MINIREVIEW

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Molecular Mechanisms of Soluble Cytokine Receptor Generation*[S]

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Soluble cytokine receptors play key roles in regulating cytokine-mediated biological events by binding and modulating the activity of target ligands in either an antagonistic or agonistic fashion. This Minireview will provide an overview of the molecular mechanisms mediating the generation of soluble cytokine receptors, which include sheddase-mediated proteolytic cleavage of cell-surface receptors, generation of soluble receptors by alternative gene splicing, transcription and translation of cytokine-binding genes, and extracellular release of membrane-bound receptors within vesicles such as exosomes.

Cytokine-mediated biological events such as host defense, immune regulation, cellular proliferation, and apoptosis are tightly controlled to prevent adverse sequelae secondary to dysregulated signaling. An important mechanism by which aberrant cytokine signaling is modulated is the generation of soluble cytokine receptors that bind target cytokines and modify their biological activity in an antagonistic or agonistic fashion. This Minireview will focus on the molecular mechanisms that modulate the generation of soluble cytokine receptors.

Proteolytic Cleavage and Shedding of Cell-surface Cytokine Receptors

TACE[2] (ADAM17), a member of the ADAM (a disintegrin and metalloproteinase) family of zinc metalloproteinases, is the prototypical sheddase of cytokine ligands and receptors (reviewed in Refs. 1–5). The ADAM family is composed of 40 named genes that encode type I transmembrane proteases containing multiple domains: an N-terminal signal sequence, a prodomain, a zinc metalloproteinase domain, a disintegrin cysteine-rich domain, an EGF-like domain, a transmembrane domain, and a cytoplasmic domain (1, 2, 4, 5). TACE was originally identified by its ability to mediate the cell-surface cleavage and shedding of TNF, which is expressed as a 26-kDa transmembrane protein and proteolytically cleaved to produce the soluble 17-kDa TNF (6, 7). Proteolytic cleavage of cell-surface TNF modulates its activity, as both the soluble and membrane forms of TNF signal and mediate distinct biological functions (2).

TACE functions as a cytokine receptor sheddase for the TNFR superfamily, the IL-1R/Toll-like receptor superfamily, and the type I cytokine receptor superfamily (supplemental Table 1). TACE also mediates the cleavage of a structurally and functionally diverse set of protein ectodomain substrates, which include cytokines, chemokines, EGFR ligands, growth factor receptors, receptor tyrosine kinases, and Notch1 (reviewed in Refs. 2, 3, 8, and 9). Furthermore, mice expressing a catalytically inactive TACE have an embryonic lethal phenotype secondary to impaired TGF-α ectodomain shedding with resultant defects in epithelial cell maturation and organization (10). The molecular mechanisms underlying TACE substrate recognition of its varied substrates remain incompletely defined (1, 3, 4). Scissile bond sequence and the length of the juxtamembrane stalk of TACE substrates may contribute to substrate specificity (11). For example, a systemic analysis of P1′ and P2′ substrate specificity using a peptide library has shown that TACE prefers lipophilic amino acids at the P1′ position, such as leucine and valine, whereas the P2′ position accommodates basic amino acids, such as arginine and lysine, as well as non-basic amino, acids, such as threonine (12).

Multiple mechanisms exist by which TACE enzymatic activity is regulated. Both the TACE prodomain and TIMP3 (tissue inhibitor of matrix metalloproteinases 3) function as inhibitors of the TACE catalytic site. TACE is synthesized as an inactive zymogen that is processed by proprotein convertases (such as furin) that cleave TACE at a putative KEX-2/furin recognition site, located between the prodomain and the catalytic domain (reviewed in Refs. 2 and 4). This occurs in the *trans*-Golgi, followed by transport of the mature enzyme to the plasma membrane (13). The TACE prodomain contains a putative cysteine-switch box, which functions as an inhibitor of catalytic activity in other ADAM proteins and matrix metalloproteinases via ligation of the cysteinyl thiol in the prodomain to the zinc ion in the catalytic site. Although Cys^{184} in the TACE prodomain ligates the catalytic zinc ion, the cysteine-switch motif is not essential for inhibition of TACE enzymatic activity (14). Instead, the TACE prodomain, which exists as a stably folded protein that tightly associates with the catalytic domain, may retain the catalytic domain in an open, inactive, conformational state (15). Furthermore, the TACE prodomain may function as an intramolecular chaperone that protects the TACE zymogen from intracellular degradation and facilitates proper intracellular trafficking. Similarly, the cleaved ADAM10 prodomain associates with mature ADAM10 and functions as a specific competitive inhibitor of catalytic activity that does not involve a cysteine-switch mechanism (16). This may represent a unique feature of TACE and ADAM10, as the cysteine switch appears to be required for the prodomains of other ADAM family mem-

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[2] The abbreviations used are: TACE, tumor necrosis factor-α-converting enzyme; EGF, epidermal growth factor; TNF, tumor necrosis factor; TNFR, TNF receptor; IL-1R, interleukin-1 receptor; EGFR, EGF receptor; TGF, transforming growth factor; SH, Src homology; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; GRP, gastrin-releasing peptide; DcR3, decoy receptor 3; IL-18BP, IL-18-binding protein; IFN-γ, interferon-γ; MVBs, multivesicular bodies.

bers (such as ADAM12) to inhibit catalytic activity (4). Attenuation of TACE catalytic activity by TIMP3 is another important regulatory mechanism that controls TNF-mediated inflammation (17). TIMP3 is the only TIMP family member that binds and inhibits TACE. TIMP3 knock-out mice display increased constitutive soluble TNF generation in the liver, which is associated with chronic hepatic inflammation. This finding is consistent with a key role for TIMP3 in modulating TACE-mediated TNF shedding and supports the concept of TACE as the main physiological TNF-processing enzyme (18).

The cytoplasmic domain of TACE, which contains SH2 and SH3 domain-binding motifs, also modulates its function (2, 4). Although the TACE cytoplasmic domain undergoes inducible phosphorylation, phorbol 12-myristate 13-acetate-mediated TACE catalytic activity can occur independently of the cytoplasmic domain (19). Instead, the TACE cytoplasmic domain may play an important role in regulating TACE protein trafficking and maturation, which are regulated by MAPKs (20). Consistent with this, phorbol 12-myristate 13-acetate stimulates the ERK-mediated phosphorylation of Thr^{735} in the TACE cytoplasmic domain, which induces the translocation of TACE from the endoplasmic reticulum to the cell surface. Furthermore, the phosphorylation state of the TACE cytoplasmic domain may be regulated by an interaction with the PDZ domain of the protein-tyrosine phosphatase PTPH1. Additional proteins that interact with the TACE cytoplasmic domain and potentially regulate its catalytic activity include MAD2 (mitotic arrest-deficient 2), SAP97 (synapse-associated protein 97), and Eve-1, a SH3 domain-containing protein that potentiates the proteolytic cleavage and shedding of EGFR ligands (8, 21). FHL2 (four-and-a-half LIM domains 2) also binds the TACE cytoplasmic domain as well as the actin cytoskeleton and may regulate TACE cellular localization and activity (22).

TACE proteolytic activity can be regulated by several additional mechanisms. First, TACE undergoes stimulation-dependent internalization, which down-regulates catalytic activity at the plasma membrane (23). Second, partitioning of TACE and its substrates within lipid raft microdomains may regulate proteolytic cleavage and cell-surface shedding. Both mature TACE and its substrates have been localized to lipid raft microdomains, although sequestration of TACE within lipid rafts has not been a universal finding (24, 25). Disruption of lipid raft microdomains by cholesterol depletion has been associated with the increased release of TACE substrates and may reflect enhanced interactions between substrates in non-raft membrane regions and TACE released from lipid rafts (24–26). Furthermore, co-localization of TACE and furin within lipid raft microdomains in the Golgi apparatus may be necessary for prodomain processing (25). Third, nardilysin (*N*-arginine dibasic convertase), a dibasic selective metalloendopetidase of the M16 family, can form a complex with TACE and enhance TACE-induced cleavage of heparin-binding EGF-like protein via a mechanism that is not dependent upon *N*-arginine dibasic convertase catalytic activity (27). Fourth, in the case of CD30 shedding, cysteine-rich domains 2 and 5 of the cleaved ectodomain may serve as a stimulus for enhanced shedding, possibly by ligand-independent aggregation that promotes enzyme binding (28).

Transactivation of TACE-mediated receptor shedding can also be modulated by cross-talk between G protein-coupled receptors and EGFRs. "Triple membrane-passing signaling" describes the activation of an ADAM by a G protein-coupled receptor, which then cleaves and releases an EGFR ligand that binds and activates its EGFR (reviewed in Ref. 2). This allows TACE to function as a molecular switch for EGFR signaling (2). Consistent with this concept, a GRP-induced signaling cascade that induces EGFR ligand cleavage has been identified recently (29). GRP stimulation induces the activation of c-Src, leading to downstream induction of phosphoinositide 3-kinase and PDK1 (phosphoinositide-dependent kinase 1), which mediates phosphorylation and translocation of TACE to the cell surface, EGFR proligand cleavage, and EGFR activation. Furthermore, GRP stimulation enhances the cytoplasmic association between c-Src and TACE, which is followed by their co-translocation to the cell membrane. Similarly, in human airway epithelial cells, neutrophil elastase activates DUOX1 (dual oxidase 1) to produce reactive oxygen species that activate TACE to cleave pro-TGF-α, which induces MUC5AC mucin expression (30). Conversely, EGFR-dependent signaling has been shown to activate TACE in response to stimulation with *Staphylococcus aureus* protein A and to induce release of soluble TNFR1 (TNFRSF1A, p55 TNFR) (31). Interaction of the IgG-binding domain of protein A with EGFR induces TACE phosphorylation and activation via a c-Src-ERK1/2 pathway. This results in the mobilization of TNFR1 from intracellular stores such as the Golgi apparatus to the cell surface, where it co-localizes with TACE and undergoes ectodomain shedding. Histamine has also been shown to mobilize Golgi-associated TNFR1 via a MEK1-p42/44 MAPK pathway that induces TNFR1 shedding (32).

Other ADAM family members possess cytokine receptor shedding activity (reviewed in Refs. 3 and 8). ADAM10 has been identified as the sheddase mediating the calcium-dependent cleavage of CD30 and the constitutive and inducible shedding of IL-6Rα (8, 33). ADAM10 also mediates the constitutive and ionomycin-inducible proteolytic shedding of the chemokine ligands fractalkine (CX3CL1) and CXCL16 (8, 34). ADAM10 has been identified as the principal sheddase for CD23, the low affinity IgE receptor (35). TNF, osteoprotegerin ligand, and Kit ligand 1 have been identified as substrates for ADAM19 (36).

Soluble cytokine receptors can also be generated via a mechanism that involves regulated intramembrane proteolysis. Cleavage of the IL-1 type II decoy receptor (IL-1RII) in an α-secretase-like fashion sheds the IL-1RII ectodomain and generates a C-terminal fragment, which undergoes γ-secretase-mediated intramembrane proteolysis (37). Furthermore, β-secretases BACE1 and BACE2 can function as IL-1RII sheddases that cleave the IL-1RII ectodomain at a site adjacent to the α-secretase site. The physiological relevance of BACE1 and BACE2 as IL-1RII sheddases is unclear, as IL-1RII shedding remains intact in cells deficient in both BACE1 and BACE2. ADAM10-mediated α-secretase cleavage of fractalkine and CXCL16 may also precede a γ-secretase cleavage that releases intracellular signaling fragments (38).

MMP9 (matrix metalloproteinase 9) has also been implicated in the shedding of IL-2Rα from activated T cells (39), whereas neutrophil elastase may participate in the shedding of TNFR2 and TNFR1 from neutrophils (40). Phosphatidylinositol-specific phospholipase C has been identified as a receptor sheddase for the cil-

iary neurotrophic factor receptor-α, which is anchored to cell membranes by a glycosylphosphatidylinositol linkage (8).

Generation of Soluble Cytokine Receptors by Alternative Splicing

The second major mechanism of soluble cytokine receptor generation is the alternative splicing of mRNA transcripts, which deletes the transmembrane domain of membrane-associated receptors. Soluble cytokine receptors that can be generated by alternative splicing include members of the TNFR superfamily, the IL-1R/Toll-like receptor superfamily, class I and II cytokine receptor superfamilies, the TGF-β receptor family, and the IL-17 receptor (supplemental Table 1).

Several cytokine receptors such as IL-1RII, IL-6Rα, IL-15Rα, and TNFR2 (TNFRSF1B) can be generated by either proteolytic cleavage of receptor ectodomains or alternative splicing events (8). For example, a soluble, differentially spliced isoform of human TNFR2 that lacks exons 7 and 8, which encode the transmembrane and cytoplasmic domains, has been described recently (41). Similarly, soluble IL-15Rα can be generated by either TACE-mediated proteolytic cleavage or alternative splicing (8, 42). Two closely homologous, alternatively spliced IL-15Rα isoforms that contain only the signal peptide and the exon 2 sushi domain, which mediates cytokine binding, have been identified. Both the proteolytically cleaved full-length IL-15Rα ectodomain and the alternatively spliced soluble IL-15Rα isoforms can form heterocomplexes with IL-15, thereby either inhibiting or promoting IL-15 activity, respectively (42, 43). Consistent with this, the alternatively spliced IL-15Rα sushi domains have been implicated in *trans*-presentation, whereby IL-15·IL-15Rα complexes present IL-15 to cells (such as natural killer and $CD8^+$ T cells) that lack IL-15Rα but express the intermediate affinity IL-15$\beta\gamma$ complex (42).

trans-Presentation of IL-15 is an example of cytokine *trans*-signaling that was initially described for soluble IL-6Rα·IL-6 complexes, which bind ubiquitously expressed membrane-bound gp130 and thereby confer IL-6 signaling capabilities to cells deficient in IL-6Rα (8, 44). *trans*-Signaling has also been reported for other IL-6 subfamily members such as IL-11 and ciliary neurotrophic factor (45). In contrast, soluble IL-6Rα·IL-6 *trans*-signaling can be attenuated by the soluble form of gp130, which competes with membrane gp130 for binding of soluble IL-6Rα·IL-6 complexes (46). Similarly, soluble forms of the leukemia inhibitory factor and oncostatin M receptors that can bind and antagonize leukemia inhibitory factor and oncostatin M in association with soluble gp130 have been identified (45, 47).

Soluble Cytokine-binding Proteins

The third mechanism of soluble cytokine receptor generation involves the transcription and translation of distinct genes that encode secreted cytokine-binding proteins that function as inhibitory decoy receptors. DcR3 (TNFRSF6B, TR6, M68) is a secreted TNFR superfamily member that contains a signal peptide and four tandem cysteine-rich domains, but lacks a transmembrane domain (reviewed in Ref. 8). DcR3 inhibits apoptosis by binding the Fas ligand (TNFSF6) and blocking its interaction with Fas (CD95, TNFRSF6). DcR3 is highly expressed by malignant cells (such as Epstein-Barr virus-positive lymphomas) as a protective mechanism against Fas ligand-dependent immune/cytotoxic attack. Furthermore, the Epstein-Barr virus immediate-early protein Rta directly binds an Rta-responsive element in the DcR3 promoter and functions as a transactivator that increases DcR3 expression in conjunction with recruitment of cAMP-responsive element-binding protein-binding protein (48). This represents a mechanism by which a viral gene product may up-regulate DcR3 expression and thereby promote viral survival and tumorigenesis. Similarly, DcR3 binds and inhibits the interaction of LIGHT (TNFSF14) with its receptors, herpesvirus entry mediator (TNFRSF14, TR2), and the lymphotoxin β-receptor (TNFRSF3), with the resultant inhibition of LIGHT-mediated tumor cell apoptosis. DcR3 also binds TL1A, an endothelial cell-derived TNF-like factor, and competitively inhibits its interaction with death receptor 3 (TNFRSF25). Thus, DcR3 may function as an angiogenic factor by inhibiting the negative proliferative effects of TL1A on endothelial cells.

Osteoprotegerin (TNFRSF11B) is another TNFR superfamily member that lacks a transmembrane domain and is expressed as a soluble decoy receptor that binds osteoprotegerin ligand (RANKL, TNFSF11) and prevents its interaction with RANK (TNFRSF11A), with the resultant inhibition of osteoclast differentiation (8). Osteoprotegerin-deficient mice develop early osteoporosis and vascular calcification (8, 49).

IL-18BP is a soluble decoy receptor for IL-18 that is structurally and functionally similar to IL-1RII (8). IL-18BP contains a single Ig-like domain and functions as a constitutively expressed and secreted inhibitor of IL-18-induced IFN-γ production and Th1 responses (50, 51). Four human IL-18BP isoforms that differ by the presence (IL-18BPa and IL-18BPc) or absence (IL-18BPb and IL-18BPd) of Ig domains, which mediate IL-18 binding and neutralization, have been identified (50). Mice overexpressing IL-18BPa demonstrate reduced lipopolysaccharide-induced IFN-γ production and are protected from concanavalin A-induced hepatotoxicity (51). IL-18BP also binds the IL-1 homolog IL-1F7 at the same engagement sites as IL-18, which may allow IL-1F7 and IL-18BP to form a ternary complex with IL-18Rb, which sequesters IL-18Rb and inhibits the formation of a functional receptor complex (52).

IL-22RA2 (IL-22Rα2, IL-22-binding protein) is a secreted member of the human class II cytokine receptor family that lacks a transmembrane domain (8). IL-22RA2 binds and inhibits IL-22, an IL-10 homolog that is secreted by T cells and induces the proliferation of acute-phase reactants. Proteinase 3 has been identified as a binding protein for IL-32, an IL-18-inducible, pro-inflammatory cytokine that up-regulates TNF, IL-8, and MIP-2 (macrophage inflammatory protein 2) production (53). Limited proteolysis of IL-32 by proteinase 3 enhances its biological activity. IL-33 is an IL-1-related cytokine that binds the IL-1R family member ST2 and activates NF-κB and MAPKs and induces Th2 cytokines such as IL-4, IL-5, and IL-13 (54). A soluble form of ST2 that functions as a soluble decoy receptor for IL-33 and blocks the anti-hypertrophic effects of IL-33 on cardiomyocytes has been identified (54, 55). Cytokine-like factor 1, a member of the class I cytokine receptor family, is another soluble receptor that forms a stable heterodimer with cardiotrophin-like cytokine prior to its secretion (56).

The important role of soluble cytokine receptors in inhibiting the activity of pro-inflammatory cytokines has been usurped by viral pathogens, which synthesize homologs of mammalian cytokine receptors as a mechanism to evade host defenses (reviewed in Ref. 57). Poxviruses such as cowpox, variola, myxoma, and Shope fibroma viruses encode viral TNFR homologs termed cytokine response modifiers (*crmB*, *crmC*, *crmD*, and *crmE*) that lack transmembrane domains and are secreted from infected cells to inhibit TNF activity (8, 57). Similarly, poxviruses (cowpox, ectromelia (mousepox)) secrete a soluble viral CD30 homolog that may function as a decoy receptor via binding of CD30L and induce reverse signaling in immune cells that express CD30L. Secreted viral cytokine receptors have also been identified for IL-1β and IFN-γ. Alternatively, viral cytokine-binding proteins, which have limited or no sequence similarity to their cellular counterparts, that interact with IFN-α/β, IFN-γ/IL-2/IL-5, macrophage colony-stimulating factor 1, granulocyte-macrophage colony-stimulating factor/IL-2, and IL-18 have been identified. Similarly, viral chemokine-binding proteins CKBP-1, CKBP-2, CKBP-3, and CKBP-4, which lack sequence similarity to host chemokine receptors, can bind and inhibit the activity of C, CC, CXC, and CX3C chemokines. The viral TNFRs CrmB (encoded by variola virus) and CrmD (encoded by orthopoxviruses) display dual functionality that enables TNF binding via cysteine-rich domains and chemokine binding via a unique SECRET (smallpox virus-encoded chemokine receptor) C-terminal domain (58).

Exosome-associated Cytokine Receptors

Cytokines and cytokine receptors may also be released to the extracellular compartment as membrane components of vesicles such as exosomes. Exosomes are small membrane vesicles (typically $<$100 nm in diameter) that are formed by reverse budding of the membrane of multivesicular bodies (MVBs), followed by MVB fusion with the plasma membrane and release of intraluminal vesicles to the extracellular space (reviewed in Refs. 59 and 60). Sorting of proteins into intraluminal vesicles within MVBs may be regulated by pathways utilizing the ESCRT (endosomal sorting complex required for transport) machinery, but ESCRT-independent mechanisms that are regulated by ceramide-mediated budding of exosome vesicles into MVBs have been identified (61).

Human vascular endothelial cells release a full-length 55-kDa TNFR1 within the membranes of exosome-like vesicles of 20–50 nm in diameter that are capable of binding TNF (62). Both the release of TNFR1 exosome-like vesicles and the proteolytic shedding of TNFR1 ectodomains appear to be regulated by pathways that mediate the translocation of intracytoplasmic TNFR1 vesicles. ARTS-1 (aminopeptidase regulator of TNFR shedding 1), also known as ERAP1 (endoplasmic reticulum-associated aminopeptidase 1), is a type II integral membrane protein that associates with TNFR1 and nucleobindin 2 prior to the commitment of TNFR1 to pathways that result in either the constitutive release of TNFR1 exosome-like vesicles or the inducible proteolytic cleavage of TNFR1 ectodomains (63, 64). A functional interaction between TNFR1 and BIG2, a brefeldin A-inhibited ARF-GEP, selectively regulates the constitutive release of TNFR1 exosome-like vesicles in an ARF1- and ARF3-dependent fashion (65). Synovial fibroblasts from patients with rheumatoid arthritis have been shown to release exosomes that express membrane-associated TNF and blunt the activation-induced cell death of $CD4^+$ T cells (66). Similarly, malignant melanoma cells have been reported to release exosomes that express TNF, TNFR1, and TNFR2 (67).

Exosomes that express EGF and macrophage migration inhibitory factor, as well as numerous protein components of MVBs and the endosomal pathway, have been found in human urine, whereas keratinocytes release exosomes that contain a full-length EGFR (ErbB1) (60, 68). Exosomes also express functionally active forms of ADAM10 and TACE that may cleave exosome-associated substrates such as CD171 (L1) (69). Furthermore, cytoplasmic cleavage products of CD44 and CD171 can accumulate in exosomes prior to extracellular release.

Microvesicle shedding from the plasma membrane, which generates vesicles that are 100–1000 nm in diameter, has been identified as a mechanism for the secretion of chemokine receptors (CCR6, CX3CR1) and IL-1β (70, 71). Similarly, Fas ligand can be released from tumor cells within microvesicles 100–200 nm in diameter as a mechanism of inducing apoptosis of Fas-sensitive lymphoid cells, with resultant impaired antitumor responses ("Fas tumor counterattack") (8).

Perspective

Soluble cytokine receptors, which can be generated by several distinct molecular mechanisms, are key regulators of inflammation, immune responses, cellular proliferation, and apoptosis. Further elucidation and characterization of the molecular pathways that mediate soluble cytokine receptor generation may identify new mechanisms of disease pathogenesis as well as future opportunities for therapeutic interventions.

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