Molecular Pathways: Targeting MALT1 paracaspase activity in lymphoma

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Abstract

MALT1 mediates the activation of NF-κB in response to antigen receptor signaling. MALT1, in association with BCL10 and CARD11, functions as a scaffolding protein to activate the IKK complex. Additionally, MALT1 is a paracaspase that targets key proteins in a feedback loop mediating termination of the NF-κB response; thus promoting activation of NF-κB signaling. Activated B-cell like diffuse large B-cell lymphomas (ABC-DLBCLs), which tend to be more resistant to chemotherapy, are often biologically dependent on MALT1 activity. Newly developed MALT1 small molecule inhibitors suppress the growth of ABC-DLBCLs in vitro and in vivo. This review highlights the recent advances in the normal and disease-related functions of MALT1. Furthermore, recent progress targeting MALT1 proteolytic activity raises the possibility of deploying MALT1 inhibitors for the treatment of B-cell lymphomas and perhaps autoimmune diseases that involve increased B or T-cell receptor signaling.
Background

MALT1 as a critical mediator of B-cell receptor signaling

The mucosa-associated lymphoid tissue lymphoma translocation 1 (MALT1) gene was first identified in the recurrent t(11;18)(q21;q21) in MALT lymphomas. Resulting fusion product contains the N-terminal portion of cellular inhibitor of apoptosis 2 (cIAP2 or API2) and the C-terminal portion of MALT1. MALT1 is also translocated to the immunoglobulin heavy-chain gene enhancer in MALT lymphomas, leading to aberrant expression of the protein. Notably MALT1 transgenic mice develop MALT lymphomas histologically and molecularly analogous to the human disease. MALT1 also plays a critical role in the activated B-cell subtype of diffuse large B-cell lymphoma (ABC-DLBCL), and indeed MALT1 transgenic mice develop an ABC-DLBCL like disease when crossed into a p53 null background.

MALT1 forms a complex with the B-Cell CLL/Lymphoma 10 (BCL10) and caspase recruitment domain family, member 11 (CARD11). As part of this CARD11-BCL10-MALT1 (CBM) complex, MALT1 transduces signals from the B-cell receptor (BCR) and T-cell receptor (TCR), NK-cell and B cell-activating factor receptors. Upon BCR engagement, a cascade of tyrosine kinase phosphorylation activates PI3K, which in turn activates PDK1 and BTK, and then PLC-γ2 to produce DAG and Ca^{2+} and activate PKC-β. PKC-β then phosphorylates CARD11 promoting a conformational change that enables interaction with BCL10 and MALT1. Once this complex is formed, TRAF6 recruitment and polyubiquitylation of MALT1 and BCL10 promote the binding of IKKγ and TAK1. Next IKKγ complexes with IKKα and IKKβ, which is phosphorylated and activated by TAK1, to phosphorylate the IκB proteins and induce their proteolytic degradation. NF-κB proteins can then translocate to the nucleus where they activate genes involved in proliferation, apoptosis inhibition and inflammation. Remarkably, c-REL nuclear translocation is dependent on MALT1 activity, while it is dispensable for RELA activation.

Accordingly, studies of MALT1 knockout mice indicated its essential role in antigen-receptor–induced NF-κB activation, cytokine production, and proliferation in T- and B-cells. MALT1 knockout mice also exhibited impaired proliferation of splenic B-cells upon LPS stimulation. Moreover BCL10 interacts with IRAK1 and transduces signaling through interaction with MALT1 upon LPS treatment in macrophages. These findings may
implicate MALT1 in Toll-like receptor (TLR) signaling, however this remains controversial as Ruland and colleagues did not observe contribution of MALT1 to LPS response\textsuperscript{16}. MALT1 also signals downstream of C-type lectin family and G-protein coupled receptors in complex with CARD9 and CARMA3, respectively\textsuperscript{9}.

**MALT1 protease activity in NF-κB signal transduction**

Structurally MALT1 has a conserved dead domain (DD), 2 Ig-like domains and a caspase-like paracaspase domain\textsuperscript{18}. The paracaspase domain was first predicted by structural similarity but its protease activity and cleavage targets remained elusive for years until the identification of MALT1 substrates Tumor Necrosis Factor Alpha-Induced Protein 3 (TNFAIP3/A20)\textsuperscript{19} and BCL10\textsuperscript{20}. Other identified targets include: Cylindromatosis (CYLD)\textsuperscript{21}, v-rel reticuloendotheliosis viral oncogene homolog B (RELB)\textsuperscript{22} and Regnase-1\textsuperscript{23}. Notably fusion of MALT1 to API2 leads to ectopic cleavage of NF-κB-inducing kinase (NIK)\textsuperscript{24} (Figure 1B). A20 and CYLD are deubiquitylases that cleave Lys-63-linked polyubiquitin chains\textsuperscript{25}. A20 deubiquitylates MALT1 decreasing its stability and attenuating the B-cell response\textsuperscript{26}. CYLD decreases JNK activity therefore inhibiting AP-1\textsuperscript{21}. RELB cleavage by MALT1 enhanced RELA- and c-REL DNA binding\textsuperscript{22}. Regnase-1 is an RNase that specifically targets and degrades mRNAs implicated in the inflammatory response such as IL-2, IL-6 and c-REL, thus attenuating signaling downstream of the TCR\textsuperscript{23}. Collectively, MALT1 cleavage of its substrate proteins enhances and prolongs NF-κB signaling downstream of the BCR and/or TCR\textsuperscript{19-24}.

**Clinical-Translational Advances**

**MALT1 protease activity inhibition in ABC-DLBCL**

DLBCLs are a heterogeneous group of diseases. Among them, the ABC subtype, characterized by constitutive NF-κB signaling, is most resistant to current chemotherapy regimens and therefore the most clinically challenging\textsuperscript{27}. MALT1 is not mutated or translocated in DLBCL, although its locus is frequently affected by copy number gain in ABC-DLBCL patients\textsuperscript{28}. An shRNA screening identified several BCR pathway components as essential for ABC-DLBCL including: MALT1, CARD11, BCL10 and IKKβ\textsuperscript{7} and led to the discovery of activating mutations in CARD11, CD79A/B and MyD88 in ABC-DLBCL patients\textsuperscript{29-31}. Exposure of ABC-DLBCLs to z-VRPR-fmk, a peptide inhibitor of MALT1 paracaspase activity was sufficient to inhibit growth of
ABC-DLBCL cells\textsuperscript{5, 6}. However, whereas z-VRPR-fmk is an excellent tool compound, it is not suitable for clinical use given that its effects can only be observed at 50-75 \(\mu\)M in cell culture, maybe due to poor cell penetrance.

In an effort to identify more clinically tractable MALT1 inhibitors, Nagel and colleagues identified the phenothiazines mepazine, thioridazine and promazine as reversible small inhibitors of MALT1 protease activity\textsuperscript{32}. These phenothiazines also inhibited MALT1 downstream signaling and proliferation of ABC-DLBCL cell lines and xenografts\textsuperscript{32}. Phenothiazines are dopamine D2 receptor antagonists and have been used as anti-psychotic and sedative drugs\textsuperscript{33}. Thioridazine, the only of these drugs still in use, is generally restricted to patients that do not respond to other anti-psychotic drugs due to concerns about cardiotoxicity and retinopathy\textsuperscript{34}. Repurposing of these drugs for treatment of ABC-DLBCL has been proposed although carries the risk of off-target effects.

Fontán and colleagues identified novel MALT1 protease inhibitors by screening small molecule libraries using an \textit{in vitro} active form of MALT1\textsuperscript{13}. The most biologically potent inhibitor identified, “MI-2”, exhibited irreversible and specific binding to MALT1 and suppressed its protease function \textit{in vitro} and \textit{in vivo}. MI-2 induced nuclear depletion of c-REL and suppressed NF-\(\kappa\)B activity\textsuperscript{13}. Most notably, MI-2 was non-toxic to mice, and displayed potent and specific activity against ABC-DLBCL cells \textit{in vitro} and xenotransplanted \textit{in vivo}\textsuperscript{13}. The compound was also specifically effective against primary human non-GCB DLBCLs \textit{ex vivo}\textsuperscript{13}. Hence MI-2 may represent a potentially clinically useful MALT1 inhibitor.

Finally, monoubiquitylation of MALT1 on Lys644 activates the protease function of MALT1. Expression of a non-ubiquitylatable MALT1(K644R) mutant reduced survival of ABC-DLBCL cell lines\textsuperscript{35}. Targeting the ubiquitin ligase responsible for this activation, currently unknown, might also disrupt MALT1 activity.

**Clinical context for translation of MALT1 targeted therapy**

Biological dependency on BCR signaling is a central feature of several types of B-cell neoplasms\textsuperscript{36} and its inhibition has been proposed as a strategy to treat lymphomas. A number of BCR pathway inhibitors are in development (\textbf{Figure 2}). Among these, Ibrutinib, an irreversible BTK inhibitor, is showing signs of efficacy in phase I and II clinical trials especially in CLL\textsuperscript{37} and MCL\textsuperscript{38}. Activity in ABC-DLBCL has also been reported\textsuperscript{39}. The SYK kinase inhibitor fostamatinib also has single agent activity in CLL and MCL\textsuperscript{40}. Efficacy of BTK and SYK inhibitors in CLL and
MCL was not necessarily expected and point towards BCR signaling as an important pathway in these tumors. MALT1 inhibition is therefore an attractive target for ABC-DLBCLs and other BCR-dependent lymphoma subtypes.

An additional level of complexity in targeting BCR signaling is conferred by the genetic heterogeneity of lymphomas. Indeed the somatic mutation landscape of a tumor will determine its response to different inhibitors, depending on mutations upstream or downstream of the targeted protein. For example, whereas MALT1 inhibitors would be expected to suppress ABC-DLBCLs with CARD11 activating mutations, drugs targeting upstream kinases SYK, PI3K, BTK or PKC may not be as effective (Figure 2). In turn, lymphomas with mutations in TAK1 or c-REL (downstream of MALT1) will potentially be less responsive to MALT1 inhibition\(^\text{13}\). Moreover MALT1 contributes to the signaling downstream of other receptors. Indeed, smoothened (SMO) a GPCR-like receptor that activates the Hedgehog (Hh) signaling pathway contributes to NF-κB activation in DLBCL independent of Hh through engagement of the CBM complex\(^\text{41}\). Additionally, some DLBCL cell lines, not classified as ABC-DLBCL although BCR-dependent, present PI3K activation that could potentially trigger the CBM complex\(^\text{42}\). MALT lymphoma patients with t(11;18)(q21;q21), that exhibit unfavorable clinical outcome\(^\text{43}\), might also benefit from MALT1 proteolytic inhibition by preventing API2-MALT1 aberrant proteolytic activation of NIK and constitutive non-canonical NF-κB signaling\(^\text{24}\).

Interestingly, MALT1 has been shown to contribute to the encephalitogenic potential of Th17 cells in a murine model of multiple sclerosis (MS)\(^\text{44}\); while MALT1 deficiency protects mice from developing clinical symptoms of MS including demyelination\(^\text{45}\). Moreover, genome-wide association studies have determined MALT1 as a risk locus for multiple sclerosis\(^\text{46}\) and type 2 diabetes\(^\text{47}\). MALT1 substrates have also been implicated in autoimmune diseases such as Crohn’s disease, rheumatoid arthritis, systemic lupus erythematosus and psoriasis, most notably A20\(^\text{48}\) and c-REL\(^\text{49}\). MALT1 paracaspase inhibition needs to be explored in these settings.

Because MALT1 is involved in the immune response\(^\text{8, 10, 11}\), it will be important to monitor immune competency after administration of inhibitors of its activity. In particular since an autosomal recessive form of combined immunodeficiency has been associated with homozygous mutation of MALT1 in one family. This mutation led to loss of protein expression\(^\text{50}\). How MALT1 paracaspase activity inhibition will influence the functioning of the immune system from the clinical standpoint is not known and will be important to evaluate when MALT1 inhibitor
trials are conducted in humans. If MALT1 inhibitors are used in a prolonged manner, especially for autoimmune conditions, it is possible that secondary malignancies could arise linked to failure of immune surveillance as has been shown for other immunomodulatory treatments.

**MALT1 inhibitors in rational combination therapies**

Lymphomas are genetically complex, with individual tumors featuring a variety of genetic alterations including gain and loss of genomic regions, translocations and point mutations\(^{36, 51}\). Hence targeting a single oncogenic pathway in these tumors is unlikely to be curative. Moreover many lymphomas exhibit genomic instability and on-going somatic hypermutation\(^ {36}\), which might increase the opportunity for acquired resistance to emerge. Both of these scenarios underline the importance of developing rational combinatorial therapy regimens to more effectively and completely eradicate lymphomas.

ABC-DLBCLs are more resistant to current chemotherapy regimens\(^ {27}\). It is possible that MALT1 inhibition could sensitize ABC-DLBCLs to R-CHOP by disrupting cell survival signaling through NF-\(\kappa\)B. It is also possible that MALT1 targeted therapy could synergistically kill lymphoma cells when combined with other more upstream BCR pathway inhibitors that might complement MALT1 inhibition. For example, inhibiting SYK or BTK could allow the inhibition of pathways parallel to NF-\(\kappa\)B like MAPK, JUNK or NFAT to further inhibit survival and proliferation signals (**Figure 2**). Other potential targets for MALT1 combination therapy in ABC-DLBCL include other oncogenes frequently deregulated in this subtype of lymphoma: \(BCL2\), \(BCL6\) and \(MYC\). \(BCL2\) is frequently amplified and overexpressed in ABC-DLBCL\(^ {28}\). Several agents have been developed to inhibit \(BCL2\) and its anti-apoptotic family members\(^ {52}\) including small molecule BH3-mimetic compounds such as ABT-737 and Obatoclax. Simultaneous inhibition of MALT1 and \(BCL2\) would be expected to reduce NF-\(\kappa\)B activation and induce apoptosis with potential synergistically killing of lymphoma cells (**Figure 2a**). The \(BCL6\) gene is also frequently translocated or mutated resulting in its deregulated expression in ABC-DLBCL, where it suppresses cell cycle checkpoint genes as well as terminal differentiation through repression of \(PRDM1\) and other genes\(^ {53, 54}\). Peptidomimetic and small molecule inhibitors of \(BCL6\) that disrupt its ability to form repression complexes have potent anti-lymphoma activity against DLBCLs including ABC-DLBCLs\(^ {55-57}\). \(BCL6\) inhibitors do not seem to induce toxic effects in animals, supporting the suitability of their use in combinatorial regimens. Concurrent inhibition of MALT1 paracaspase activity and \(BCL6\) would be expected to simultaneously attenuate NF-\(\kappa\)B activation.
and promote checkpoint growth suppression and apoptosis (Figure 2b). MYC is frequently overexpressed in DLBCL\textsuperscript{58}. Deregulated expression of MYC affects many cellular processes including proliferation, differentiation, and metabolism\textsuperscript{58}. An inhibitor of the Bromodomain-containing protein 4 (BRD4), JQ1 downregulates MYC transcription resulting in downregulation of MYC-induced target genes. JQ1 caused cell-cycle arrest and cellular senescence in multiple myeloma\textsuperscript{59}, Burkitt lymphoma and acute myeloid leukemia\textsuperscript{60}. Combination of MALT1 inhibition with JQ1 is expected to synergistically collaborate to kill lymphoma by concomitantly affecting fundamental pathways for cell proliferation.

**Conclusions**

Reported native MALT1 paracaspase activity targets are part of the negative feedback program of the BCR pathway and accordingly MALT1 mediated cleavage of these proteins potentiates NF-κB activation, proliferation and survival. One exclusive target of API2-MALT1 has also been reported, NIK that becomes constitutively active after cleavage promoting aberrant non-canonical NF-κB activation. Hence, MALT1 protease activity inhibition constitutes a therapeutic target in lymphoma. Recent advances in the development of anti-paracaspase drugs have yielded small molecules that inhibit MALT1 *in vivo* and suppress ABC-DLBCL in xenograft experiments and patient samples *ex vivo*\textsuperscript{13,32}. MALT1 paracaspase inhibitors are of particular interest in treating BCR-dependent lymphomas, especially those with mutations impeding response to SYK and BTK inhibitors like CARD11, and may also benefit patients with CLL, MCL and those with certain autoimmune diseases. Finally, rational combination of MALT1 paracaspase inhibitors with other drugs could serve as the basis for more definitive targeted therapy based regimens for eradication of lymphomas with less toxic side effects.
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References


Figure legends

Figure 1. B-cell receptor induced activation of NF-κB through the CBM complex. (A) MALT1 (highlighted in orange) is a mediator of NF-κB signaling in B-lymphocytes. BCR stimulation leads to a cascade of tyrosine phosphorylations that activate SYK and then PI3K. This activates PDK1 and BTK, which subsequently promotes PLCγ activation and DAG and Ca²⁺ production, to finally activate PKC. PKC phosphorylates CARD11, which allows formation of the CBM complex. TRAF6 and TAK1 are then recruited to the CBM complex and activate the IKK signalosome through poly-ubiquitination and phosphorylation to finally engage canonical NF-κB signaling. Red and bold with asterisk, denote frequently mutated genes in ABC-DLBCL. (B) Targets of the paracaspase activity of MALT1 and API2-MALT1 and effects of their cleavage. In red, inhibitory actions; in green, activating actions.
Figure 2. Rational combinatorial therapy of MALT1 inhibitors in ABC-DLBCL. Rational combination will include other BCR signaling inhibitors (left panel) or inhibition of complementary pathways (right panel). **Left panel:** BCR signaling pathway with therapeutically targetable proteins for which one or more inhibitors are currently in clinical trials involving lymphoma patients listed in www.clinicaltrials.gov are highlighted in red. Dashed lines indicate pathways parallel to NF-κB signaling also activated through BCR engagement. **Right panel:** Other pathways, important for ABC-DLBCL survival, could complement MALT1 inhibition and favor apoptosis to kill lymphoma. **a)** BCL2 prevents Cytochrome C release and apoptosis. **b)** BCL6 inhibits the expression of checkpoint sentinels ATR, CHEK1, TP53 or CDKN1A protecting cells from DNA-damage induced apoptosis. **c)** MYC promotes cell growth and enhances cellular metabolism. Inhibition of MYC by BRD4 promoted cell cycle arrest and senescence.
Rational combinatorial therapy

BCR signaling

+ Complementary pathway

- Apoptosis
  - Cytoplasm
  - Bcl-2
  - Obatoclax
- Bcl-6
  - Nucleus
  - Bcl-2
  - ATR, CHEK1, TP53, CDKN1A
- Apoptosis
  - Nucleus
  - JQ1
- Cell growth, metabolism
  - MYC

- Cell proliferation, survival, inflammation
  - AP-1
  - NFKB
  - REL

- Ca2+
- Akt
- eNOS
- PKC
- mTOR
- PLCγ2
- BTK

- FGFR
- MAPK
- IgM
- CD79a/b*
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