1-mediated apoptosis induction, but also non-apoptotic signalling like activation of NF- κ B. Moreover, these effects were not mediated by the cytoplasmic domain of TRAIL-R4 (see Sect. 2.3.3.4), i.e., signalling crosstalk, because a cytoplasmically truncated TRAIL-R4 mutant showed comparable effects. In addition, the results from mathematical modelling clearly showed that the classical decoy mechanism must be neglectable under the experimental conditions chosen. As both, intracellular signalling by TRAIL-R4 and the decoy mechanism could be ruled out to be effective, the formation of heteromeric complexes was proposed to cause the dominant negative effects of TRAIL-R4 on TRAIL-R1 signalling (Fig. 2.3).

2.3.3.4 Activation of Pro-Survival Pathways by TRAIL-R4

TRAIL and its receptors create a highly complex signalling system not only because of the presence of multiple receptors, but also caused by the fact that individual TRAIL receptors can not only induce apoptosis but are also known to initiate survival pathways. The induction of survival pathways, like e.g., the activation of NF- κ B and Akt, has been suggested as an additional mechanism contributing to the inhibitory effects of TRAIL-R4, mediated by its truncated DD suggested to be incapable to induce apoptosis. There exist conflicting results regarding the role of TRAIL-R4 in activation of the transcription factor NF- κ B, as initial studies by Marsters and colleagues showed that removal of the intracellular domain of TRAIL-R4 had no effects on TRAIL-mediated apoptosis induction and NF- κ B activation. Accordingly, it was proposed that the truncated DD of TRAIL-R4 is not functional and does not play any role in the inhibitory function of this receptor [29]. However, another group reported that TRAIL-R4, alike TRAIL-R1/R2 is capable to activate the NF- κ B pathway via its cytoplasmic domain although the precise molecular mechanism remained unclear [30]. It was therefore proposed that NF- κ B activation and subsequent transcription of various anti-apoptotic proteins could play a role in TRAIL-R4-mediated resistance against TRAIL. In our experimental systems, however, TRAIL-R1-mediated phosphorylation of inhibitor of NF- κ B (I κ B α), a central step in the activation of the classical NF- κ B pathway, was inhibited by overexpression of both functional and cytoplasmatically deleted TRAIL-R4 [154]. Although it is generally accepted that TRAIL is not a potent inducer of NF- κ B activation, this signalling pathway may be controlled in a cell specific manner similar to the activation of Akt where again conflicting results exist. TRAIL-R4 expression in HeLa cells was shown to protect from TRAIL-induced apoptosis and enhanced cell proliferation and these effects could be reversed by inhibiting Akt phosphorylation [166]. Contrary to these results we could not observe any difference in the phosphorylation of Akt in HeLa cells overexpressing TRAIL-R4 [154].

More recent data open a new facet in the field of negative TRAIL receptor interaction. In most studies which have been investigated, the interference between apoptotic and non-apoptotic TRAIL receptors took only the amounts of membrane-expressed receptor molecules into account. It is now clear, however, that TRAIL receptors including TRAIL-R3 and TRAIL-R4 also occur in intracellular compartments and may act there in a still largely undefined manner [5, 96, 119, 167]. It is, therefore, possible that the ligand TRAIL may act also as a stimulus to induce a relocalization of receptors, thereby shifting their function. Further intricate studies will be necessary to verify this hypothesis.

Taken together, three possible mechanisms have been proposed up to now that may contribute to TRAIL-resistance being mediated by TRAIL-R3/TRAIL-R4. These are the classical decoy mechanism which does occur but has been convincingly shown to play no major role under typical experimental conditions. In special (patho)physiological situations this effect could be of significance. The formation of ligand/receptor signalling clusters comprising a mixture of signal competent and incompetent receptors (TRAIL-R1/R2 and TRAIL-R3/R4, respectively) as the

second proposed mechanism are believed to have a strong impact (Fig. 2.3). Molecular interactions and parameters guiding their formation are complex, however, including (co)expression levels, distribution in microdomains, PLAD-mediated interaction and finally ligand/receptor interactions. The third possible mechanism, intracellular signal induction mediated by TRAIL-R3 and TRAIL-R4, is again believed to occur in a special context only. Whereas a signalling capacity by the GPI moiety-anchored TRAIL-R3 appears speculative, the biological function of the intracellular domain of TRAIL-R4 needs to be further investigated.

2.4 Outlook

Coming to the insight that the TRAIL system is extremely complex and the role of TRAIL receptors and their crosstalk in cancer are highly undefined, it is evident that we still need much more data from experimental systems and from the clinic. Nevertheless, it appears to be a valuable therapeutic approach to induce apoptosis/necroptosis in tumour cells upon stimulation of TRAIL-R1 and/or TRAIL-R2. To successfully follow this pathway, we need strong agonists like targeted, multimerized TRAIL fusion proteins or receptor-specific agonistic antibody constructs. A multitude of such molecules has been already tested in animal models and clinical studies. In all these approaches it appears beneficial to spare the inhibitory receptors TRAIL-R3 and TRAIL-R4, which can be easily obtained using agonistic antibodies, but also by the construction of receptor-selective mutants of TRAIL.

References

1. Aggarwal BB, Gupta SC, Kim JH (2012) Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* 119(3):651–665. doi:10.1182/blood-2011-04-325225
2. Croft M, Benedict CA, Ware CF (2013) Clinical targeting of the TNF and TNFR superfamilies. *Nat Rev Drug Discov* 12(2):147–168. doi:10.1038/nrd3930
3. Wajant H, Moosmayer D, Wuest T, Bartke T, Gerlach E, Schonherr U, Peters N, Scheurich P, Pfizenmaier K (2001) Differential activation of TRAIL-R1 and -2 by soluble and membrane TRAIL allows selective surface antigen-directed activation of TRAIL-R2 by a soluble TRAIL derivative. *Oncogene* 20(30):4101–4106. doi:10.1038/sj.onc.1204558
4. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, Smith C, Smolak P, Goodwin RG, Rauch CT, Schuh JC, Lynch DH (1999) Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med* 5(2):157–163. doi:10.1038/5517
5. Haselmann V, Kurz A, Bertsch U, Hubner S, Olempska-Muller M, Fritsch J, Hasler R, Pickl A, Fritsche H, Annawanter F, Engler C, Fleig B, Bernt A, Roder C, Schmidt H, Gelhaus C, Hauser C, Egberts JH, Heneweier C, Rohde AM, Boger C, Knippschild U, Rocken C, Adam D, Walczak H, Schutze S, Janssen O, Wulczyn FG, Wajant H, Kalthoff H, Trauzold A (2014) Nuclear death receptor TRAIL-R2 inhibits maturation of let-7 and promotes proliferation of pancreatic and other tumor cells. *Gastroenterology* 146(1):278–290. doi:10.1053/j.gastro.2013.10.009

6. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA et al (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3(6):673–682
7. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A (1996) Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem* 271(22):12687–12690
8. Krieg A, Krieg T, Wenzel M, Schmitt M, Ramp U, Fang B, Gabbert HE, Gerharz CD, Mahotka C (2003) TRAIL-beta and TRAIL-gamma: two novel splice variants of the human TNF-related apoptosis-inducing ligand (TRAIL) without apoptotic potential. *Br J Cancer* 88(6):918–927. doi:10.1038/sj.bjc.6600772
9. Wang P, Lu Y, Li C, Li N, Yu P, Ma D (2011) Novel transcript variants of TRAIL show different activities in activation of NF-kappaB and apoptosis. *Life Sci* 89(23–24):839–846. doi:10.1016/j.lfs.2011.09.003
10. Krieg A, Mersch S, Wolf N, Stoecklein NH, Verde PE, am Esch JS 2nd, Heikaus S, Gabbert HE, Knoefel WT, Mahotka C (2013) Expression of TRAIL-splice variants in gastric carcinomas: identification of TRAIL-gamma as a prognostic marker. *BMC Cancer* 13:384. doi:10.1186/1471-2407-13-384
11. Spierings DC, de Vries EG, Vellenga E, van den Heuvel FA, Koomstra JJ, Wesseling J, Hollema H, de Jong S (2004) Tissue distribution of the death ligand TRAIL and its receptors. *J Histochem Cytochem* 52(6):821–831
12. Hymowitz SG, O'Connell MP, Ultsch MH, Hurst A, Totpal K, Ashkenazi A, de Vos AM, Kelley RF (2000) A unique zinc-binding site revealed by a high-resolution X-ray structure of homotrimeric Apo2L/TRAIL. *Biochemistry* 39(4):633–640
13. Mariani SM, Krammer PH (1998) Differential regulation of TRAIL and CD95 ligand in transformed cells of the T and B lymphocyte lineage. *Eur J Immunol* 28(3):973–982. doi:10.1002/(SICI)1521-4141(199803)28:03<973::AID-IMMU973>3.0.CO;2-T
14. Muhlenbeck F, Schneider P, Bodmer JL, Schwenzer R, Hauser A, Schubert G, Scheurich P, Moosmayer D, Tschopp J, Wajant H (2000) The tumor necrosis factor-related apoptosis-inducing ligand receptors TRAIL-R1 and TRAIL-R2 have distinct cross-linking requirements for initiation of apoptosis and are non-redundant in JNK activation. *J Biol Chem* 275(41):32208–32213. doi:10.1074/jbc.M000482200
15. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM (1997) The receptor for the cytotoxic ligand TRAIL. *Science* 276(5309):111–113
16. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI, Goddard AD, Godowski P, Ashkenazi A (1997) Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 277(5327):818–821
17. Screaton GR, Mongkolsapaya J, Xu XN, Cowper AE, McMichael AJ, Bell JI (1997) TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL. *Curr Biol* 7(9):693–696
18. Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM (1997) An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* 277(5327):815–818
19. MacFarlane M, Ahmad M, Srinivasula SM, Fernandes-Alnemri T, Cohen GM, Alnemri ES (1997) Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. *J Biol Chem* 272(41):25417–25420
20. Schneider P, Bodmer JL, Thome M, Hofmann K, Holler N, Tschopp J (1997) Characterization of two receptors for TRAIL. *FEBS Lett* 416(3):329–334
21. Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L (1997) Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-kappaB pathway. *Immunity* 7(6):821–830
22. Wu GS, Burns TF, McDonald ER 3rd, Jiang W, Meng R, Krantz ID, Kao G, Gan DD, Zhou JY, Muschel R, Hamilton SR, Spinner NB, Markowitz S, Wu G, el-Deiry WS (1997) KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nat Genet* 17(2):141–143. doi:10.1038/ng1097-141

23. Picarda G, Surget S, Guiho R, Teletchea S, Berreur M, Tirode F, Pellat-Deceunynck C, Heymann D, Trichet V, Redini F (2012) A functional, new short isoform of death receptor 4 in Ewing's sarcoma cell lines may be involved in TRAIL sensitivity/resistance mechanisms. *Mol Cancer Res* 10(3):336–346. doi:10.1158/1541-7786.MCR-11-0390
24. van Roosmalen IA, Quax WJ, Kruyt FA (2014) Two death-inducing human TRAIL receptors to target in cancer: similar or distinct regulation and function? *Biochem Pharmacol* 91(4):447–456. doi:10.1016/j.bcp.2014.08.010
25. Bertsch U, Roder C, Kalthoff H, Trauzold A (2014) Compartmentalization of TNF-related apoptosis-inducing ligand (TRAIL) death receptor functions: emerging role of nuclear TRAIL-R2. *Cell Death Dis* 5:e1390. doi:10.1038/cddis.2014.351
26. von Karstedt S, Conti A, Nobis M, Montinaro A, Hartwig T, Lemke J, Legler K, Annewanter F, Campbell AD, Taraborrelli L, Grosse-Wilde A, Coy JF, El-Bahrawy MA, Bergmann F, Koschny R, Werner J, Ganten TM, Schweiger T, Hoetzenecker K, Kenessey I, Hegedus B, Bergmann M, Hauser C, Egberts JH, Becker T, Rocken C, Kalthoff H, Trauzold A, Anderson KI, Sansom OJ, Walczak H (2015) Cancer cell-autonomous TRAIL-R signaling promotes KRAS-driven cancer progression, invasion, and metastasis. *Cancer Cell* 27(4):561–573. doi:10.1016/j.ccell.2015.02.014
27. Degli-Esposti MA, Smolak PJ, Walczak H, Waugh J, Huang CP, DuBose RF, Goodwin RG, Smith CA (1997) Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J Exp Med* 186(7):1165–1170
28. Mongkolsapaya J, Cowper AE, Xu XN, Morris G, McMichael AJ, Bell JI, Screaton GR (1998) Lymphocyte inhibitor of TRAIL (TNF-related apoptosis-inducing ligand): a new receptor protecting lymphocytes from the death ligand TRAIL. *J Immunol* 160(1):3–6
29. Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, Yuan J, Gurney A, Goddard AD, Godowski P, Ashkenazi A (1997) A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol* 7(12):1003–1006
30. Degli-Esposti MA, Dougall WC, Smolak PJ, Waugh JY, Smith CA, Goodwin RG (1997) The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. *Immunity* 7(6):813–820
31. Pan G, Ni J, Yu G, Wei YF, Dixit VM (1998) TRUNDD, a new member of the TRAIL receptor family that antagonizes TRAIL signalling. *FEBS Lett* 424(1–2):41–45
32. Kimberley FC, Screaton GR (2004) Following a TRAIL: update on a ligand and its five receptors. *Cell Res* 14(5):359–372. doi:10.1038/sj.cr.7290236
33. Krieg A, Schulte am Esch J 2nd, Ramp U, Hosch SB, Knoefel WT, Gabbert HE, Mahotka C (2006) TRAIL-R4-beta: a new splice variant of TRAIL-receptor 4 lacking the cysteine rich domain 1. *Biochem Biophys Res Commun* 349(1):115–121. doi:10.1016/j.bbrc.2006.08.031
34. Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. *Nature* 423(6937):337–342. doi:10.1038/nature01658
35. Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC, Young PR (1998) Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem* 273(23):14363–14367
36. Candido R (2014) The osteoprotegerin/tumor necrosis factor related apoptosis-inducing ligand axis in the kidney. *Curr Opin Nephrol Hypertens* 23(1):69–74. doi:10.1097/01.mnh.0000437611.42417.7a
37. Pitti RM, Marsters SA, Lawrence DA, Roy M, Kischkel FC, Dowd P, Huang A, Donahue CJ, Sherwood SW, Baldwin DT, Godowski PJ, Wood WI, Gurney AL, Hillan KJ, Cohen RL, Goddard AD, Botstein D, Ashkenazi A (1998) Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. *Nature* 396(6712):699–703. doi:10.1038/25387
38. Arakawa Y, Tachibana O, Hasegawa M, Miyamori T, Yamashita J, Hayashi Y (2005) Frequent gene amplification and overexpression of decoy receptor 3 in glioblastoma. *Acta Neuropathol* 109(3):294–298. doi:10.1007/s00401-004-0956-6

39. Yu KY, Kwon B, Ni J, Zhai Y, Ebner R, Kwon BS (1999) A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. *J Biol Chem* 274(20):13733–13736
40. Migone TS, Zhang J, Luo X, Zhuang L, Chen C, Hu B, Hong JS, Perry JW, Chen SF, Zhou JX, Cho YH, Ullrich S, Kanakaraj P, Carrell J, Boyd E, Olsen HS, Hu G, Pukac L, Liu D, Ni J, Kim S, Gentz R, Feng P, Moore PA, Ruben SM, Wei P (2002) TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. *Immunity* 16(3):479–492
41. Wang W, Zhang M, Sun W, Yang S, Su Y, Zhang H, Liu C, Li X, Lin L, Kim S, Okunieff P, Zhang Z, Zhang L (2013) Reduction of decoy receptor 3 enhances TRAIL-mediated apoptosis in pancreatic cancer. *PLoS One* 8(10):e74272. doi:10.1371/journal.pone.0074272
42. Zhou J, Song S, He S, Wang Z, Zhang B, Li D, Zhu D (2013) Silencing of decoy receptor 3 (DcR3) expression by siRNA in pancreatic carcinoma cells induces Fas ligand-mediated apoptosis in vitro and in vivo. *Int J Mol Med* 32(3):653–660. doi:10.3892/ijmm.2013.1437
43. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26(4):239–257
44. Davidovich P, Kearney CJ, Martin SJ (2014) Inflammatory outcomes of apoptosis, necrosis and necroptosis. *Biol Chem* 395(10):1163–1171. doi:10.1515/hsz-2014-0164
45. Renahan AG, Booth C, Potten CS (2001) What is apoptosis, and why is it important? *BMJ* 322(7301):1536–1538
46. Elmore S (2007) Apoptosis: a review of programmed cell death. *Toxicol Pathol* 35(4):495–516. doi:10.1080/01926230701320337
47. Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, Bao JK (2012) Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif* 45(6):487–498. doi:10.1111/j.1365-2184.2012.00845.x
48. Chen D, Yu J, Zhang L (2016) Necroptosis: an alternative cell death program defending against cancer. *Biochim Biophys Acta* 1865(2):228–236. doi:10.1016/j.bbcan.2016.03.003
49. Azijli K, Weyhenmeyer B, Peters GJ, de Jong S, Kruyt FA (2013) Non-canonical kinase signaling by the death ligand TRAIL in cancer cells: discord in the death receptor family. *Cell Death Differ* 20(7):858–868. doi:10.1038/cdd.2013.28
50. Varfolomeev E, Maecker H, Sharp D, Lawrence D, Renz M, Vucic D, Ashkenazi A (2005) Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J Biol Chem* 280(49):40599–40608. doi:10.1074/jbc.M509560200
51. Grunert M, Gottschalk K, Kapahnke J, Gundisch S, Kieser A, Jeremias I (2012) The adaptor protein FADD and the initiator caspase-8 mediate activation of NF-kappaB by TRAIL. *Cell Death Dis* 3:e414. doi:10.1038/cddis.2012.154
52. Zhang L, Blackwell K, Workman LM, Chen S, Pope MR, Janz S, Habelhah H (2015) RIP1 cleavage in the kinase domain regulates TRAIL-induced NF-kappaB activation and lymphoma survival. *Mol Cell Biol* 35(19):3324–3338. doi:10.1128/MCB.00692-15
53. Song JH, Tse MC, Bellail A, Phuphanich S, Khuri F, Kneteman NM, Hao C (2007) Lipid rafts and nonrafts mediate tumor necrosis factor related apoptosis-inducing ligand induced apoptotic and nonapoptotic signals in non small cell lung carcinoma cells. *Cancer Res* 67(14):6946–6955. doi:10.1158/0008-5472.CAN-06-3896
54. Collison A, Foster PS, Mattes J (2009) Emerging role of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) as a key regulator of inflammatory responses. *Clin Exp Pharmacol Physiol* 36(11):1049–1053. doi:10.1111/j.1440-1681.2009.05258.x
55. Mizrahi K, Askenasy N (2014) Physiological functions of TNF family receptor/ligand interactions in hematopoiesis and transplantation. *Blood* 124(2):176–183. doi:10.1182/blood-2014-03-559641
56. Sato K, Hida S, Takayanagi H, Yokochi T, Kayagaki N, Takeda K, Yagita H, Okumura K, Tanaka N, Taniguchi T, Ogasawara K (2001) Antiviral response by natural killer cells through TRAIL gene induction by IFN-alpha/beta. *Eur J Immunol* 31(11):3138–3146. doi:10.1002/1521-4141(200111)31:11<3138::AID-IMMU3138>3.0.CO;2-B

57. Stegmann KA, Bjorkstrom NK, Veber H, Ciesek S, Riese P, Wiegand J, Hadem J, Suneetha PV, Jaroszewicz J, Wang C, Schlaphoff V, Fytily P, Cornberg M, Manns MP, Geffers R, Pietschmann T, Guzman CA, Ljunggren HG, Wedemeyer H (2010) Interferon-alpha-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. *Gastroenterology* 138(5):1885–1897. doi:10.1053/j.gastro.2010.01.051
58. Schuster IS, Wikstrom ME, Brizard G, Coudert JD, Estcourt MJ, Manzur M, O'Reilly LA, Smyth MJ, Trapani JA, Hill GR, Andoniou CE, Degli-Esposti MA (2014) TRAIL+ NK cells control CD4+ T cell responses during chronic viral infection to limit autoimmunity. *Immunity* 41(4):646–656. doi:10.1016/j.immuni.2014.09.013
59. Cook KD, Kline HC, Whitmire JK (2015) NK cells inhibit humoral immunity by reducing the abundance of CD4+ T follicular helper cells during a chronic virus infection. *J Leukoc Biol* 98(2):153–162. doi:10.1189/jlb.4HI1214-594R
60. Waggoner SN, Cornberg M, Selin LK, Welsh RM (2011) Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 481(7381):394–398. doi:10.1038/nature10624
61. Lang PA, Lang KS, Xu HC, Grusdat M, Parish IA, Recher M, Elford AR, Dhanji S, Shaabani N, Tran CW, Dissanayake D, Rahbar R, Ghazarian M, Brustle A, Fine J, Chen P, Weaver CT, Klose C, Diefenbach A, Haussinger D, Carlyle JR, Kaech SM, Mak TW, Ohashi PS (2012) Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. *Proc Natl Acad Sci U S A* 109(4):1210–1215. doi:10.1073/pnas.1118834109
62. Peppas D, Gill US, Reynolds G, Easom NJ, Pallett LJ, Schurich A, Micco L, Nebbia G, Singh HD, Adams DH, Kennedy PT, Maini MK (2013) Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. *J Exp Med* 210(1):99–114. doi:10.1084/jem.20121172
63. Jeremias I, Herr I, Boehler T, Debatin KM (1998) TRAIL/Apo-2-ligand-induced apoptosis in human T cells. *Eur J Immunol* 28(1):143–152. doi:10.1002/(SICI)1521-4141(199801)28:01<143::AID-IMMU143>3.0.CO;2-3
64. Kayagaki N, Yamaguchi N, Nakayama M, Eto H, Okumura K, Yagita H (1999) Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: a novel mechanism for the antitumor effects of type I IFNs. *J Exp Med* 189(9):1451–1460
65. Zhang XR, Zhang LY, Devadas S, Li L, Keegan AD, Shi YF (2003) Reciprocal expression of TRAIL and CD95L in Th1 and Th2 cells: role of apoptosis in T helper subset differentiation. *Cell Death Differ* 10(2):203–210. doi:10.1038/sj.cdd.4401138
66. Roberts AI, Devadas S, Zhang X, Zhang L, Keegan A, Greenelth K, Solomon J, Wei L, Das J, Sun E, Liu C, Yuan Z, Zhou JN, Shi Y (2003) The role of activation-induced cell death in the differentiation of T-helper-cell subsets. *Immunol Res* 28(3):285–293. doi:10.1385/IR:28:3:285
67. Weckmann M, Collison A, Simpson JL, Kopp MV, Wark PA, Smyth MJ, Yagita H, Matthaei KI, Hansbro N, Whitehead B, Gibson PG, Foster PS, Mattes J (2007) Critical link between TRAIL and CCL20 for the activation of TH2 cells and the expression of allergic airway disease. *Nat Med* 13(11):1308–1315. doi:10.1038/nm1660
68. Mirandola P, Ponti C, Gobbi G, Sponzilli I, Vaccarezza M, Cocco L, Zauli G, Secchiero P, Manzoli FA, Vitale M (2004) Activated human NK and CD8+ T cells express both TNF-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors but are resistant to TRAIL-mediated cytotoxicity. *Blood* 104(8):2418–2424. doi:10.1182/blood-2004-04-1294
69. Brincks EL, Gurung P, Langlois RA, Hemann EA, Legge KL, Griffith TS (2011) The magnitude of the T cell response to a clinically significant dose of influenza virus is regulated by TRAIL. *J Immunol* 187(9):4581–4588. doi:10.4049/jimmunol.1002241
70. Brincks EL, Katewa A, Kucaba TA, Griffith TS, Legge KL (2008) CD8 T cells utilize TRAIL to control influenza virus infection. *J Immunol* 181(7):4918–4925
71. Herold S, Steinmueller M, von Wulffen W, Cakarova L, Pinto R, Pleschka S, Mack M, Kuziel WA, Corazza N, Brunner T, Seeger W, Lohmeyer J (2008) Lung epithelial apoptosis

- in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand. *J Exp Med* 205(13):3065–3077. doi:10.1084/jem.20080201
72. Gurung P, Rai D, Condotta SA, Babcock JC, Badovinac VP, Griffith TS (2011) Immune unresponsiveness to secondary heterologous bacterial infection after sepsis induction is TRAIL dependent. *J Immunol* 187(5):2148–2154. doi:10.4049/jimmunol.1101180
 73. Beyer K, Poetschke C, Partecke LI, von Bernstorff W, Maier S, Broecker BM, Heidecke CD (2014) TRAIL induces neutrophil apoptosis and dampens sepsis-induced organ injury in murine colon ascendens stent peritonitis. *PLoS One* 9(6):e97451. doi:10.1371/journal.pone.0097451
 74. Falschlehner C, Schaefer U, Walczak H (2009) Following TRAIL's path in the immune system. *Immunology* 127(2):145–154. doi:10.1111/j.1365-2567.2009.03058.x
 75. Fanger NA, Maliszewski CR, Schooley K, Griffith TS (1999) Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J Exp Med* 190(8):1155–1164
 76. Griffith TS, Wiley SR, Kubin MZ, Sedger LM, Maliszewski CR, Fanger NA (1999) Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL. *J Exp Med* 189(8):1343–1354
 77. Takeda K, Hayakawa Y, Smyth MJ, Kayagaki N, Yamaguchi N, Kakuta S, Iwakura Y, Yagita H, Okumura K (2001) Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nat Med* 7(1):94–100. doi:10.1038/83416
 78. Lelaidier M, Diaz-Rodriguez Y, Cordeau M, Cordeiro P, Haddad E, Herblot S, Duval M (2015) TRAIL-mediated killing of acute lymphoblastic leukemia by plasmacytoid dendritic cell-activated natural killer cells. *Oncotarget* 6(30):29440–29455. doi:10.18632/oncotarget.4984
 79. Tecchio C, Scapini P, Pizzolo G, Cassatella MA (2013) On the cytokines produced by human neutrophils in tumors. *Semin Cancer Biol* 23(3):159–170. doi:10.1016/j.semcancer.2013.02.004
 80. Lin YC, Richburg JH (2014) Characterization of the role of tumor necrosis factor apoptosis inducing ligand (TRAIL) in spermatogenesis through the evaluation of trail gene-deficient mice. *PLoS One* 9(4):e93926. doi:10.1371/journal.pone.0093926
 81. Zauli G, Celeghini C, Monasta L, Martinelli M, Luppi S, Gonelli A, Grill V, Ricci G, Secchiero P (2014) Soluble TRAIL is present at high concentrations in seminal plasma and promotes spermatozoa survival. *Reproduction* 148(2):191–198. doi:10.1530/REP-14-0144
 82. Zhang XD, Nguyen T, Thomas WD, Sanders JE, Hersey P (2000) Mechanisms of resistance of normal cells to TRAIL induced apoptosis vary between different cell types. *FEBS Lett* 482(3):193–199
 83. Gochuico BR, Zhang J, Ma BY, Marshak-Rothstein A, Fine A (2000) TRAIL expression in vascular smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 278(5):L1045–L1050
 84. Secchiero P, Gonelli A, Carnevale E, Milani D, Pandolfi A, Zella D, Zauli G (2003) TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. *Circulation* 107(17):2250–2256. doi:10.1161/01.CIR.0000062702.60708.C4
 85. Secchiero P, Corallini F, di Iasio MG, Gonelli A, Barbarotto E, Zauli G (2005) TRAIL counteracts the proadhesive activity of inflammatory cytokines in endothelial cells by downmodulating CCL8 and CXCL10 chemokine expression and release. *Blood* 105(9):3413–3419. doi:10.1182/blood-2004-10-4111
 86. Zauli G, Pandolfi A, Gonelli A, Di Pietro R, Guarnieri S, Ciabattini G, Rana R, Vitale M, Secchiero P (2003) Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells. *Circ Res* 92(7):732–740. doi:10.1161/01.RES.0000067928.83455.9C
 87. Di Pietro R, Mariggio MA, Guarnieri S, Sancilio S, Giardinelli A, Di Silvestre S, Consoli A, Zauli G, Pandolfi A (2006) Tumor necrosis factor-related apoptosis-inducing ligand

- (TRAIL) regulates endothelial nitric oxide synthase (eNOS) activity and its localization within the human vein endothelial cells (HUVEC) in culture. *J Cell Biochem* 97(4):782–794. doi:10.1002/jcb.20686
88. Baudino TA (2015) Targeted cancer therapy: the next generation of cancer treatment. *Curr Drug Discov Technol* 12(1):3–20
 89. Johnston PG (2014) Identification of clinically relevant molecular subtypes in colorectal cancer: the dawning of a new era. *Oncologist* 19(5):568–573. doi:10.1634/theoncologist.2014-038
 90. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A (2009) Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 101(19):1308–1324. doi:10.1093/jnci/djp280
 91. Strater J, Hinz U, Walczak H, Mechttersheimer G, Koretz K, Herfarth C, Moller P, Lehnert T (2002) Expression of TRAIL and TRAIL receptors in colon carcinoma: TRAIL-R1 is an independent prognostic parameter. *Clin Cancer Res* 8(12):3734–3740
 92. McCarthy MM, Szoln M, DiVito KA, Camp RL, Rimm DL, Kluger HM (2005) Evaluating the expression and prognostic value of TRAIL-R1 and TRAIL-R2 in breast cancer. *Clin Cancer Res* 11(14):5188–5194. doi:10.1158/1078-0432.CCR-05-0158
 93. van Geelen CM, Westra JL, de Vries EG, Boersma-van Ek W, Zwart N, Hollema H, Boezen HM, Mulder NH, Plukker JT, de Jong S, Kleibeuker JH, Koornstra JJ (2006) Prognostic significance of tumor necrosis factor-related apoptosis-inducing ligand and its receptors in adjuvantly treated stage III colon cancer patients. *J Clin Oncol Off J Am Soc Clin Oncol* 24(31):4998–5004. doi:10.1200/JCO.2006.06.8809
 94. Oikonomou E, Kosmidou V, Katseli A, Kothonidis K, Mourtzoukou D, Kontogeorgos G, Andera L, Zografos G, Pintzas A (2009) TRAIL receptor upregulation and the implication of KRAS/BRAF mutations in human colon cancer tumors. *Int J Cancer* 125(9):2127–2135. doi:10.1002/ijc.24613
 95. Duiker EW, van der Zee AG, de Graeff P, Boersma-van Ek W, Hollema H, de Bock GH, de Jong S, de Vries EG (2010) The extrinsic apoptosis pathway and its prognostic impact in ovarian cancer. *Gynecol Oncol* 116(3):549–555. doi:10.1016/j.ygyno.2009.09.014
 96. Gallmeier E, Bader DC, Kriegl L, Berezowska S, Seeliger H, Goke B, Kirchner T, Bruns C, De Toni EN (2013) Loss of TRAIL-receptors is a recurrent feature in pancreatic cancer and determines the prognosis of patients with no nodal metastasis after surgery. *PLoS One* 8(2):e56760. doi:10.1371/journal.pone.0056760
 97. Shin MS, Kim HS, Lee SH, Park WS, Kim SY, Park JY, Lee JH, Lee SK, Lee SN, Jung SS, Han JY, Kim H, Lee JY, Yoo NJ (2001) Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. *Cancer Res* 61(13):4942–4946
 98. Jeng YM, Hsu HC (2002) Mutation of the DR5/TRAIL receptor 2 gene is infrequent in hepatocellular carcinoma. *Cancer Lett* 181(2):205–208
 99. Teng MS, Brandwein-Gensler MS, Teixeira MS, Martignetti JA, Duffey DC (2005) A study of TRAIL receptors in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 131(5):407–412. doi:10.1001/archotol.131.5.407
 100. Pai SI, Wu GS, Ozoren N, Wu L, Jen J, Sidransky D, El-Deiry WS (1998) Rare loss-of-function mutation of a death receptor gene in head and neck cancer. *Cancer Res* 58(16):3513–3518
 101. Martinez-Ferrandis JI, Rodriguez-Lopez R, Milne RL, Gonzalez E, Cebolla E, Chirivella I, Zamora P, Arias JI, Palacios S, Cervantes A, Diez O, Benitez J, Armengod ME (2007) Polymorphisms in TRAIL receptor genes and risk of breast cancer in Spanish women. *Cancer Biomark* 3(2):89–93
 102. Jamshidi M, Fagerholm R, Khan S, Aittomaki K, Czene K, Darabi H, Li J, Andrulis IL, Chang-Claude J, Devilee P, Fasching PA, Michailidou K, Bolla MK, Dennis J, Wang Q, Guo Q, Rhenius V, Cornelissen S, Rudolph A, Knight JA, Loehberg CR, Burwinkel B, Marme F, Hopper JL, Southey MC, Bojesen SE, Flyger H, Brenner H, Holleccek B, Margolin S, Mannermaa A, Kosma VM, kConFab I, Van Dyck L, Nevelsteen I, Couch FJ, Olson JE, Giles GG, McLean C, Haiman CA, Henderson BE, Winqvist R, Pylkas K, Tollenaar RA, Garcia-Closas M, Figueroa J, Hooning MJ, Martens JW, Cox A, Cross SS, Simard J, Dunning AM,

- Easton DF, Pharoah PD, Hall P, Blomqvist C, Schmidt MK, Nevanlinna H (2015) SNP-SNP interaction analysis of NF-kappaB signaling pathway on breast cancer survival. *Oncotarget* 6(35):37979–37994. doi:10.18632/oncotarget.4991
103. Min YJ, Lee JH, Choi SJ, Chi HS, Lee JS, Kim WK, Lee KH (2004) Prognostic significance of Fas (CD95) and TRAIL receptors (DR4/DR5) expression in acute myelogenous leukemia. *Leuk Res* 28(4):359–365. doi:10.1016/j.leukres.2003.08.015
 104. Koornstra JJ, Kleibeuker JH, van Geelen CM, Rijcken FE, Hollema H, de Vries EG, de Jong S (2003) Expression of TRAIL (TNF-related apoptosis-inducing ligand) and its receptors in normal colonic mucosa, adenomas, and carcinomas. *J Pathol* 200(3):327–335. doi:10.1002/path.1364
 105. Sanlioglu AD, Korcum AF, Pestereli E, Erdogan G, Karaveli S, Savas B, Griffith TS, Sanlioglu S (2007) TRAIL death receptor-4 expression positively correlates with the tumor grade in breast cancer patients with invasive ductal carcinoma. *Int J Radiat Oncol Biol Phys* 69(3):716–723. doi:10.1016/j.ijrobp.2007.03.057
 106. Li Y, Jin X, Li J, Jin X, Yu J, Sun X, Chu Y, Xu C, Li X, Wang X, Kakehi Y, Wu X (2012) Expression of TRAIL, DR4, and DR5 in bladder cancer: correlation with response to adjuvant therapy and implications of prognosis. *Urology* 79(4):968.e967–968.e915. doi:10.1016/j.urology.2011.11.011
 107. Ganten TM, Sykora J, Koschny R, Batke E, Aulmann S, Mansmann U, Stremmel W, Sinn HP, Walczak H (2009) Prognostic significance of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor expression in patients with breast cancer. *J Mol Med* 87(10):995–1007. doi:10.1007/s00109-009-0510-z
 108. Koschny R, Brost S, Hinz U, Sykora J, Batke EM, Singer S, Breuhahn K, Stremmel W, Walczak H, Schemmer P, Schirmacher P, Ganten TM (2013) Cytosolic and nuclear caspase-8 have opposite impact on survival after liver resection for hepatocellular carcinoma. *BMC Cancer* 13:532. doi:10.1186/1471-2407-13-532
 109. Macher-Goeppinger S, Aulmann S, Tagscherer KE, Wagener N, Haferkamp A, Penzel R, Brauckhoff A, Hohenfellner M, Sykora J, Walczak H, Teh BT, Autschbach F, Herpel E, Schirmacher P, Roth W (2009) Prognostic value of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors in renal cell cancer. *Clin Cancer Res* 15(2):650–659. doi:10.1158/1078-0432.CCR-08-0284
 110. Yoldas B, Ozer C, Ozen O, Canpolat T, Dogan I, Griffith TS, Sanlioglu S, Ozluoglu LN (2011) Clinical significance of TRAIL and TRAIL receptors in patients with head and neck cancer. *Head Neck* 33(9):1278–1284. doi:10.1002/hed.21598
 111. Spierings DC, de Vries EG, Timens W, Groen HJ, Boezen HM, de Jong S (2003) Expression of TRAIL and TRAIL death receptors in stage III non-small cell lung cancer tumors. *Clin Cancer Res* 9(9):3397–3405
 112. Cooper WA, Kohonen-Corish MR, Zhuang L, McCaughan B, Kennedy C, Screaton G, Sutherland RL, Lee CS (2008) Role and prognostic significance of tumor necrosis factor-related apoptosis-inducing ligand death receptor DR5 in nonsmall-cell lung cancer and precursor lesions. *Cancer* 113(1):135–142. doi:10.1002/cncr.23528
 113. Vigneswaran N, Baucum DC, Wu J, Lou Y, Bouquot J, Muller S, Zacharias W (2007) Repression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) but not its receptors during oral cancer progression. *BMC Cancer* 7:108. doi:10.1186/1471-2407-7-108
 114. Hernandez-Cueto A, Hernandez-Cueto D, Antonio-Andres G, Mendoza-Marin M, Jimenez-Gutierrez C, Sandoval-Mejia AL, Mora-Campos R, Gonzalez-Bonilla C, Vega MI, Bonavida B, Huerta-Yepez S (2014) Death receptor 5 expression is inversely correlated with prostate cancer progression. *Mol Med Rep* 10(5):2279–2286. doi:10.3892/mmr.2014.2504
 115. Bavi P, Prabhakaran SE, Abubaker J, Qadri Z, George T, Al-Sanea N, Abduljabbar A, Ashari LH, Alhomoud S, Al-Dayel F, Hussain AR, Uddin S, Al-Kuraya KS (2010) Prognostic significance of TRAIL death receptors in middle eastern colorectal carcinomas and their correlation to oncogenic KRAS alterations. *Mol Cancer* 9:203. doi:10.1186/1476-4598-9-203
 116. Yao Q, Du J, Lin J, Luo Y, Wang Y, Liu Y, Zhang B, Ren C, Liu C (2016) Prognostic significance of TRAIL signalling molecules in cervical squamous cell carcinoma. *J Clin Pathol* 69(2):122–127. doi:10.1136/jclinpath-2014-202811

117. Kuijlen JM, Mooij JJ, Platteel I, Hoving EW, van der Graaf WT, Span MM, Hollema H, den Dunnen WF (2006) TRAIL-receptor expression is an independent prognostic factor for survival in patients with a primary glioblastoma multiforme. *J Neurooncol* 78(2):161–171. doi:10.1007/s11060-005-9081-1
118. Kriegl L, Jung A, Horst D, Rizzani A, Jackstadt R, Hermeking H, Gallmeier E, Gerbes AL, Kirchner T, Goke B, De Toni EN (2012) Microsatellite instability, KRAS mutations and cellular distribution of TRAIL-receptors in early stage colorectal cancer. *PLoS One* 7(12):e51654. doi:10.1371/journal.pone.0051654
119. Chen JJ, Shen HC, Rivera Rosado LA, Zhang Y, Di X, Zhang B (2012) Mislocalization of death receptors correlates with cellular resistance to their cognate ligands in human breast cancer cells. *Oncotarget* 3(8):833–842. doi:10.18632/oncotarget.542
120. Koksai IT, Sanlioglu AD, Karacay B, Griffith TS, Sanlioglu S (2008) Tumor necrosis factor-related apoptosis inducing ligand-R4 decoy receptor expression is correlated with high Gleason scores, prostate-specific antigen recurrence, and decreased survival in patients with prostate carcinoma. *Urol Oncol* 26(2):158–165. doi:10.1016/j.urolonc.2007.01.022
121. Sanlioglu AD, Dirice E, Elpek O, Korum AF, Ozdogan M, Suleymanlar I, Balci MK, Griffith TS, Sanlioglu S (2009) High TRAIL death receptor 4 and decoy receptor 2 expression correlates with significant cell death in pancreatic ductal adenocarcinoma patients. *Pancreas* 38(2):154–160. doi:10.1097/MPA.0b013e31818db9e3
122. Koschny R, Krupp W, Xu LX, Mueller WC, Bauer M, Sinn P, Keller M, Koschny T, Walczak H, Bruckner T, Ganten TM, Holland H (2015) WHO grade related expression of TRAIL-receptors and apoptosis regulators in meningioma. *Pathol Res Pract* 211(2):109–116. doi:10.1016/j.prp.2014.11.002
123. Granci V, Bibeau F, Kramar A, Boissiere-Michot F, Thezenas S, Thirion A, Gongora C, Martineau P, Del Rio M, Ychou M (2008) Prognostic significance of TRAIL-R1 and TRAIL-R3 expression in metastatic colorectal carcinomas. *Eur J Cancer* 44(15):2312–2318. doi:10.1016/j.ejca.2008.06.042
124. Erkul E, Kucukodaci Z, Pinar D, Gungor A, Alparslan Babayigit M, Kurt O, Cincik H (2016) TRAIL and TRAIL receptors in patients with laryngeal cancer. *Head Neck* 38(Suppl 1):E535–E541. doi:10.1002/hed.24035
125. Bolkun L, Lemancewicz D, Jablonska E, Szumowska A, Bolkun-Skornicka U, Moniuszko M, Dzieciol J, Kloczko J (2015) Prognostic significance of ligands belonging to tumour necrosis factor superfamily in acute lymphoblastic leukaemia. *Leuk Res* 39(3):290–295. doi:10.1016/j.leukres.2014.12.012
126. Bolkun L, Lemancewicz D, Jablonska E, Szumowska A, Bolkun-Skornicka U, Ratajczak-Wrona W, Dzieciol J, Kloczko J (2015) The impact of TNF superfamily molecules on overall survival in acute myeloid leukaemia: correlation with biological and clinical features. *Ann Hematol* 94(1):35–43. doi:10.1007/s00277-014-2178-x
127. Toiyama D, Takaha N, Shinnoh M, Ueda T, Kimura Y, Nakamura T, Hongo F, Mikami K, Kamoi K, Kawauchi A, Miki T (2013) Significance of serum tumor necrosis factor-related apoptosis-inducing ligand as a prognostic biomarker for renal cell carcinoma. *Mol Clin Oncol* 1(1):69–74. doi:10.3892/mco.2012.35
128. McLornan DP, Barrett HL, Cummins R, McDermott U, McDowell C, Conlon SJ, Coyle VM, Van Schaeybroeck S, Wilson R, Kay EW, Longley DB, Johnston PG (2010) Prognostic significance of TRAIL signaling molecules in stage II and III colorectal cancer. *Clin Cancer Res* 16(13):3442–3451. doi:10.1158/1078-0432.CCR-10-0052
129. de Miguel D, Lemke J, Anel A, Walczak H, Martinez-Lostao L (2016) Onto better TRAILS for cancer treatment. *Cell Death Differ* 23(5):733–747. doi:10.1038/cdd.2015.174
130. Kelley SK, Harris LA, Xie D, Deforge L, Totpal K, Bussiere J, Fox JA (2001) Preclinical studies to predict the disposition of Apo2L/tumor necrosis factor-related apoptosis-inducing ligand in humans: characterization of in vivo efficacy, pharmacokinetics, and safety. *J Pharmacol Exp Ther* 299(1):31–38
131. Ganten TM, Koschny R, Sykora J, Schulze-Bergkamen H, Buchler P, Haas TL, Schader MB, Untergasser A, Stremmel W, Walczak H (2006) Preclinical differentiation between appar-

- ently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs. *Clin Cancer Res* 12(8):2640–2646. doi:10.1158/1078-0432.CCR-05-2635
132. Berg D, Lehne M, Muller N, Siegmund D, Munkel S, Sebald W, Pfizenmaier K, Wajant H (2007) Enforced covalent trimerization increases the activity of the TNF ligand family members TRAIL and CD95L. *Cell Death Differ* 14(12):2021–2034. doi:10.1038/sj.cdd.4402213
 133. Schneider B, Munkel S, Krippner-Heidenreich A, Grunwald I, Wels WS, Wajant H, Pfizenmaier K, Gerspach J (2010) Potent antitumoral activity of TRAIL through generation of tumor-targeted single-chain fusion proteins. *Cell Death Dis* 1:e68. doi:10.1038/cddis.2010.45
 134. Gieffers C, Kluge M, Merz C, Sykora J, Thiemann M, Schaal R, Fischer C, Branschadel M, Abhari BA, Hohenberger P, Fulda S, Fricke H, Hill O (2013) APG350 induces superior clustering of TRAIL receptors and shows therapeutic antitumor efficacy independent of cross-linking via Fcγ receptors. *Mol Cancer Ther* 12(12):2735–2747. doi:10.1158/1535-7163.MCT-13-0323
 135. Siegmund M, Pollak N, Seifert O, Wahl K, Hanak K, Vogel A, Nussler AK, Gottsch D, Munkel S, Bantel H, Kontermann RE, Pfizenmaier K (2012) Superior antitumoral activity of dimerized targeted single-chain TRAIL fusion proteins under retention of tumor selectivity. *Cell Death Dis* 3:e295. doi:10.1038/cddis.2012.29
 136. de Bruyn M, Wei Y, Wiersma VR, Samplonius DF, Klip HG, van der Zee AG, Yang B, Helfrich W, Bremer E (2011) Cell surface delivery of TRAIL strongly augments the tumoricidal activity of T cells. *Clin Cancer Res* 17(17):5626–5637. doi:10.1158/1078-0432.CCR-11-0303
 137. El-Mesery M, Trebing J, Schafer V, Weisenberger D, Siegmund D, Wajant H (2013) CD40-directed scFv-TRAIL fusion proteins induce CD40-restricted tumor cell death and activate dendritic cells. *Cell Death Dis* 4:e916. doi:10.1038/cddis.2013.402
 138. Wiersma VR, de Bruyn M, Shi C, Gooden MJ, Wouters MC, Samplonius DF, Hendriks D, Nijman HW, Wei Y, Zhou J, Helfrich W, Bremer E (2015) C-type lectin-like molecule-1 (CLL1)-targeted TRAIL augments the tumoricidal activity of granulocytes and potentiates therapeutic antibody-dependent cell-mediated cytotoxicity. *MAbs* 7(2):321–330. doi:10.1080/19420862.2015.1007811
 139. Lemke J, von Karstedt S, Zinngrebe J, Walczak H (2014) Getting TRAIL back on track for cancer therapy. *Cell Death Differ* 21(9):1350–1364. doi:10.1038/cdd.2014.81
 140. Yang TM, Barbone D, Fennell DA, Broaddus VC (2009) Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. *Am J Respir Cell Mol Biol* 41(1):14–23. doi:10.1165/rcmb.2008-0320OC
 141. Chandrasekaran S, Marshall JR, Messing JA, Hsu JW, King MR (2014) TRAIL-mediated apoptosis in breast cancer cells cultured as 3D spheroids. *PLoS One* 9(10):e111487. doi:10.1371/journal.pone.0111487
 142. Newsom-Davis T, Prieske S, Walczak H (2009) Is TRAIL the holy grail of cancer therapy? *Apoptosis* 14(4):607–623. doi:10.1007/s10495-009-0321-2
 143. Bernard D, Quatannens B, Vandenbunder B, Abbadie C (2001) Rel/NF-κB transcription factors protect against tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by up-regulating the TRAIL decoy receptor DcR1. *J Biol Chem* 276(29):27322–27328. doi:10.1074/jbc.M01183200
 144. Sanlioglu AD, Dirice E, Aydin C, Erin N, Koksoy S, Sanlioglu S (2005) Surface TRAIL decoy receptor-4 expression is correlated with TRAIL resistance in MCF7 breast cancer cells. *BMC Cancer* 5:54. doi:10.1186/1471-2407-5-54
 145. Riccioni R, Pasquini L, Mariani G, Saulle E, Rossini A, Diverio D, Pelosi E, Vitale A, Chierichini A, Cedrone M, Foa R, Lo Coco F, Peschle C, Testa U (2005) TRAIL decoy receptors mediate resistance of acute myeloid leukemia cells to TRAIL. *Haematologica* 90(5):612–624
 146. Sanlioglu AD, Karacay B, Koksai IT, Griffith TS, Sanlioglu S (2007) DcR2 (TRAIL-R4) siRNA and adenovirus delivery of TRAIL (Ad5hTRAIL) break down in vitro tumorigenic

- potential of prostate carcinoma cells. *Cancer Gene Ther* 14(12):976–984. doi:10.1038/sj.cgt.7701087
147. Chamuleau ME, Ossenkoppele GJ, van Rhenen A, van Dreunen L, Jirka SM, Zevenbergen A, Schuurhuis GJ, van de Loosdrecht AA (2011) High TRAIL-R3 expression on leukemic blasts is associated with poor outcome and induces apoptosis-resistance which can be overcome by targeting TRAIL-R2. *Leuk Res* 35(6):741–749. doi:10.1016/j.leukres.2010.12.032
 148. Ashkenazi A, Dixit VM (1999) Apoptosis control by death and decoy receptors. *Curr Opin Cell Biol* 11(2):255–260
 149. Morizot A, Merino D, Lalaoui N, Jacquemin G, Granci V, Iessi E, Lanneau D, Bouyer F, Solary E, Chauffert B, Saas P, Garrido C, Micheau O (2011) Chemotherapy overcomes TRAIL-R4-mediated TRAIL resistance at the DISC level. *Cell Death Differ* 18(4):700–711. doi:10.1038/cdd.2010.144
 150. Wada S, Manabe N, Nakayama M, Inou N, Matsui T, Miyamoto H (2002) TRAIL-decoy receptor 1 plays inhibitory role in apoptosis of granulosa cells from pig ovarian follicles. *J Vet Med Sci* 64(5):435–439
 151. Griffith TS, Rauch CT, Smolak PJ, Waugh JY, Boiani N, Lynch DH, Smith CA, Goodwin RG, Kubin MZ (1999) Functional analysis of TRAIL receptors using monoclonal antibodies. *J Immunol* 162(5):2597–2605
 152. Zhang XD, Franco A, Myers K, Gray C, Nguyen T, Hersey P (1999) Relation of TNF-related apoptosis-inducing ligand (TRAIL) receptor and FLICE-inhibitory protein expression to TRAIL-induced apoptosis of melanoma. *Cancer Res* 59(11):2747–2753
 153. Wuchter C, Krappmann D, Cai Z, Ruppert V, Scheidereit C, Dorken B, Ludwig WD, Karawajew L (2001) In vitro susceptibility to TRAIL-induced apoptosis of acute leukemia cells in the context of TRAIL receptor gene expression and constitutive NF-kappa B activity. *Leukemia* 15(6):921–928
 154. Neumann S, Hasenauer J, Pollak N, Scheurich P (2014) Dominant negative effects of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 4 on TRAIL receptor 1 signaling by formation of heteromeric complexes. *J Biol Chem* 289(23):16576–16587. doi:10.1074/jbc.M114.559468
 155. Truneh A, Sharma S, Silverman C, Khandekar S, Reddy MP, Deen KC, McLaughlin MM, Srinivasula SM, Livi GP, Marshall LA, Alnemri ES, Williams WV, Doyle ML (2000) Temperature-sensitive differential affinity of TRAIL for its receptors. DR5 is the highest affinity receptor. *J Biol Chem* 275(30):23319–23325. doi:10.1074/jbc.M910438199
 156. Clancy L, Mruk K, Archer K, Woelfel M, Mongkolsapaya J, Sreaton G, Lenardo MJ, Chan FK (2005) Preligand assembly domain-mediated ligand-independent association between TRAIL receptor 4 (TR4) and TR2 regulates TRAIL-induced apoptosis. *Proc Natl Acad Sci U S A* 102(50):18099–18104. doi:10.1073/pnas.0507329102
 157. Merino D, Lalaoui N, Morizot A, Schneider P, Solary E, Micheau O (2006) Differential inhibition of TRAIL-mediated DR5-DISC formation by decoy receptors 1 and 2. *Mol Cell Biol* 26(19):7046–7055. doi:10.1128/MCB.00520-06
 158. Valley CC, Lewis AK, Mudaliar DJ, Perlmutter JD, Braun AR, Karim CB, Thomas DD, Brody JR, Sachs JN (2012) Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces death receptor 5 networks that are highly organized. *J Biol Chem* 287(25):21265–21278. doi:10.1074/jbc.M111.306480
 159. Chan FK (2000) The pre-ligand binding assembly domain: a potential target of inhibition of tumour necrosis factor receptor function. *Ann Rheum Dis* 59(Suppl 1):i50–i53
 160. Siegel RM, Frederiksen JK, Zacharias DA, Chan FK, Johnson M, Lynch D, Tsien RY, Lenardo MJ (2000) Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 288(5475):2354–2357
 161. Branschdel M, Aird A, Zappe A, Tietz C, Krippner-Heidenreich A, Scheurich P (2010) Dual function of cysteine rich domain (CRD) 1 of TNF receptor type 1: conformational stabilization of CRD2 and control of receptor responsiveness. *Cell Signal* 22(3):404–414. doi:10.1016/j.cellsig.2009.10.011

162. Lewis AK, Valley CC, Sachs JN (2012) TNFR1 signaling is associated with backbone conformational changes of receptor dimers consistent with overactivation in the R92Q TRAPS mutant. *Biochemistry* 51(33):6545–6555. doi:10.1021/bi3006626
163. Lewis AK, James ZM, McCaffrey JE, Braun AR, Karim CB, Thomas DD, Sachs JN (2014) Open and closed conformations of the isolated transmembrane domain of death receptor 5 support a new model of activation. *Biophys J* 106(6):L21–L24. doi:10.1016/j.bpj.2014.01.044
164. Winkel C, Neumann S, Surulescu C, Scheurich P (2012) A minimal mathematical model for the initial molecular interactions of death receptor signalling. *Math Biosci Eng* 9(3):663–683. doi:10.3934/mbe.2012.9.663
165. Lee HW, Lee SH, Lee HW, Ryu YW, Kwon MH, Kim YS (2005) Homomeric and heteromeric interactions of the extracellular domains of death receptors and death decoy receptors. *Biochem Biophys Res Commun* 330(4):1205–1212. doi:10.1016/j.bbrc.2005.03.101
166. Lalaoui N, Morle A, Merino D, Jacquemin G, Iessi E, Morizot A, Shirley S, Robert B, Solary E, Garrido C, Micheau O (2011) TRAIL-R4 promotes tumor growth and resistance to apoptosis in cervical carcinoma HeLa cells through AKT. *PLoS One* 6(5):e19679. doi:10.1371/journal.pone.0019679
167. Zhang XD, Franco AV, Nguyen T, Gray CP, Hersey P (2000) Differential localization and regulation of death and decoy receptors for TNF-related apoptosis-inducing ligand (TRAIL) in human melanoma cells. *J Immunol* 164(8):3961–3970

TRAIL, Fas Ligand, TNF and TLR3 in Cancer

Micheau, O. (Ed.)

2017, XIII, 317 p. 40 illus., 35 illus. in color., Hardcover

ISBN: 978-3-319-56804-1