MECHANISMS OF B-CELL LYMPHOMA PATHOGENESIS

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Abstract | Chromosomal translocations involving the immunoglobulin loci are a hallmark of many types of B-cell lymphoma. Other factors, however, also have important roles in the pathogenesis of B-cell malignancies. Most B-cell lymphomas depend on the expression of a B-cell receptor (BCR) for survival, and in several B-cell malignancies antigen activation of lymphoma cells through BCR signalling seems to be an important factor for lymphoma pathogenesis. Recent insights into the lymphomagenic role of factors supplied by the microenvironment also offer new therapeutic strategies.

CD79A AND CD79B
Components of the B-cell receptor that mediate signalling following crosslinking.

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In the Western world, about 20 new cases of lymphoma are diagnosed per 100,000 people per year[1]. About 95% of the lymphomas are of B-cell origin, the rest are T-cell malignancies. This might be surprising at first glance, given the similar frequency of B and T cells in the human body, but is understandable considering the specific factors that influence the pathogenesis of B-cell lymphomas. About 15 types of B-cell lymphoma are distinguished in the current World Health Organization lymphoma classification[2] (TABLE 1). The distinction of these lymphomas is not only relevant in terms of lymphoma pathogenesis, but also regarding the consequences for treatment of the patients. This is because the various types of B-cell lymphoma can have very different clinical behaviours, and therefore require diverse treatment strategies.

Exciting progress has been made in the past 20 years to elucidate the cellular origin of human B-cell lymphomas and the identification of key transforming events, in particular the role of chromosomal translocations in lymphoma pathogenesis. However, it is becoming clear that B-cell tumours are not as autonomous as previously thought — key factors that are crucial for normal B-cell differentiation and survival are also required for the malignant growth of most B-cell lymphomas. What is the cellular origin of B-cell lymphomas and what are the main transforming events? How do antigen activation of the B-cell receptor (BCR) and the cellular microenvironment contribute to the pathogenesis of B-cell lymphomas?

Cellular origin of B-cell lymphomas

B-cell development takes place in distinct differentiation steps that are characterized by the specific structure of the BCR. The BCR is composed of two identical heavy-chain and two identical light-chain immunoglobulin (Ig) polypeptides that are covalently linked by disulphide bridges. Other components of the BCR are the CD79A AND CD79B molecules, which contain cytoplasmic immunoreceptor tyrosine-based activation motifs. These motifs transmit signals following BCR crosslinking. The intracellular signalling components activated by BCR crosslinking include several tyrosine kinases. Depending on the differentiation stage of the B cell that recognizes an antigen and on the activation of other B-cell surface receptors that modulate BCR signalling, the activated B cell might be induced to proliferate and/or undergo further differentiation steps[3].

Early B-cell development, which occurs in the bone marrow, concludes when a B-cell precursor successfully rearranges Ig heavy- and light-chain genes and is equipped with a functional surface antigen receptor (FIG. 1). Cells that express a functional (and non-autoreactive) BCR differentiate into mature naive B cells and leave the bone marrow, whereas B-cell precursors that fail to express a BCR undergo apoptosis[3]. Mature naive B cells can be activated by antigen binding to the BCR and participate in immune responses. In T-cell-dependent immune responses, antigen-activated B cells undergo clonal expansion in structures called 'germinal centres'

Summary

- A hallmark of many types of B-cell lymphoma is reciprocal chromosomal translocations involving one of the immunoglobulin loci and a proto-oncogene. As a consequence of such translocations, the oncogene comes under the control of an active immunoglobulin locus, causing deregulated, constitutive expression of the translocated gene.
- Normal B cells depend on B-cell receptor (BCR) expression for survival. The selection for expression of a BCR also seems to operate in most malignant B cells.
- Although there is strong evidence that most B-cell lymphomas depend on BCR expression, there are a few exceptions — namely classical Hodgkin's lymphoma, primary mediastinal B-cell lymphoma, some post-transplant lymphomas, and the rare primary effusion lymphomas.
- In several lymphomas, there is a strong indication that the lymphoma cells recognize an antigen and that stimulation by antigen binding contributes to the survival and proliferation of lymphoma cells.
- In many lymphomas, such as follicular lymphoma, mucosa-associated lymphoid tissue lymphomas and classical Hodgkin's lymphoma, the tumour microenvironment seems to be important for the survival and/or proliferation of the lymphoma cells.
- The recognition that the survival and/or proliferation of many B-cell lymphomas depends on their interaction with other cells in the microenvironment, as well as on expression of the B-cell receptor and, sometimes, antigen activation, might lead to novel treatment options for B-cell lymphomas.

(GCs), where the the Ig genes are modified by somatic hypermutation and class-switch recombination (FIGS 1,2).

As distinct stages of B-cell development and differentiation are characterized by the particular structure of the BCR and expression patterns of differentiation markers, and as these processes often take place in specific histological structures, analysis of these features was used to determine the origin of the various human B-cell lymphomas[4,5] (TABLE 1). The rationale for such a classification of B-cell lymphomas is based on the observation that malignant B cells seem to be 'frozen' at a particular differentiation stage, which reflects their origin[4,6,7]. One of the main concepts emerging from these studies is that most types of B-cell lymphoma are derived from GC or post-GC B cells[4,5] (BOX 1).

The cellular origin of B-cell lymphomas was further clarified, and previously unrecognized distinct lymphoma subtypes were also identified, by gene-expression profiling of human B-cell lymphomas and normal B-cell subsets. Such studies identified, for example, a GC B-cell gene-expression signature that is associated with follicular lymphoma, Burkitt's lymphoma and a subset of diffuse large B-cell lymphomas[8]. These findings supported the GC B-cell origin of these tumours. Gene-expression profiling studies of other malignancies also revealed unexpected relationships, in terms of gene-expression patterns. For example, in addition to B-cell chronic lymphocytic leukaemia (B-CLL) cells with mutated Ig variable (V)-region genes, B-CLL cells with unmutated Ig V-region genes showed greatest similarity to memory B cells that had undergone somatic hypermutation, indicating that both subtypes of B-CLL are related to memory B cells[9]. Moreover, a subset of diffuse large B-cell lymphomas was identified that, among the various B-cell subsets included in the analysis, most closely resembled *in-vitro*-activated B cells[8]. In these cancer cells, the transformation process might have been associated with an alteration of the gene-expression profile, masking the signature of the cell of origin, as seems to be the case in classical Hodgkin's lymphoma (see below). It is also possible that the normal B-cell counterpart of some cancer types might not have been identified yet. In the activated B-cell type of diffuse large B-cell lymphoma, the normal counterpart could be a poorly defined, small subset of GC B cells that is undergoing plasmacytoid differentiation, or a post-GC immunoblast population[7].

Transforming events

Reciprocal chromosomal translocations involving one of the Ig loci and a proto-oncogene are a hallmark of many types of B-cell lymphoma[10,11] (TABLE 2). As a consequence of such translocations, the oncogene comes under the control of the active Ig locus, causing a deregulated, constitutive expression of the oncogene. Three types of breakpoints can be distinguished in the Ig loci. Some translocations, such as the *BCL2–IgH* translocation associated with follicular lymphoma, have breakpoints that are directly adjacent to Ig heavy chain J-region (J_H) gene segments or that are adjacent to regions where the Ig heavy chain D-region (D_H) joins the J-region (D_HJ_H) (FIG. 1). As the breakpoints also often show loss of nucleotides at the end of the J_H or D_H segments and the addition of non-germline-encoded nucleotides — typical features of V(D)J recombination — it is likely that these translocations happen as mistakes during V(D)J recombination in early B-cell development in the bone marrow[12–14]. In other translocations, the breakpoints are found within or adjacent to rearranged V(D)J genes, and these V-region genes are always somatically mutated. These and additional features indicate that such translocations occur as by-products of the somatic hypermutation process[10,15], which is associated with DNA strand breaks[15–17]. The third type of translocation is characterized by breakpoints in the *IgH* constant region switch regions, in which DNA breaks are introduced during class switching. This indicates that these events occur during class-switch recombination.

The causes for the generation of DNA strand breaks in the oncogenes involved in Ig-associated translocations are less clear[10]. Some of these genes, however, undergo aberrant somatic hypermutation, and therefore acquire DNA strand breaks in the same regions where the chromosomal breakpoints are located (see below)[18]. Regarding the *BCL2–IgH* translocations associated with follicular lymphoma, it was recently shown that the DNA in the major breakpoint region of the *BCL2* gene often acquires an altered structure that is cut by the RAG nucleases, which mediate V(D)J recombination. This finding indicates that in these translocations, RAG-mediated DNA cleavage is responsible for the DNA breaks in both partners involved in the translocation[19]. RAG enzymes might also be involved in chromosomal translocations through another mechanism — RAGs have been shown to possess transposase activity, so some translocation events could be explained by double-ended transposition events[20,21].

Table 1 | **Human mature B-cell lymphomas**

Lymphoma	Features	Frequency among lymphomas (%)*	Proposed cellular origin
B-cell chronic lymphocytic leukaemia (B-CLL)	Leukaemia of small B cells that express the CD5 antigen, involving peripheral-blood and bone-marrow cells. Common in elderly patients. Called 'small lymphocytic lymphoma' when lymph-node cells are predominantly involved. Patients with leukaemia cells that lack variable (V)-region gene mutations have a worse prognosis than patients with mutations in V-region genes.	7	Memory B cell? Naive B cell? Marginal-zone B cell?
Mantle-cell lymphoma	Lymphoma arises from cells that populate the mantle zone of follicles, express CD5 and show aberration in cyclin-D1 expression. Nearly all cases are associated with *BCL1–IgH* translocation.	5	$CD5^+$ mantle-zone B cell
B-cell prolymphocytic leukaemia	Chronic B-cell malignancy related to B-CLL. Over 50% of cancer cells represent prolymphocytes (large lymphocytes with clumped chromatin and prominent nucleolus).	<1	Memory B cell
Follicular lymphoma	A nodal lymphoma with a follicular growth pattern. Lymphoma cells morphologically and phenotypically resemble GC B cells. Most cases are associated with *BCL2–IgH* translocation.	20	GC B cell
Hairy-cell leukaemia	Chronic B-cell malignancy involving spleen and bone marrow. Very few circulating leukaemia cells. Tumour cells form 'hairy' projections.	<1	Memory B cell
MALT lymphoma	Extranodal marginal-zone B-cell lymphoma. Develops mostly in aquired lymphoid structures.	7	Marginal-zone B cell
Nodal marginal-zone lymphoma	Lymphoma with primary presentation in lymph nodes. Lymphoma cells resemble marginal-zone or monocytoid B cells, but often have heterogenous cytology, which ranges from small to large lymphocytes and includes plasma cells.	2	Marginal-zone B cell? Monocytoid B cell?
Splenic marginal-zone lymphoma	Micronodular lymphoid infiltration in the splenic white pulp. Mostly small IgD^+ lymphoma cells that replace normal follicles and the marginal-zone region. Frequently involves infiltration into bone marrow and circulation.	1	Subset of naive B cells that have partially differentiated into marginal-zone B cells?
Burkitt's lymphoma	Fast growing. Mostly extranodal. Characterized by a *MYC–Ig* translocation. Patients with endemic form are EBV-positive in nearly all cases. Patients with sporadic form are EBV-positive in about 30% of cases.	2	GC B cell
Diffuse large B-cell lymphoma	Heterogenous group of lymphomas characterized by large B cells. Several subtypes are recognized. Morphological variants include centroblasts and immunoblasts.	30–40	GC or post-GC B cell
Primary mediastinal B-cell lymphoma	Subtype of diffuse large B-cell lymphoma located in the mediastinum. Tumour cells are large B cells but also show a number of similarities to Reed–Sternberg cells of classical Hodgkin's lymphoma. Most frequently occurs in young women.	2	Thymic B cell
Post-transplant lymphoma	Mostly of the diffuse large-cell lymphoma type. Lymphomas that arise in patients after organ transplantation. Immunosuppressive treatment confers risk of uncontrolled proliferation of EBV-infected B cells that can develop into lymphomas.	<1	GC B cell
Primary effusion lymphoma	Frequently occurs in patients with AIDS or patients who have received organ transplants. Lymphoma cells are found as effusions in serous cavities, such as pleura, pericardium or peritoneum.	<0.5	(Post) GC B cell
Lymphoplasmacytic lymphoma	Involves lymph nodes, bone marrow and spleen. The tumour-cell population is composed of small B cells, plasmacytoid lymphocytes and plasma cells. Most patients present with a serum monoclonal protein, usually of the IgM type.	1	(Post) GC B cell
Multiple myeloma	Neoplastic proliferation of plasma cells in the bone marrow.	10	Plasma cell
Classical Hodgkin's lymphoma	Characterized by bizarre, large tumour cells. Hodgkin and Reed–Sternberg cells account for less than 1% of cells in the tumour, and are admixed with various non-neoplastic cell types. Tumour cells show a phenotype not characteristic of any normal haematopoietic cell type.	10	Defective GC B cell
Lymphocyte-predominant Hodgkin's lymphoma	Rare indolent subtype of Hodgkin's lymphoma. Lymphoma cells show a B-cell phenotype, represent a small population in the tissue, and grow in association with follicular dendritic cells and T-helper cells. Good prognosis.	0.5	GC B cell

*These numbers refer to the frequencies in Europe and North America. AIDS, acquired immune deficiency syndrome; EBV, Epstein–Barr virus; Ig, immunoglobulin; MALT, mucosa-associated lymphoid tissue; GC, germinal centre.

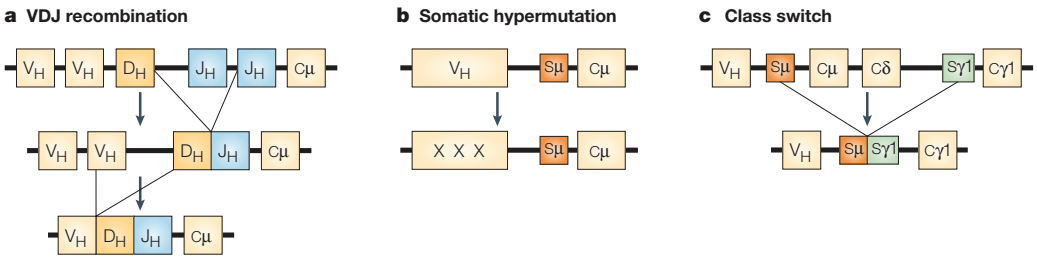

Figure 1 | **Molecular processes that remodel immunoglobulin genes.** Immunoglobulins (Igs) are expressed by B cells and consist of variable (V) regions, which interact with antigen, and constant (C) regions, which mediate the effector functions of Igs. To create a functional Ig, B cells must rearrange DNA segments that encode the heavy (H)- and light-chain (not shown) regions of the variable genes. **a** | First, through a process called 'V(D)J recombination', three gene segments, V_H, D_H and J_H, are joined to encode the H-chain variable region. The V regions of the κ- and λ-light chains, alternatively, are each encoded by two gene segments — the V_L and J_L genes (not shown). B-cell precursors first carry out D_H–J_H rearrangements in H-chain genes. These D_H–J_H rearrangements are followed by V_H–D_HJ_H rearrangements, resulting in the expression of a pre-B-cell receptor if the rearrangement is productive[3]. About 50 functional V_H gene segments, 27 D_H segments and 6 J_H segments are available in the germline, allowing the generation of a diverse repertoire of V_H gene rearrangements. The diversity is further increased by the addition or removal of nucleotides at the joining sites of the gene segments[3]. The cells then carry out rearrangements at their L-chain loci (not shown). The V-region of the Ig gene is ultimately connected to the C-region of the Ig gene (Cμ of IgM in diagram) **b** | The process of somatic hypermutation is activated when B cells reach the germinal centre (GC, shown in more details in FIG. 2). This process leads to the introduction of point mutations, deletions or duplications in the rearranged V-region of Ig genes (denoted by 'Xs' in the figure)[102]. These mutations occur in the V-region of Ig genes — not in the downstream Cμ region. **c** | Class switching results in the replacement of the originally expressed H-chain C-region gene with that of another Ig gene. In the diagram, the C-region for IgM (Cμ) and IgD (Cδ) are exchanged for the C-region of IgG (Cγ1) by recombination at the switch regions for these genes (Sμ and Sγ1, respectively). This results in an antibody with different effector functions but the same antigen-binding domain.

The process of somatic hypermutation contributes to lymphoma pathogenesis not only by causing chromosomal translocations, but probably also by targeting non-Ig genes. Two situations have to be distinguished. The genes encoding BCL6 and CD95 (also known as FAS) were found to contain mutations in a considerable fraction of normal GC and memory B cells, indicating that these genes are often targeted by the hypermutation machinery in normal B cells[22–24]. In rare instances, such mutations might promote the development of lymphomas. For example, inactivating mutations of *CD95* are found in about 20% of (post) GC B-cell lymphomas and could protect lymphoma cells from death induction by CD95-ligand-expressing cells[25]. In the case of the *BCL6* gene, the frequent occurrence of hypermutation might also cause translocations of this gene into Ig- as well as non-Ig-encoding loci. This possibility was indicated by the finding that the 5′ region of *BCL6*, which is the site of hypermutation, is also the region where chromosomal-translocation breakpoints are mostly found[18,23]. In diffuse large B-cell lymphomas, aberrant hypermutation of multiple oncogenes has been reported, which might also represent an important mechanism of pathogenesis[18].

Two of the molecular processes that could cause chromosome translocations or mutations in non-Ig genes occur exclusively (or at least mainly) in the GC — somatic hypermutation and class-switch recombination[26]. This could be one of the reasons that most B-cell lymphomas derive from GC B cells or their descendents. Class switching and somatic hypermutation do not occur in the DNA of T cells, which could also partly explain why B cells are more prone to undergo malignant transformation than T cells.

Whereas chromosome translocations involving Ig loci are clearly a hallmark of many types of B-cell lymphoma, many other transforming events have also been implicated in the pathogenesis of lymphomas, such as mutations in tumour-suppressor genes (such as *TP53* and the gene encoding IκBα), genomic amplifications (such as *REL*) and translocations not involving Ig loci (*API2*–*MALT1*) (TABLE 2).

Finally, viruses might also be involved in the transformation of B cells. The most well-known example is Epstein–Barr virus (EBV), which is found in nearly all endemic Burkitt's lymphomas, in many post-transplant and primary effusion lymphomas, and in about 40% of cases of classical Hodgkin's lymphoma (see REFS 27–30 for reviews) (TABLE 2). Another member of the herpes-virus family, human herpes virus 8, is implicated in the pathogenesis of primary effusion lymphomas[31]. The oncogenic features of herpes virus 8 are not well understood, but it was recently shown that the viral protein FLIP activates the transcription factor NF-κB, which is an important survival factor in primary effusion lymphoma cells[32].

Role of the BCR in B-cell lymphomas

Role of the BCR in the survival of normal B cells. Throughout their lives, B cells undergo stringent selection for expression of the appropriate BCR. Pre-B cells are selected for a pre-BCR (composed of Ig heavy chains and surrogate light chains), and immature B cells are selected for expression of a non-autoreactive, functional BCR. After these steps, GC B cells are only able to survive the GC reaction and differentiate into memory or plasma cells if somatic mutations in their V-region genes result in expression of a BCR with increased affinity for a

CD95
Cell-surface receptor that mediates apoptosis signalling.

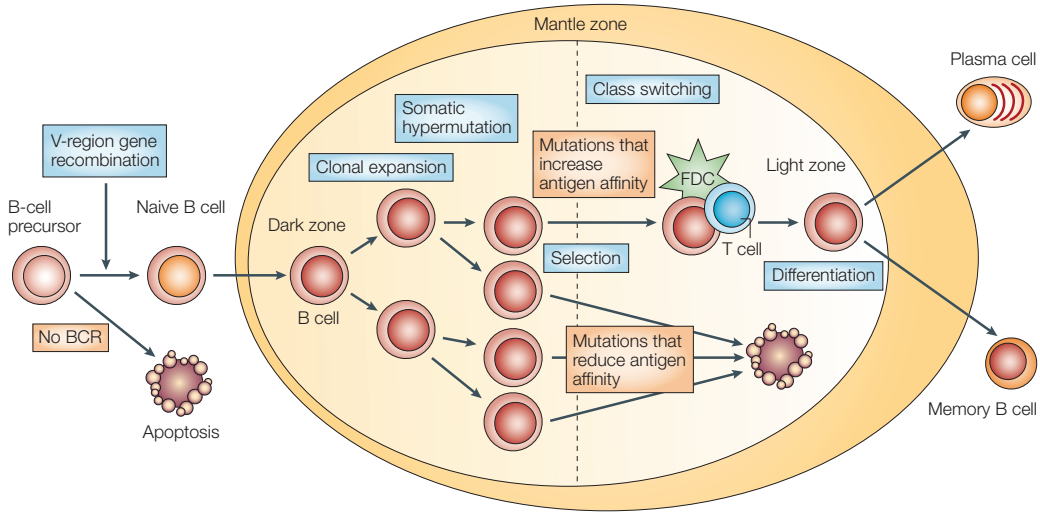

Figure 2 | **B-cell differentiation in the germinal-centre reaction.** Mature (naive) antigen-activated B cells that receive signals known as 'T-cell help' are driven into primary B-cell follicles in secondary lymphoid organs such as lymph nodes, where they establish germinal centres (GCs; lightest yellow region) [103]. The naive IgM^+IgD^+ B cells that constitute the primary B-cell follicle are replaced by the proliferating GC B cells and displaced to the outside of the follicle, where they form a mantle zone around the GC. In the GC, a dark zone and a light zone can be distinguished (left and right sides, respectively). The dark zone mainly consists of proliferating GC B cells, whereas the GC B cells in the light zone are resting[103]. In proliferating GC B cells, the process of somatic hypermutation is activated, which leads to the introduction of mutations at a high rate into the rearranged Ig variable (V)-region genes of the B cells[102]. Most mutations are disadvantagous for the cells— such as those that lead to reduced affinity of the BCR for antigen and cause cells to undergo apoptosis. A few GC B cells will acquire mutations in the BCR that increase their affinity for antigen, and these cells will be positively selected. The selection process presumably takes mainly place in the light zone, where the GC B cells are in close contact with $CD4^+$ T cells and follicular dendritic cells (FDCs). A fraction of these GC B cells undergo class-switch recombination[104]. Finally, GC B cells differentiate into memory B cells or plasma cells and leave the GC microenvironment.

cognate antigen[3]. Even mature resting B cells are constantly under selective pressure to express the BCR — ablation of BCR expression in mice leads to the apoptotic death of BCR-negative B cells[33,34]. So, it seems that this BCR dependency is a main determinant of B-cell survival. It is still debated whether the survival signal supplied by the BCR is an autonomous signal or is initiated by low-level BCR activation by antigen.

BCR dependency of B-cell lymphomas. The selection for expression of a BCR also seems to occur in malignant B cells. Indeed, most B-cell lymphomas still express a BCR, although sometimes at relatively low levels[35–37] (BOX 2). The proposal that there is a need for BCR-derived survival signals is indirectly supported by the observation that translocations into the Ig-loci are virtually always found on the non-productively rearranged Ig loci, with a few exceptions[38]. As the three Ig-gene-remodelling processes that are implicated in the generation of these translocations — V-region gene recombination, class switching and somatic hypermutation (FIG. 1) — principally occur in both Ig alleles, translocation events should happen at nearly equal frequency on the expressed Ig allele and the non-expressed allele. However, as the expressed Ig alleles are not found to be inactivated by translocation events, it seems that at least at the time that the translocations happened, the inability to form a BCR was incompatible with survival of the cells and development into a B-cell tumour.

Further evidence that the BCR supplies important survival signals to B-cell lymphoma cells is provided by the observation that treatment of patients who have follicular lymphoma with ANTI-IDIOTYPIC ANTIBODIES did not result in the emergence of BCR-negative lymphoma variants — either through downregulation of BCR expression or by selected outgrowth of clones with inactivating Ig V-region gene mutations[39,40]. Finally, several types of lymphoma show ongoing V-region gene mutation during tumour clone expansion[39,41–44]. As a considerable fraction of mutations would interfere with BCR expression or function, such as nonsense mutations or replacement mutations that prevent proper heavy- and light-chain pairing, it is notable that such lymphomas also retain BCR expression[35,37]. Indeed, it has been determined that two types of destructive somatic mutation — nonsense mutations and deletions or duplications causing reading-frame shifts — account for nearly 10% of mutation events, if mutations accumulate under non-selective conditions[15,45]. So, the rare occurence of BCR-loss variants of lymphomas with ongoing somatic hypermutation, such as follicular lymphoma, Burkitt's lymphoma, lymphocyte-predominant Hodgkin's lymphoma or mucosa-associated lymphoid tissue (MALT) lymphomas, is a strong indication that lymphoma cells undergo selection for BCR expression. Therefore, the survival signals supplied by BCR expression in normal B cells might also promote survival of B-cell lymphoma cells.

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