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Protease activity of the API2–MALT1 fusion oncoprotein in MALT lymphoma development and treatment

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Abstract

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a prototypical cancer that occurs in the setting of chronic inflammation and an important model for understanding how deregulated NF-κB transcriptional activity contributes to malignancy. Most gastric MALT lymphomas require ongoing antigenic stimulation for continued tumor growth, and Stage I disease is usually cured by eradicating the causative microorganism, *Helicobacter pylori*, with antibiotics. However, in a subset of MALT lymphomas, chromosomal translocations are acquired that render the lymphoma antigen-independent. The recurrent translocation t(11;18)(q21;q21) is associated with failure to respond to antibiotic therapy and increased rate of dissemination. This translocation creates the API2–MALT1 fusion oncoprotein, which comprises the amino terminus of inhibitor of apoptosis 2 (API2 or cIAP2) fused to the carboxy terminus of MALT1. A common characteristic of chromosomal translocations in MALT lymphoma, including t(11;18), is that genes involved in the regulation of the NF-κB transcription factor are targeted by the translocations, and these genetic perturbations thereby result in deregulated, constitutive NF-κB stimulation. In the last decade, great insights into the roles of API2 and MALT1 in NF-κB signaling have been made. For example, recent pivotal studies have uncovered the long sought-after proteolytic activity of MALT1 and have demonstrated its critical involvement in the survival of certain lymphomas. Here, we review the current understanding of the role of MALT1 in normal lymphocyte function and lymphomagenesis. We then highlight our recent work that has revealed an intriguing link between the proteolytic activity of the API2–MALT1 fusion and its ability to influence lymphomagenesis by cleaving a key NF-κB regulatory protein, NF-κB-inducing kinase.

Keywords

A20; API2–MALT1; Bcl10; chromosomal translocation; lymphoma; MALT1 protease; NIK

Normal lymphocyte signaling is perturbed in MALT Lymphoma

Lymphoma presents as a solid tumor comprising cells of the immune system, affecting lymph nodes and/or extranodal sites, including mucosal tissues and other organs. Marginal

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zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) is the most common extranodal lymphoma and accounts for up to 8% of non-Hodgkin lymphomas [1]. MALT lymphoma typically arises at mucosal sites that lack lymphoid tissue but acquire lymphoid structure in response to chronic infection, such as *Helicobacter pylori* gastritis, or autoimmune disease, such as Hashimoto's thyroiditis or Sjögren syndrome [2]. During sustained antigenic signaling, abnormal B-cell clones can acquire genetic alterations that enhance and, in some cases, supplant the lymphoproliferative response. These clones then overgrow the reactive B cells at the inflammatory site, ultimately leading to the development of lymphoma. Indeed, several chromosomal translocations have been identified and are associated exclusively with the pathogenesis of a subset of MALT lymphomas, and have been recently reviewed in detail [3].

A common chromosomal translocation associated with MALT lymphoma, t(11;18)(q21;q21), creates a fusion of two proteins: a member of the inhibitor of apoptosis (IAP) protein family, apoptosis inhibitor 2 (API2; also termed cIAP2), and the paracaspase MALT lymphoma-translocation gene 1 (MALT1). While many chromosomal breakpoints have been characterized, the resulting API2–MALT1 fusion proteins invariably contain the amino terminus of API2, comprising three intact baculovirus IAP repeat domains that are essential for protein interactions, and the carboxyl terminus of MALT1, including the caspase-like protease domain (Figure 1A) [4].

Since its discovery more than a decade ago, wild-type MALT1 has emerged as a critical component of lymphocyte signaling. Specifically, MALT1 mediates antigen-induced canonical NF- κ B activation – a pathway crucial to lymphocyte survival and proliferation – following its auto-oligomerization induced by upstream factors CARMA1 and Bcl10 [5,6]. Oligomerized MALT1 can stimulate the multisubunit inhibitor of NF- κ B (I κ B) kinase (IKK), which phosphorylates the NF- κ B inhibitor, I κ B α . Phosphorylation of I κ B α targets it for degradation, freeing sequestered p50/RelA NF- κ B dimers, which then translocate to the nucleus, and transcriptionally activate genes involved in innate and adaptive immune responses [7]. Interestingly, because the API2 moiety of the fusion protein can mediate auto-oligomerization, API2–MALT1 can activate NF- κ B independently of antigenic signals [8,9]. This may explain why t(11;18)-positive MALT lymphomas are not dependent on chronic inflammation for progression, whereas t(11;18)-negative tumors require ongoing antigenic stimulation. This phenomenon is illustrated in gastric MALT lymphomas, the majority of which arise in the context of chronic *H. pylori* gastritis and are cured by antibiotic therapy. In contrast, t(11;18)-positive gastric tumors do not respond to *H. pylori* eradication and are associated with advanced-stage disease [1,4].

Aberrant MALT1 protease activity is associated with B-cell lymphomagenesis

The MALT1 protein contains several protein interaction domains, which allow MALT1 to bind NF- κ B signaling modulators, and a caspase-like domain that shares homology with metacaspases, which are arginine/lysine-specific cysteine proteases found in plants, fungi and protozoa (Figure 1A) [10]. Although early efforts to establish caspase-like protease activity were unsuccessful [11], mutation of the predicted active-site cysteine in MALT1 impaired its ability to activate NF- κ B [5,10], suggesting a possible role for enzymatic activity. Finally, in 2008, two independent studies identified the first MALT1 protease substrates, Bcl10 and A20 [12,13]. Cleavage of Bcl10, although not essential for NF- κ B activation in stimulated T lymphocytes, was important for T-cell adhesion, suggesting that MALT1-dependent cleavage causes Bcl10 to gain a specific function. By contrast, cleavage of A20, a well-characterized negative regulator of NF- κ B activation, resulted in loss of its inhibitory function, leading to an enhanced NF- κ B-dependent cellular response. Thus, these

newly identified substrates of MALT1 protease activity influence normal lymphocyte function, and perturbation of these activities could be associated with inflammatory disease and malignancy. Indeed, aberrant MALT1 protease activity has been identified in activated B cell-like diffuse large B-cell lymphoma (ABC-DLBCL) [14,15]. Two pivotal studies established that constitutive MALT1 protease activity is associated with enhanced survival and proliferation of ABC-DLBCL cell lines, and that targeted inhibition of MALT1 protease activity is selectively toxic to these cells, suggesting a possible novel therapeutic approach for the treatment of lymphoma with aberrant MALT1 protease activity.

As stated previously, the API2–MALT1 fusion protein is capable of activating NF- κ B in the absence of antigenic stimulation, and this effect is dependent, in part, on the protease activity of the MALT1 moiety [5,10]. Previous work demonstrated that API2–MALT1 could also cause A20 cleavage [13], suggesting a common link of aberrant protease activity between t(11;18)-positive MALT lymphoma and ABC-DLBCL. Work from our laboratory has identified a new proteolytic substrate of API2–MALT1, NF- κ B-inducing kinase (NIK) [16].

NF- κ B-inducing kinase is proteolytically cleaved by the API2–MALT1 fusion oncoprotein

Although NIK was originally identified as a candidate mediator of canonical NF- κ B induced by TNF receptor family signaling [17], studies using mice with targeted genetic disruption confirmed that NIK is a pivotal regulator of noncanonical NF- κ B signaling [18]. This pathway is critical for lymphocyte survival and development of secondary lymphoid organs, and requires NIK kinase activity. NIK phosphorylates and activates a subunit of the IKK complex, IKK α , triggering phosphorylation and partial proteasomal processing of NF- κ B2/p100 to p52, thereby allowing nuclear translocation of p52–RelB NF- κ B dimers, in contrast to canonical p50–RelA NF- κ B dimers, which promote expression of distinct genes. In the absence of noncanonical NF- κ B-activating stimuli, such as lymphotoxin- β and B-cell activating factor belonging to the TNF family (BAFF), NIK protein is maintained at low levels owing to its association with a complex of proteins, including TNF receptor-associated factors (TRAFs)2 and 3, cIAP1 and cIAP2 (API2), which ubiquitinates NIK and targets it for degradation via the proteasome [19,20]. Binding of either lymphotoxin- β or BAFF to their cognate receptors disrupts this complex, allowing NIK levels to stabilize, thereby activating the noncanonical NF- κ B pathway [21].

We discovered that, in addition to activating canonical NF- κ B, expression of API2–MALT1 also activated noncanonical NF- κ B through NIK. Because API2–MALT1 lacks the API2 ubiquitin ligase domain, which is responsible for NIK degradation, we hypothesized that NIK stability would be enhanced due to API2–MALT1 competitively inhibiting API2-mediated NIK degradation. Instead, we discovered that API2–MALT1 caused proteolytic cleavage of NIK. Importantly, we found that the cleavage reaction represents a gain-of-function activity for the fusion protein, because cellular activation of the wild-type MALT1 protease is unable to induce NIK cleavage. Disruption of MALT1 protease activity within API2–MALT1, via mutation of the active site cysteine or treatment with a specific MALT1 protease inhibitor, blocked API2–MALT1-induced NIK cleavage. These observations suggest that NIK, although not a target of wild-type MALT1 protease activity, becomes an available MALT1 substrate in the context of the API2–MALT1 fusion protein. Indeed, we demonstrated that the API2 moiety interacts with NIK and presumably juxtaposes NIK with the caspase-like domain of the MALT1 moiety of API2–MALT1.

How can API2–MALT1-dependent cleavage of NIK be reconciled with the observation that the fusion protein stimulates noncanonical NF- κ B through NIK? The answer lies in the fact

that API2–MALT1-dependent cleavage of NIK generates an active carboxy terminal fragment that retains full kinase activity, but cannot bind the TRAF2/3–cIAP1/2 complex, thus creating a stable, uninhibited NF- κ B activator (Figure 1B). We determined that this carboxy terminal NIK fragment is critical for API2–MALT1-induced cellular responses, including resistance to apoptotic stimuli and enhanced lymphocyte adhesion. Furthermore, cleaved NIK was found in t(11;18)-positive MALT lymphoma patient specimens, but not in translocation-negative samples, and correlated with enhanced activation of noncanonical NF- κ B signaling and target gene expression. This suggested that API2–MALT1-dependent cleavage of NIK and generation of an unrestrained, functionally replete NIK kinase plays a role in the pathogenesis of t(11;18)-positive MALT lymphomas.

Interestingly, deregulated NIK activity has been associated with other B-cell malignancies. For example, in a case of multiple myeloma, investigators identified a chromosomal translocation that creates a fusion protein comprising the amino terminus of elongation factor Tu guanosine triphosphate binding domain containing 2 (EFTUD2), a protein of unknown function, fused to the kinase domain of NIK [22]. Similar to the API2–MALT1-induced NIK cleavage fragment, in which the TRAF3-binding domain is detached from the NIK kinase domain, the EFTUD2–NIK fusion lacks the region of NIK that interacts with the TRAF2/3–cIAP1/2 complex, thus stabilizing the fusion and deregulating NF- κ B activation. In addition, mice genetically engineered for B cell-specific expression of a TRAF3 binding-deficient mutant of NIK demonstrate high levels of the stable mutant NIK protein, enhanced noncanonical NF- κ B activation, and severe B-cell hyperplasia [23]. These observations, coupled with our findings, suggest an intriguingly convergent mechanism for perturbing normal NIK function in B-cell malignancies – separating the TRAF3-binding domain on NIK from the active NIK kinase domain.

Future perspective

While major advances in our understanding of the mechanism of action of the API2–MALT1 fusion oncoprotein and the molecular pathogenesis of MALT lymphoma have been made over the past decade, much remains to be learned. As one significant example, while mice engineered to ectopically express API2–MALT1 in the B-cell compartment demonstrate expansion of splenic marginal zone B cells, the mice do not develop frank lymphoma, suggesting that additional undefined events may play a crucial role t(11;18)-positive MALT lymphomagenesis [24]. Future work will be aimed at identifying these as yet unknown factors that collaborate with API2–MALT1 in promoting MALT lymphoma.

The API2–MALT1 fusion protein comprises two potentially targetable protein moieties for therapeutic intervention. First, it will be interesting to determine if inhibiting MALT1 protease activity to prevent continued NIK cleavage in the context of t(11;18)-positive MALT lymphoma has either cytostatic or cytotoxic effects, similar to the effects of MALT1 inhibition in ABC-DLBCL. Second, interventions targeting the API2 moiety could also prevent NIK cleavage. For example, identification of compounds that disrupt API2 moiety-mediated NIK binding or auto-oligomerization could be of great value. Our studies also suggest that deregulated NIK kinase activity likely plays a role in sustaining MALT lymphomagenesis; a kinase dead mutant of NIK is able to suppress API2–MALT1-induced NF- κ B activation. Notably, a recent study identified two inhibitors of NIK kinase activity [25]. Testing the effects of these compounds, alone or in combination with other blockers of API2–MALT1 function, may provide a means to treat t(11;18)-positive MALT lymphoma.

Executive summary

Perturbation of normal lymphocyte signaling in B-cell malignancy

- Proper regulation of the NF- κ B transcription factor is critical to normal lymphocyte function, and deregulated, constitutive NF- κ B activation promotes lymphomagenesis.
- Recurrent chromosomal translocations that target genes involved in NF- κ B regulation have been identified in mucosa-associated lymphoid tissue (MALT) B-cell lymphoma.
- The recurrent t(11;18)(q21;q21) chromosomal translocation in MALT lymphoma is associated with resistance to standard treatment and increased rate of tumor dissemination. This translocation creates API2–MALT1, a fusion oncoprotein that constitutively stimulates NF- κ B.

Aberrant MALT protease activity in lymphoma

- Wild-type MALT1 is a critical regulator of NF- κ B activity in lymphocytes.
- Wild-type MALT1 possesses protease activity, and two proteolytic substrates, A20 and Bcl10, have been identified thus far.
- Constitutive MALT1 protease activity is present in ABC-DLBCL cells and is associated with enhanced survival and proliferation. Targeted inhibition of MALT1 protease activity is selectively toxic to ABC-DLBCL cells.
- The API2–MALT1 fusion oncoprotein in MALT lymphoma, but not wild-type MALT1, proteolytically cleaves the protein NIK, thereby generating a degradation-resistant, constitutively active C-terminal NIK fragment that stimulates the noncanonical NF- κ B signaling pathway.

Conclusion

- Targeted inhibition of MALT1 protease may represent a novel therapeutic approach for the treatment of lymphoma with aberrant MALT1 protease activity, including activated B cell-like diffuse large B-cell lymphoma and t(11;18)-positive MALT lymphoma.
- Interventions aimed at disrupting the interaction of NIK with the API2 moiety of the API2–MALT1 fusion oncoprotein, or inhibiting NIK kinase activity, could also prove to be novel approaches to treating t(11;18)-positive MALT lymphoma.

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Figure 1. The API2–MALT1 fusion oncoprotein

(A) Structural domains in API2/cIAP2, MALT1 and API2–MALT1. The most commonly occurring API2–MALT1 fusion protein is depicted. (B) Model for NF-κB inducing kinase cleavage by API2–MALT1. The API2 moiety recruits NIK, making it an available substrate for the MALT1 caspase-like protease domain (shown in yellow). Cleavage of NIK separates the TNF receptor associated factor-3 binding domain from the KD (shown in green), thereby generating a stable, uninhibited carboxy terminal fragment that is capable of activating noncanonical NF-κB signaling.

BIR: Baculovirus IAP repeats; CARD: Caspase associated recruitment domain; DD: Death domain; IAP: Inhibitor of apoptosis protein; Ig: Immunoglobulin-like domain; KD: Kinase domain; RING: Really interesting new gene domain; TRAF: TNF receptor-associated factors; UBA: Ubiquitin associated domain.