Role of ubiquitination and proteolysis in the regulation of pro- and anti-apoptotic TNF-R1 signaling ☆

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ABSTRACT

Tumor Necrosis Factor Receptor 1 (TNF-R1) transmits various intracellular signaling cascades leading to diverse biological outcomes, ranging from proliferation, differentiation, survival to the induction of various forms of cell death (i.e. apoptosis, necrosis, necroptosis). These signaling pathways have to be tightly regulated. Proteolysis is an important regulatory mechanism in TNF-R1 pro-apoptotic as well as anti-apoptotic/pro-inflammatory signaling. Some key players in these signaling cascades are known (mainly the caspase-family of proteases and a previously unrecognized "lysosomal death pathway" involving cathepsins), however the interaction of proteases in the regulation of TNF signaling is still enigmatic. Ubiquitination of proteins, both non-degradative degradative, which either results in proteolytic degradation of target substrates or regulates their biological function, represents another layer of regulation in this signaling cascade.

We and others found out that the differences in signal quality depend on the localization of the receptors. Plasma membrane resident receptors activate survival signals, while endocytosed receptors can induce cell death. In this article we will review the role of ubiquitination and proteolysis in these diverse events focusing on our own contributions to the lysosomal apoptotic pathway linked to the subcellular compartmentalization of TNF-R1. This article is part of a Special Issue entitled: Proteolysis as a Regulatory Event in Pathophysiology edited by Stefan Rose-John.

1. Introduction

During the last years it became evident, that activated receptors cannot only signal from the plasma membrane (PM), but also from intracellular organelles (e.g. endosomes). Thus, internalization is not only a mechanism to switch off unwanted signaling by degradation of surface receptors in the lysosomes. It rather serves as a temporal and spatial regulatory mechanism for the recruitment of discrete signaling platforms, allowing the diversification of various receptor-mediated signaling pathways (see recent reviews [1,2]). Activated by their specific ligands, most receptors undergo post translational modifications e.g. phosphorylation, ubiquitination or lipidation, resulting in internalization of the ligand/receptor complexes, enabling them to recruit the endocytotic machinery [1,3,4].

The family of death receptors (DR) currently comprises TNF-R1, CD95, TRAIL-R1, TRAIL-R2, death receptor-3 (DR3), death receptor 6 (DR6).Studies of our own group and also others showed that the signaling quality of the death receptors TNF-R1, CD95 and partially also the TRAIL-receptors 1 and 2 depends on their subcellular localization:

Plasma membrane bound receptors mediate pro-inflammatory and proliferative signals via the activation of NF-κB, while endocytosed receptors can induce cell death by recruiting the "death inducing signaling complex" (DISC) to their cytoplasmic death domain [5–15].

First evidence for the role of TNF-R1 internalization in apoptosis signaling came from our initial observation that blocking of TNF receptor endocytosis by a pharmacological inhibitor, Monodansylcadaverine (MDC), K$^+$-depletion or low temperature resulted in prevention of apoptotic cell death [16]. Of note, also TNF-activation of some enzyme systems like acid sphingomyelinase (A-SMase) and c-Jun terminal kinase (JNK) were also blocked, others like neutral sphingomyelinase (N-SMase) and mitogen-activated kinases (MAP-K) were still induced under these conditions, indicating a functional role of TNF-R1 compartmentalization for selective signal transduction. Further studies identified the internalization site in TNF-R1, termed "TNF Receptor Internalization Domain" (TRID). Mutations of the tyrosine-based internalization motif (YQRW, aa207-210) within the TRID resulted in complete prevention of TNF-induced apoptosis [12]. These internalization-defective receptors were unable to recruit the

DISC-proteins TRADD, FADD and caspase-8, still allowing the recruitment of RIP1 and TRAF2 and transmission of signaling for NF-κB activation.

In an independent approach, TNF-R1 internalization, recruitment of the DISC and apoptosis-induction could also be prevented after infection of cells with adenoviruses or ectopic expression of the adenovirus E3-14K protein [11], again leaving the recruitment of TRADD and TRAF2 as well as NF-κB activation unimpeded. Inhibition of TNF-R1 endocytosis by adenovirus E3-14K was effected by the lack of co-ordination in the assembly of the endocytotic machinery at the site of activated TNF-R1, i.e. missing essential components such as Rab5 and Dynamin 2. This study described a novel molecular mechanism of a virus to escape immunosurveillance by preventing TNF-induced apoptosis due to inhibition of DISC formation and caspase activation by selectively targeting TNF-R1 endocytosis. In an independent study it was shown that the infection of epithelial cells with Chlamydia trachomatis inhibits TNF-induced apoptosis also at the level of receptor internalization while leaving non-apoptotic signaling intact [17].

The physiological significance of the internalization of TNF-R1 for apoptosis signaling was further demonstrated in a cooperative project with T. Futermann, Weizmann-Institute of Science, Rehovot, Israel, analyzing the effect of the ceramide acyl chain length by studying TNF-R1-mediated apoptosis in a ceramide synthase 2 (CerS2) null mouse, which cannot synthesize very-long acyl chain ceramides. CerS2 null mice were resistant to LPS/galactosamine mediated fulminant hepatic failure even though TNFα secretion from macrophages was unaffected. Cultured hepatocytes were also insensitive to TNFα-mediated apoptosis. Additionally, in both liver and in hepatocytes, caspase activity was not elevated, consistent with inhibition of TNF-R1 pro-apoptotic signaling. Caspase activation was blocked due to the inability of CerS2 null mice to internalize the TNF-R1. While TNF-R1 was internalized to a perinuclear region in hepatocytes from wild-type mice, no internalization was detected in CerS2 null mice. These results confirm all our previous in-vitro data on the role of TNF-R1 internalization in an in-vivo mouse model by showing that altering the acyl chain composition of sphingolipids inhibits TNF-R1 internalization and selective pro-apoptotic downstream signaling for apoptosis [5].

2. Regulation of TNF-R1 at the plasma membrane

TNF can bind to two structurally distinct TNF receptors, TNF-R1 and TNF-R2, each initiating separate signaling pathways. Based on its death domain, only TNF-R1 can induce apoptosis via recruitment of the adaptor proteins TRADD, FADD and caspase-8. However, TNF-R2 can also regulate TNF-R1 mediated cell death by recruiting the anti-apoptotic proteins TRAF2 and the TRAF2-associated proteins cIAP1 and cIAP2, resulting in the limited availability of these proteins for other binding partners. By this, supporting the pro-apoptotic pathway of TNF-R1 [18–20]. TNF-R1 Pro-survival and pro-inflammatory signaling to the nucleus by TNF-R1 is mediated via the classical NF-κB pathway [21]. TNF-R2 is a rather weak inducer of the classical NF-κB signaling but is able to induce the alternative NF-κB pathway [18]. Of note, TNF-R2 can also internalize via a di-leucine motif, but TNF-induced internalization of TNF-R2 is dispensable for activation of the NF-κB pathway [22], similar to our findings with TNF-R1, where a blockade of internalization still allowed for NF-κB signaling [5,11–13]. Although it was believed that TNF-R2 is responsible for pro-survival functions and TNF-R1 signaling for cell death, recent reports discovered some degree of overlap in both TNF receptor signaling functions (for review see [23]).

Ligand binding to TNF-R1 initially triggers formation of a signaling complex at the plasma membrane composed of TRADD (TNF-receptor associated death domain), TRAF2 and TRAF5 (TNF-receptor associated factor 2 and 5), cIAP1/2 (cellular inhibitor of apoptosis protein 1/2) and RIP1 (receptor interacting serine/threonine-protein kinase 1) (complex I) [19]. RIP1 is ubiquitinated and recruits TAK1 (transforming growth factor-beta-activated kinase 1) [20]. Complex I signals for the classical pathway of NF-κB (nuclear factor κB) activation and JNK (c-Jun N-terminal kinase) activation [21]. Subsequently, the expression of anti-apoptotic proteins, like FILPL (FLICE-like inhibitory protein) is upregulated, which inhibits caspase-8 and caspase-10 activation [22,23].

Proteolysis is important in the regulation of TNF-R1 signaling from the cell surface by the control of the expression and biological functions of TNF and TNF-R1. Typically, transmembrane anchored signaling molecules are initially processed by a shedding protease, which removes the ectodomain, and subsequently by an intramembrane-cleaving protease which leads to the liberation of the intracellular domain (ICD) for signaling to the nucleus. TNF is synthesized as a type-II transmembrane protein and can be cleaved by the shedding protease a disintegrin and metalloproteinase 17 (ADAM17) from the cell surface [24–27] and by regulated intramembrane proteolysis (RIP) mediated by the γ − secretase-like aspartyl protease SPPL2a and SPPL2b [28,29], resulting in liberation of soluble TNF into the extracellular space and the intracellular domain (ICD) of TNF into the cytosol. The TNF-ICD contains a nuclear localization signal which directs this protein to the nucleus [30] and mediates reverse signaling by triggering IL-12 production i.e. in activated dendritic cells [29].

Transmembrane and soluble TNF apparently have different functions [31,32], soluble TNF is the main ligand for binding and activating TNF-R1, inducing NF-κB signaling from cell surface TNF-R1 and apoptosis from internalized TNF-R1, while transmembrane TNF predominantly binds and activates cell surface TNF-R2 [33]. A very recent report by Jiang demonstrated that transmembrane TNF can also induce death-domain independent apoptosis after binding to cell surface TNF-R1 by recruiting STAT1 to a region spanning amino acids 319–337 [34]. Soluble TNF induces apoptosis only after internalization of TNF-R1 by recruiting the death inducing complex to the death domain of TNF-R1, in line with our own reports [7,11,12].

TNF receptors are class I membrane bound proteins, whose extracellular part also can be released from the cell surface. Like the ligand TNF, both TNF-R1 and TNF-R2 can be shed by the protease ADAM17 [26,27], releasing the receptors from the cell surface and by this influencing their biological functions. Recently it was shown that ADAM17 deficiency promotes artherosclerosis by enhancing TNF-R2 signaling in a mouse-model [35]. Based on its pleiotropic and broad biological function of ADAM17 in various inflammatory diseases, this enzyme has to be tightly regulated. A recent report from the Karina Reiss group demonstrated that the TNF-induced endothelial cell death can be impaired by inducing ADAM17 activation and TNF-R1 shedding by exogenous acid sphingomyelinase [36].

Following ADAM17-mediated ectodomain shedding of TNF-R1, the membrane-bound TNF-R1 fragment can subsequently be subjected to regulated intramembrane proteolysis mediated by γ-secretase to generate a cytosolic TNF-R1 intracellular domain [37]. Prerequisite for effective γ-secretase activity and intramembrane cleavage is the clathrin-mediated internalization of TNF-R1. Loss of presenilin expression - the catalytic subunit of the γ − secretase complex - and loss of γ-secretase activity antagonized TNF-mediated JNK/MAPK activation, reduced the assembly of the pro-apoptotic complex II assembly, and inhibited TNF-induced caspase activation and apoptosis. These interesting findings are again in line of our own results on the importance of TNF-R1 internalization for complex II formation and apoptosis signaling from intracellular compartments, discussed in the next chapters.

The role of ubiquitination and proteolysis in NF-κB activation from Complex I had been extensively investigated and described in various comprehensive reviews [38–42]. Thus this review will not focus on this pathway.

The mechanisms beyond TNF-R1 internalization, intracellular trafficking and maturation and its biological outcomes became clearer during the last years and will be the main subject of this review in the following chapters.