LYMPHOID MALIGNANCIES: THE DARK SIDE OF B-CELL DIFFERENTIATION

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When the regulation of B-cell differentiation and activation is disrupted, lymphomas and leukaemias can occur. The processes that normally create immunoglobulin diversity might be misdirected, resulting in oncogenic chromosomal translocations that block differentiation, prevent apoptosis and/or promote proliferation. Prolonged or unregulated antigenic stimulation might contribute further to the development and progression of some malignancies. Lymphoid malignancies often resemble normal stages of B-cell differentiation, as shown by molecular techniques such as gene-expression profiling. The similarities and differences between malignant and normal B cells indicate strategies for the treatment of these cancers.

DECISION MAKING IN THE IMMUNE SYSTEM

V(D)J RECOMBINATION
The somatic rearrangement of variable (V), diversity (D) and joining (J) regions of antigen-receptor genes, which leads to the repertoire diversity of both

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doi:10.1038/nri953

Normal lymphocyte differentiation is, in some sense, a disaster waiting to happen. B cells put their genomic integrity in danger during the formation and revision of their antigen receptors. A second potentially dangerous event is the response to antigen. When this response functions normally, the clonal expansion of B cells is regulated tightly by homeostatic controls. However, chronic infections can wreak havoc on lymphocyte homeostasis, as can abnormal responses to self-antigens, and both of these mechanisms might contribute to lymphoid malignancies. Finally, many of the oncogenic events that occur in lymphomas and leukaemias disrupt the molecular pathways that regulate B-cell differentiation, proliferation and apoptosis.

A confounding issue in the study of human lymphoid malignancies has been imprecision of diagnosis. Recent studies using gene-expression profiling and genomic mutational analysis have shown that lymphomas and leukaemias that are difficult to distinguish histologically can nevertheless be molecularly distinct diseases. Using more-precise disease definitions, malignancies can be related often to distinct stages of B-cell differentiation. In this review, we focus on advances in the molecular definition of mature B-cell malignancies and discuss how the relationship between a lymphoma and its normal B-cell counterpart might be exploited to understand and treat these cancers. We discuss also how oncogenic alterations in these cancers subvert homeostatic regulation of lymphocyte responses.

The perils of normal B-cell differentiation

The first dangerous hurdle in B-cell differentiation is rearrangement of the immunoglobulin genes of B-cell precursors in the bone marrow to form a B-cell receptor (BCR). This molecular process, V(D)J RECOMBINATION, involves double-stranded DNA breaks that are initiated by recombination-activating genes (RAG1 and RAG2) and resolved by the non-homologous end-joining repair apparatus[1]. Occasionally, these breaks are resolved aberrantly, leading to chromosomal translocations. In lymphomas, chromosomal translocations typically replace the normal regulatory sequence of a gene with heterologous regulatory elements that drive inappropriate gene expression near the breakpoints. Clear examples of such mistakes in V(D)J recombination are t(14;18) — that is, a translocation between chromosomes 14 and 18 — which involves the *BCL2* gene and the immunoglobulin heavy-chain (IgH) locus in follicular lym-

T- and B-cell receptors.

GERMINAL CENTRE
The structure that is formed by the clonal expansion of antigen-activated B-cell blasts that have migrated to the follicles of lymph nodes. The B cells in these structures proliferate and their immunoglobulin genes undergo somatic hypermutation, before the cells leave as plasma cells or

phoma (BOX 1), and t(11;14), which involves the gene encoding cyclin D1 and the IgH locus in mantle-cell lymphoma (BOX 1). The structures of the recombination breakpoints in these translocations are consistent with RAG-mediated cleavage of the IgH locus, guided by recombination signal sequences (RSSs). However, the recombination sites in the partner genes lack clear RSSs and do not have cryptic sequences that could function as RSSs[2], although they sometimes involve DNA regions with altered structure[3]. The IgH breaks of t(11;14) in mantle-cell lymphoma seem to occur before heavy-chain diversity and joining segment (D_H–J_H) rearrangement, which indicates that this translocation occurs early in B-cell differentiation[4]. As RAGs are not expressed after the immature B-cell stage[5,6], t(14;18) might occur in a pre-GERMINAL CENTRE (GC) B cell as well.

Box 1 | Mature B-cell malignancies

Follicular lymphoma
An often indolent B-cell lymphoma with a follicular growth pattern. Most are characterized by the overexpression of BCL-2, owing to t(14;18). They comprise ~22% of non-Hodgkin lymphomas (NHLs). They cannot be cured by conventional chemotherapy and the survival rate is 73% at 10 years.

Mantle-cell lymphoma
A B-cell lymphoma that localizes to the mantle region of secondary follicles. Mantle-cell lymphoma (MCL) is associated with t(11;14), which results in the overexpression of cyclin D1. MCLs comprise 6% of all NHLs, have a male predominance and occur at a median age of 60. With current chemotherapy regimens, patients with MCL can achieve complete remission, but long-term remission is rare and median survival is 3–5 years.

Burkitt lymphoma
An aggressive B-cell lymphoma of children and young adults that is associated invariably with translocations of *c-MYC*. The endemic form involves Epstein–Barr virus (EBV) infection of malignant cells, whereas the sporadic form is EBV independent. These lymphomas can be cured in more than 80% of cases.

Multiple myeloma
An incurable malignancy of plasma cells with a median survival of three years. Multiple myeloma constitutes ~10% of all haematological malignancies, with a median age at diagnosis of ~65. Neoplastic cells are located in the bone marrow, and osteolytic bone lesions are characteristic. Reciprocal chromosomal translocations between one of the immunoglobulin loci and various other genes, including those that encode cyclin D1, cyclin D3, c-MAF, MMSET (multiple myeloma SET-domain protein) or fibroblast growth factor receptor 3 (FGFR3), are considered to be primary oncogenic events.

Diffuse large B-cell lymphoma
Diffuse large B-cell lymphoma (DLBCL) is the most common type of NHL (30–40% of cases). Up to one third of cases have abnormalities of *BCL6*, and ~20% of cases have translocations of *BCL2*. DLBCLs are clinically, morphologically and molecularly heterogeneous. 40% of patients with DLBCL can be cured by conventional chemotherapy.

Hodgkin lymphoma
This type of lymphoma accounts for ~10% of all lymphoid malignancies, and it usually arises in the lymph nodes of young adults. It can be subdivided into a classical subtype and a less common nodular lymphocyte predominant subtype. Cure rates of more than 80% can be achieved with present therapies.

Lymphoplasmacytic lymphoma
This is a rare form of NHL that comprises ~1.5% of nodal lymphomas. It is usually indolent and frequently involves bone marrow, lymph nodes and spleen. Most patients have monoclonal immunoglobulin M in their serum, and the tumour cells have a plasmacytic morphology. A subset of lymphoplasmacytic lymphomas is characterized by recurrent t(9;14), which involves the *PAX5* (paired box gene 5) and immunoglobulin heavy-chain loci.

Marginal-zone lymphoma
This extranodal lymphoma occurs in organs that normally lack organized lymphoid tissue (such as the stomach, salivary glands, lungs and thyroid glands), and it comprises 7–8% of all B-cell lymphomas. In many cases, chronic inflammation or an autoimmune process precedes development of the lymphoma. Gastric mucosal-associated lymphoid tissue (MALT) lymphoma, the most common type, is associated with *Helicobacter pylori* infection, and 70% of patients at early stages have complete remission after eradication of this bacterium. At later stages, the acquisition of genetic abnormalities might lead to *H. pylori*-independent growth of the tumour cells or to transformation to an aggressive DLBCL.

Chronic lymphocytic leukaemia
The most common type of leukaemia, chronic lymphocytic leukaemia (CLL), is often an indolent disease with a median age of onset of 65. CLL is molecularly and clinically related to a nodal lymphoma known as small lymphocytic lymphoma. Current therapy can reduce symptoms, but it is not curative and does not prolong survival.

memory B cells.

FOLLICULAR DENDRITIC CELLS (FDCs). Cells with a dendritic morphology that are present in lymph nodes, where they present intact antigens held in immune complexes to B cells.

PLASMABLAST
A dividing B cell that is committed to plasma-cell differentiation.

CLASS-SWITCH RECOMBINATION
DNA rearrangement of the VDJ region from immunoglobulin M to any of the IgG, IgA or IgE constant genes at the heavy-chain locus. Recombination occurs in repetitive sequences of DNA that are located upstream of each constant gene.

SOMATIC HYPERMUTATION
The substitution of 'untemplated' nucleotides or small deletions targeted to a rearranged VDJ or VJ segment, which occurs only in B cells. The mutations are found between the promoter and enhancer of the rearranged gene (including non-coding regions), but they are found at the highest frequency in 'hotspots' (RGYW) that are located in the complementarity-determining regions.

This possibility is interesting because follicular lymphomas seem to be arrested at the GC stage of differentiation (see later), which indicates that a naive B cell that has acquired a *BCL2* translocation can nevertheless participate in an antigen-driven GC response.

After antigen encounter, naive B cells follow one of three pathways: they can enter the GC microenvironment, where they interact with T cells, FOLLICULAR DENDRITIC CELLS and antigen[7]; they can differentiate into short-lived PLASMABLASTS outside of the GC[8]; or they can enter an unresponsive state known as anergy (FIG. 1a). In the GC, two molecular processes remodel DNA — immunoglobulin CLASS-SWITCH RECOMBINATION (CSR) and immunoglobulin SOMATIC HYPERMUTATION (SHM).

Both CSR and SHM generate DNA breaks[9–11] and are, therefore, dangerous mechanisms that might predispose to chromosomal translocations. The DNA breaks that are induced by CSR and SHM coincide with the sites of chromosomal translocations that involve the IgH locus in certain lymphoid malignancies. SHM is probably involved in t(8;14) in endemic Burkitt lymphomas (BOX 1) because the *c-MYC* gene is often joined to the IgH locus in a rearranged and somatically mutated IgH variable (V) region[12–14]. SHM can also target non-immunoglobulin loci, such as *BCL6* (REFS 15–17), and the involvement of these genes in translocations is probably a byproduct of this process. CSR is the culprit in many of the translocations that occur in multiple myeloma (BOX 1) and sporadic Burkitt lymphoma, because the translocation breakpoints occur in IgH switch regions[18,19].

Cell of origin

Historically, the relationship between normal B-cell subpopulations and types of lymphoma has been assessed by a combination of microscopic appearance and immunophenotype. By these criteria, most mature B-cell malignancies seem to be 'trapped' at particular stages of normal B-cell development. Follicular lymphomas, for example, have growth patterns that resemble those of normal GC B cells, and they are infiltrated with follicular dendritic cells and T cells. The tumour cells also express the membrane metalloendopeptidase CD10, which is a hallmark of human GC B cells, leaving little doubt that follicular lymphoma is a disease of GC B cells. However, in some lymphomas, the tumour cells show a spectrum of morphological differentiation, ranging from GC-like cells to plasmacytic cells, which indicates that the block in differentiation is not complete.

When we speak of cell of origin we are, by necessity, referring to the relationship between the phenotype of the tumour on clinical presentation and a normal stage of B-cell differentiation. We cannot observe human lymphoid tumours during their natural evolution from a normal B cell. Therefore, as mentioned earlier, oncogenic translocations might occur at an early stage of B-cell differentiation, after which the transformed B cell might differentiate further and arrest at a later stage of differentiation. The important point is that the phenotype of the tumour at clinical presentation will influence its clinical behaviour and responsiveness to therapy.

Several possible mechanisms could account for the apparent developmental arrest in many lymphoid malignancies. First, oncogenic alterations could interfere with regulatory networks that control lymphocyte differentiation. As discussed later, translocation of *BCL6* might cause lymphomas, in part, by blocking plasmacytic differentiation. Second, the malignant lymphocyte might lose responsiveness to external cues, such as antigen or other immune cells that regulate normal differentiation. Third, it is conceivable that an oncogenic event might activate pathways that mimic a particular stage of normal differentiation. This possibility seems less likely in some lymphoid malignancies, as described later, that share extensive gene-expression profiles and biological functions with particular stages of B-cell differentiation.

The analysis of somatic mutations in the rearranged immunoglobulin loci of lymphoid malignancies shows that there are clear differences between the diagnostic categories (TABLE 1). Most types of non-Hodgkin lymphoma have highly mutated immunoglobulin genes that bear the hallmarks of SHM. A prominent exception to this rule might be mantle-cell lymphoma, which indicates that this lymphoma might be pre-GC in origin. Of course, the mere presence of immunoglobulin mutations in a lymphoid malignancy only indicates that the cell that gave rise to the tumour had passed through a stage of B-cell differentiation during which SHM occurs. In some lymphomas, however, individual tumour cells in the malignant clone have distinct immunoglobulin sequences, which indicates that the tumour is frozen at a stage of differentiation at which SHM is ongoing (TABLE 1).

The presence of immunoglobulin mutations in lymphoid malignancies is usually taken as evidence that the cell of origin of the tumour passed through the GC microenvironment. Although most SHM takes place in GCs[20], recent work indicates that it can occur also outside of classical GC structures. Signalling through CD40 is required to initiate and maintain the GC reaction[21,22], and studies of hyper-IgM patients with genetic deficiencies in CD40 ligand (CD40L) have shown that some SHM can take place in the absence of CD40 signalling[23]. In particular, a $CD27^{+}IgM^{+}IgD^{+}$ subpopulation of somatically mutated memory B cells is retained in the peripheral blood of these patients, whereas other memory B-cell subpopulations are absent. These studies indicate that SHM can occur outside of the GC, and they are reminiscent of earlier work in lymphotoxin-α-deficient mice, which lack GCs but can initiate SHM after several immunizations[24]. A direct observation of SHM outside of GCs was reported recently using a transgenic mouse engineered to synthesize anti-IgG antibodies (rheumatoid factors)[25]. Clonal expansion of anti-IgG-specific B cells was observed in the T-zone–red-pulp border of the spleen, and the B cells in these proliferative foci were shown to have ongoing SHM at a rate similar to that seen in GC B cells. Given the possibility of extra-GC SHM, the presence of immunoglobulin mutations

in a lymphoid malignancy cannot be taken as definitive evidence for a GC or post-GC cell of origin.

Recently, the relationship of B-cell malignancies to normal stages of B-cell differentiation and activation has been clarified using genomic-scale gene-expression profiling. A unique gene-expression signature distinguishes GC B cells from other stages of B-cell differentiation, including resting naive and memory blood B cells and mitogenically activated blood B cells[26,27] (FIG. 1b). The GC B-cell signature contains several hundred genes, including well-known GC markers (such as the genes encoding CD10, CD77 synthase and BCL-6) and many new genes of unknown function that were identified by high-throughput sequencing of complementary DNA libraries from normal GC B cells[28]. Expression of the GC B-cell signature genes is maintained in some lymphoma cell lines[26], which indicates that this signature is a stable change in gene expression and does not require the cellular interactions that are present in the GC microenvironment to be maintained. So, the GC B cell is at a discrete stage of B-cell differentiation and is not just a specialized type of activated lymphocyte.

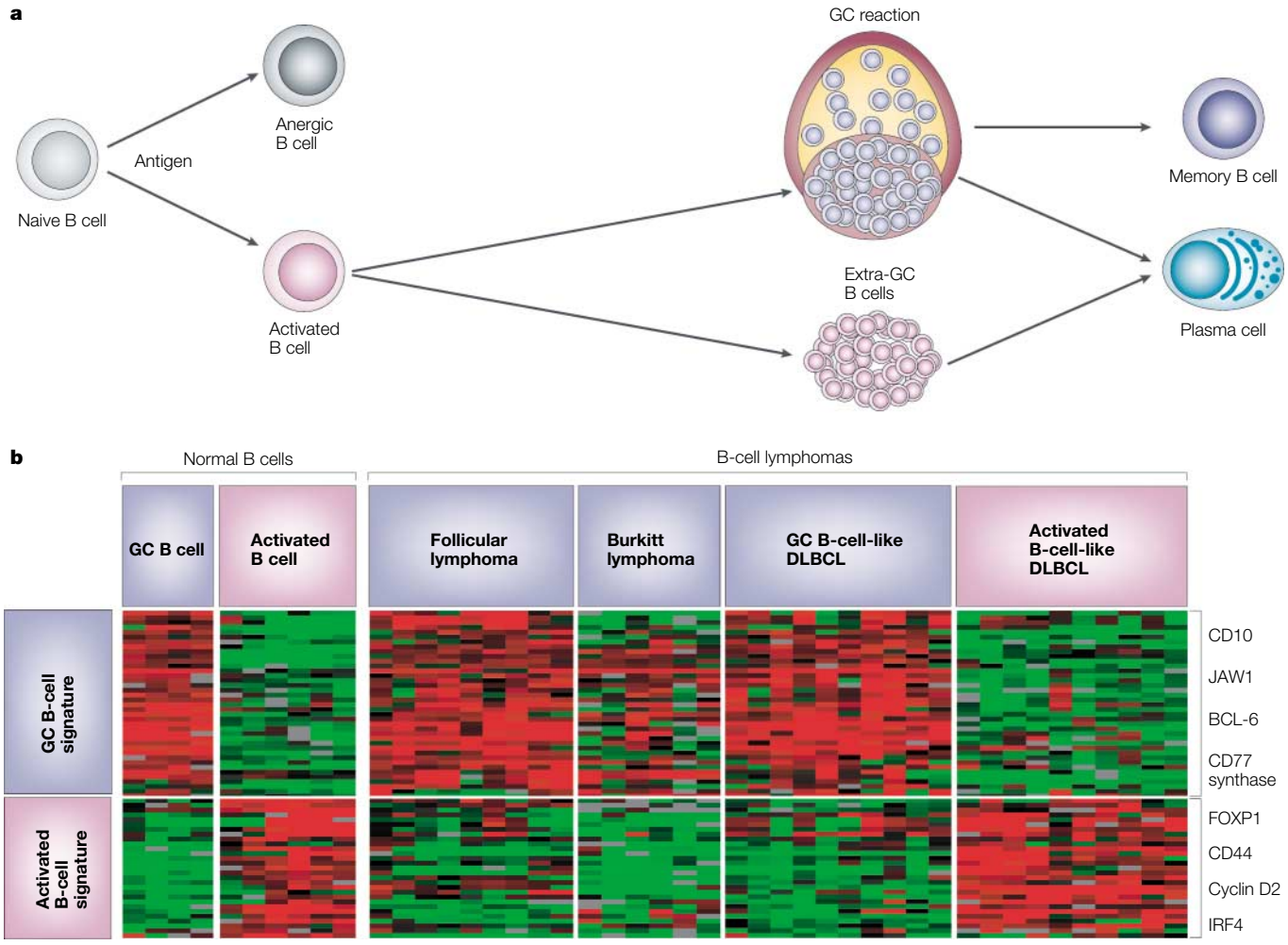

Figure 1 | **Mature B-cell lymphomas: cell of origin. a** | When naive B cells encounter antigen they become activated and face three cell fates: clonal expansion and selection in a germinal centre (GC), clonal expansion and differentiation at extra-GC sites, or anergy. Eventually, B cells either die or differentiate to memory B cells or antibody-secreting plasma cells. **b** | Gene-expression profiling shows a relationship between stages of B-cell differentiation and several types of mature B-cell lymphoma. Each column represents the results of gene-expression profiling from a single messenger RNA sample of normal or malignant B cells. Each row represents the expression of a single gene. Genes were chosen on the basis of their ability to distinguish between diffuse large B-cell lymphomas (DLBCLs) of GC and non-GC phenotype. Samples are compared with a common reference RNA pool, and relative gene expression is shown using a colour scale in which shades of red indicate genes that are expressed at a higher than median level, shades of green indicate genes that are expressed at a lower than median level, and black indicates genes that are expressed at the median level across all samples. A 16-fold range of gene expression is shown. Germinal-centre B-cell signature genes — for example, those that encode CD10, JAW1, BCL-6 and CD77 synthase — relate normal GC B cells to some lymphomas (follicular lymphomas, Burkitt lymphomas and the GC B-cell-like DLBCL subgroup). Genes that are expressed at a higher level in mitogenically activated peripheral-blood B cells than in GC B cells — for example, those that encode FOXP1 (forkhead box P1), CD44, cyclin D2 and IRF4 (interferon-regulatory factor 4) — uniquely identify the activated B-cell-like DLBCL subgroup. Potential cell types of origin for these lymphomas are indicated in pink (activated B cell) and blue (GC B cell) in part **a**.

Table 1 | **Characteristics of mature B-cell malignancies**

Malignancy	SHM	Ongoing SHM	GC B-cell expression profile[27,71,99,130]	Putative cell of origin*
Mantle-cell lymphoma	No (except for a small percentage[131])	No	No	Pre-GC B cell
Chronic lymphocytic leukaemia (CLL)‡	Yes and no	No	No	Antigen-experienced B cell (pre- or post-GC)
Burkitt lymphoma	Yes	No	Yes	GC B cell
Follicular lymphoma	Yes	Yes	Yes	GC B cell
Marginal-zone lymphoma — nodal, extranodal (MALT) and splenic	Yes (except for some splenic variants[132])	Yes (prevalent in MALT lymphomas[133])	No§	GC B cell or post-GC B cell
GC B-cell-like DLBCL	Yes	Yes	Yes	GC B cell
Activated B-cell-like DLBCL	Yes	No	No	GC B-cell subset or extra-GC mutated B cell[23,25]
Lymphoplasmacytic lymphoma (LPL)	Yes	Yes	No¶	Post-GC B cell
Multiple myeloma	Yes	No	No	Post-GC B cell
Hodgkin lymphoma (classical type)	Yes	No	No	GC or post-GC B cell
Hodgkin lymphoma (nodular lymphocyte pre-dominant type)	Yes	Yes	Yes	GC B cell

*Based on the presence or absence of somatic hypermutation (SHM) and the gene-expression profile. ‡CLL consists of two clinically distinct subtypes, one with SHM and one without[134]. In some CLLs, subclones can accumulate additional mutations through SHM or another mutational process. §The gene-expression profile of marginal-zone lymphomas (MZLs) has yet to be determined, but MZL B cells lack germinal-centre (GC) markers (such as CD10 and BCL-6) and express marginal-zone markers (such as CD21 and CD35)[86]. ¶The gene-expression profile has yet to be determined, but LPL has characteristics that relate it to post-GC plasma cells (such as cytoplasmic immunoglobulin)[97]. DLBCL, diffuse large B-cell lymphoma; MALT, mucosal-associated lymphoid tissue.

Several types of B-cell lymphoma express GC B-cell signature genes, including follicular lymphomas, Burkitt lymphomas and a subgroup of diffuse large B-cell lymphomas (DLBCLs)[26,29] (BOX 1 and FIG. 1). This finding establishes that these malignancies are derived from a GC B cell *per se* and not from a post-GC somatically mutated B cell. Although these malignancies retain expression of most of the GC B-cell signature genes, the lymphoma from an individual patient might have lost expression of any one GC B-cell marker. Furthermore, expression of a single gene is usually insufficient to establish the relationship between a malignancy and its normal counterpart, because many of the gene-expression differences between stages of differentiation are quantitative, not qualitative, in nature. Therefore, a 'diagnosis' of a GC B-cell origin must be based on the expression of several GC B-cell signature genes to be accurate. By contrast, other types of lymphoid malignancy fail to express these GC B-cell genes, and they have their own gene-expression signatures that relate them to other stages of B-cell differentiation (TABLE 1).

About half of all DLBCLs fall into a gene-expression subgroup known as GC B-cell-like DLBCLs (GCB DLBCLs), which have a gene-expression profile that closely resembles that of normal GC B cells[26,29] (FIG. 1 and BOX 2). Furthermore, these lymphomas have highly mutated immunoglobulin genes and SHM is ongoing in malignant clones[30]. Gene-expression profiling indicates also that most GCB DLBCLs have undergone immunoglobulin class switching[29] (A.R. and L.M.S., unpublished observations). Together, these observations point to a GC B cell as the cell of origin for GCB DLBCLs, and they show that these tumours are trapped at this stage of differentiation.

Another subgroup of DLBCLs, representing ~30% of cases, are known as activated B-cell-like DLBCLs (ABC DLBCLs), because these lymphomas resemble mitogenically activated peripheral B cells, and not GC B cells, in their gene-expression profile[26,29] (FIG. 1). An important feature of ABC DLBCLs is the high level of expression of nuclear factor-κB (NF-κB) target genes, including those that encode BCL-2, interferon regulatory factor 4 (IRF4), CD44, FLIP (FLICE-like inhibitory protein) and cyclin D2 (see below)[31]. These lymphomas have a high level of immunoglobulin somatic mutations, but they do not have ongoing SHM[30]. Nearly all ABC DLBCLs express a high level of IgM[29] (A.R. and L.M.S., unpublished observations), which indicates that they have not undergone immunoglobulin class-switch recombination, a finding that is unexplained so far.

The cell of origin for ABC DLBCLs is less clear than for GCB DLBCLs, although the absence of the GC B-cell gene-expression signature and the lack of ongoing SHM do not indicate a GC B-cell origin. ABC DLBCLs resemble pre-plasma cells in terms of gene expression in that they have higher levels of expression of immunoglobulin, X-box binding protein 1 (XBP1), IRF4 and other plasma-cell genes than GCB DLBCLs, and a lower level of expression of BCL-6 (REF. 29; A.R. and L.M.S., unpublished observations). GCs contain a subpopulation

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