Masking MALT1: the paracaspase's potential for cancer therapy

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A key feature of aggressive B cell lymphomas is constitutive NF-κB activation, which requires signals from the CARD11–BCL-10–MALT1 (CMB) complex. The unique enzymatic activity of MALT1 degrades one of its binding partners, BCL-10, as well as the NF-κB inhibitor A20. New data shows that targeting MALT1 protease activity may be a promising therapeutic strategy for treating aggressive B cell lymphomas.

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MALT1 biology

Since its identification a decade ago, MALT1 has emerged as a critical mediator of T and B cell receptor signaling (Uren et al., 2000; Thome, 2008). MALT1 constitutively associates with a CARD-containing protein called Bcl-10. After antigen receptor engagement, the CARD-containing scaffold adaptor protein CARMA1/CARD11 is phosphorylated by protein kinase C (PKCβ or PKCΘ), allowing MALT1/BCL-10 to bind (Fig. 1; Rawlings et al., 2006; Thome, 2008). The subsequent recruitment of TRAF6 and Nemo/IKKγ to the complex results in NF-κB–dependent gene transcription, which is necessary for lymphocyte activation (Rawlings et al., 2006; Thome, 2008). Studies using gene-targeted mice reveal the functional importance of the CARMA1–Bcl-10–MALT1 complex in the adaptive immune response, as lymphocytes from mice deficient in any one of these proteins exhibit defective proliferation and cytokine production upon antigen receptor engagement caused by defective NF-κB activation (Ruland et al., 2001, 2003; Egawa et al., 2003; Hara et al., 2003; Newton and Dixit, 2003; Ruefli-Brasse et al., 2003). The central role of MALT1 in regulating NF-κB activation is highlighted by the functional consequences of the chromosomal translocation t(11;18)(q21;q21), which is found in MALT lymphomas. This translocation produces a protein consisting of the N-terminal portion of cellular inhibitor of apoptosis 2 (c-IAP2) fused to the immunoglobulin (Ig)-like and caspase-like segments of MALT1 (Akagi et al., 1999; Dierlamm et al., 1999). Expression of the fusion protein leads to constitutive activation of the NF-kB pathway, positioning MALT1 as a potential key factor in the development of inflammation-associated tumors (Uren et al., 2000; Lucas et al., 2001). On p. 2313 of this issue, Ferch et al. (2009) demonstrate that the MALT1 substrates A20 and BCL-11 are constitutively processed in activated B cell–like diffuse large B cell lymphoma (ABC-DLBCL) cells, exposing the protease activity of MALT1 as an attractive pharmacological target for treating these lymphomas.

MALT1-mediated signaling

MALT1 possesses an N-terminal death domain, two Ig-like domains, a centrally located caspase-like domain, and another Ig-like domain at its C terminus (Fig. 2). The presence of multiple protein interaction domains enables MALT1 to engage with many potential binding partners. In addition to forming a constitutive complex with Bcl-10, MALT1 also binds TRAF6 and Nemo (Thome, 2008). Its associations with TRAF6 and/or Nemo are postulated to promote ubiquitination events that are essential for NF-κB activation. Spatiotemporal models of these interactions invoke MALT1-dependent recruitment and oligomerization of TRAF6 to promote autoubiquitination of TRAF6 and/or ubiquitination of Nemo, Bcl-10, and MALT1, as well as the ubiquitin ligase activity of MALT1 itself (Thome, 2008). Thus, ubiquitination is accepted as a critical component of MALT1-mediated NF-κB activation.

When the caspase-like domain of MALT1 was first reported, it was noted that it closely resembled metacaspases present in plants and single-cell organisms (Uren et al., 2000). In the years following, there were unsuccessful attempts to show a caspase-like activity for MALT1 (Snipas et al., 2004). Because mutations in the predicted active site cysteine greatly diminished the ability of MALT1 to induce NF-κB activation, it was tempting to suspect that this protein harbored enzymatic activity (Lucas et al., 2001; Uren et al., 2000). The conundrum was resolved last year when two groups identified Bcl-10 and the ubiquitin-editing enzyme A20 as substrates for MALT1 protease activity (Coornaert et al., 2008; Rebeaud et al., 2008). A20 inhibits NF-κB activation (Malynn and Ma, 2009), so its cleavage by MALT1 could diminish those inhibitory effects and result in persistent NF-κB activation. Likewise, MALT1-dependent cleavage of Bcl-10 controls integrin-dependent T cell activation, and silencing either

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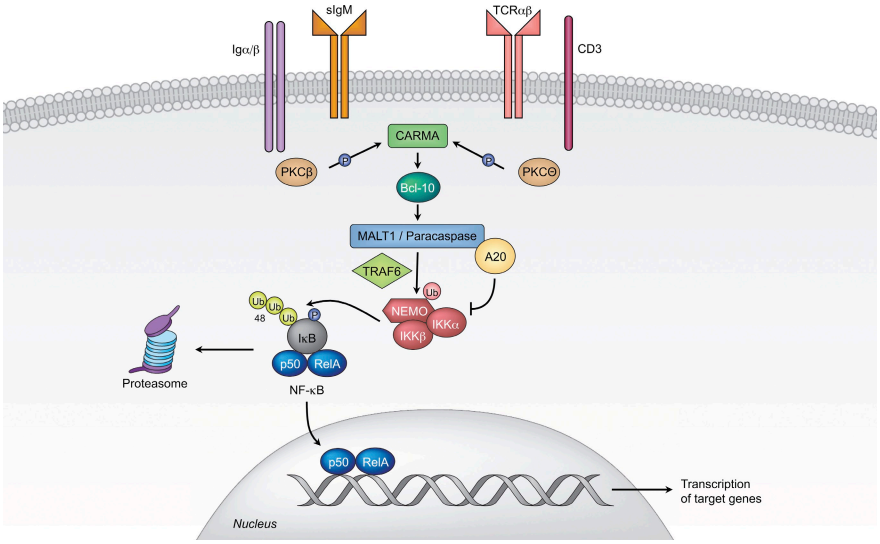

Figure 1. The role of MALT1 in antigen receptor–stimulated activation of NF-κB pathway. The engagement of antigen receptors results in the activation of protein kinase C (PKCβ or PKCΘ), which leads to phosphorylation and a conformational change of the scaffold adaptor protein CARMA1. This change allows the formation of a signaling complex consisting of CARMA1, Bcl-10, and MALT1, and enables recruitment of TRAF6 and Nemo, leading to the stimulation of NF-κB and B cell and T cell activation.

protein diminishes activation-induced adhesion of T cells (Rebeaud et al., 2008). Therefore, Bcl-10 cleavage by MALT1 could attenuate integrin-dependent adhesion of MALT lymphomas. The mechanistic aspects of these proteolytic events need further examination, but they seem to play an important role in MALT1-dependent NF-κB activation and cellular proliferation. In the new study, Ferch et al. (2009) demonstrate that ABC-DLBCL cells contain preassembled MALT1–Bcl-10–CARMA1 complexes and constitutively process the MALT1 substrates BCL-10 and A20. This finding suggests a growth-promoting role for MALT1 in these lymphomas.

Central regulators make attractive therapeutic targets

Although the critical role of MALT1 in the generation and progression of MALT lymphomas is well established, it has not been not apparent if or how MALT1 could be targeted for anticancer therapy. However, its attractiveness as a potential candidate drug target is revealed in several studies. First, gene ablation studies in mice show that MALT1 is a key mediator of NF-κB activation, cytokine production, and proliferation after antigen receptor engagement (Ruefli-Brasse et al., 2003; Ruland et al., 2003; Ferch et al., 2007). This makes it an attractive target for the treatment of cancers in which NF-κB dysregulation is a hallmark of disease. Second, MALT1-null animals are fertile and generally healthy (Ruefli-Brasse et al., 2003; Ruland et al., 2003), indicating that neutralization of MALT1 may be safely tolerated. Finally, MALT1 plays a prominent role in lymphomas. As mentioned, it is a target of chromosomal translocations associated with MALT lymphomas (Akagi et al., 1999; Dierlamm et al., 1999), and its central role in regulating the growth of ABC-DBCLs is confirmed in this issue by Ferch et al. (2009; Ngo et al., 2006). Thus, its elevated expression in many lymphatic malignancies, its central role regulating the NF-κB survival pathway, and the identification of a tumor suppressor (A20) as one of its major substrates makes MALT1 an attractive target in cancer therapy.

High hopes for protease inhibitors

Given the role of MALT1 in certain lymphoid malignancies, its cysteine-dependent arginine-directed protease activity warrants consideration as a therapeutic target (Coornaert et al., 2008; Rebeaud et al., 2008). Cleavage of Bcl-10 and A20 in the presence of candidate proteins could provide an efficient screening assay to facilitate drug discovery. In addition, the small inhibitory peptide reported to abolish MALT1 proteolytic activity and MALT1-dependent NF-κB activation (Rebeaud et al., 2008; Ferch et al., 2009) is available as a positive control for these screens (because of their inherent proteolytic susceptibility and short systemic half-life, such small inhibitory peptide are not suitable as therapeutic agents).

However, there are several hurdles in specifically targeting MALT1 protease therapeutically, including its validation as a suitable target. The generation of a gene-targeted mouse expressing only catalytically inactive MALT1 would accomplish this. The phenotype of this animal would prove definitively whether MALT1 protease activity is necessary for antigen receptor–mediated NF-κB signaling and the development of B cell lymphomas. The caveat of the studies published to date is that the MALT1 catalytic mutant has not been examined in the complete absence of endogenous wild-type MALT1. For example, studies reporting the cleavage of Bcl-10 and A20 showed that inhibition of MALT1 proteolytic activity, either with small peptide inhibitors or active site mutation, resulted in an incomplete blockade in antigen receptor–stimulated signaling (Coornaert et al., 2008; Rebeaud et al., 2008). The residual presence of endogenous MALT1 caused by incomplete small interfering (si) RNA silencing or by suboptimal pharmacokinetic properties of the small peptide inhibitors may explain the partial effect, but a catalytically inactive MALT1 knockin mouse would directly address this uncertainty.

Another missing link for effective targeting of MALT1 protease activity is the absence of structural data on the caspase-like domain (Uren et al., 2000), which imposes limitations for small molecule development and hinders optimization of initial "hits." The benefits

of structural information are exemplified by the development of IAP antagonists. The critical role of IAP proteins in the inhibition of apoptosis, tumor maintenance, and therapeutic resistance to anticancer agents were known for years, but there were no evident targeting strategies. It was a structural discovery that revealed that four amino acids in the active form of the proapoptotic protein SMAC were sufficient for binding and antagonizing IAP proteins. These data enabled the development of the high-affinity IAP antagonists that are currently undergoing clinical evaluation (Flygare and Vucic, 2009). Therefore, future structural studies on the caspase-like domain of MALT1 should be viewed as a priority for accelerating development of a potent inhibitor.

Finally, one should consider the historic perspective and the data on targeting proteases for drug discovery (Turk, 2006). Although there are some remarkable success stories, such as inhibitors of angiotensin-converting enzyme, there are unfortunately many more tales of woe, such as the failed clinical development of inhibitors of matrix metalloproteases. Thus, one should be extremely cautious when considering the development of MALT1 protease inhibitors. In addition, although Bcl-10 and A20 are likely true targets for the proteolytic activity of MALT1 (Coornaert et al., 2008; Rebeaud et al., 2008; Ferch et al., 2009), there may be additional functional targets yet to be identified, which may have undesired consequences. Systematic proteomic analysis for MALT1 substrates might identify additional targets of potential consequence to the therapeutic index of the inhibitor.

Other targeting modalities for MALT1

Conventional wisdom in drug discovery is to inhibit a well-defined activity, such as an enzymatic function like a protease activity. However, recent descriptions of small molecules that antagonize Bcl-2 and IAP proteins counter this traditional view and suggest that disruption of protein–protein interactions deserves serious consideration as a therapeutic strategy (Oltersdorf et al., 2005; Flygare and Vucic, 2009; Fuentes et al., 2009). Thus, an alternative approach would be to determine the key protein–protein interactions of MALT1 for antigen receptor signaling. Although associations with TRAF6, Nemo and a few other signaling proteins have been reported, interaction with Bcl-10 is undoubtedly important for MALT1 function (Fig. 1). Without Bcl-10, MALT1 cannot participate in B or T cell receptor signaling. Targeting the Bcl-10–MALT1 interaction would require a well-defined binding interface amenable to disruption by small molecules. An obvious exception to this targeting strategy is the c-IAP2/MALT1 fusion protein, which constitutively activates NF-κB in a Bcl-10– and stimulus-independent fashion (Uren et al., 2000; Ruland et al., 2003; Varfolomeev et al., 2006).

Finally, the activity of MALT1 could also be blocked using antisense oligonucleotides that down-regulate MALT1 by targeting its native mRNA. Knocking down MALT1 expression with siRNA or short hairpin RNA impairs cleavage of Bcl-10 and A20, subsequently affecting receptor signaling (Coornaert et al., 2008; Rebeaud et al., 2008). Thus, efficient silencing of MALT1 expression could potentially dampen T cell– and B cell–mediated NF-κB activation and cell proliferation. However, as with other strategies, siRNA approaches have several technical issues, including poor permeability, cellular uptake, and short in vivo half-life of antisense oligonucleotides (Tamm, 2006).

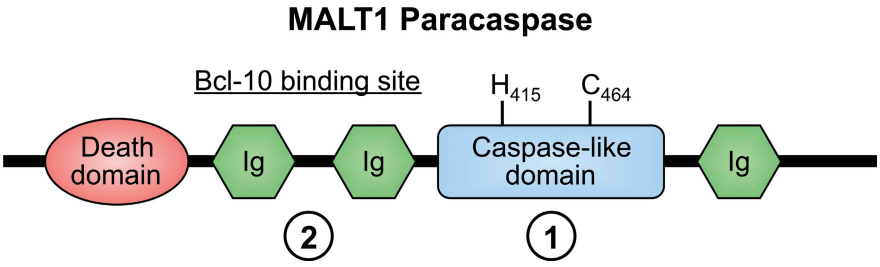

Figure 2. Schematic representation of MALT1 protein. MALT1 contains an N-terminal death domain and two Ig-like domains, a centrally located caspase-like domain, and another Ig-like domain at the C-terminal end of the molecule. The critical active site residues His415 and Cys464 in caspase-like domain and the Bcl-10–interacting region of MALT1 are indicated. Potential MALT1-targeting strategies involve inhibition of the caspase-like protease activity (1) or inhibition of interaction with its crucial binding partner and key adaptor of antigen receptor signaling, Bcl-10 (2).

The fascinating aspect of MALT1 is its uniqueness. Most proteins belong to a family or to a structurally or functionally related group of proteins. However, MALT1 is the sole human paracaspase, suggesting that MALT1-targeting molecules may be remarkably specific, although experimental verification of such an assumption is lacking at the present time.

Overall, the prominent expression of MALT1 in lymphatic malignancies, together with its critical role in the regulation of survival signaling pathways, makes MALT1 a potentially attractive target for the development of novel cancer therapeutics. A deeper mechanistic understanding of MALT1 should enable identification of a therapeutically efficacious inhibitor.

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