Constitutive NF-κB (nuclear factor κB) activation in B-cell lymphomas relies greatly on the CARMA1 [CARD (caspase recruitment domain)-containing MAGUK (membrane-associated guanylate kinase) 1]–Bcl10–MALT1 (mucosa-associated lymphoid tissue translocation gene 1) signalling complex. Within this protein complex, MALT1 possesses a rather unique enzymatic activity, which allows it to cleave Bcl10, RelB and CYLD, among other substrates. The catalytic activity of MALT1 promotes activation of canonical and non-canonical NF-κB as well as other signalling pathways. However, even after a decade of intense research on MALT1, many mechanistic aspects of its enzymatic activity remain elusive. A recent article by Hachmann, Snipas, van Raam, Cancino, Houlihan, Poreba, Kasperkiewicz, Drag and Salvesen ([2012] Biochem. J. 443, 287–295) provides novel insight into the activation mechanism and the substrate specificity of MALT1. These intriguing findings convincingly demonstrate the importance of MALT1 dimerization for its catalytic activity and pave the way for novel therapeutic approaches that target this crucial regulator of lymphoma survival and proliferation.

Key words: dimerization, lymphoma, mucosa-associated lymphoid tissue translocation gene 1 (MALT1), nuclear factor κB (NF-κB), protease, substrate specificity.

INTRODUCTION

MALT1 (mucosa-associated lymphoid tissue translocation gene 1) or paracaspase is a multi-domain protein that is composed of a caspase-like domain, an N-terminal death domain and three Ig-like domains [1,2]. Through constitutive association with the CARD (caspase recruitment domain)-containing protein Bcl10, MALT1 is recruited to the antigen-receptor-activated adaptor protein CARMA1 [CARD-containing MAGUK (membrane-associated guanylate kinase) 1]; also known as CARD11] [1]. CARMA1, Bcl10 and MALT1 comprise a docking platform for the assembly of signalling complexes that involve TRAF6 [TNF (tumour necrosis factor)-receptor-associated factor 2], TAK1 [TGF (transforming growth factor)-β-activated kinase 1], NEMO [NF-κB (nuclear factor κB) essential modulator] and other proteins [3,4]. This leads to the induction of NF-κB activation and results in lymphocyte stimulation. In agreement with their importance for lymphocyte activation, genetic ablation of CARMA1, Bcl10 or MALT1 causes a deficiency in adaptive immune responses owing to defective NF-κB activation [5,6]. Further evidence supporting the instrumental role of MALT1 in the regulation of NF-κB activation is the high occurrence of the chromosomal translocation t(11;18)(q21;q21) that fuses the N-terminal region of εCASP2 (cellular IAP (inhibitor of apoptosis) 2) with the Ig-like and caspase-like domains of MALT1 [7]. This genetic modification, which is quite frequent in MALT1 lymphomas, enables constitutive NF-κB activation and highlights MALT1 as a key factor for the development of inflammation-associated tumours [2,7,8].

MALT1 INTERACTIONS

MALT1 utilizes its different protein interaction domains for association with multiple protein partners (e.g. Bcl10 and TRAF6). This allows MALT1 to participate in various signalling complexes with distinct functional activities [3,4]. For example, MALT1 association with TRAF6 and NEMO is postulated to stimulate ubiquitination, leading to NF-κB activation. Similarly, engagement of TAK1 in MALT1-associated signalling complexes can promote TAK1-dependent phosphorylation and activation of the canonical NF-κB pathway. However, these activities are probably not directly linked to the catalytic domain of MALT1. Although initial attempts to show a caspase-like activity of MALT1 were unsuccessful, mutations in the predicted active-site cysteine residue reduced its biological activity, suggesting a prominent role for MALT1-mediated proteolysis [2,9]. Just when everyone was ready to give up on the protease activity of the caspase-like domain of MALT1, a series of studies reported several substrates of paracaspase MALT1. The list of substrates is still growing, but it now includes Bcl10, A20, CYLD, NIK (NF-κB-inducing kinase) and RelB [1,3,4]. Interestingly, all of these substrates are involved in regulating NF-κB activation; A20 and CYLD are de-ubiquitinating enzymes that are capable of restricting canonical NF-κB activation; Bcl10 is a critical adaptor for the CBM (CARMA1–Bcl10–MALT1) signalling complex; NIK phosphorylates IKKα (inhibitory κB kinase) and regulates non-canonical NF-κB signalling; whereas RelB is an NF-κB binding protein that participates in the activation of NF-κB-mediated gene expression. MALT1 was shown to be an arginine-specific protease, and cleavage of its substrates can have profound effects on signalling pathways [1,3,4]. For example, MALT1-dependent cleavage and inactivation of the deubiquitinases A20 and CYLD can lead to persistent NF-κB activation, whereas cleavage of Bcl10 can diminish integrin-dependent adhesion of MALT lymphomas. Collectively, these findings suggest a prominent role of MALT1 protease activity in the regulation of NF-κB activity and cellular proliferation. Nevertheless, many mechanistic aspects of MALT1 catalytic activity remained obscure.

Abbreviations used: CARD, caspase recruitment domain; CARMA1, CARD-containing MAGUK (membrane-associated guanylate kinase) 1; CBM complex, CARMA1–Bcl10–MALT1 complex; cIAP2, cellular inhibitor of apoptosis 2; IAP, inhibitor of apoptosis; MALT, mucosa-associated lymphoid tissue translocation gene; NEMO, NF-κB (nuclear factor κB) essential modulator; NIK, NF-κB (nuclear factor κB)-inducing kinase; NF-κB, nuclear factor κB; TAK1, TGF (transforming growth factor)-β-activated kinase 1; TNF, tumour necrosis factor; TRAF, TNF-receptor-associated factor.

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MALT1 ACTIVITY AND DIMERIZATION

The recent paper by Hachmann et al. [10] provides intriguing new insight into the activation mechanism and the substrate specificity of MALT1. Their findings demonstrate that enzyme activity is dependent on the formation of MALT1 dimers, reminiscent of the activation mechanism of apical caspsases (i.e. caspsases 8, 9 and 10). Purified recombinant MALT1, similar to apical caspsases, has a propensity to remain in the monomeric enzymatically inactive form [10,11]. Hachmann et al. [10] applied lessons learned from the work on caspsases 8 and 9 conducted in the Salvesen laboratory, in that ‘protein ordering’ kosmotropic salts of the Hofmeister series can force dimer formation [12]. They observed that addition of the kosmotrope sodium citrate increased the enzymatic activity of MALT1 by more than 1000-fold, commensurate with MALT1 dimer formation. In an elegant experiment in which wild-type MALT1 was mixed with increasing concentrations of enzymatically inactivated MALT1 (CA64A), these investigators were able to demonstrate that the kosmotrope-mediated activity increase was mainly driven by dimerization. Their conclusions are entirely consistent with the dimeric arrangement of MALT1 catalytic domains observed in the recent crystal structure [11]. Thus it appears that MALT1 preserves the activation mechanism and structural features of apical caspsases, despite having fundamentally different substrate specificity and biological functions.

As high kosmotrope concentrations are not present in cells, what then is driving dimerization in vivo? Dimer formation of apical caspsases 8 and 9 is promoted by the assembly of monomers into large protein complexes [FADD (Fas-associated death domain)-mediated recruitment to death receptor complexes for caspase-8 and Apaf1-mediated apotosome formation for caspase-9] that facilitate dimerization by inducing proximity. The study by Hachmann et al. [10] would suggest that MALT1 is activated in a similar fashion, except that the CBM complex functions as the assembly platform. In their enzymatic assays, the MALT1 catalytic domain alone dimerized in a similar fashion as the full-length form, suggesting that the N-terminal death domain and the two Ig-like domains may not be essential for kosmotrope-enhanced dimerization when the other CBM complex constituents Bcl10 and CARMA1 are absent. For the cIAP2–MALT1 fusion protein, the first of the three BIR (baculovirus IAP repeat) domains of cIAP2 provides the dimerization function, most likely without the need for other cellular partners such as TRAF2 [1,8]. In the case of MALT1 alone, its constitutive interaction with Bcl10 is obligatory for MALT1 function as it provides dimerization and/or oligomerization modalities through the recruitment to antigen-receptor-stimulated signalling complexes. The N-terminal domains of MALT1 mediate binding of MALT1 to Bcl10. Therefore it is somewhat puzzling that the MALT1 Ig1 and Ig2 domains themselves can form multimers as shown in a recent study by Qiu et al. [13], whereas the experiments by Hachmann et al. [10] did not demonstrate spontaneous dimerization of full-length MALT1. This highlights the need for a more complete structural understanding of the molecular interactions between the protein partners in the CBM complex.

MALT1 AS A PROTEASE

The five presently known MALT1 substrates, Bcl10, A20, CYLD, NIK and RelB, are cleaved after an arginine residue (arginine = P1 residue). Using a combinatorial peptide library, Hachmann et al. [10] confirmed the strong preference of arginine at the P1 position. This is consistent with structural features of the MALT1 substrate specificity pocket, which is ideally suited to accommodate an arginine side chain [11]. The consensus sequence LVSR (P4–P1) was a perfect match to the recently identified MALT1 substrate RelB [14], yet MALT1 catalytic efficiency (kcat/Km) towards the derived LVSR fluorogenic substrate was relatively low (10⁻¹⁰⁻¹⁻¹). This could mean that MALT1 is not a very efficient protease, a notion that would be consistent with the retention of the uncleaved L2 loop away from the active site [10,11]. Alternatively, one could speculate that the in vitro conditions are missing some important constituents that enhance activity in vivo.

It is also interesting to note that MALT1 and caspase-8, which share the mechanistic modality of activation (both through dimerization), also share at least one substrate – the de-ubiquitinating enzyme CYLD. However, the cellular signalling pathways that lead to CYLD cleavage, as well as the biological consequences of the cleavages (MALT1 cleaves CYLD after Arg324 and caspase 8 after Asp215) are quite different for caspase 8 and MALT1. Caspase-8-mediated cleavage of CYLD was reported to block necrotic cell death and allow survival of cells following TNF stimulation [15], MALT1, on the other hand, proteolytically inactivates CYLD during T-cell receptor activation, and this enzymatic activity allows JNK (c-Jun N-terminal kinase) activation [16]. In the future, it would be interesting to explore the detailed kinetics and robustness of these proteolytic events to determine whether they can occur simultaneously, and whether they affect each other with regards to the fate of their substrate and biological outcome of signalling stimuli that lead to the activation of these proteases.

CONCLUSIONS

From a therapeutic perspective, the work by Hachmann et al. [10] could open the door for the discovery of MALT1 inhibitors. The establishment of a sensitive enzymatic assay with recombinant MALT1 could be the key to unlock screening of chemical compound libraries and eventual identification of MALT1 active-site inhibitors. The availability of the MALT1 catalytic domain structure [11] should be of great value in guiding lead optimization. Generally, efforts to synthesize protease active-site inhibitors are plagued by the problem of specificity owing to the high structural conservation of active sites within a protease family. MALT1 seems to be a lucky exception; even though it shares the catalytic domain fold with the members of the caspase family, its active site is distinctly different, since it accommodates a basic amino acid residue at the P1 position (arginine, instead of the caspase-family-defining P1 residue aspartate). Thus there is hope that one could obtain MALT1-specific inhibitors that, in light of the relatively mild phenotype of MALT1-deficient mice, might also be relatively safe [6]. Other potential MALT1 target sites remain to be defined further, including substrate-binding exosites as well as the exact binding site for Bcl10 located in the MALT1 N-terminal region [1]. Such protein–protein interaction sites are notorious for their intractability by traditional synthetic chemistry. However, there are examples of successful targeting of protein–protein interactions, such as IAP and Bcl2 antagonists that prevent binding of these anti-apoptotic proteins to the activators and executioners of cell death signalling [17]. Although there are many questions remaining concerning the (patho-) physiological role of MALT1, CBM assembly and regulation, there is great hope and expectation that the breakthrough progress made in unravelling the biology, structure and enzyme function of MALT1 will translate into future therapeutic treatments of MALT1-associated human diseases. We believe that the Hachmann et al. [10] study on the activation mechanism and the substrate specificity of MALT1 represents an important step in that direction.
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