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MALT LYMPHOMA: MANY ROADS LEAD TO NF-κB ACTIVATION

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ABSTRACT

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is genetically characterised by several recurrent, but mutually exclusive chromosome translocations. To date, it has been shown that at least the oncogenic products of t(1;14)(p22;q32)/*BCL10-IGH*, t(14;18)(q32;21)/*IGH-MALT1* and t(11;18)(q21;q21)/*API2-MALT1* activate the NF-κB activation pathway. Recently, A20, an essential global NF-κB inhibitor, was found to be inactivated by somatic deletion and/or mutation in translocation negative MALT lymphomas. However, these genetic abnormalities alone are not sufficient for malignant transformation and thus need to cooperate with other factors in MALT lymphomagenesis. Recent studies have shown steady exciting progresses in our understanding of the biological functions of BCL10, MALT1 and A20 in the regulation of the NF-κB activation pathways and the biology of lymphocytes. This review discusses the implication of these recent advances in the molecular pathogenesis of MALT lymphoma, and explores how the above genetic abnormalities cooperate with immunological stimulation in the lymphoma development.

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INTRODUCTION

NF-κB is a master transcription factor critical for a number of biological processes involved in both innate and adaptive immunity. The NF-kB transcription factor family consists of NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (p65), RelB and c-Rel. All of the NF-κB family members contain an N-terminal REL homology domain (RHD) and can form various homo- or heterodimers through RHD-RHD interaction. The NF-κB dimmers are kept inactive in the cytoplasm by association with one of the three inhibitors (IκBα, IκBβ and IκBε) or in its dormant precursor forms. A number of surface receptors are linked to the NF-κB activation pathway.

Canonical NF-κB pathway

The signalling from the antigen receptor (BCR or TCR), TLR, IL-1R and TNFR leads to the activation of the canonical NF-κB pathway, which is characterised by activation of the IκB kinase

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(IKK) complex, consisting of two catalytic subunits IKKα and IKKβ, and the regulatory subunit IKKγ, also known as NEMO (NF-κB essential modulator). The activated IKK complex phosphorylates IκB, triggering its K48-liked polyubiquitination and subsequent degradation by the 26S proteasome (Figure 1). This releases the NF-κB dimmers, exposes their nuclear localisation signal and thus permits their nuclear translocation and transcriptional activation of their target genes.

Although all signals from the aforementioned receptors converge on the IKK complex, the upstream events leading to IKK activation are distinct and involve different adaptor molecules. For example, the proximal antigen receptor signalling triggers the recruitment of the scaffolding adaptor CARMA1 (CARD11) and causes phosphorylation in its PKC-regulated domain (PRD). This induces conformational changes of CARMA1 and enables its association with BCL10 and self-oligomerisation, subsequent assembly of the CARMA1/BCL10/MALT1 complex (CBM complex).[1,2] The CBM complex interacts with TRAF6, activates its E3 ligase activity, resulting in the K63-linked ubiquitination (non degradative) of TRAF6 and NEMO (Figure 1).[3] The activated TRAF6 also recruits TAB2/TAK1, which phosphorylates IKKβ.[3] These concert events activate the IKK complex.[1] Likewise, IL-1R / TLR signalling triggers sequential recruitment of MyD88, IRAK and TRAF6 (Figure 1), and this causes TRAF6 auto-K63-linked polyubiquitylation and thereby its activation.[4,5]

Noncanonical NF-κB pathway

The signalling from CD40, BAFFR (B-cell activating factor receptor) and LTβR (lymphotoxin β receptor) activates the noncanonical NF-κB pathway, which is characterised by sequential activation of the NF-κB inducible kinase (NIK) and IKKα (Figure 1). The activated IKKα phosphorylates NF-κB2/p100 and induces its partial proteolysis to generate the functional active form of p52. The activated p52, often in association with RelB, and translocates to the nucleus and transactivates their target genes.

Negative regulation of NF-κB activation pathway

The NF-κB activation pathway is also governed by a number of negative regulators such as IκBα, A20, CYLD (cylindromatosis) and TRAF3 (Figure 1).[6,7] Interestingly, IκBα and A20 are targets of NF-κB, thus their expression following NF-κB activation could act as an auto-negative feedback.

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The newly synthesized IκBα can enter the nucleus, dissociate NF-κB from DNA, and export it back to the cytoplasm. A20, also known as TNFα inducible protein 3 (TNFAIP3), can specifically inactivate several proteins critical for the NF-κB signalling, such as RIP1/2, TRAF6, NEMO and TAK1 (Figure 1). A20 removes the K63-linked ubiquitin chain that mediates protein function, and also catalyses the K48-linked polyubiquitin that targets protein for proteasome degradation.[6,8]

NF-κB transactivates more than 200 genes encoding cell cycle regulators, growth factors, immunoregulatory cytokines, apoptosis inhibitors, negative regulators of the NF-κB pathway etc. In general, NF-κB activation promotes cellular activation and proliferation. Activation of NF-κB is normally transient and plays a critical role in lymphocyte development, activation and differentiation. There is now growing evidence that NF-κB is constitutively activated in several lymphoma subtypes and many of the aforementioned NF-κB regulators are targeted by genetic alterations in B-cell lymphomas including MALT lymphoma. The incidences of these genetic abnormalities in MALT lymphoma and their clinical relevance have been reviewed elsewhere.[9,10] This review instead focuses on the recent advances in our understanding of the genetic abnormalities and immune receptor signalling that underpin NF-κB activation in MALT lymphoma.

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GENETIC ABNORMALITIES IN MALT LYMPHOMA THAT TARGET THE NF-κB PATHWAY

A number of chromosome translocations have been reported in MALT lymphoma and among these, t(1;14)(p22;q32), t(14;18)(q32;q21), t(11;18)(q21;q21) and t(3;14)(p13;q32) are recurrent although at considerably variable frequencies in MALT lymphoma of different sites. Despite that these chromosome translocations involve different genes, at least the first three chromosome translocations commonly involve genes encoding for NF-κB positive regulators. Recent studies have also shown that NF-κB negative regulator is also targeted by genetic deletion and inactivating mutations in MALT lymphoma, preferentially in those lacking the above chromosome translocations.

Chromosome translocation

<u>t(1;14)(p22;q32):</u> The translocation brings the entire *BCL10* gene under the regulatory control of the *IG* gene enhancer and hence causes its over-expression.[11,12] BCL10 contains an N-terminal CARD (caspase recruitment domain) and a C-terminal Ser/Thr rich domain (Figure 2). Studies of Bcl10-/-

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mice show that Bcl10 deficiency causes a considerable reduction of all mature B-cell subsets including marginal zone B-cells, and a profound immune-deficiency including both T-cell dependent and T-cell independent responses.[13,14] These defects are essentially due to the role of BCL10 in the antigen receptor and TLR mediated NF-κB activation.[13,14] Biochemical investigations demonstrate that over-expression of BCL10 alone is capable of activating of the NF-κB pathway and c-Jun N-terminal kinase (JNK), and this depends on BCL10 oligomerisation mediated by its CARD domain.[15-19]

Physiologically, BCL10 acts as an adaptor protein linking its upstream protein CARMA1 to its downstream molecule MALT1.[2] The binding between CARMA1 and BCL10 is through interaction of their respective CARD domain,[20-22] while the interaction between BCL10 and MALT1 is via a short region (amino acids 107-119) downstream of the BCL10 CARD and the two N-terminal Ig-like domains of MALT1.[23] The interaction among these proteins leads to formation of the CBM complex, which relays the signalling from the antigen receptor, TLR and GPCR (G protein –coupled receptors) to the canonical NF-κB activation pathway.[24-26] It is likely that there are further receptors that link to the CBM complex.

In addition to the role of BCL10 in the canonical NF-κB pathway, there is now evidence that BCL10 also activates the non-canonical NF-κB pathway. It has been shown that BCL10 silencing abrogated the sulphated polysaccharide carrageenan induced NIK phospharylation and p52 production, the hallmark of the non-canonical pathway activation, in mouse embryonic fibroblasts and in human colonic epithelial cells.[27] In line with this, an early study showed that the BCL10 mediated NF-κB activation could be abrogated by co-expression of a dominant negative form of NIK.[28] It remains to be investigated whether this is also a feature in B-cells. Interestingly, the Eu-BCL10 mice show constitutive activation of both canonical and noncanonical NF-κB pathways in their splenic marginal zone B -cells.[29] Nonetheless, the constitutive activation of the noncanonical pathway in Eu-BCL10 mice might be secondary to the increased expression of BAFF, which is an NF-κB target and can activate both the canonical and noncanonical NF-κB pathways.[29] An increased expression of BAFF is also seen in human gastric MALT lymphoma.[30] Thus, BAFF expression may serve as an autocrine positive feedback.

In line with the known physiological role of BCL10 in normal lymphocytes, the protein is typically expressed essentially in the cytoplasm of normal B-cells including marginal zone B- cells,[31] the

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normal cell counterpart of MALT lymphoma. Intriguingly, the protein is expressed strongly in the nuclei of lymphoma cells with t(1;14)(q22;q32)/BCL10-IGH and moderately in those with t(11;18)(q21;q21).[31-34] Strong nuclear BCL10 expression is also a feature of the marginal zone B-cells of Eu-BCL10 mice.[29] BCL10 does not contain any known nuclear localisation signal. How BCL10 sub-cellular localisation is regulated is not totally understood although several factors that regulate BCL10 stability or its shuttling between the nucleus and cytoplasm have been identified. Firstly, it has been shown that API2 is an E3 ubiquitin ligase of BCL10 and targets it for degradation, thereby limiting the antigen receptor mediated NF-κB activation.[35] In line with this, the level of BCL10 protein is much higher in MALT lymphoma with t(11;18)(q21;q21)/*API2-MALT1* than those without chromosome translocation since one *API2* allele is fused with *MALT1* and the API2 mediated BCL10 degradation is likely compromised in lymphoma cells with the translocation.[35] Secondly, a previous study shows that MALT1 contains nuclear export signals and can export BCL10 from the nucleus to the cytoplasm in COS7 cells, and the sub-cellular localisation of BCL10 depends on the relative ratio of BCL10 and MALT1 expression.[36] Intriguingly, MALT1 does not contain any known nuclear localisation signals and no nuclear expression of the protein has been seen in normal B-cells or lymphoma cells including those with MALT1 involved translocation.[36,37] It remains uncertain whether MALT1 mediated BCL10 cytoplasmic export is operational *in vivo.* Lastly, TNFα up-regulates BCL10 expression, induces its association with BCL3 and subsequent nuclear translocation in MCF7 cells.[38] The above findings from both studies of primary MALT lymphoma and *in vitro* experiments consistently suggest that BCL10 nuclear expression is strongly associated with its increased expression. The function of nuclear BCL10 is unknown. In view of its association with BCL3, a co-activator of NF-κB, nuclear BCL10 may be involved in the regulation of the NF-κB transcriptional activities.

<u>t(14;18)(q32;q21):</u> The translocation brings the entire *MALT1* gene under the regulatory control of the *IGH* gene and hence deregulates its expression. MALT1 belongs to the paracapase family and contains an N-terminal death domain, followed by two Ig-like domains, and a caspase-like domain and a third Ig-like domain in its C-terminus. Studies of Malt1 deficiency mice demonstrate that Malt1 is important for antigen receptor and TLR mediated NF-κB activation in B and T-cells, particularly the latter, operating downstream of Bcl10.[39,40] In addition, studies of Malt1 deficiency B-cells also show that Malt1 is essential for BAFF mediated activation of the noncanonical NF-κB pathway and this is most likely due to Malt1 dependent degradation of TRAF3, an inhibitor of NIK.[41] Nonetheless, expression of MALT1 alone *in vitro* does not activate NF-κB,[23,42] and this is

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likely due to a lack of structural domain that can mediate MALT1 self-oligomerisation. Despite this, MALT1 is synergistic with BCL10 in the activation of NF-κB. As mentioned above, BCL10 binds to the two N-terminal Ig-like domains of MALT1, and mediates its oligomerisation and activation.[23] The activated MALT1 binds to TRAF6, induces TRAF6 oligomerisation, and thus its activation, which leads to the activation of the IKK complex as described above.[3,43] Three TRAF6 binding motifs have been identified in MALT1, one within the second Ig-like domain and the other two in its C-terminal region (Figure 2).[3,43]

It has also been shown recently that MALT1 can directly activate caspase-8 through hetero-dimerisation and direct its function to activate the NF-κB pathway rather than apoptosis pathway upon antigen receptor stimulation in T-cells.[44] This functional feature of MALT1 is unlikely restricted to T-cells since caspase-8 is also important for the B-cell receptor and NK-cell receptor signalling in human.[45] Nonetheless, how caspase-8 activation leads to NF-κB activation remains poorly understood.

In addition, several recent studies have shown that MALT1 also regulates NF-κB activation through its protease activities. Despite the recognition of a caspase-like domain in the C-terminal of MALT1, it is only shown until recently that MALT1 indeed possesses protease activities. To date, two MALT1 substrates, namely BCL10 and A20, have been identified. MALT1 cleaves BCL10 at Arg228 and this is required for T-cell receptor induced cell adhesion to fibronectin.[46] It remains to be investigated whether BCL10 cleavage occurs in B-cells and if so whether it regulates B-cell adhesion, migration and retention to inflammation sites. MALT1 cleaves human A20 at Arg439 between its first and second zinc fingers, and abolishes its NF-κB inhibitory function.[47,48] The MALT1 protease activity is required for optimal NF-κB activation, although inhibition of the proteolytic activity does not affect IκB phosphorylation by IKK.[46,48] By attenuating the activity of the "global" NF-κB inhibitor A20, MALT1 likely maximizes and prolongs NF-κB activation. In the case of MALT lymphoma cells with t(1;14)(p22;q32)/*BCL10-IGH* or t(14;18)(q32;q21)/*IGH-MALT1* where MALT1 is constitutively activated, the proteolytic degradation of the newly synthesised A20 may eliminate such physiological auto-negative feedback and lead to relentless NF-κB activation.

<u>t(11;18)(q21;q21):</u> The translocation fuses the N-terminal region of the *API2* to the C-terminal region of the *MALT1* and generates a functional chimeric fusion (Figure 2),[49-51] which gains the

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ability to activate the NF-κB pathway.[23,42] It has been shown that the API2-MALT1 fusion oligomerises through heterotypic interaction between the BIR1 of the API2 moiety and the C-terminal region of MALT1 in absence of any upstream stimulation, and such oligomerisation is critical for API2-MALT1 mediated NF-κB activation.[52] In analogue to the BCL10 transgenic mice, the Eμ-API2-MALT1 mice show expansion of splenic marginal zone B-cells that are characterised by increased NF-κB activities.[53]

API2-MALT1 directly binds to both TRAF2 and TRAF6. Disruption of either of these interactions significantly impairs the capacity of API2-MALT1 mediated NF-κB activation.[23,43,54] It is highly likely that API2-MALT1 requires both TRAF2 and TRAF6 to maximise its capacity of NF-κB activation. The motif of API2-MALT1, which binds to TRAF2, is in the BIR1 domain of the API2 moiety, and this is distinct from the region of BIR1 domain responsible for API2-MALT1 oligomerisaiton.[52] The downstream of molecular events on how the interaction between API2-MALT1 and TRAF2 leads to NF-κB activation remains to be investigated. The motif of API2-MALT1, which binds to TRAF6, is within the moiety of C-terminal MALT1, as mentioned above.[3,43] Majority of the API2-MALT1 fusions in MALT lymphoma retain all the three TRAF6 binding sites, with all fusions containing the two C-terminal binding sites (Figure 2).[43,55] The interaction between API2-MALT1 and TRAF6 likely mediates TRAF6 oligomerisation and activates its E3 ubiquitin ligase activity, which leads to the activation of the IKK complex.[3,43]

All the API2-MALT1 fusion products also contain the intact caspase-like domain of the C-terminal MALT1 and thus possess its protease activities. Like MALT1, expression of API2-MALT1 also results in A20 cleavage.[47] By proteolytic inactivation of this global NF-κB inhibitor, constitutive expression of API2-MALT1 may result in persistent NF-κB activation. In addition, API2-MALT1 itself is a target of NF-κB and thus the API2-MALT1 induced NF-κB activation may enhance its own expression, potentially forming a positive feedback loop.[56] In keeping with the above notion, high levels of polyubiquitination of NEMO are seen in both MALT lymphomas with t(11;18)(q21;q21)/*API2-MALT1* and splenic marginal zone B-cells of Eμ-API2-MALT1 mice.[53,57]

<u>t(3;14)(p13;q32):</u> This translocation deregulates the expression of the *FOXP1* gene.[58-60] FOXP1 contains an N-terminal polyglutamine domain, followed by a glutamine-rich domain, a zinc finger, a leucine zipper, a forkhead domain and an acid rich domain (Figure 1). FOXP1 is involved in regulation of the Rag1 and Rag2 expression, thus essential for B-cell development.[61] Apart from this,

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the biological function of FOXP1 is largely unknown. There are several FOXP1 isoforms and the smaller isoforms are expressed at a much higher level in the activated B-cell like diffuse large B-cell lymphoma (ABC-DLBCL) than the germinal centre B-cell like diffuse large B-cell lymphoma (GCB-DLBCL).[62] Given that ABC-DLBCL is characterised by constitutive NF-κB activation, it has been long speculated that FOXP1 may regulate NF-κB activation. In support of this speculation, various FOXP1 isoforms, particularly the smaller isoforms, were capable of activating the NF-κB reporter in both B and T-cell lines (Appert, Goatly and Du, unpublished results).

Other newly identified chromosomal translocations in MALT lymphoma: Several novel chromosome translocations have been recently described in MALT lymphoma and these include t(X;14)(p11.4;q32)/GPR34-IGH, [63] t(1;14)(p21;q32)/CNN3-IGH, t(5;14)(q34;q32)/ODZ2-IGH and t(9;14)(p24;q32)/JMJD2C-IGH.[64] Like classic chromosome translocation involving the *IG* gene loci, the expression of these genes is markedly increased in cases with the respective translocation.[63,64] The function of these potential oncogenes is currently unknown. Nonetheless, expression of GPR34 in Hela cells activated both the canonical NF-κB pathway and the MAP kinase pathway,[63] further extending the list of genetic abnormalities in MALT lymphoma, which target the NF-κB pathway.

A20 inactivation:

The majority of MALT lymphomas, particularly those from the ocular adnexa, salivary gland and thyroid, are negative for the above chromosome translocations. In an attempt to characterise the genetics of translocation negative MALT lymphomas, we and others identified *A20* as the target of 6q23 deletion in ocular adnexal MALT lymphoma by array comparative genomic hybridisation.[65-67] Subsequent studies show that A20 is frequently inactivated by somatic deletion and/or mutation in MALT lymphoma, DLBCL, Hodgkin lymphoma and primary mediastinal B-cell lymphoma.[68-73] In ocular adnexal MALT lymphoma, complete A20 inactivation was associated with poor lymphoma free survival,[65,68] and the clinical impact of A20 inactivation in other lymphoma subtypes is currently unknown. In addition, *A20* is linked to a range of chronic inflammatory disorders including autoimmune diseases such as systemic lupus erythematosis and rheumatoid arthritis,[74-78] which associates with a significantly increased risk of lymphoma.[79]

A20 is a "global" essential negative regulator of the NF-κB activation pathway and can attenuate the NF-κB activity triggered by a number of surface receptors.[74,80,81] A20 restricts NF-κB activities by

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inactivating several proteins that are critical to NF-κB signalling, such as RIP1/2, TRAF6, NEMO, and TAK1.[74,80,81] A20 can specifically remove the K63-linked ubiquitin chain that is crucial to protein function, and this is essentially mediated by the N-terminal OTU domain that belongs to the family of deubiquitinating cysteine proteases.[6,8] Additionally, A20 catalyses the K48-linked polyubiquitin that targets proteins for proteasome degradation, and this is mediated by the C-terminal ZF domains that possess E3 ligase activity.[6,8] By inhibiting NF-κB pathway activation, A20 acts as a tumour suppressor in lymphoma.[70-73]

As a molecular "brake" of the NF-κB activation pathways, A20 inactivation alone clearly is not sufficient for malignant transformation. In fact, A20 deficiency mice die shortly after birth due to massive multiorgan inflammation.[82] As mentioned above, *A20* genetic abnormalities are mutually exclusive from the chromosome translocations seen in MALT lymphoma. Nonetheless, there is a significant association between *A20* genetic abnormalities and a low copy number gain of 6p21 containing the *TNFA/B/C loci*.[65,68] It is highly likely that A20 mediated oncogenic activities in MALT lymphoma depends on the NF-κB activation triggered by TNF and others yet to be identified.

ROLE OF IMMUNOLOGICAL DRIVE IN TRANSLOCATION POSITIVE MALT LYMPHOMA

There is overwhelming evidence indicating that chromosome translocation alone including those seen in MALT lymphoma is not sufficient for full malignant transformation. Both Eμ-BCL10 and Eμ-API2-MALT1 mice develop splenic marginal zone hyperplasia but not lymphoma.[29,53] Thus, additional factors are required to cooperate with these chromosome translocations in MALT lymphoma development. T(11;18)(q21;q21)/*API2-MALT1,* the most frequent translocation in MALT lymphoma, often occurs as a sole cytogenetic aberration,[83,84] and comparative genomic hybridisation analyses also show that t(11;18)(q21;q21) positive MALT lymphoma has few genomic copy number alterations.[85] In addition, translocation positive MALT lymphomas do not harbour *A20* gene deletion or mutation.[65,68] Although the full genetic abnormalities in translocation positive MALT lymphoma remain to be investigated, there is now increasing evidence suggesting that the oncogenic product of MALT lymphoma associated translocation cooperates with immunological stimulation in oncogenesis.

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Stimulation of surface immune receptors such as CD40 and antigen receptor can enhance the NF-κB activation by expression of API2-MALT1, MALT1 and BCL10 *in vitro*.[19,86] Several other surface receptors such as CCR2 and TLR6, and the apoptosis inhibitor BCL2 are also more abundantly and homogeneously expressed in MALT lymphoma with chromosome translocation than those without the translocation as shown recently by gene expression profiling analyses.[87] Interestingly, these genes themselves are the transcriptional targets of NF-κB and expression ofTLR6, in presence of its heterodimer partner TLR2, can enhance both API2-MALT1 and BCL10 mediated NF-κB activation *in vitro.* [87] Although it remains to be investigated, it is also likely that the enhanced CCR2 expression may augment the NF-κB activation by MALT lymphoma associated oncogenic products. Thus, the enhanced expression of these surface receptors may act as a positive feedback to the NF-κB activation by the MALT lymphoma associated translocations (Figure 3). This, together with the aforementioned proteolytic degradation of the NF-κB inhibitor A20 by MALT1 or API2-MALT1, would lead to relentless constitutive NF-κB activation (Figure 3).

The above speculated cooperation between MALT lymphoma associated translocation and surface receptor signalling is likely operational *in vivo*. The notion is supported by several strands of evidence based on studies of gastric MALT lymphoma. Firstly, in gastric MALT lymphoma, the translocation positive cases, like those without translocation, are invariably associated with *H. pylori* infection, and the milieu of immunological stimulation is present although this may not be as strong as that in translocation negative MALT lymphoma; Secondly, there are rare cases of translocation positive gastric MALT lymphoma that responded to *H. pylori* eradication,[9] suggesting a role of *H. pylori* generated immune responses in the lymphoma cell survival; Finally, stimulation of Eμ-API2-MALT1 mice with Freund's complete adjuvant leads to development of splenic marginal zone lymphoma-like lesion.[88]

ROLE OF IMMUNOLOGICAL DRIVE IN TRANSLOCATION NEGATIVE MALT LYMPHOMA

Most of the translocation negative gastric MALT lymphomas can be cured by *H. pylori* eradication alone,[55] and this strongly indicates that the survival of the lymphoma cells critically depends on the microbe generated immune responses. There is consistent evidence *from in vitro* studies that the growth of malignant B-cells depends on tumour infiltrating *H. pylori* specific T-cells and this requires interaction between B and T cells involving CD40 and CD40L co-stimulatory molecules.[89-

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[91] Despite this, the gastric MALT lymphoma-derived Ig is autoreactive rather than recognising *H. pylori* antigen.[92] Furthermore, the tumour infiltrating T-cells are polyclonal and react to a wide spectrum of *H. pylori* derived antigens.[93] How could these polyclonal *H. pylori* reactive tumour infiltrating T-cells provide growth help to the clonal malignant B-cell population that recognises a completely different antigen? This is clearly against the dogma of classic T dependent B-cell activation, which requires recognition of a common antigen and interaction of co-stimulatory molecules i.e. CD40-CD40L between the two cells. The findings that the switched memory B-cells could be stimulated by newly activated bystander T-cells provide pertinent explanation of this dilemma.[94]

In the case of gastric MALT lymphoma, *H. pylori* infection constantly results in T-cell dependent responses through the classic germinal centre reaction, and thus generates reactive B and T-cells. The *H. pylori* specific T-cells raised in the reactive component then migrate to the marginal zone / tumour area and provide noncognate help to auto-reactive malignant B-cells, which may involve stimulation of CD40 and other surface receptors by soluble ligands and cytokines (Figure 3). This together with direct antigen receptor stimulation may sustain the growth of malignant B-cells. This notion is supported by studies of *H. felis* induced murine MALT lymphoma, in which the growth of the lymphoma cells was found to be driven by signalling from both antigen receptor stimulation by self-antigen and noncognate help of tumour infiltrating T-cells.[95]

In line with the above speculation, gene profiling analysis showed that translocation negative gastric MALT lymphoma is characterised by enriched expression of proinflammatory cytokines such as IL8 and IL1β, molecules involved in B and T-cell interaction such as CD86, CD28 and ICOS.[87] These findings suggest that there is an active immune response to *H. pylori* infection in translocation-negative gastric MALT lymphoma, and this most likely underscores the tumour cell survival and proliferation, and thus determines their response to *H. pylori* eradication.

LESSONS LEARNT FROM MALT LYMPHOMA

Several other lymphoma subtypes such as ABC-DLBCL,[96,97] primary mediastinal large B-cell lymphoma (PMBL),[98,99] multiple myeloma (MM),[100] and classical Hodgkin lymphoma (HL),[101] and adult T cell leukemia/ lymphoma[102] are also characterised by constitutive NF-κB activation. The survival of these lymphoma cells critically depends on constitutive NF-kB activities as demonstrated

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by both *in vitro* and *in vivo* investigations.[96,97,100,103-105] The genetic basis underlying the constitutive NF-κB activities in these lymphoma subtypes is now under extensive investigations. *A20* mutation and deletion, initially identified in translocation negative ocular adnexal MALT lymphoma, are also frequently seen in ABC-DLBCL (24-54%), Hodgkin lymphoma (26-44%) and PMBL lymphoma (36%).[70-73] In addition, genetic abnormalities have been reported in a number of other NF-κB pathway regulators in DLBCL [71,73,106,107] and MM[108,109] although at low frequencies. The advance in such genetic characterisation in one lymphoma will shed lights into the investigation of other lymphoma subtypes. In this context, MALT lymphoma may offer a unique advantage. Given it is an indolent low grade B-cell lymphoma, its genetic make-up may be relatively less complex than those of DLBCL and MM, and a single genetic abnormality, as shown by t(11;18)(q21;q21)/*API2-MALT1* in gastric MALT lymphoma and *A20* deletion/mutation in ocular adnexal MALT lymphoma as discussed above, may have a clear impact on clinicopathological presentation. These should facilitate the identification of potential "driver" mutations from the "passenger" mutations.

The causative relationship between MALT lymphoma and microbial infection provides a paradigm in characterisation and illustration of the critical role of immunological drive in the lymphoma pathogenesis. As discussed above, the immunological responses to *H. pylori* infection not only directly and indirectly stimulate malignant B-cells, but are also likely to cooperate with the genetic events in the development of gastric MALT lymphoma. The knowledge learnt from MALT lymphoma has stimulated the research in characterisation of the role of tumour microenvironment in other B-cell lymphoma subtypes and will continue providing important insights into these investigations. Such investigations may also lead to the development of novel therapeutic strategies in other lymphoma subtypes,[110] as fruitfully demonstrated in gastric MALT lymphoma.

CONCLUSIONS

As discussed above, various genetic abnormalities seen in MALT lymphoma target the NF-κB activation pathway. The oncogentic products of t(1;14)(p22;q32)/*BCL10-IGH*, t(14;18)(q32;21)/*IGH-MALT1* and t(11;18)(q21;q21)/*API2-MALT1* are all potent activators of the NF-κB activation pathway. They activate the canonical NF-κB activation pathway, and also potentially trigger directly and/or indirectly activation of the non-canonical NF-κB pathway. Most likely, they cooperate with signalling from several surface receptors including the antigen receptor, TLR, CCR2, and CD40, and consequently cause constitutive NF-κB activation. Similarly, the

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inactivation of the global NF-κB inhibitor A20 also impacts on multiple signalling pathways leading to NF-κB activation and thus potentially exacerbates the effect of stimulation of several surface receptors such as the antigen receptor, TNFR and TLR. In this regard, there is a considerable overlap in the molecular mechanism between MALT lymphoma with and without such genetic abnormalities. Nonetheless, there is a significant difference in the extent of immunological drive between gastric MALT lymphoma with and without chromosome translocation, and this underscores their different responses to *H. pylori* eradication.

Despite the exciting progress in our understanding of the molecular mechanism of MALT lymphoma, there are still many open questions to be answered. In the immediate context of MALT lymphoma pathogenesis, several outstanding questions remain to be investigated. Firstly, what is the function of nuclear BCL10? Paradoxical to the well descried function of BCL10 in the cytoplasm, the protein is expressed strongly in the nuclei of MALT lymphoma cells with t(1;14)(p22;q32)/*BCL10-IGH* and moderately in those with t(11;18)(q21;q21)/*API2-MALT1* .[31-34] High levels of BCL10 nuclear expression are also seen in the splenic marginal B-cells of Eu-BCL10 mice.[29] These, together with the finding that BCL10 binds to BCL3, a co-activator of NF-κB, strongly argue for a potential role of nuclear BCL10 in regulation of NF-κB transcription activities. Secondly, what is the full spectrum of genetic abnormalities of the NF-κB regulators in MALT lymphomas? As shown in DLBCL and multiple myeloma, there are diverse NF-κB regulators that are targeted by genetic alterations.[70,71,73,106-109] The current finding of A20 deletion /somatic mutation in MALT lymphoma may only represents the start of expedition into the world of NF-κB pathway genetic abnormalities.

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References:

1. Rawlings DJ, Sommer K, Moreno-Garcia ME. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. *Nat Rev Immunol.* 2006;**6;**799-812.

2. Thome M, Weil R. Post-translational modifications regulate distinct functions of CARMA1 and BCL10. *Trends Immunol.* 2007;**28;**281-288.

3. Sun L, Deng L, Ea CK, Xia ZP, Chen ZJ. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell* 2004;**14;**289-301.

4. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol.* 2004;**4;**499-511.

5. Skaug B, Jiang X, Chen ZJ. The role of ubiquitin in NF-kappaB regulatory pathways. *Annu. Rev Biochem.* 2009;**78;**769-796.

6. Sun SC. Deubiquitylation and regulation of the immune response. *Nat Rev Immunol.* 2008;**8;**501-511.

7. Sun SC, Ley SC. New insights into NF-kappaB regulation and function. *Trends Immunol.* 2008;**29;**469-478.

8. Coornaert B, Carpentier I, Beyaert R. A20: central gatekeeper in inflammation and immunity. *J. Biol. Chem.* 2009;**284;**8217-8221.

9. Du MQ, Atherton JC. Molecular subtyping of gastric MALT lymphomas: implications for prognosis and management. *Gut* 2006;**55;**886-893.

10. Du MQ. MALT lymphoma : recent advances in aetiology and molecular genetics. *J. Clin. Exp. Hematop.* 2007;**47;**31-42.

11. Willis TG, Jadayel DM, Du MQ *et al.* Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. *Cell* 1999;**96;**35-45.

12. Zhang Q, Siebert R, Yan M *et al.* Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). *Nat. Genet.* 1999;**22;**63-68.

13. Ruland J, Duncan GS, Elia A *et al.* Bcl10 is a positive regulator of antigen receptor-induced activation of NF-kappaB and neural tube closure. *Cell* 2001;**104;**33-42.

14. Xue L, Morris SW, Orihuela C *et al.* Defective development and function of Bcl10-deficient follicular, marginal zone and B1 B cells. *Nat. Immunol.* 2003;**4;**857-865.

15. Yan M, Lee J, Schilbach S, Goddard A, Dixit V. mE10, a novel caspase recruitment domain-containing proapoptotic molecule. *J. Biol. Chem.* 1999;**274;**10287-10292.

16. Koseki T, Inohara N, Chen S *et al.* CIPER, a novel NF kappaB-activating protein containing a caspase recruitment domain with homology to Herpesvirus-2 protein E10. *J. Biol. Chem.* 1999;**274;**9955-9961.

17. Thome M, Martinon F, Hofmann K *et al.* Equine herpesvirus-2 E10 gene product, but not its cellular homologue, activates NF-kappaB transcription factor and c-Jun N-terminal kinase. *J. Biol. Chem.* 1999;**274;**9962-9968.

18. Srinivasula SM, Ahmad M, Lin JH *et al.* CLAP, a novel caspase recruitment domain-containing protein in the tumor necrosis factor receptor pathway, regulates NF-kappaB activation and apoptosis. *J. Biol. Chem.* 1999;**274;**17946-17954.

19. Tian MT, Gonzalez G, Scheer B, DeFranco AL. Bcl10 can promote survival of antigen-stimulated B lymphocytes. *Blood* 2005.

For Peer Review

20. Gaide O, Martinon F, Micheau O *et al.* Carma1, a CARD-containing binding partner of Bcl10, induces Bcl10 phosphorylation and NF-kappaB activation(1). *FEBS Lett. 2001. May. 11. ;496. (2. -3.):121. -7.* 2001;**496;**121-127.

21. Bertin J, Wang L, Guo Y *et al.* CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. *J. Biol. Chem.* 2001;**276;**11877-11882.

22. McAllister-Lucas LM, Inohara N, Lucas PC *et al.* Bimp1, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-kappaB induction. *J. Biol. Chem.* 2001;**276;**30589-30597.

23. Lucas PC, Yonezumi M, Inohara N *et al.* Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. *J. Biol. Chem.* 2001;**276;**19012-19019.

24. Liu Y, Dong W, Chen L *et al.* BCL10 mediates lipopolysaccharide/toll-like receptor-4 signaling through interaction with Pellino2. *J. Biol. Chem.* 2004;**279;**37436-37444.

25. Klemm S, Zimmermann S, Peschel C, Mak TW, Ruland J. Bcl10 and Malt1 control lysophosphatidic acid-induced NF-kappaB activation and cytokine production. *Proc. Natl. Acad. Sci. U. S. A* 2007;**104;**134-138.

26. Wang D, You Y, Lin PC *et al.* Bcl10 plays a critical role in NF-kappaB activation induced by G protein-coupled receptors. *Proc. Natl. Acad. Sci. U. S. A* 2007;**104;**145-150.

27. Bhattacharyya S, Borthakur A, Tyagi S *et al.* B-cell CLL/lymphoma 10 (BCL10) is required for NF-kappaB production by both canonical and noncanonical pathways and for NF-kappaB-inducing kinase (NIK) phosphorylation. *J. Biol. Chem.* 2010;**285;**522-530.

28. Costanzo A, Guiet C, Vito P. c-E10 Is a Caspase-recruiting Domain-containing Protein That Interacts with Components of Death Receptors Signaling Pathway and Activates Nuclear Factor-kappaB. *J. Biol. Chem.* 1999;**274;**20127-20132.

29. Li Z, Wang H, Xue L *et al.* Emu-BCL10 mice exhibit constitutive activation of both canonical and noncanonical NF-kappaB pathways generating marginal zone (MZ) B-cell expansion as a precursor to splenic MZ lymphoma. *Blood* 2009;**114;**4158-4168.

30. Kuo SH, Yeh PY, Chen LT *et al.* Overexpression of B cell-activating factor of TNF family (BAFF) is associated with Helicobacter pylori-independent growth of gastric diffuse large B-cell lymphoma with histologic evidence of MALT lymphoma. *Blood* 2008;**112;**2927-2934.

31. Ye H, Dogan A, Karran L *et al.* BCL10 expression in normal and neoplastic lymphoid tissue : nuclear localization in MALT lymphoma. *Am. J. Pathol.* 2000;**157;**1147-1154.

32. Liu H, Ye H, Dogan A *et al.* T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. *Blood.* 2001;**98;**1182-1187.

33. Maes B, Demunter A, Peeters B, Wolf-Peeters C. BCL10 mutation does not represent an important pathogenic mechanism in gastric MALT-type lymphoma, and the presence of the API2-MLT fusion is associated with aberrant nuclear BCL10 expression. *Blood* 2002;**99;**1398-1404.

34. Ye H, Liu H, Attygalle A *et al.* Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of H pylori in gastric MALT lymphoma. *Blood* 2003;**102;**1012-1018.

35. Hu S, Du MQ, Park SM *et al.* cIAP2 is a ubiquitin protein ligase for BCL10 and is dysregulated in mucosa-associated lymphoid tissue lymphomas. *J. Clin. Invest* 2006;**116;**174-181.

36. Nakagawa M, Hosokawa Y, Yonezumi M *et al.* MALT1 contains nuclear export signals and regulates cytoplasmic localization of BCL10. *Blood* 2005;**106;**4210-4216.

For Peer Review

37. Ye H, Gong L, Liu H *et al.* MALT lymphoma with t(14;18)(q32;q21)/IGH-MALT1 is characterized by strong cytoplasmic MALT1 and BCL10 expression. *J. Pathol.* 2005;**205;**293-301.

38. Yeh PY, Kuo SH, Yeh KH *et al.* A pathway for tumor necrosis factor-alpha-induced Bcl10 nuclear translocation. Bcl10 is up-regulated by NF-kappaB and phosphorylated by Akt1 and then complexes with Bcl3 to enter the nucleus. *J. Biol. Chem.* 2006;**281;**167-175.

39. Ruefli-Brasse AA, French DM, Dixit VM. Regulation of NF-kappaB-dependent lymphocyte activation and development by paracaspase. *Science* 2003;**302;**1581-1584.

40. Ruland J, Duncan GS, Wakeham A, Mak TW. Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity.* 2003;**19;**749-758.

41. Tusche MW, Ward LA, Vu F *et al.* Differential requirement of MALT1 for BAFF-induced outcomes in B cell subsets. *J. Exp. Med.* 2009;**206;**2671-2683.

42. Uren GA, O'Rourke K, Aravind L *et al.* Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol. Cell.* 2000;**6;**961-967.

43. Noels H, Van Loo G, Hagens S *et al.* A Novel TRAF6 binding site in MALT1 defines distinct mechanisms of NF-kappaB activation by API2middle dotMALT1 fusions. *J. Biol. Chem.* 2007;**282;**10180-10189.

44. Kawadler H, Gantz MA, Riley JL, Yang X. The paracaspase MALT1 controls caspase-8 activation during lymphocyte proliferation. *Mol. Cell* 2008;**31;**415-421.

45. Su H, Bidere N, Zheng L *et al.* Requirement for caspase-8 in NF-kappaB activation by antigen receptor. *Science* 2005;**307;**1465-1468.

46. Rebeaud F, Hailfinger S, Posevitz-Fejfar A *et al.* The proteolytic activity of the paracaspase MALT1 is key in T cell activation. *Nat Immunol.* 2008;**9;**272-281.

47. Coornaert B, Baens M, Heyninck K *et al.* T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-kappaB inhibitor A20. *Nat Immunol.* 2008;**9;**263-271.

48. Duwel M, Welteke V, Oeckinghaus A *et al.* A20 negatively regulates T cell receptor signaling to NF-kappaB by cleaving Malt1 ubiquitin chains. *J. Immunol.* 2009;**182;**7718-7728.

49. Dierlamm J, Baens M, Wlodarska I *et al.* The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. *Blood* 1999;**93;**3601-3609.

50. Akagi T, Motegi M, Tamura A *et al.* A novel gene, MALT1 at 18q21, is involved in t(11;18) (q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. *Oncogene* 1999;**18;**5785-5794.

51. Morgan JA, Yin Y, Borowsky AD *et al.* Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma lie within or near the previously undescribed gene MALT1 in chromosome 18. *Cancer Res.* 1999;**59;**6205-6213.

52. Lucas PC, Kuffa P, Gu S *et al.* A dual role for the API2 moiety in API2-MALT1-dependent NF-kappaB activation: heterotypic oligomerization and TRAF2 recruitment. *Oncogene* 2007;**26;**5643-5654.

53. Baens M, Fevery S, Sagaert X *et al.* Selective expansion of marginal zone B cells in Emicro-API2-MALT1 mice is linked to enhanced IkappaB kinase gamma polyubiquitination. *Cancer Res.* 2006;**66;**5270-5277.

54. Garrison JB, Samuel T, Reed JC. TRAF2-binding BIR1 domain of c-IAP2/MALT1 fusion protein is essential for activation of NF-kappaB. *Oncogene* 2009;**28;**1584-1593.

55. Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. *Nat Rev Cancer* 2004;**4;**644-653.

For Peer Review

56. Hosokawa Y, Suzuki H, Nakagawa M, Lee TH, Seto M. API2-MALT1 fusion protein induces transcriptional activation of the API2 gene through NF-kappaB binding elements: evidence for a positive feed-back loop pathway resulting in unremitting NF-kappaB activation. *Biochem. Biophys. Res. Commun.* 2005;**334;**51-60.

57. Zhou H, Du MQ, Dixit VM. Constitutive NF-kappaB activation by the t(11;18)(q21;q21) product in MALT lymphoma is linked to deregulated ubiquitin ligase activity. *Cancer Cell* 2005;**7;**425-431.

58. Streubel B, Vinatzer U, Lamprecht A, Raderer M, Chott A. T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. *Leukemia* 2005;**19;**652-658.

59. Wlodarska I, Veyt E, De Paepe P *et al.* FOXP1, a gene highly expressed in a subset of diffuse large B-cell lymphoma, is recurrently targeted by genomic aberrations. *Leukemia* 2005.

60. Fenton JA, Schuuring E, Barrans SL *et al.* t(3;14)(p14;q32) results in aberrant expression of FOXP1 in a case of diffuse large B-cell lymphoma. *Genes Chromosomes. Cancer* 2006;**45;**164-168.

61. Hu H, Wang B, Borde M *et al.* Foxp1 is an essential transcriptional regulator of B cell development. *Nat. Immunol.* 2006;**7;**819-826.

62. Brown PJ, Ashe SL, Leich E *et al.* Potentially oncogenic B-cell activation-induced smaller isoforms of FOXP1 are highly expressed in the activated B cell-like subtype of DLBCL. *Blood* 2008;**111;**2816-2824.

63. Novak A, Akasaka T, Manske M *et al.* Elevated expression of GPR34 and its association with a novel translocation t(X;14)(p11;q32) involving IGHS and GPR34 in MALT lymphoma. *Blood* 2008; **112**[11], 785.

64. Vinatzer U, Gollinger M, Mullauer L *et al.* Mucosa-associated lymphoid tissue lymphoma: novel translocations including rearrangements of ODZ2, JMJD2C, and CNN3. *Clin. Cancer Res.* 2008;**14;**6426-6431.

65. Chanudet E, Ye H, Ferry J *et al.* A20 deletion is associated with copy number gain at the TNFA/B/C locus and occurs preferentially in translocation-negative MALT lymphoma of the ocular adnexa and salivary glands. *J. Pathol* 2009;**217;**420-430.

66. Kim WS, Honma K, Karnan S *et al.* Genome-wide array-based comparative genomic hybridization of ocular marginal zone B cell lymphoma: comparison with pulmonary and nodal marginal zone B cell lymphoma. *Genes Chromosomes. Cancer* 2007;**46;**776-783.

67. Honma K, Tsuzuki S, Nakagawa M *et al.* TNFAIP3 is the target gene of chromosome band 6q23.3-q24.1 loss in ocular adnexal marginal zone B cell lymphoma. *Genes Chromosomes. Cancer* 2008;**47;**1-7.

68. Chanudet E, Huang Y, Ichimura K *et al.* A20 is targeted by promoter methylation, deletion and inactivating mutation in MALT lymphoma. *Leukemia* 2010;**24;**483-487.

69. Novak U, Rinaldi A, Kwee I *et al.* The NF-{kappa}B negative regulator TNFAIP3 (A20) is inactivated by somatic mutations and genomic deletions in marginal zone lymphomas. *Blood* 2009;**113;**4918-4921.

70. Kato M, Sanada M, Kato I *et al.* Frequent inactivation of A20 in B-cell lymphomas. *Nature* 2009;**459;**712-716.

71. Compagno M, Lim WK, Grunn A *et al.* Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* 2009;**459;**717-721.

72. Schmitz R, Hansmann ML, Bohle V *et al.* TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma. *J. Exp. Med.* 2009;**206;**981-989.

73. Honma K, Tsuzuki S, Nakagawa M *et al.* TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. *Blood* 2009.

74. Vereecke L, Beyaert R, Van Loo G. The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. *Trends Immunol.* 2009;**30;**383-391.

For Peer Review

75. Graham RR, Cotsapas C, Davies L *et al.* Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet.* 2008;**40;**1059-1061.

76. Dieguez-Gonzalez R, Calaza M, Perez-Pampin E *et al.* Analysis of TNFAIP3, a feedback inhibitor of nuclear factor-kappaB and the neighbor intergenic 6q23 region in rheumatoid arthritis susceptibility. *Arthritis Res. Ther.* 2009;**11;**R42.

77. Plenge RM, Cotsapas C, Davies L *et al.* Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nat Genet.* 2007;**39;**1477-1482.

78. Musone SL, Taylor KE, Lu TT *et al.* Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. *Nat Genet.* 2008;**40;**1062-1064.

79. Kiss E, Kovacs L, Szodoray P. Malignancies in systemic lupus erythematosus. *Autoimmun. Rev* 2010;**9;**195-199.

80. Boone DL, Turer EE, Lee EG *et al.* The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol.* 2004;**5;**1052-1060.

81. Thome M, Tschopp J. TCR-induced NF-kappaB activation: a crucial role for Carma1, Bcl10 and MALT1. *Trends Immunol.* 2003;**24;**419-424.

82. Lee EG, Boone DL, Chai S *et al.* Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000;**289;**2350-2354.

83. Ott G, Katzenberger T, Greiner A *et al.* The t(11;18)(q21;q21) chromosome translocation is a frequent and specific aberration in low-grade but not high-grade malignant non- Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT-) type. *Cancer Res.* 1997;**57;**3944-3948.

84. Auer IA, Gascoyne RD, Connors JM *et al.* t(11;18)(q21;q21) is the most common translocation in MALT lymphomas. *Ann. Oncol.* 1997;**8;**979-985.

85. Zhou Y, Ye H, Martin-Subero JI *et al.* Distinct comparative genomic hybridisation profiles in gastric mucosa-associated lymphoid tissue lymphomas with and without t(11;18)(q21;q21). *Br. J. Haematol.* 2006;**133;**35-42.

86. Ho L, Davis RE, Conne B *et al.* MALT1 and the API2-MALT1 fusion act between CD40 and IKK and confer NF-kappa B-dependent proliferative advantage and resistance against FAS-induced cell death in B cells. *Blood* 2005;**105;**2891-2899.

87. Hamoudi RA, Appert A, Ye H *et al.* Differential expression of NF-kappaB target genes in MALT lymphoma with and without chromosome translocation: insights into molecular mechanism. *Leukemia* 2010;**24;**1487-1497.

88. Sagaert X, Theys T, Wolf-Peeters C, Marynen P, Baens M. Splenic marginal zone lymphoma-like features in API2-MALT1 transgenic mice that are exposed to antigenic stimulation. *Haematologica* 2006;**91;**1693-1696.

89. Hussell T, Isaacson PG, Crabtree JE, Spencer J. The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to Helicobacter pylori. *Lancet.* 1993;**342;**571-574.

90. Hussell T, Isaacson PG, Crabtree JE, Spencer J. Helicobacter pylori-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B cells in low- grade gastric lymphoma of mucosa-associated lymphoid tissue. *J. Pathol.* 1996;**178;**122-127.

91. Greiner A, Knorr C, Qin Y *et al.* Low-grade B cell lymphomas of mucosa-associated lymphoid tissue (MALT-type) require CD40-mediated signaling and Th2-type cytokines for in vitro growth and differentiation. *Am. J. Pathol.* 1997;**150;**1583-1593.

92. Hussell T, Isaacson PG, Crabtree JE, Dogan A, Spencer J. Immunoglobulin specificity of low grade B cell gastrointestinal lymphoma of mucosa-associated lymphoid tissue (MALT) type. *Am. J. Pathol.* 1993;**142;**285-292.

For Peer Review

93. D'Elios MM, Amedei A, Manghetti M *et al.* Impaired T-cell regulation of B-cell growth in Helicobacter pylori--related gastric low-grade MALT lymphoma. *GASTROENTEROLOGY* 1999;**117;**1105-1112.

94. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 2002;**298;**2199-2202.

95. Craig VJ, Cogliatti SB, Arnold I *et al.* B-cell receptor signaling and CD40 ligand-independent T cell help cooperate in Helicobacter-induced MALT lymphomagenesis. *Leukemia* 2010;**24;**1186-1196.

96. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J. Exp. Med.* 2001;**194;**1861-1874.

97. Feuerhake F, Kutok JL, Monti S *et al.* NFkappaB activity, function, and target-gene signatures in primary mediastinal large B-cell lymphoma and diffuse large B-cell lymphoma subtypes. *Blood* 2005;**106;**1392-1399.

98. Rosenwald A, Wright G, Leroy K *et al.* Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J. Exp. Med.* 2003;**198;**851-862.

99. Savage KJ, Monti S, Kutok JL *et al.* The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood* 2003;**102;**3871-3879.

100. Rajkumar SV, Richardson PG, Hideshima T, Anderson KC. Proteasome inhibition as a novel therapeutic target in human cancer. *J. Clin. Oncol.* 2005;**23;**630-639.

101. Hinz M, Lemke P, Anagnostopoulos I *et al.* Nuclear factor kappaB-dependent gene expression profiling of Hodgkin's disease tumor cells, pathogenetic significance, and link to constitutive signal transducer and activator of transcription 5a activity. *J. Exp. Med.* 2002;**196;**605-617.

102. Horie R. NF-kappaB in pathogenesis and treatment of adult T-cell leukemia/lymphoma. *Int. Rev Immunol.* 2007;**26;**269-281.

103. Lam LT, Davis RE, Pierce J *et al.* Small molecule inhibitors of IkappaB kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. *Clin. Cancer Res.* 2005;**11;**28-40.

104. Ngo VN, Davis RE, Lamy L *et al.* A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* 2006;**441;**106-110.

105. Jost PJ, Ruland J. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. *Blood* 2007;**109;**2700-2707.

106. Lenz G, Davis RE, Ngo VN *et al.* Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science* 2008;**319;**1676-1679.

107. Davis RE, Ngo VN, Lenz G *et al.* Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature* 2010;**463;**88-92.

108. Annunziata CM, Davis RE, Demchenko Y *et al.* Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* 2007;**12;**115-130.

109. Keats JJ, Fonseca R, Chesi M *et al.* Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell* 2007;**12;**131-144.

110. Aldinucci D, Gloghini A, Pinto A, De Filippi R, Carbone A. The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. *J. Pathol* 2010;**221;**248-263.

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FIGURE LEGENDS:

Figure 1. Canonical and noncanonical NF-κB activation pathways. The signalling from the TRAFR, TLR, IL-1R, and antigen receptor activates the canonical NF-κB pathway, which is characterised by activation of the IKK complex, phosphorylation and degradation of IκB. The signalling from CD40, BAFFR and LTβR activates the noncanonical NF-κB pathway, which is featured by activation of NIK, p100 processing and generation of functional active p52. Both canonical and noncanonical pathways are governed by several negative regulators, of which A20 and TRAF3 are typical representatives.

TNFR: tumour necrosis factor receptor; TLR: toll like receptor; IL-1R: interleukin 1 receptor; BCR: B-cell receptor; TCR: T-cell receptor; TRAF: TNF associated factor; RIP1: receptor interacting protein 1; TAK1: transforming growth factor β activating kinase; TAB: TAK binding protein; IKK: inhibitor of NF-κB kinase; NEMO: NF-κB essential modulator; IκB: inhibitor of NF-κB; BAFFR: B cell activating factor receptor; LTβR: lymphotoxin β receptor; NIK: NF-κB inducing kinase. K63Ub: K63 linked ubiquitin chain; K48Ub: K48 linked ubiquitin chain.

Figure 2. Key features of MALT lymphoma associated oncogenes or tumour suppresser genes.

Figure 3. The proposed model of molecular pathogenesis of gastric MALT lymphoma with and without chromosome translocation.

The oncogentic products of t(1;14)(p22;q32)/*BCL10-IGH*, t(14;18)(q32;21)/*IGH-MALT1* and t(11;18)(q21;q21)/*API2-MALT1* are potent activator of the canonical NF-κB activation pathway. They may augment their mediated NF-κB activation by enhanced expression of surface receptors TLR6 and CCR2, as well as proteolytic cleavage of the negative inhibitor A20. These oncogenic products may also potentially cooperate with the signalling from BCR, BAFFR and CD40, via helps of bystander T-cells generated in the *H. pylori* mediated reactive component.

The growth of translocation negative MALT lymphoma is largely driven by *H. pylori* generated immune responses including signalling from CD40 and CD86 through bystander T cell helps, and direct triggering of TLR and BCR by *H. pylori* associated lipopolysaccharides and autoantigen respectively. This underscores that most of translocation negative gastric MALT lymphomas can be cured by *H. pylori* eradication.

TLR: toll like receptor; BCR: B-cell receptor; MAPK: MAP kinafe; IκB: inhibitor of NF-κB; BAFFR: B cell activating factor receptor; K48Ub: K48 linked ubiquitin chain.

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Figure 1

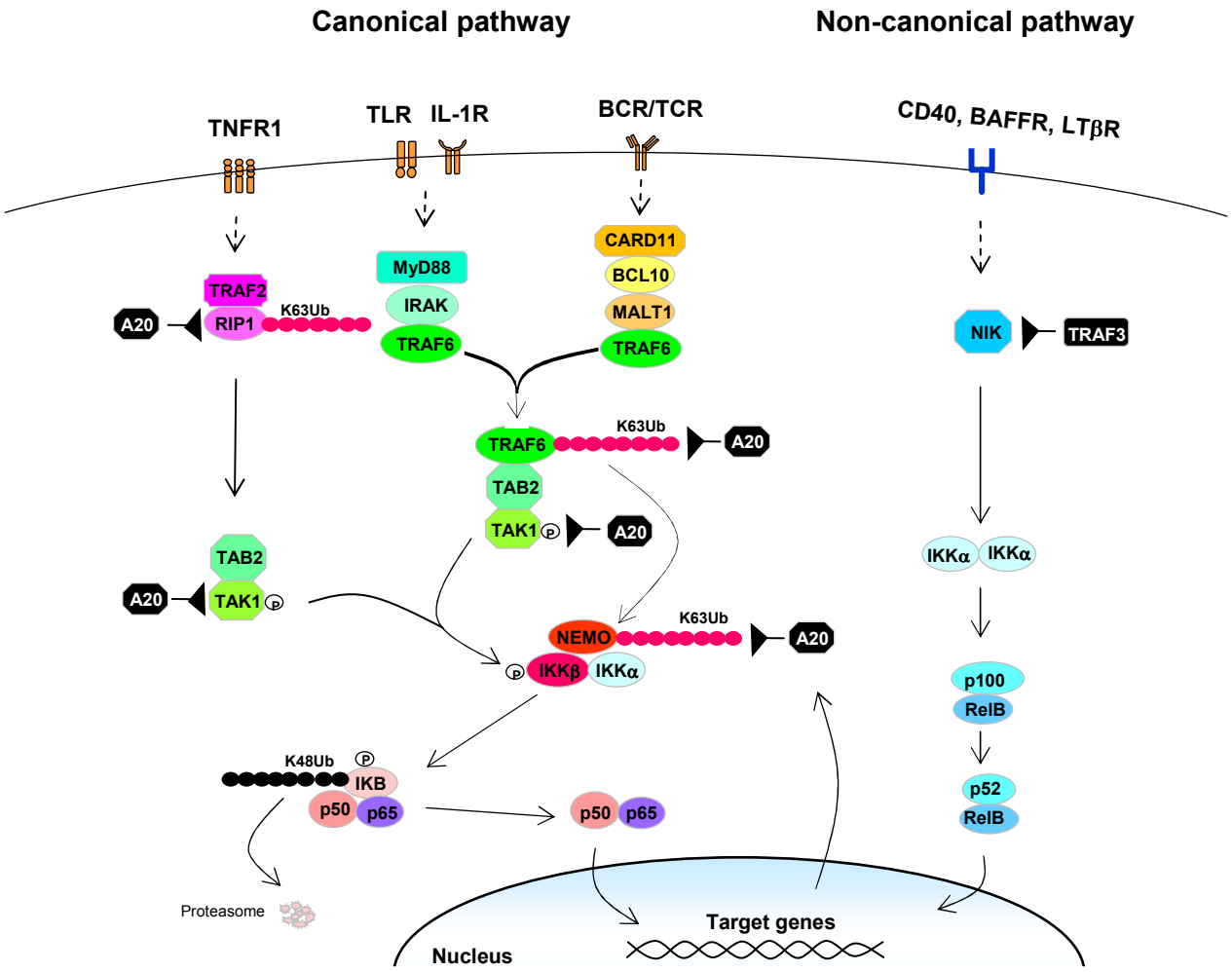

Canonical pathway
Non-canonical pathway
TNFR1
TLR
IL-1R
BCR/TCR
CD40, BAFFR, LTβR
TRAF2
RIP1
K63Ub
A20
MyD88
IRAK
TRAF6
CARD11
BCL10
MALT1
TRAF6
NIK
TRAF3
TRAF6
K63Ub
A20
TAB2
TAK1
A20
TAB2
TAK1
A20
NEMO
K63Ub
A20
IKKβ
IKKα
IKKα
IKKα
p100
RelB
p52
RelB
K48Ub
IKB
p50
p65
p50
p65
Proteasome
Target genes
Nucleus

Figure 2

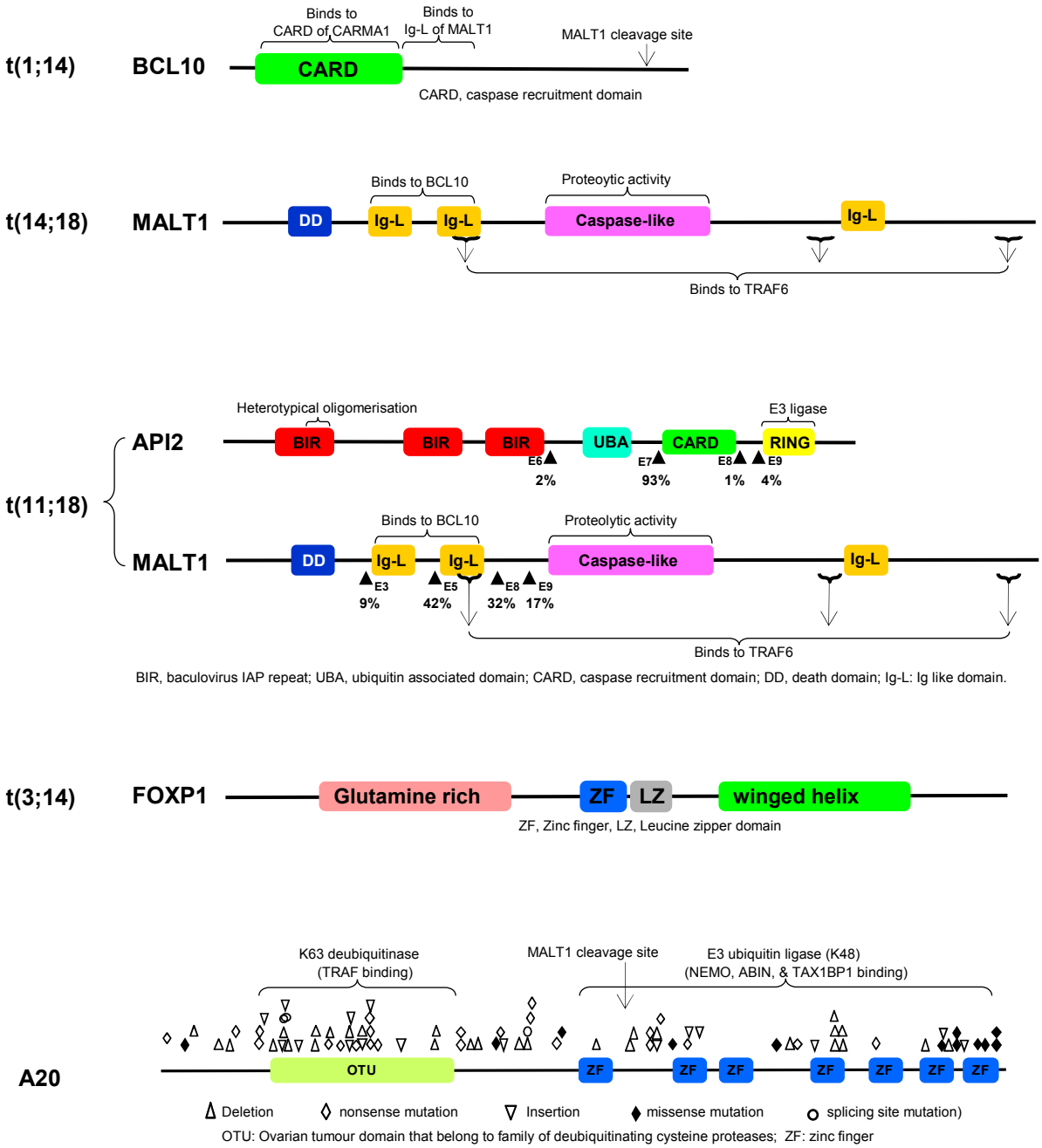
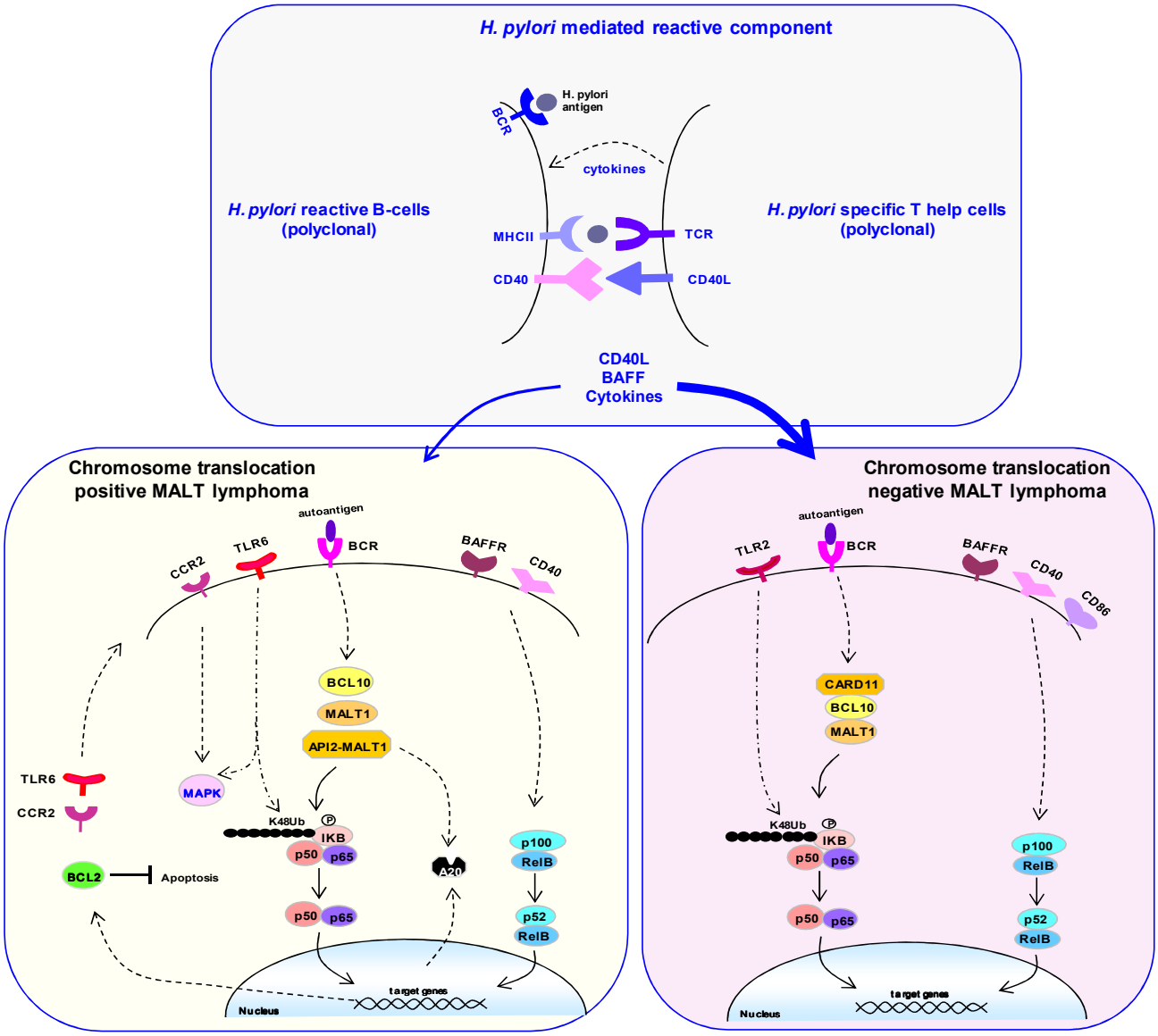

t(1;14)
BCL10
Binds to CARD of CARMA1
Binds to Ig-L of MALT1
MALT1 cleavage site
CARD
CARD, caspase recruitment domain
t(14;18)
MALT1
Binds to BCL10
Proteoytic activity
DD
Ig-L
Ig-L
Caspase-like
Ig-L
Binds to TRAF6
t(11;18)
API2
Heterotypical oligomerisation
E3 ligase
BIR
BIR
BIR
UBA
CARD
RING
E6
2%
E7
93%
E8
1%
E9
4%
MALT1
Binds to BCL10
Proteolytic activity
DD
Ig-L
Ig-L
Caspase-like
Ig-L
E3
9%
E5
42%
E8
32%
E9
17%
Binds to TRAF6
BIR, baculovirus IAP repeat; UBA, ubiquitin associated domain; CARD, caspase recruitment domain; DD, death domain; Ig-L: Ig like domain.
t(3;14)
FOXP1
Glutamine rich
ZF
LZ
winged helix
ZF, Zinc finger, LZ, Leucine zipper domain
A20
K63 deubiquitinase (TRAF binding)
MALT1 cleavage site
E3 ubiquitin ligase (K48) (NEMO, ABIN, & TAX1BP1 binding)
OTU
ZF
ZF
ZF
ZF
ZF
ZF
ZF
Deletion
nonsense mutation
Insertion
missense mutation
splicing site mutation)
OTU: Ovarian tumour domain that belong to family of deubiquitinating cysteine proteases; ZF: zinc finger

Figure 3


H. pylori mediated reactive component
H. pylori antigen
BCR
cytokines
H. pylori reactive B-cells (polyclonal)
MHCII
TCR
CD40
CD40L
H. pylori specific T help cells (polyclonal)
CD40L
BAFF
Cytokines
Chromosome translocation positive MALT lymphoma
autoantigen
CCR2
TLR6
BCR
BAFFR
CD40
BCL10
MALT1
API2-MALT1
TLR6
CCR2
MAPK
K48Ub
IKB
p50
p65
BCL2
Apoptosis
A20
p100
RelB
p52
RelB
target genes
Nucleus
Chromosome translocation negative MALT lymphoma
autoantigen
TLR2
BCR
BAFFR
CD40
CD86
CARD11
BCL10
MALT1
K48Ub
IKB
p50
p65
p100
RelB
p52
RelB
target genes
Nucleus