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MULTI-AUTHOR REVIEW

Control of death receptor ligand activity by posttranslational modifications

R. Weinlich · T. Brunner · G. P. Amarante-Mendes

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Abstract The death receptor ligands are involved in many physiological and pathological processes involving triggering of apoptosis, inflammation, proliferation, and activation. The expression of these molecules is reported to be tightly regulated at the transcriptional level. However, over the last few years, an increasing number of data demonstrated that the control of transcription is only one of the mechanisms that manage the expression of the death receptor ligands. Thus, this review is focused on posttranslational regulation of the three main members of this family, namely FasL, TNF-α, and TRAIL. We discuss here the importance of distribution, storage, and degranulation of these molecules, as well as their shedding by proteases on the control of death receptor ligands expression and activity.

Keywords FasL · TNF · TRAIL · Rafts · Lysosomes · Degranulation · ADAM · Metalloprotease · Shedding

R. Weinlich · G. P. Amarante-Mendes
Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

R. Weinlich · G. P. Amarante-Mendes
Instituto de Investigação em Imunologia, Instituto Nacional de Ciência e Tecnologia (INCT), São Paulo, Brazil

T. Brunner
Division of Immunopathology, Institute of Pathology, University of Bern, Bern, Switzerland

Present Address:
R. Weinlich (✉)
Department of Immunology, St. Jude Children's Research Hospital, Mail Stop 351. 262 Danny Thomas Place, Memphis, TN 38105, USA
e-mail: Ricardo.Weinlich@stjude.org

Introduction

The family of death receptors (DRs) belongs to the larger superfamily of tumor necrosis factor (TNF) receptors. Members of this subfamily, such as Fas (CD95/APO-1), TNF-R1, TNF-R2, TRAMP (DR3), TRAIL-R1 (DR4), TRAIL-R2 (DR5), DR6, EDAR, and p75NTR, contain an exclusive a 80 amino acid-long domain called "death domain", that is essential for apoptosis induction [1, 2]. They are activated by their cognate ligands, which belong to the TNF-α protein family and are usually called "death receptor ligands" [3, 4]. These molecules are comprised of a C-terminal extracellular portion (which interacts with DRs), a transmembrane domain, and an N-terminal domain [5, 6]. An exception is lymphotoxin-α (LTα), which is produced as a soluble protein. The most studied and well-described members of the death receptor ligands family are the FasL (CD95L), tumor necrosis factor alpha (TNF-α) and the TNF-related apoptosis-inducing ligand (TRAIL).

FasL is the prototypical death receptor ligand. It is mostly expressed in the hematopoietic compartment, including dendritic cells and NK, B, and T lymphocytes but can also be found in immune privileged sites, such as retina, testis, and ovary as well as in chronically inflamed tissues [7, 8]. FasL is a well-known apoptosis inducer and therefore it is involved in many physiological and pathological situations that result in cell death. FasL expression on effector cells can trigger death of target cells. However, when expressed in T cells, it can also induce a suicide process called activation-induced cell death (AICD) [9], involved in the deletion of autoreactive or chronically stimulated T cells and thereby contributing to the maintenance of the homeostasis of the immune system [10]. In non-hematopoietic tissues, FasL expression is important for the protection of immune privileged sites from damage

inflicted by infiltrating immune/inflammatory cells [11]. In addition to its proapoptotic role, FasL has also been shown to induce T cell activation and proliferation [12].

TNF-α is better known for its pro-inflammatory activity. It is involved in a myriad of physiological and pathological processes, including inflammation, reproduction, metabolism, and immune responses. TNF-α not only induces cell death but also proliferation, differentiation, and activation of immune and non-immune cells [13]. TNF-α expression is tightly regulated and occurs mainly in hematopoietic cells, such as macrophages, monocytes, neutrophils, and lymphocytes, although it can also be found in some non-hematopoietic cells, like the microglia, fibroblasts, and muscle cells [13]. In regard to its pro-apoptotic function, TNF-α is known to be the major mediator of macrophage-induced cytotoxicity but is also important for T cell-mediated killing [14] .

In contrast to FasL and TNF-α, TRAIL is widely and constitutively expressed, both in non-hematopoietic tissues, like colon, placenta, and small intestine, and in hematopoietic tissues including spleen, thymus, and lymph nodes [15]. In some cell types, especially immune cells, TRAIL is upregulated in response to diverse stimuli, such as TCR stimulation and IFN type I for T cells, LPS for monocytes and macrophages, and IFN type I, IL-12, and IL-15 for NKs [16–19]. Although TRAIL is reported to have different functions, including inhibition of proliferation [20], regulation of hematopoiesis [21], and killing of virus-infected cells [22], the most studied role of this protein is the induction of apoptosis in tumors. Differently from TNF-α and FasL, TRAIL specifically induces apoptosis in a vast number of tumors without killing non-transformed cells [23–26]. Furthermore, it synergizes with radiotherapy, chemotherapeutic drugs, and other death receptor ligands to promote tumor cell death [27–30]. However, TRAIL may induce liver damage under some stress and/or inflammatory conditions [31, 32].

Although primarily produced as transmembrane proteins, the death receptor ligands can be released by proteolytic cleavage, which may alter their biological activities. Whereas transmembrane ligands in general possess potent pro-apoptotic activities, soluble proteins may maintain apoptosis activities, acquire antagonistic properties, or become pro-inflammatory cytokines [3, 6, 33, 34]. Thus, given the important biological functions of death receptor ligands, it is not surprising that their expression and availability are tightly regulated at both the transcriptional and posttranslational levels. Besides the fundamental role of death receptor ligands modulation at the transcriptional level, there is an increasing body of evidence pointing towards a critical role of posttranscriptional mechanisms in the regulation of death receptor ligands' availability and activity. We will focus on posttranslational control of FasL, TNF-α, and TRAIL, and discuss the role of sorting, storage, and distribution of these proteins in the membranes, as well as their shedding by proteolysis, on the regulation of death receptor ligand activity. For transcriptional regulation of death receptors ligands, please refer to [35–38].

Sorting and storage

In some cell types, de novo synthesized FasL is directly targeted to the plasma membrane, as is the case in the retina, placenta, testis, and tumor cells such as colon and breast cancer cells. Direct translocation of de novo synthesized FasL to the plasma membrane is normally associated with immune privileged sites or malignancies [39–43]. In other cells, mostly from the haematopoietic lineages, like cytotoxic T cells and NK cells, FasL can either be directly transported to the plasma membrane or stored in granule-like structure, normally described as secretory lysosomes [44–46]. Interestingly, intracellular storage of FasL is usually observed in cell types with inducible FasL expression, e.g., T cells and NK cells [45, 47, 48]. Stimulation of these cells not only induces de novo synthesis and direct transportation of FasL to the cell surface but also promotes degranulation of preformed FasL, which further contributes to engagement of the Fas receptor on the target cells and induction of target cell killing [45, 49]. Thus, storage of FasL in secretory lysosomes adds an additional level of control of this dangerous death-inducing ligand.

Secretory lysosomes are acidic double-membrane organelles similar to conventional lysosomes in the sense that they have all the protease machinery requested for degradative processes [50, 51]; however, they also function as storage compartments for secreted proteins, including histamine, perforins, granzymes, and FasL [52–54]. Upon appropriated stimuli, like the recognition of a target cell, the vesicles are directed to the site of interaction where they fuse with the plasma membrane, exposing previously stored membrane-attached proteins and also releasing their soluble content [55].

Although initial data indicated that storage and degranulation of FasL is similar to the general mechanism described for other lysosomal proteins, like granzymes, currently it is accepted that FasL is sorted and released by different mechanisms. In 1999, Bossi and Griffiths showed that FasL usually co-localizes with markers of secretory lysosomes—CD63, perforin, and granzyme—in CTLs and NKs as well as in activated CD4+ T cells [45]. Additional support for the co-localization of FasL and perforin was provided by Kojima et al. [56], who showed that FasL stained at the periphery of the same granules whose cores stained for perforin. However, later work by Kassahn et al.

showed that ectopically-expressed FasL in Jurkat cells was rarely found in the same granules as CD63 and CD107a, both markers of secretory lysosomes [57], and the same pattern was also described for a murine CTL clone [49]. Indeed, a recent paper from Schmidt et al. [58] revealed that, upon extensive fractioning of the cytolytic granules, FasL was enriched in a different fraction than perforin, granzymes, and other conventional secretory lysosomes markers. They speculated that distinct secretory lysosomes subtypes may form from an initial multivesicular complex, resulting in two independent compartments. The characterization of specific markers for these lysosome subsets might shed light on these contradictory data concerning FasL subcellular localization.

In accordance with the distinct FasL storage site, FasL is transported to secretory lysosomes by an unconventional pathway, which is dependent on the FasL proline-rich domain (PRD) and independent of the well-established di-leucine/tyrosine-based domains, which are lacking in the FasL cytoplasmic tail [44, 59, 60]. In the absence of the PRD domain, FasL travels by default to the plasma membrane indicating that this domain is critical for sorting FasL to the secretory lysomes. While FasL devoid of this PRD goes directly to the cell surface, its integration into distinct sites of the plasma membrane (like membrane rafts) is also disturbed (further discussed below) [44, 61].

Molecular modeling of FasL PRD predicted that this domain could interact with proteins that contain Src-homology 3 (SH3) domains or WW motifs [44], and further studies showed that, at least in vitro, FasL PRD can promiscuously interact with a large list of SH3 and/or WW-containing proteins [62–64]. These include the Fyn, Lyn, and Fgr kinases, the adaptor proteins Nck, Grb2, and PSTPIP, and the Pombe Cdc15 homology (PHC) proteins PACSIN1-3, FPB17, CIP4, and CD2BP1 [46, 64–67]. Thus, overexpression of PHC proteins in nonhematopoietic cells promotes, at least partially, the sorting of FasL to the secretory lysosomes instead of to the plasma membrane [46]. The same was found for PSTPIP, which also caused a reduction of FasL-mediated cytotoxicity due to a reduced FasL surface expression [68]. Interestingly, the internalization of FasL to the secretory lysosomes is dependent on SH3-containing Src kinases phosphorilation of FasL PRD and also the mono-ubiquitylation of this domain [69]. Finally, FasL PRD also plays a role during degranulation. Thornhill et al. investigated the participation of the adaptor protein Grb2 and found that it is important to sort FasL to the plasma membrane through its connection to adaptin-β, a well-established molecule involved in protein trafficking. In cells with reduced levels of GrB2, FasL accumulated in intracellular vesicles [70]. Finally, Nck may be involved in the degranulation of vesicle-stored FasL into the immunological synapse of TCR-stimulated T cells, by a mechanism dependent on actin cytoskeleton and on WASP/WIP proteins [67].

As TNF-α and TRAIL have a much smaller cytoplasmic tail and do not present the PRD domain [63], it is believed that they are not trafficked to secretory lysosomes the same way as FasL. TNF-α is thought to be targeted to the secretory lysosomes after a transient exposure in the plasma membrane followed by re-endocytosis [71]. In rodents, N-linked glycosylation of mannose-6-phosphate residues is required for proper sorting [72], while in humans, the TNF-α cytoplasmic tail is dispensable [71]. In fact, the accumulation of TNF-α is practically restricted to mast cells [73, 74], while in the other cell types, TNF-α is synthesized as a 26-kDa transmembrane protein, and later on proteolytically processed on the plasma membrane to a 17-kDa soluble form [75]. Although some papers have shown that TRAIL accumulates in diverse granular structures, like secretory lysosomes, the mechanism of TRAIL trafficking is still unknown [76–80]. Interestingly, de novo synthesized TRAIL molecules in neutrophils are stored in slightly different vesicles, indicating that more than one mechanism is involved in TRAIL sorting [76, 77].

Distribution in the membrane

Membrane rafts are plasma membrane compartments enriched for sphingolipids and cholesterol [81]. They are less fluid and more ordered than the phospholipid-rich portions of the plasma membrane, conferring them great lateral mobility without losing their integrity [82]. They usually contain various receptors and molecules involved in several signaling pathways and, as they can aggregate in larger structures, they are involved in controlling the intensity and efficiency of the response to diverse cellular stimuli, including TCR, BCR, and FcεR engagement [83]. Thus, membrane rafts often represent signaling platforms. They have also been reported to be involved in regulating death-receptor-mediated induction of apoptosis [84, 85].

Recently, two independent groups demonstrated that FasL recruitment to the membrane rafts is an important posttranscriptional event, which strongly enhances the killing activity of this molecule [61, 86]. Indeed, the disruption of membrane rafts in T cells by various means, e.g., cholesterol depletion, cholesterol sequestration, or inhibition of cholesterol synthesis, strongly inhibits FasL-induced target cell killing [61]. Further support for these results is provided by two other publications, which show that enforced expression of FasL in HeLa cells resulted in its translocation to the rafts [87], and in Jurkat cells, FasL was recruited to the membrane microdomains upon aplidin-mediated chemotherapy [88]. It is also important to point out that high-stability supramolecular Fas–FasL

clusters are formed in the contact site between effector and target cells, which may be partially mediated by recruitment of FasL into the membrane rafts [87].

The mechanism for enhanced FasL killing activity by clustering in membrane rafts may be explained by the fact that the proper formation of death-inducing signaling complex (DISC) during Fas triggering is dependent on (at least) two adjacent FasL molecules [89] and that, upon interaction with Fas, FasL may become aggregated within the membrane rafts [86]. In fact, different reports have shown that raft localization is important for activation of downstream signaling events and apoptosis induction [90–92]. It is thus feasible to speculate that, in the absence of proper clustering of FasL, killing of the target cell might be limited. In contrast, the non-polarized distribution of FasL throughout the plasma membrane could also result in uncontrolled bystander killing of unrelated neighboring cells. Thus, accumulation and aggregation of FasL in membrane rafts of cytotoxic lymphocytes may assure specific and efficient killing of the target cells by focusing the signal towards them and by promoting, in target cells, the clustering of Fas receptor in membrane rafts.

Substantial data relating to TNF-α and TRAIL distribution in the different plasma membrane microdomains is still lacking, but it is interesting to note that the recruitment of the TNF-R1 into the membrane rafts results in the opposite effect than Fas receptor clustering. Rather than enhancing apoptosis induction, membrane raft-clustered TNF-R1 preferentially induces NF-κB activation, resulting in enhanced pro-inflammatory and pro-survival responses [84, 91, 93]. Also, TRAIL-R1 and -R2 can be recruited to the membrane rafts, enhancing the susceptibility to apoptosis induction [88, 94].

Different mechanisms for the recruitment of molecules into the membrane rafts have been described, including phosphorylation, myristoylation, palmitoylation, and double acetylation [94–97], as well as the interaction with trafficking proteins or proteins anchored in the rafts [70]. In the case of FasL, the domain responsible for raft localization was mapped as being the proline rich domain (PRD). Deletion of this domain strongly reduced the recruitment of FasL into the rafts and, as a consequence, diminished FasL-dependent killing of target cells. In contrast, the deletion of a casein kinase II target site, named SxxS motif, did not affect raft recruitment [61]. As described in the previous section, the PRD domain is also involved in the trafficking of FasL to secretory lysosomes and to the plasma membrane, indicating that this domain has a broad role on FasL transportation. The PRD domain enables interactions with proteins containing SH3 or WW motifs. Among them Fyn and Lck seemed to be the best candidates to modulate the trafficking of FasL to the membrane rafts, since they physically interact with FasL and reside inside this structure [64, 66]. However, to date, none of these molecules has been convincingly shown to be involved in FasL recruitment to membrane rafts. On the contrary, Nachbur and colleagues did not find more FasL in membrane rafts in 293T cells ectopically expressing Lck and Fyn [61].

Taking into the consideration that: (1) depending on cell type, FasL can be mostly stored in secretory lysosomes, distributed throughout the membrane or concentrated in membrane rafts, (2) PRD domain seems to be involved in all trafficking events, and (3) different maturation stages or different stimuli can alter FasL distribution, we speculate that different sets of PRD-interacting proteins may control FasL storage and distribution, and that their expression and availability will affect the final result. Therefore, further studies, focusing on different moments in the differentiation and/or maturation of a FasL-expressing cell as well as different activating signals, are necessary to unravel the underlying mechanisms that govern intracellular storage and surface expression of FasL.

Shedding by proteolytic activity

Another critical aspect about the regulation of death receptor ligand activity is the conversion of the transmembrane molecule into a soluble ligand by a proteolytic process called shedding. Membrane FasL (mFasL) is considered to be the primarily pro-apoptotic version of FasL while secreted FasL (sFasL) has either no activity or is rather anti-apoptotic [6, 34, 98]. Still, some controversy remains, as sFasL is also described as an inducer of cell death [99–101]. Recently, a study by O'Reilly et al. [102] shone new light on this matter. They generate gene-targeted mice that selectively lack either secreted or transmembrane FasL and demonstrated that, while the former is apparently normal and its cells exhibit normal FasL-mediated cytotoxicity activity, the latter develops lymphadenopathy and autoimmunity, similar to the FasL-deficient *gld* mice. Moreover, the inhibition of the proteases involved in FasL shedding enhanced CD4+ T cell-mediated cytotoxicity [103], and the expression of a non-cleavable form of FasL in ovarian and cervical carcinoma cells significantly decreased cell survival [104].

Although the first form of TNF-α discovered was the soluble cytokine, subsequent studies identified the 26-kDa transmembrane form of TNF-α as one of the cytotoxic molecules used by activated T cells and macrophages to kill target cells [14, 105]. In fact, similarly to FasL, TNF-α is produced as a transmembrane molecule. Membrane bound TNF-α is then converted to its soluble form by ADAM17 (TACE, TNF-alpha converting enzyme), a member of the ADAM (a disintegrin and metalloprotease) family of metalloproteases. Using a non-cleavable transmembrane, TNF-α, Perez et al. [106] showed that processing of TNF-α

is not required to the pro-apoptotic effect of this molecule and that these cells could kill virus-infected target cells by cell–cell contact. Also, transplanted T cells that express only the noncleavable TNF-α presented a decreased graft-versus-host disease without impairing its anti-tumor activity [107]. However, only sTNF-α sensitizes T cells for enhanced AICD [108].

Shedding of death receptor ligands is not only important to regulate their pro-apoptotic functions but it also modulates the nonapoptogenic functions of these molecules. sTNF-α is a pro-inflammatory cytokine involved in almost all immune responses and also has a critical role in a great variety of inflammatory and/or auto-immune diseases [109]. For example, mTNF-α transgenic mice are protected from LPS-induced death [110] similarly to the animals treated with an inhibitor of TNF-α sheddase [111], highlighting the importance of a tight control of sTNF-α generation. sFasL also has a pro-inflammatory and tumorigenic effect, as depicted by mice expressing only the secreted form of FasL, which develop SLE-like syndromes and histiocytic sarcomas [102].

To date, two members of the ADAM family of metalloproteases were described as being involved in shedding of the death receptor ligands, namely ADAM17 and ADAM10. ADAM17 and ADAM10 share the highest amino-acid identity in comparison to the other ADAMs, and both possess an extracellular zinc-dependent metalloprotease catalytic domain, which is responsible for the shedding activity [112, 113].

ADAM17 was implicated for the first time as the main TNF-α sheddase in 1997 in a study that showed that mice with non-functional ADAM17 lacks 90% of its ability to process precursor TNF-α [112]. Since then, its role has been confirmed by different groups, but it is still a matter of debate whether ADAM17 is the sole TNF-α-processing ADAM [113–115]. Antisense-mediated reduction of ADAM10 expression in THP-1 cells did not alter the quantity of secreted sTNF-α [116], and ADAM10 overexpression in MEFs and CHO cells did not enhance TNF-α shedding [115]. On the other hand, human and bovine ADAM10 can cleave recombinant TNF-α [117, 118], and ADAM10 is the main TNF-α sheddase in ADAM17-deficient fibroblasts [119]. Recently, Le Gall et al. have shown that ADAM10 can, over time, compensate for ADAM17 activity in cells chronically treated with ADAM17-specific inhibitors. They propose that the accumulation of some but not all ADAM17 substrates could result in their leakage to compartments where ADAM10 is more active [120], and in this case, ADAM10 could act as a sheddase for these molecules. Taken together, these observations may suggest that ADAM10-dependent TNF-α cleavage depends on cell type and TNF-α-inducing stimuli, and also on the subcellular localization/storage of ADAM10 and TNF-α.

On the other hand, FasL shedding is totally dependent on ADAM10. Incubation of FasL with recombinant ADAM10 but not with ADAM17 resulted in FasL proteolysis and ADAM10$^{-/-}$ but not ADAM17$^{-/-}$ MEFs being completely deficient in the generation of soluble FasL [103]. Furthermore, CD4+ T cells treated with ADAM10 inhibitors enhanced membrane FasL expression, increasing FasL-mediated cytotoxicity and AICD [103]. ADAM10-deficient mice die early during embryogenesis, probably due to the lack of the Notch signaling pathway [121]; thus, the relevance of ADAM10-mediated FasL shedding in vivo has yet to be determined.

In addition to the ADAMs, some other metalloproteases are also involved in FasL and TNF-α shedding, as is the case for MMP7 (matrilysin) [122, 123] and MMP3 (stromelysin-1) [124]. However, this activity seems to be accessory to ADAM-mediated FasL and TNF-α shedding since ADAM "loss-of-function" promotes a complete blocking of FasL/TNF-α cleavage while metalloprotease inhibitors normally result in a smaller effect on death receptor ligand shedding [103, 115, 124].

The modulation of the metalloprotease activity involved in TNF-α and FasL shedding is still not completely understood, but it seems that they are induced by addition of phorbol esters and calcium influx [113, 125]. A family of tissue inhibitors of metalloproteinases (TIMPs), which comprises four different members TIMP-1, -2, -3, and -4, seems to be the mainly endogenous inhibitors of MMPs and ADAM metalloprotease activity [126]. TIMP-3 is reported to be the major inhibitor of TNF-α processing by ADAM17 [127]. While rADAM17 is blocked by TIMP-3, neither TIMP-1 and TIMP-2 showed any effect on its activity. Although the N-terminal domain of TIMP-4 has a strong ability to inhibit TNF-α processing, the full-length protein presents negligible activity in inhibiting ADAM17 [128]. TIMP-1 and TIMP-3 are capable of inhibiting ADAM10 activity, although, to date, nobody has formally demonstrated that TIMP-1 and TIMP-3 block ADAM10-mediated FasL shedding.

In agreement with its ability to impair ADAM10 and ADAM17 activities in the majority of in vitro models, TIMP-3 induces apoptosis or renders the cells more susceptible to FasL/TNF-α/TRAIL-induced death [129–131]. This was not observed upon overexpression of TIMP-1, which is normally described as an anti-apoptotic molecule [132]. Its pro-survival effect is independent of the TIMP-1-mediated MMP/ADAM inhibition [133, 134] and further studies focusing on FasL-induced cell death may clarify whether TIMP-1 has a pro-apoptotic role by its ability to inhibit ADAM10-mediated FasL shedding.

To date, little is known about TRAIL shedding. Mariani and Krammer showed that metalloprotease inhibitors did not enhance surface TRAIL expression in any cell type

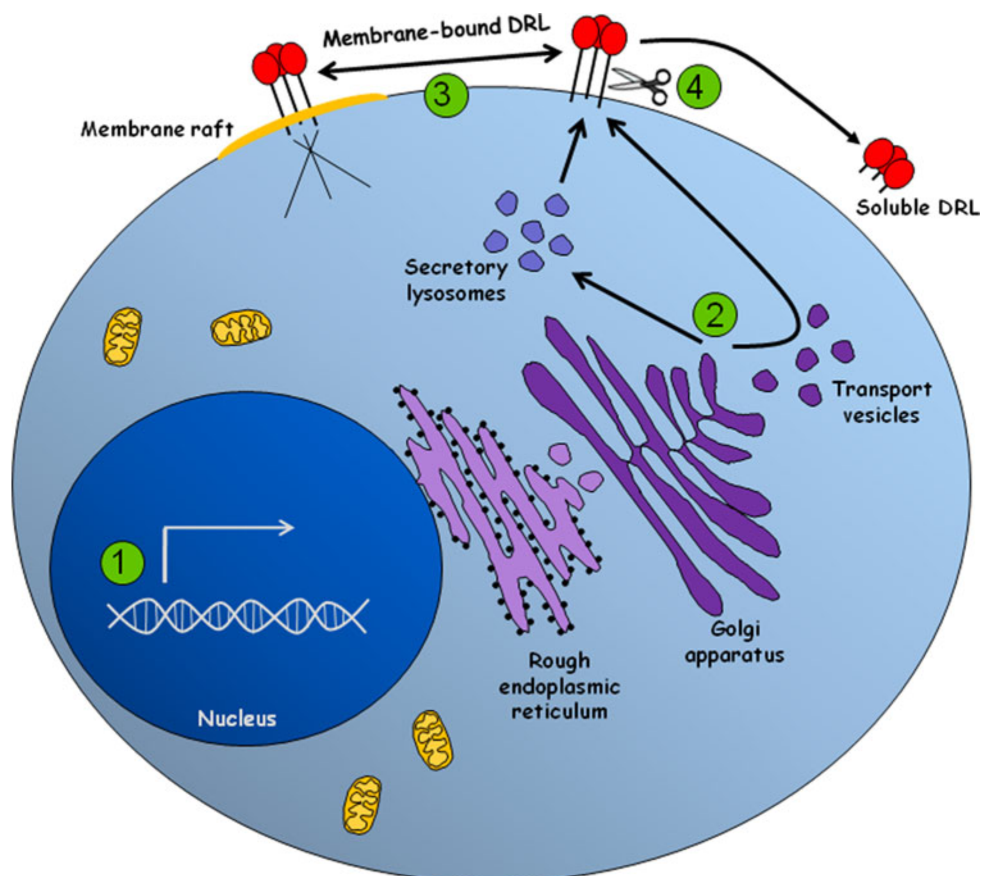

Fig. 1 The expression of death receptor ligands is tightly modulated at different levels. *1* Transcription of death receptor ligands is modulated by groups of transcriptional activators or repressors that are activated and/or de novo expressed in response to a variety of stimuli. *2* FasL is either sorted directly to the plasma membrane or stored in secretory lysosomes. Sorting of FasL is dependent on its cytoplasmic PRD domain, which interacts with proteins that contain SH3 domains or WW motifs, including Nck, Grb2, PSTPIP and members of the PHC family of proteins. Similarly, TNF-α, which is normally delivered to the cell surface, can also be stored in secretory lysosomes. However, TNF-α storage depends on a brief exposure at the plasma membrane followed by re-endocytosis. TRAIL can also be found in secretory lysosomes although its sorting mechanism still remains to be elucidated. *3* Recruitment of FasL to membrane rafts boost its killing potential, probably by enhancing the clustering of Fas in target cells. Trafficking of FasL into the rafts is dependent on the cytoplasmic PRD domain of FasL and independent of the SxxS motif. To date, TRAIL and TNF-α redistribution to the membrane rafts is still unclear. *4* Once in the cell surface, death receptor ligands can be proteolytically shed, being converted into soluble molecules. The soluble forms of these ligands can acquire different or even opposing functions, including the induction of proliferation, inflammation and survival. The main enzymes involved in TNF-α and FasL shedding are ADAM-17, ADAM-10, MMP7, and MMP3. TRAIL shedding seems to involve cysteine protease activity, although no specific enzyme has been already described. Regulation of the shedding activity involves a family of proteins called TIMP

tested [135], and that the TIMP-1-mediated inhibition of TRAIL-induced apoptosis is independent of its ability to inhibit MMPs and ADAMs [134], indicating that MMP and ADAM are not the major players in TRAIL cleavage. In vitro assays indicated that TRAIL shedding can be mediated by cysteine proteases [135], but the identity of the proteases critically involved in TRAIL shedding still remains to be elucidated.

Conclusions and perspectives

Posttranslational mechanisms involved in modulation of death receptor ligand activity comprise sorting to and storage in different subcellular compartments, including secretory lysosomes and specialized cell membrane microdomains, as well as shedding from the cell membrane by proteolytic activity. These multiple mechanisms are predominant, and of particular relevance, in hematopoietic cells, especially in T lymphocytes and NK cells, where death receptors ligands are used as both death effector and homeostatic mechanisms.

Death receptor ligands were shown to be important to the immune response against tumors and intracellular microorganism-infected cells and to participate in the immune privilege and in the homeostatic control of the immune response, as well as being involved in a diverse array of pathologies, including autoimmune diseases. For

all these reasons, they have been the subject of much attention aiming to develop novel pharmacological and genetic therapeutic approaches to different diseases.

The systemic administration of recombinant FasL as an antitumor treatment was proved to be unfeasible; even very low doses of this molecule induced massive liver apoptosis, and in many cases resulted in death [136, 137]. Recently, a FasL-base pro-drug was described as a promising treatment against transformed cells. This pro-drug consists of an engineered inactive form of FasL which is activated by tumor-expressed metalloproteases [138]. One of the major difficulties with this treatment is the proper delivery to tumor cells, which was achieved by addition of a tumor antigen-specific single-chain antibody to the construct. The obvious caveat is that the tumor cell has to express the specific tumor antigen [139]. Also, this technique would be useful not only for FasL but also for TRAIL-based treatments. As the main concern about TRAIL-based therapy is its potential hepatocytotoxicity, a tumor-directed TRAIL may be a safer option for tumor treatment.

Shedding of death receptor ligands from the cell surface, limiting their proapoptotic effect and/or changing their range of action and functions, seems to be another relevant point of therapeutical intervention. Indeed, ADAM17 has been recognized as an important drug target, as TNF-α is involved in a myriad of proinflammatory diseases [140, 141]. Conditional ADAM17-deficient mice are strongly protected against endotoxic shock by reduced TNF-α release [142], and ADAM17 inhibition downmodulates LPS-induced TNF-α secretion, reducing collagen- and adjuvant-induced arthritis [143]. Also, inhibitors of TNF-α-processing were already shown to ameliorate diverse immunological diseases, like insulin resistance, diabetes, myelodysplastic syndrome, cancer, and sepsis; some of them are already being tested in clinical trials [111, 144–148]. ADAM10 is another interesting therapeutical target due to its FasL sheddase activity. Many tumors express relatively high amounts of the transmembrane form of FasL and use this mechanism as a form of counter-attacking the infiltrating T cells [149]. It would be attractive to investigate if ADAM10 is downmodulated in these malignant tissues and if the enforced expression of ADAM10 in these cells would reduce their tumorigenic potential. Another interesting idea would be to enforce the expression of a FasL molecule resistant to ADAM10/MMP7 proteolytic cleavage in transplanted organs. This may mimic immune privileged tissues, where immune responses are abrogated due to the killing of infiltrating cells through a FasL/Fas-dependent mechanism. In this sense, when Langerhans cells were transplanted together with syngeneic FasL-expressing myoblasts they survived longer [150].

Our present state of knowledge of posttranslational modulation of death receptor ligands is summarized in Fig. 1. Taken together, it is now clear that future studies are needed to improve this knowledge, aiming to devise novel molecular approaches that can be used for clinical applications.

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