MALT1 Protease: A New Therapeutic Target in B Lymphoma and Beyond?

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Abstract

The identification of mucosa-associated lymphoid tissue lymphoma translocation 1 (MALT1) as a gene that is perturbed in the B-cell neoplasm MALT lymphoma, already more than a decade ago, was the starting point for an intense area of research. The fascination with MALT1 was fueled further by the observation that it contains a domain homologous to the catalytic domain of caspases and thus, potentially, could function as a protease. Discoveries since then initially revealed that MALT1 is a key adaptor molecule in antigen receptor signaling to the transcription factor NF-κB, which is crucial for lymphocyte function. However, recent discoveries show that this function of MALT1 is not restricted to lymphocytes, witnessed by the ever-increasing list of receptors from cells within and outside of the immune system that require MALT1 for NF-κB activation. Yet, a role for MALT1 protease activity was shown only recently in immune signaling, and its importance was then further strengthened by the dependency of NF-κB–addicted B-cell lymphomas on this proteolytic activity. Therapeutic targeting of MALT1 protease activity might, therefore, become a useful approach for the treatment of these lymphomas and, additionally, an effective strategy for treating other neoplastic and inflammatory disorders associated with deregulated NF-κB signaling. Clin Cancer Res; 17(21); 6623–31. ©2011 AACR.

Background

MALT1, a regulator of NF-κB signaling

The mucosa-associated lymphoid tissue lymphoma translocation 1 (MALT1) gene was first identified by virtue of its involvement in the recurrent t(11;18)(q21;q21) chromosomal translocation in the B-cell neoplasm MALT lymphoma (1–3). This translocation creates a fusion oncprotein consisting of the carboxy terminus of MALT1 linked to the amino terminus of cellular inhibitor of apoptosis 2 (abbreviated as cIAP2 or API2; Fig. 1A). MALT1 was noted to contain a putative proteolytic domain that bears similarity to the active site of the caspase family of cysteine proteases (3–5). However, structural analyses suggested that, in contrast to caspases, MALT1 would show specificity for substrates with a basic or uncharged amino acid in the P1 position (amino-terminal to the cleavage site). Hence, MALT1 has been classified as a paracaspase to distinguish it from caspases and to recognize its similarity to other paracaspase family members found in zebrafish and Dicystostelium (5).

The discovery that expression of API2-MALT1 results in constitutive activation of the canonical NF-κB pathway (4, 5) led to numerous investigations of the role of MALT1 as a regulator of the NF-κB family of transcription factors. MALT1 binds to the adaptor protein BCL10 and synergizes with BCL10 in promoting canonical NF-κB activation (4, 5). Notably, MALT1 and BCL10 can each be deregulated via the recurrent t(14;18)(q32;q21) and t(1;14)(p22;q32) translocations in MALT lymphoma, respectively, because these translocations bring the MALT1 or BCL10 gene under the control of the immunoglobulin (Ig) heavy-chain gene enhancer, thus leading to inappropriately enhanced expression (6). In addition to its caspase-like proteolytic domain, MALT1 contains an amino-terminal death domain and 3 Ig-like protein–protein interaction domains (Fig. 1A). BCL10 binds to a region of MALT1 that comprises the death domain and first Ig domain, and this interaction induces the oligomerization of MALT1 (4, 7). MALT1 oligomerization triggers activation of the inhibitor of kappa B kinase (IKK) complex, leading to phosphorylation-dependent degradation of inhibitor of kappa Bα (IkBα) and release of transcriptionally active NF-κB dimers, composed primarily of p65(relA) and p50, into the nucleus (Fig. 1B; refs. 4, 8). In the case of API2-MALT1, API2 moiety-mediated auto-oligomerization allows for constitutive NF-κB activation in the absence of BCL10 (9, 10), and API2 moiety-mediated recruitment of other NF-κB signaling proteins, such as TNF receptor–associated...
Figure 1. MALT1 protease and its known proteolytic substrates. A, domain structure of MALT1, API2-MALT1, and MALT1 proteolytic domain substrates. Several API2-MALT1 fusion variants, resulting from varied breakpoints within the API2 and MALT1 genes, have been identified, and the most commonly occurring variant is shown. All reported API2-MALT1 fusions retain the 3 API2 baculovirus inhibitor of apoptosis repeat (BIR) domains and the MALT1 caspase-like proteolytic domain. Studies thus far indicate that NIK cleavage within the cell can be carried out by API2-MALT1 but not by wild-type MALT1. DD, death domain; caspase-like, proteolytic domain that bears resemblance to the proteolytic domain of caspases; UBA, ubiquitin-associated domain; Zn, zinc finger; CAP-Gly, cytoskeletal-associated protein-glycine conserved domain; BD, binding domain. B, summary of MALT1-mediated signaling. The 4 known substrates of the MALT1 proteolytic domain (Bcl10, A20, CYLD, and NIK) are indicated by yellow stars numbered 1 to 4. Left, the API2-MALT1 fusion oncoprotein stimulates activation of both the canonical and noncanonical NF-κB signaling pathways. Like wild-type MALT1, API2-MALT1 proteolytically cleaves A20 and Bcl10 to activate the canonical NF-κB signaling pathway. Right, API2-MALT1 induces ubiquitination of A20 and CYLD, which results in proteasomal degradation and activation of the noncanonical NF-κB signaling pathway. © 2011 American Association for Cancer Research.
factor 2 (TRAF2). It also contributes to API2-MALT1-dependent NF-κB activation (7, 9, 11). The precise mechanism by which MALT1 or API2-MALT1 stimulates IKK activation is not completely understood, but it involves recruitment of the ubiquitin ligase TRAF6 (7, 8) and the TRAF6-directed, K63-linked ubiquitination of several proteins, including IKKγ, BCL10, and MALT1 itself (12–14). Early studies suggested that the MALT1 caspase-like domain plays a role in NF-κB signaling, because substitution of the predicted active-site cysteine caused a reduction in NF-κB activation by BCL10-MALT1 or API2-MALT1 (4, 5).

Analysis of MALT1 knockout mice revealed that MALT1 plays a critical role in the adaptive immune response by mediating antigen-dependent NF-κB activation in lymphocytes (15, 16). Biochemical studies then elucidated the specific molecular mechanisms by which MALT1 can be activated in response to antigen receptor engagement. T-cell receptor (TCR) or B-cell receptor (BCR) stimulation induces protein kinase C (PKC)–dependent phosphorylation of caspase recruitment domain (CARD) domain–containing membrane-associated guanylate kinase (MAGUK) protein (CARMA1), a member of the MAGUK family of scaffolding proteins. This phosphorylation induces a conformational change that allows CARMA1 to recruit BCL10-MALT1 to the receptor and form the CARMA1–BCL10–MALT1 (CBM) complex (Fig. 1B: refs. 17, 18). In the T cell, formation of the CBM complex is assisted by the coordinated engagement of the CD28 coreceptor, which stimulates phosphoinositide 3-kinase–dependent phosphorylation of 3-phosphoinositide–dependent protein kinase 1 (PDK1). Activated PDK1 then stimulates PKCθ and serves to scaffold the TCR with the CBM complex (19, 20). CBM-mediated recruitment of multiple signaling proteins, such as TRAF6 and the IKK complex, then sets in motion canonical NF-κB activation, leading to cytokine production and cellular proliferation in response to antigen. This precise role for MALT1 is less firmly established in B cells compared with T cells. Although MALT1-deficient T cells fail to induce IKK-dependent stimulation of NF-κB, proliferate, and secrete interleukin 2 (IL-2) in response to TCR stimulation (15, 16), the nature of the relationship between BCR stimulation, MALT1-dependent NF-κB signaling, and B-cell activation remains less well understood.

Recent studies suggest that MALT1 may be specifically required only for activation of the c-Rel NF-κB subunit; it may not be essential for IKK recruitment or activation after BCR engagement (21).

In addition to its role in lymphocyte antigen receptor signaling, MALT1 has now been shown to have a critical function downstream of a variety of other cell-surface receptors, both within and outside of the immune system (Table 1). For example, MALT1 mediates B-cell activation factor of the TNF family (BAFF)–dependent antiapoptotic gene induction in marginal zone B cells (22) and IgE FcεRI receptor–mediated secretion of TNFα and IL-6 in mast cells (23). In macrophages, MALT1 has been implicated in mediating NF-κB activation downstream of the innate immune and/or pattern recognition receptors toll-like receptor 4 (TLR4) and dc-R1 and -2 (24–26), and in natural killer cells, MALT1 operates downstream of multiple immunoreceptor tyrosine-based activation motif (ITAM)–coupled receptors (27). Together, these studies show that MALT1 plays an important and complex role in multiple aspects of immunity.

Moreover, recent studies reveal that the MALT1-containing CBM complex also plays a role downstream of G-protein–coupled receptors (GPCR) in a variety of nonimmune cell types. In these cases, GPCR-dependent PKC activation triggers the assembly of a CBM complex containing CARMA3, a homolog of CARMA1 that is expressed in cells outside of the hematopoietic system (Fig. 1B). Thus far, evidence suggests that MALT1 plays a critical role in mediating NF-κB activation downstream of the GPCRs for lysophosphatidic acid (LPA), angiotensin II, cysteine X cysteine chemokine ligand 8 (CXCL8)/IL-8, CXCL12/stromal cell–derived factor-1 α (SDF-1α), and thrombin (28–32). As a mediator of GPCR-dependent signaling, MALT1 may play a critical role in the pathophysiology of NF-κB–driven carcinomas and inflammatory diseases. For example, LPA stimulates the proliferation, migration, and survival of ovarian cancer cells, and this effect is due, in part, to upregulation of urokinase plasminogen activator (uPA). MALT1 silencing inhibits LPA-mediated uPA upregulation, suggesting that MALT1 may play a role in ovarian cancer pathogenesis (33). Similarly, MALT1 silencing in oral squamous cell carcinoma inhibits cellular invasion and CYLD.

In addition, the API2 moiety of API2-MALT1 recruits NIK, thereby making NIK available as a proteolytic substrate for the MALT1 protease domain within API2-MALT1. API2-MALT1–dependent NIK cleavage separates the TRAF3 binding site from the active NIK kinase domain, and the stabilized NIK fragment containing the kinase domain promotes deregulated noncanonical NF-κB signaling. The fact that NIK cleavage is required for API2-MALT1–induced protection of B cells from apoptosis and B-cell adhesion to the endothelium suggests that NIK cleavage is likely critical to API2-MALT1–dependent B lymphomagenesis. Center, MALT1 mediates antigen-dependent NF-κB signaling in lymphocytes. TCR or BCR stimulation leads to PKC-dependent phosphorylation of CARMA1, which allows for the formation of a CBM-signaling complex. TCR-dependent activation of the canonical NF-κB pathway requires CD28 coreceptor stimulation and recruitment and activation of PDK1. TCR stimulation leads to MALT1–dependent cleavage of BCL10, A20, and CYLD. Bcl10 cleavage may be required for T-cell adhesion to fibronectin. Cleavage of A20 prevents A20–mediated inhibition of TCR–dependent NF-κB activation. CYLD cleavage may be required for TCR–induced JNK activation. CYLD cleavage may be required for TCR–induced JNK activation. The hollow line indicates that the mechanisms by which stimulation of the BCR leads to activation of a CBM complex are less well understood. An important role for MALT1–dependent signaling in B lymphomagenesis is suggested by the findings that ABC–DLBCL cells contain preassembled CBM complexes and constitutive MALT1–dependent cleavage of A20 and BCL10. Furthermore, activating somatic mutations of the BCR subunits CD79A and CD79B and CARMA1 are commonly present in ABC–DLBCL cells, and inhibition of MALT1 proteolytic activity in these cells impairs cell proliferation and survival. Right, a CARMA3–BCL10–MALT1 complex mediates canonical NF-κB activation downstream of several GPCRs in a variety of cell types (see text). The specific G-protein subunits involved in coupling GPCR to CBM-dependent signaling have not yet been determined, but Gαq/11 can mediate GPCR–dependent PKC activation. In contrast to TCR signaling, PDK1 is not necessary for CBM activation, but instead, β arrestins can serve as a scaffold linking GPCR to the CBM complex. The role of MALT1 proteolytic activity in GPCR-dependent signaling is not yet known.
Table 1. Summary of cell-surface receptors known to induce MALT1-dependent signaling

<table>
<thead>
<tr>
<th>Cell classification</th>
<th>Receptor</th>
<th>Ligand</th>
<th>Specific cell type</th>
<th>Upstream MALT1 adaptor</th>
<th>MALT1 proteolytic substrate</th>
<th>Effect of MALT1-dependent proteolytic cleavage</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Lymphoid cells</td>
<td>TCR and/or BCR</td>
<td>Antigen</td>
<td>T and/or B lymphocyte</td>
<td>CARMA1-BCL10</td>
<td>A20</td>
<td>Canonical NF-κB activation</td>
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<tr>
<td></td>
<td>None</td>
<td>None</td>
<td>AP12-MALT1</td>
<td>None</td>
<td>None</td>
<td>Integrin-dependent T-cell adhesion</td>
<td>36</td>
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<tr>
<td></td>
<td>ITAM-coupled NK receptors NK1.1, Ly49D, Ly49H, and NKG2D</td>
<td>Varied</td>
<td>NK</td>
<td>CARMA1-BCL10</td>
<td>ND</td>
<td>Noncanonical NF-κB activation</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>BAFF-R</td>
<td>BAFF</td>
<td>B lymphocyte</td>
<td>TRAF3</td>
<td>ND</td>
<td>ND</td>
<td>22</td>
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<tr>
<td>Myeloid cells</td>
<td>FcεRI</td>
<td>IgE</td>
<td>Mast</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>23</td>
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<td></td>
<td>TLR4</td>
<td>LPS</td>
<td>Macrophage</td>
<td>IRAK1-BCL10</td>
<td>ND</td>
<td>ND</td>
<td>24</td>
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<tr>
<td></td>
<td>Dectin-1</td>
<td>Zymosan/β-glucan</td>
<td>BM-derived dendritic</td>
<td>CARD9-BCL10</td>
<td>ND</td>
<td>ND</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Dectins-1 and -2</td>
<td>Curdlan/Ab cross-linking</td>
<td>Human primary dendritic</td>
<td>CARD9-BCL10</td>
<td>ND</td>
<td>Selective c-rel activation</td>
<td>26</td>
</tr>
<tr>
<td>Nonimmune cells</td>
<td>Edg subfamily of GPCRs</td>
<td>LPA</td>
<td>MEF</td>
<td>CARMA3-BCL10</td>
<td>ND</td>
<td>ND</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>AT1R</td>
<td>Ang II</td>
<td>Ovarian cancer Hepatocyte Vascular smooth muscle</td>
<td>CARMA3-BCL10</td>
<td>ND</td>
<td>ND</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>CXCR2</td>
<td>CXCL8/IL-8</td>
<td>Endothelial</td>
<td>CARMA3-BCL10</td>
<td>ND</td>
<td>ND</td>
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<td>Thrombin</td>
<td>Endothelial</td>
<td>CARMA3-BCL10</td>
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Abbreviations: ND, not determined; NK, natural killer; LPS, lipopolysaccharide; BM, bone marrow; MEF, mouse embryonic fibroblast.
in response to CXCL12/SDF-1α (31). MALT1 is also expressed in vascular smooth muscle cells and endothelial cells, where it is required for CXCL8-dependent upregulation of the proangiogenic growth factor VEGF and for thrombin-dependent adhesion of monocytes, suggesting that MALT1 has a role in neovascularization, endothelial dysfunction, and atherosclerosis (30, 32, 34). These ongoing investigations into GPCR-dependent MALT1-mediated signaling indicate that, in addition to its classic role in lymphocyte function and lymphomagenesis, MALT1 may play a substantial role in solid tumor biology and vascular pathophysiology.

**MALT1 is a protease**

Despite early recognition of the structural similarity between the MALT1 paracaspase and the caspase family of cysteine proteases, attempts to show MALT1 protease activity were initially unsuccessful (5, 35). Several years later, the MALT1-binding partner BCL10 and the ubiquitin-editing protein A20 were identified as MALT1 proteolytic substrates. In both cases, mapping of the specific site of cleavage showed that, in contrast to caspases that cleave their substrates after a negatively charged Asp residue, MALT1 cleaves protein substrates after a positively charged Arg residue (36, 37). This finding was consistent with predictions that had been made years earlier, based on the amino acid composition of the presumed substrate recognition pocket (5). MALT1-mediated BCL10 cleavage is induced following stimulation of the TCR or BCR, and cleavage occurs at a single site 5 amino acids from the carboxy-terminal end of the protein, between Arg228 and Thr229. Interestingly, BCL10 cleavage is not required for antigen-dependent NF-κB activation, but it may play a role in integrin-mediated T-cell adhesion (Fig. 1B; ref. 36).

A20, an ubiquitin-editing enzyme that negatively regulates NF-κB, possesses dual functions. The enzyme removes K63-linked polyubiquitin from its substrates and can then catalyze K48-linked polyubiquitinization, thus targeting its substrates for proteasome-mediated degradation (38). In T cells, A20 inhibits antigen receptor–mediated NF-κB activation, likely via its ability to remove K63-linked polyubiquitin chains from multiple substrates, including TRAF2, TRAF6, receptor interacting protein 1 (RIP1), BCL10, the IKK-γ subunit, and even MALT1 itself (37, 39, 40). A20 deficiency protects B cells from cell death, and A20 is frequently inactivated in B-lineage lymphomas, indicating its role as a critical tumor suppressor (41–43). In response to TCR or BCR stimulation, MALT1 proteolytically cleaves human A20 after Arg439, thereby separating the deubiquitinase (DUB) domain from the C-terminal zinc finger substrate-interaction–ubiquitin ligase domain, and this cleavage results in loss of A20's NF-κB inhibitory function (Fig. 1A; ref. 37). It is speculated that separation of the N-terminal DUB domain from the C-terminal substrate interaction domain prevents the removal of K63-linked polyubiquitin from A20 substrates, thereby preserving activating ubiquitination events and promoting NF-κB activity (Fig. 1B; ref. 37). In this way, MALT1 protease activity amplifies the degree of NF-κB–dependent gene expression. A later report showed that 2 other potential MALT1 cleavage sites are present within A20, and cleavage by MALT1 induces the cytosolic release of an N-terminal A20 fragment, suggesting that changes in cellular compartmentalization may also be a mechanism by which MALT1-mediated cleavage alters A20 activity (44).

A third MALT1 proteolytic substrate, cylindromatosis (CYLD), was recently identified (45). Like A20, CYLD is a tumor suppressor–deubiquitinating enzyme that negatively regulates NF-κB (40). CYLD was originally identified as a gene that is mutated in familial cylindromatosis, a disorder in which mutations resulting in truncated CYLD proteins lacking DUB activity are associated with benign tumors of the skin adnexa (46). Subsequently, CYLD has been more broadly implicated as a tumor suppressor for multiple types of cancer including the B-cell neoplasm multiple myeloma (40). Interestingly, CYLD deubiquitinates many of the same substrates as A20, including TRAF2, TRAF6, RIP1, and IKKγ (40). In response to TCR stimulation, MALT1 cleaves CYLD after Arg324 (Fig. 1B; Table 1). Surprisingly, a noncleavable CYLD mutant does not impair TCR-dependent canonical NF-κB activation, but it does block activation of c-Jun-NH₂-kinase (JNK), a critical step in TCR-induced stimulation of multiple transcription factors and production of IL-2 (45).

A new study now suggests that, in addition to its role in adaptive immunity, MALT1 protease activity also contributes to the innate immune response by mediating dectin-dependent signaling in human primary dendritic cells (26). Dectins-1 and -2 are C-type lectins that interact with the carbohydrate structures present in the cell wall of fungi and, thereby, play an important role in antifungal immune responses. Blockade of MALT1 proteolytic activity with the cell-permeable peptide inhibitor z-VRPR-FMK (36) specifically prevents nuclear translocation and DNA binding of c-Rel in response to dectins-1 or -2 stimulation without affecting other NF-κB subunits. Interestingly, a related observation has been made in B cells in which MALT1 deficiency selectively impairs c-Rel nuclear translocation following BCR stimulation without affecting RelA/p65 (21). Together, these results imply that the MALT1 protease may cleave a substrate, both in dendritic cells and in B cells, that is somehow involved in c-Rel but not in RelA activation. The identity of this substrate and the specific mechanism whereby MALT1 protease activity selectively influences c-Rel are not yet known.

**Clinical-Translational Advances**

**MALT1 protease activity promotes B lymphomagenesis**

In addition to its well-established role in MALT lymphomagenesis, a central role for MALT1 in the pathogenesis of another B-cell malignancy, diffuse large B-cell lymphoma (DLBCL), has more recently emerged. Constitutive NF-κB activity is characteristic of activated B-cell–like (ABC) DLBCL, a subtype of DLBCL that is associated with a
particularly poor prognosis (47). A shRNA-based "Achilles’ heel" screen recently identified CARMA1, BCL10, and MALT1 as essential to the survival of ABC-DLBCL cells (48). Subsequent studies identified activating oncogenic mutations in CARMA1 in about 10% of ABC DLBCLs, further confirming the critical role of the CBM complex in this malignancy (41, 43, 49). Moreover, approximately 20% of ABC DLBCLs harbor mutations in the BCR subunit CD79A or CD79B, and these mutations are associated with increased BCR expression and enhanced BCR-CBM–dependent NF-κB signaling (50). These findings prompted investigation into the role of MALT1 protease activity in ABC DLBCL. Two studies showed that, in contrast to germinal center B-cell–like (GCB)–DLBCL cells, ABC-DLBCL cells contain preassembled CBM complexes that constitutively process the MALT1 substrates BCL10 and A20 (Fig. 1B; refs. 51, 52). Furthermore, treatment of ABC-DLBCL cells with the MALT1 protease inhibitor z-VRPR-FMK blocks A20 and BCL10 cleavage, reduces NF-κB–dependent gene transcription, and impairs cell proliferation and survival. These pivotal findings thus provided the first indication that pharmacologic inhibition of MALT1 protease activity could potentially represent a promising new approach for the treatment of specific subtypes of lymphoma.

Importantly, A20 deletion or inactivation occurs in 10% to 30% of DLBCLs (41–43). Thus far, the ability of MALT1 protease inhibitor to impair cell growth and survival has been shown in 4 distinct ABC-DLBCL cell lines, and each of these lines contains at least 1 wild-type A20 allele (51, 52). If blockade of MALT1-dependent A20 cleavage is essential for MALT1 protease inhibitor to impair cell survival and proliferation, then inhibiting MALT1 protease activity may not represent an effective treatment approach for DLBCLs lacking A20. Also, activating mutations in MYD88, an adaptor protein that mediates Toll-like and IL-1 receptor signaling, have been identified in 37% of ABC-DLBCL tumors, and in some of these cases, abnormalities in A20, CD79B/A, and CARMA1 are also present (53). Whether MALT1 protease inhibition impairs cell growth and survival in cells harboring MYD88 mutation has not yet been investigated.

Our group recently showed that the MALT1 protease domain within the API2-MALT1 fusion oncprotein cleaves NF-κB–inducing kinase (NIK) after Arg 325 (Table 1; ref. 54). Importantly, API2-MALT1–dependent cleavage of NIK requires the concerted actions of the API2 moiety, which recruits NIK, and the MALT1 protease domain, which cleaves NIK. NIK is an essential mediator of noncanonical NF-κB signaling, a pathway critical to the development of secondary lymphoid organs and lymphocyte survival, which is stimulated downstream of a subset of TNF receptor family members (55). In this pathway, receptor stimulation leads to NIK-dependent phosphorylation and activation of Iκκα, which then triggers the phosphorylation of NF-κB/p100, leading to proteasome-mediated partial degradation of p100 to p52 and generation of transcriptionally active p52/RelB NF-κB dimers. Under resting conditions, NIK associates with the adaptor protein TRAF3 via an N-terminal NIK domain, and this interaction targets NIK for proteasomal degradation. Basal NIK levels are thus very low in B cells, and receptor stimulation leads to enhanced NIK stabilization and noncanonical NF-κB activation by triggering dissociation of NIK from TRAF3 (56, 57). API2-MALT1–dependent NIK cleavage separates the C-terminal NIK kinase domain from the N-terminal TRAF3-binding domain of NIK, thereby generating a C-terminal NIK fragment that retains kinase activity and is resistant to proteasomal degradation, and this leads to deregulated noncanonical NF-κB activity (Fig. 1). This active C-terminal NIK fragment is required for API2-MALT1–dependent protection of B cells from dexamethasone-induced cell death and API2-MALT1–induced adhesion of B cells to endothelium. We speculate that these consequences of API2-MALT1–dependent NIK cleavage contribute to the treatment resistance and higher rate of tumor spread that is associated with API2-MALT1–expressing t(11;18)-positive MALT lymphomas. Our findings suggest that, as in ABC DLBCL, MALT1 protease inhibition may also represent a novel pharmacological approach for the treatment of refractory t(11;18)-positive MALT lymphomas.

**Beyond B-cell lymphoma: MALT1 protease inhibition as treatment for other diseases?**

Inhibition of MALT1 protease activity may represent a novel treatment approach for other neoplastic disorders and for inflammatory and/or autoimmune diseases. Significantly, impairing MALT1 protease activity in T cells in vitro, either via mutation of the catalytic cysteine within the proteolytic site or treatment with the z-VRPR-FMK inhibitor, results in loss of TCR-induced secretion of IL-2 (36, 37), a cytokine that plays a central role in immunity and inflammation. Circulating IL-2 and T-cell IL-2 receptor α (IL-2Rα) are present only at low concentrations under basal conditions, but following TCR activation, expression of IL-2Rα is induced along with IL-2, forming an autocrine-paracrine signaling loop that promotes T-cell proliferation (58). Thus, blocking IL-2 signaling is potentially a powerful approach for immunosuppressive therapy. Indeed, IL-2Rα blockers have been used with some success in preventing organ transplant rejection, managing specific autoimmune conditions, and treating adult T-cell leukemia (59). It will be of great interest to determine whether blockade of MALT1 protease-dependent IL-2 production can be achieved in vivo and whether such an approach is effective in treating these disorders.

One important caveat, however, is that IL-2 also plays a critical role in the thymic development of regulatory T (Treg) cells and self-tolerance. Somewhat paradoxically, failure to produce normal IL-2 upon TCR activation is associated with severe autoimmune disease in mice and systemic lupus erythematosus in humans (60). It is thought that precise control of both systemic and local amounts of IL-2 is required to achieve the necessary balance between T-effector activity and Treg function. Genetic ablation of BCL10 or CARMA1 in mice is associated with impaired...
Treg differentiation, suggesting that MALT1 likely plays a role in this process as well (61). It will be important to investigate how inhibition of MALT1 protease-dependent IL-2 production might affect this critical balance.

Blockade of MALT1 protease activity has recently been found to also inhibit the production of IL-1β and IL-23 by human dendritic cells and thereby block the ability of dendritic cells to induce a T-helper 1 (Th17) cellular response to fungi (26). Th17 cells are a newly recognized subset of T-helper cells that secrete IL-17, a cytokine that stimulates the mobilization of neutrophils. Although Th17 cells perform a beneficial role in host defense against extracellular bacteria and fungi, Th17 cells are thought to play a detrimental role in the development of a variety of inflammatory disorders, including atopic dermatitis, psoriasis, multiple sclerosis, rheumatoid arthritis, and others, and blockade of Th17 signaling has shown promise as an effective treatment approach in mice and humans with these conditions (62). Therapeutic inhibition of MALT1 protease may have a future role in the treatment of these disorders, but how blocking MALT1 protease activity would disrupt the delicate balance between Th17-mediated host defense and Th17-mediated autoimmunity remains to be investigated.

Finally, in addition to its potential role in B-cell lymphoma and inflammatory disorders, deregulated MALT1 is implicated in contributing to various disease processes linked to inappropriate GPCR stimulation. For example, recent studies suggested that MALT1 is required for ovarian and oral squamous cancer cell invasion in response to GPCR stimulation by LPA and CXCL12, respectively (31, 33). However, whether MALT1 proteolytic activity is stimulated following ligation of the LPA receptor or cysteine X cysteine chemokine receptor 4 (CXCR4) or whether cleavage of specific MALT1 proteolytic substrates contributes to GPCR-induced malignant phenotypes has not yet been addressed. Similarly, several reports implicate MALT1 in mediating GPCR-induced endothelial dysfunction and atherosclerosis (30, 32, 34), but the role of MALT1 protease activity in these processes has not been investigated. The contribution of MALT1 protease activity to GPCR-dependent signaling, both in normal physiology and in disease, is certain to become a topic of intense investigation.

Conclusions

Thus far, only peptide inhibitors of the MALT1 protease, z-VRPR-fmk and Ac-LSSR-CHO, have been reported (36, 54), and it is not yet known whether pharmaceutically suitable compounds that can inhibit MALT1 protease activity in vivo can be safely administered to patients will be identified. Only 3 proteolytic substrates for wild-type MALT1 (BCL10, A20, and CYLD) and 1 unique substrate for API2-MALT1 (NIK) have been identified, and little information is available about the structural requirements for MALT1 protease interaction with its substrates. To date, MALT1 protease activity has been implicated in 3 distinct aspects of NF-κB signaling: canonical NF-κB signaling in T cells; c-Rel activation in B and dendritic cells; and noncanonical NF-κB activation in B cells expressing the API2-MALT1 fusion oncoprotein (26, 37, 51, 54). In addition, MALT1-mediated cleavage of BCL10 and CYLD in T cells has been implicated in contributing to integrin-mediated adhesion and JNK signaling, respectively (36, 45). Clearly, much remains to be learned in order to determine whether targeting MALT1 protease activity will represent a viable therapeutic approach for specific B-cell lymphomas or other diseases.

Several features of the MALT1 protease suggest that it could potentially represent a promising new drug target. First, unlike most proteins, which belong to a family of structurally and functionally related proteins, MALT1 is the only human paracaspase (5). This characteristic suggests that designing pharmaceuticals that specifically target MALT1 and do not cause unwanted off-target effects by interacting with other related proteins may be possible. Second, MALT1-deficient animals are fertile and fairly healthy (15, 16), suggesting that inhibition of MALT1 activity may be tolerable in human patients. Third, although MALT1-deficient T cells are defective in antigen receptor–induced cytokine production and proliferation, blockade of MALT1 proteolytic activity only partially inhibits TCR-induced cytokine production by 40% to 60% (36). It has been suggested that this partial effect on cytokine production may indicate that the scaffolding function of MALT1 is still preserved when MALT1 enzymatic activity is inhibited. Therefore, the effect of MALT1 protease inhibition on lymphocyte function may be less severe than the effect of MALT1 deficiency, and the degree of immune suppression may be acceptable (52). Fourth, the many investigations described in this review indicate that MALT1 protease activity plays a prominent role in both MALT lymphoma and ABC DLBCL and likely contributes to other neoplastic and inflammatory disorders.

Pharmacologic inhibition of protease activity as a strategy for drug development has some record of success. Of the approximately 400 known human proteases, about 15% are under investigation as drug targets (63). Two prominent examples include HIV protease inhibition in the prevention and treatment of AIDS and proteosome inhibition in the treatment of multiple myeloma and other B lymphoproliferations. Many other diseases, including coagulopathy, hypertension, osteoporosis, neurodegeneration, metastatic carcinoma, and bacterial and parasitic infection, are also being treated successfully with protease inhibitors (64). Perhaps the MALT1 protease, a protein that was discovered as a target of recurrent chromosomal translocation in MALT lymphoma, will join the ranks of the many proteases that can be successfully targeted for the treatment of human disease. Future studies of MALT1 may lead to identification of new proteolytic substrates, elucidation of the structural requirements for MALT1 catalytic activity, further characterization of the biologic role of MALT1, and improvement of treatment strategies for B-cell lymphoma and beyond.
Addendum

Following the initial online posting of this article, a new report identifying a fourth proteolytic substrate for wild-type MALT1 was published (65). The authors demonstrated that MALT1 cleaves the RelB NF-κB-subunit after Arg-85. MALT1-dependent RelB cleavage is induced following either TCR or BCR stimulation and results in RelB proteasomal degradation. Interestingly, overexpression of RelB or, to an even greater extent, the noncleavable RelB mutant inhibits TCR-dependent NF-κB activation and IL-2 production. The data suggest that this effect may occur via competitive inhibition of RelA and c-Rel DNA-binding by RelB. Consistent with the fact that ABC-DLBCL cells contain preassembled CBM complexes with constitutive MALT1 proteolytic activity, constitutive RelB cleavage and degradation was observed in ABC-DLBCL cells, and in these cells, overexpression of RelB leads to reduced expression of canonical NF-κB target genes and impairs survival. Together, these new findings suggest that RelB inhibits canonical NF-κB transcriptional activity in antigen-stimulated lymphocytes, and that MALT1-dependent cleavage and downregulation of RelB may play a critical role in canonical NF-κB stimulation, lymphocyte activation, and survival of ABC-DLBCL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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