

Pathogenesis of lymphomas

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Genetic alterations lead to development of lymphomas

Cancer is, in essence, a genetic disease in which an accumulation of mutations leads to activation of oncogenic pathways and impairment of normal controls in growth and survival. Recent developments in high throughput sequencing technologies have produced an increasingly detailed record of the genomic alterations present in malignant lymphomas. Acquired (somatic) genetic alterations seen in malignancies include single nucleotide variants (SNV), small insertions and deletions (indels), copy number variations (CNV), and other large structural variations. Epigenetic alterations are heritable changes in gene expression that are due to mechanisms other than changes in the DNA sequence and include DNA methylation, histone modifications, and altered expression of non-coding RNA. Every tumor has acquired genetic and epigenetic alterations that lead to its pathogenesis and dictate most of its behavior including metastases, response to therapy, and resistance. A study of these genomic alterations has led to novel approaches in the treatment of cancers including lymphomas.

Most of these changes are ‘passenger’ mutations that occur incidentally. A very small fraction of these alterations are ‘driver’ mutations that are defined in a broad sense as cell-autonomous or non-cell-autonomous alterations that contribute to tumor evolution at any stage – including

initiation, progression, metastasis, and resistance to therapy – by promoting a variety of functions including proliferation, survival, invasion, or immune evasion [1]. Only about 1–2% of human genes have been shown to act as drivers [2]. Alterations in these driver genes affect a limited number of pathways that control cell proliferation, differentiation, and apoptosis.

The frequency of genetic alterations varies widely among lymphoid malignancies. Hairy cell leukemia (HCL) and Waldenström's macroglobulinemia (WM) are characterized by low mutation load but are highly specific; *BRAF V600E* and *MYD88 L265P* are seen in most cases of HCL and WM, respectively. Burkitt lymphoma (BL) and splenic marginal zone lymphoma (SMZL) have approximately 25–30 non-silent mutations per case [3,4] whereas 'high mutators' such as diffuse large B-cell lymphoma (DLBCL) have increased mutational load (approximately 50–100 non-silent mutations per case) [5–7].

It has been proposed that the accumulation of genetic alterations occurs in three phases [2]. In the breakthrough phase, a cell acquires a driver gene mutation and begins to expand and survive abnormally, undergoing many rounds of cell division, typically over many years. In the second expansion phase, secondary driver gene mutations enable the cells to expand in the local environment even under adverse conditions. In the invasive phase, additional mutations enable cells to invade normal tissue leading to distorted architecture and widespread dissemination, which are, by definition, features of a malignancy. An analogous situation has been described in the pathogenesis of malignant lymphomas. In follicular lymphoma (FL), the initial driver mutation, which is the t(14; 18) (*IGH*;*BCL2*) translocation occurs in the bone marrow during early B-cell development. Increased *BCL2* expression enables the precursor cell to survive multiple rounds of cell division in the germinal center – these t(14;18)-positive cells now circulate in the peripheral blood and are seen in up to 50% of the normal adult population [8]. Colonization of lymph nodes leads to in-situ FL and accumulation of further mutations results in development of FL. Similarly, in-situ mantle cell lymphoma (MCL) and monoclonal B-cell lymphocytosis have been described as precursors of MCL and chronic lymphocytic leukemia (CLL), respectively. Malignant

lymphoma, therefore, represents the clinically detectable late stages in a continuum of increasing genetic complexity, which also raises the question of when it would be most appropriate to therapeutically intervene in the oncogenic process.

Mechanisms of genetic alterations in B-cell lymphomas

The B-cell receptor in the pre-B cell is assembled from various combinations of variable (V), diversity (D), and joining (J) gene segments. Two additional processes, somatic hypermutation (SHM) and class switching (CS), which occur in the germinal centers of secondary lymphoid organs, generate additional structural and functional diversity. The generation of a large antibody repertoire necessary for immunity involves DNA strand breaks and nucleotide alterations, which increase the risk for oncogenic alterations. These are described in the following section.

The variable region of the heavy chain (Vh) is assembled from approximately 40 variable, 23 diversity, and 6 joining gene segments and the variable region of the light chains is assembled from approximately 30–35 variable and 5 joining gene segments. These segments are rearranged by a process of double-stranded DNA breaks followed by recombination mediated by the recombination activating gene (RAG) enzymes. Aberrant recombination leads to chromosomal translocations, as exemplified by the RAG-mediated t(14;18) translocation in FL and t(11;14) translocation in MCL. In most cases, the coding region of the oncogene is not altered but the tightly regulated expression pattern is altered by association with the partner gene. Further development of the B cell occurs in the germinal center where the affinity of the B-cell receptor is further improved by selection of cells that have undergone SHM of the IgV region genes mediated by the activation-induced cytidine deaminase (AID) enzyme. Heavy chain class switching alters the functional properties of the antibody molecule. Both of these processes also create double-stranded breaks and aberrant chromosomal translocations. Somatic mutations mediated by AID may also affect non-immunoglobulin genes in germinal center B cells. Up to 10% of actively transcribed genes may be affected by aberrant somatic hypermutation.

The various B-cell development stages described above have been histogenetically correlated with various B-cell lymphomas as shown in Figure 2.1.

Signaling pathways involved in development of B-cell lymphomas

In this section, the major physiologic signaling pathways in B cells and examples of their alteration in lymphomas are described. An understanding of these pathways in lymphomas will enable rational therapeutic intervention.

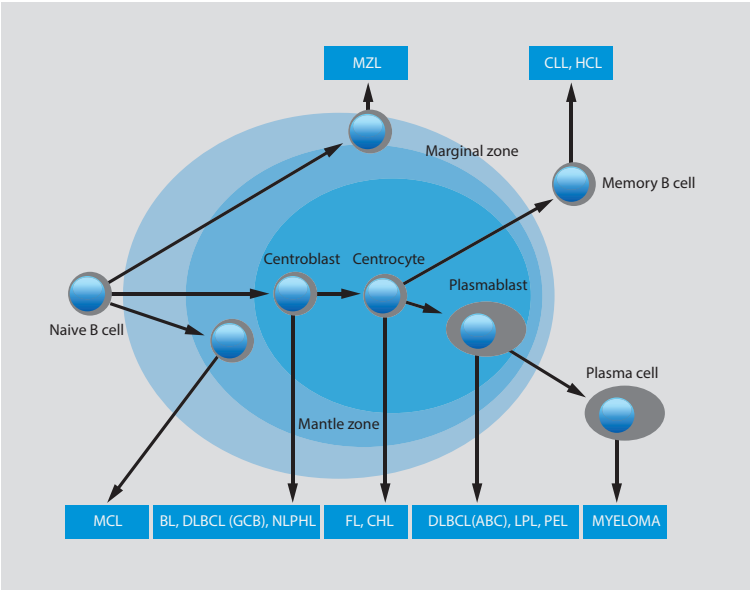


Figure 2.1 B-cell differentiation and relationship to major B-cell neoplasms.

Lymphoblasts undergo VDJ recombination in the bone marrow. After maturation, naive B cells traverse to the germinal center of secondary lymphoid organs where two additional processes, somatic hypermutation and class switching, occur and generate additional structural and functional diversity. The vast majority of B-cell neoplasms arise from germinal center B cells with the remaining arising from mantle zone cells, marginal zone cells, or memory B cells. ABC, activated B cell; BL, Burkitt lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell; HCL, Hairy cell leukemia; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; PEL, primary effusion lymphoma; VDJ, variable, diversity, and joining gene segments.

B-cell receptor pathway

The B-cell receptor complex consists of the immunoglobulin molecule linked to a signaling subunit, which is a heterodimer of Ig-alpha (CD79A) and Ig-beta (CD79B) proteins. Antigen binding to the Ig molecule leads to phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAM) in CD79A and CD79B by src family kinases such as LYN followed by activation of spleen tyrosine kinase (SYK), Bruton's tyrosine kinase (BTK), phospholipase C gamma (PLC γ), and protein kinase C beta (PKC β) (Figure 2.2).

Activation of PKC β leads to caspase recruitment domain family, member 11 (CARD11) phosphorylation, recruitment of mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) and B-cell lymphoma/leukemia 10 (BCL10) into the CARD11-BCL10-MALT1 (CBM) multi-protein complex and nuclear factor-kappa B (NF- κ B) pathway activation. Mature B cells require 'tonic' signaling from the BCR complex for survival throughout their lifespan [9]. BCR signaling is also critical for survival and proliferation of B-cell lymphomas. A prominent feature of the activated B-cell type of DLBCL (ABC-DLBCL) is the constitutive activation of NF- κ B signaling pathway and a 'chronic active' form of BCR signaling reminiscent of antigen-stimulated B cells that signal through the CBM complex to activate NF- κ B. More than 20% of ABC-DLBCL patients harbor somatic mutations in *CD79B* and to a lesser extent in *CD79A* [10], and approximately 9% have oncogenic mutations in the *CARD11* gene [11]. Interruption of the BCR signaling pathway is specifically toxic to ABC-DLBCL cells in vitro [10]. In FL, it has been shown that the 'protective' allele that encodes the expressed B-cell receptor is not involved in the translocation with the *BCL2* gene and maintains BCR signaling [12].

The transcription factor 3 protein (TCF3) modulates germinal center gene expression and regulates survival and proliferation of lymphoid cells through the BCR and PI3K signaling pathway and by modulating cell cycle regulators such as CCND3. TCF3 also induces its own inhibitor, called inhibitor of DNA binding 3 (ID3), creating an autoregulatory loop that inactivates TCF3 in the resting state (Figure 2.2). Release of TCF3 from the ID3-TCF3 complex promotes BCR signaling by upregulating

the expression of immunoglobulin heavy and light chain genes and repression of the BCR signaling inhibitor src homology region 2 domain-containing phosphatase 1 (SHP1). Most patients with BL (70%) harbor *ID3* and *TCF3* mutations leading to the activation of the TCF3 pathway, which ultimately results in intensification of ‘tonic’ BCR signaling [13].

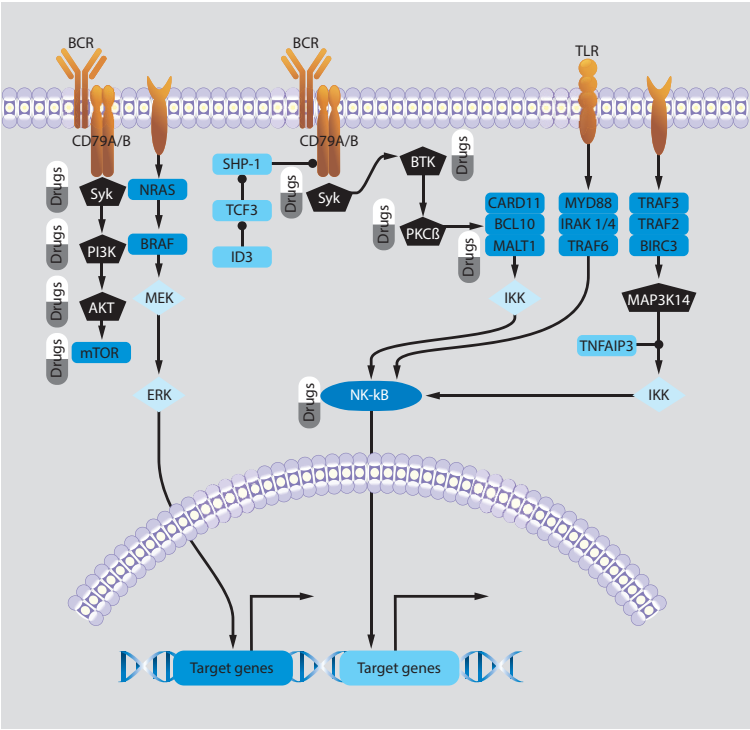


Figure 2.2 Intracellular signaling pathways involved in B-cell lymphoproliferative disorders and therapeutic targets: see text for details. AKT1, v-akt murine thymoma viral oncogene homolog 1; BCL10, B-cell CLL/lymphoma 10; BCR, B-cell receptor; BIRC3, baculoviral IAP repeat containing 3; BRAF, B-Raf proto-oncogene, serine/threonine kinase; BTK, Bruton's tyrosine kinase; CARD11, caspase recruitment domain-containing protein 11; ERK/MAPK, extracellular signal-regulated kinase/mitogen-activated protein kinase; ID3, inhibitor of DNA binding 3; IKK, inhibitor of NF-κB kinase; IRAK, interleukin-1 receptor-associated kinase 1; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MAP3K14, mitogen-activated protein kinase kinase kinase 14; MEK, mitogen-activated protein kinase kinase kinase 1; mTOR, mechanistic target of rapamycin; MYD88, myeloid differentiation primary response 88; NF-κB, nuclear factor of kappa light polypeptide gene enhancer in B cells; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; PI3K, phosphoinositide 3-kinase; PKCβ, protein kinase C β; SHP-1, Src homology region 2 domain-containing phosphatase-1; SYK, spleen tyrosine kinase; TCF3, transcription factor 3; TLR, toll-like receptor; TNFAIP3, TNF alpha-induced protein 3; TRAF, TNF receptor-associated factor.

Toll-like receptor pathway

Toll-like receptors (TLRs) recognize a variety of pathogen-associated molecular patterns derived from bacteria, viruses, and fungi in a BCR-independent manner. Ligand binding causes aggregation of TLRs and activation of cytoplasmic adapters such as *myeloid differentiation primary response gene 88* (MYD88) and triggering NF- κ B pathway activation (Figure 2.2). The MYD88 pathway can also be activated by cytokines such as interleukin 1 (IL-1). MYD88 mutations are seen in approximately 90% of all WM, approximately 30% of ABC-DLBCL, and 10% of SMZL [4,7,14,15].

Notch signaling

Notch receptor proteins are transmembrane receptors that function as ligand-activated transcription factors. Ligand binding causes proteolytic cleavage of the Notch intracellular domain, which then translocates to the nucleus and induces transcription of target genes including MYC and NF- κ B pathway components. Notch signaling plays an important role in a number of cellular functions including cell fate specification, proliferation and apoptosis, and the physiological development of the splenic marginal zone. The C-terminal proline, glutamic acid, serine, and threonine-rich (PEST) domain of the Notch protein is recognized by ubiquitin ligase leading to proteolytic degradation. Most mutations in lymphoma target the PEST domain resulting in truncation with impaired degradation and dysregulated Notch signaling. Notch pathway mutations have been identified in SMZL, CLL, and MCL. *Notch 1* mutations are seen in 5–10% of patients with CLL and this number increases with relapse and transformation. F-box and WD repeat domain-containing 7 (FBXW7) is an F-Box protein that forms one of the subunits of the ubiquitin ligase complex and promotes proteolytic degradation of Notch protein; loss of function mutations are seen in ~2% of patients with CLL [16,17].

Nuclear factor-kappa B pathway

NF- κ B is a homo or heterodimer of Rel-like domain-containing proteins including RELA/p65, RELB, c-Rel, NF- κ B1, and NF- κ B2 that function downstream of the BCR, TLR, and Notch signaling pathways. NF- κ B proteins are bound in a complex with an inhibitor protein, I κ B alpha,

which keeps them in the cytoplasm. Activation of I κ B kinase (IKK) by external stimuli leads to phosphorylation of I κ B alpha and its subsequent ubiquitination and proteasomal degradation lead to the release of NF- κ B transcription factors that translocate to the nucleus and regulate gene transcription (Figure 2.2).

The role of the NF- κ B pathway in ABC-DLBCL and SMZL has been discussed earlier. Negative regulators of NF- κ B signaling are inactivated by mutations/deletions in many B-cell lymphomas as described below. TNFAIP3 is homozygously inactivated or deleted in 30% of patients with ABC-DLBCL [18]. BIRC3, TRAF2, and TRAF3 are negative regulatory proteins that form an inhibitory complex with the enzyme MAP3K14 (Figure 2.2) in association with the CD40 and BAFF receptors that mediate the non-canonical NF- κ B signaling pathway. Activation of these receptors leads to disruption of the complex and release and stabilization of MAP3K14, which in turn activates IKK α . Loss of function mutations in *TRAF3* and *BIRC3* are detected in SMZL leading to activation of MAP3K14 and NF- κ B signaling [19]. The *BIRC3* gene is inactivated in CLL by mutations and *BIRC3* mutations correlate with the clinical course and chemo refractoriness in CLL [20]. Activator mutations such as *CARD11* mutations are present in ABC-DLBCL and have been described above.

G-protein coupled receptors

G-protein coupled receptors (GPCR) are the largest family of cell surface receptors involved in signal transduction with over 800 members. In conjunction with their cognate heterotrimeric G proteins, GPCRs activate multiple downstream targets that regulate cells survival, proliferation, and differentiation. Sequencing studies have revealed mutations or copy number alterations in members of the *SIPR2*-*GNA13* (encodes the G α 13 G-protein)-*RHOA* pathway in DLBCL as well as in BL [5,13]. Impairment of this pathway affects B-cell localization and migration and leads to simultaneous activation of the AKT pathway [21]. *GNA13* knockout mice develop DLBCL supporting the importance of this pathway in lymphomagenesis [22].

Mitogen-activated protein kinase signaling pathway

The mitogen-activated protein kinase pathway (MAPK) consists of receptor tyrosine kinases transmitting a variety of signals from the external environment to the nucleus. MAPK signaling in B cells is initiated at the BCR and propagated through RAS and RAF proteins. The *BRAF* V600E mutation is the pathognomonic genetic event for HCL. *BRAF* is also frequently mutated in Langerhans cell histiocytosis [23].

PI3k-AKT1-MTOR signaling pathway

The PI3K-AKT1-MTOR (phosphatidylinositol 3'-kinase/v-akt murine thymoma viral oncogene homolog 1/mechanistic target of rapamycin) pathway is a key component of tonic BCR signaling, a process that is required for B-cell survival. Conditional inactivation of the B-cell receptor leads to profound reduction of peripheral B cells, which can be rescued by transgenic expression of PI3K [24]. B-cell receptor signaling in BL activates the PI3K pathway, as demonstrated by phosphorylation of AKT1 and p70S6 kinase [25]. Activating mutations in *TCF3* as well as deleterious mutations in the *TCF3* inhibitor *ID3* potentiates B-cell receptor signaling by repression of SHP1 and enhancement of Ig production (Figure 2.2). 70% of BL cases have mutations in *TCF3* or *ID3* proteins, as described previously. Activation of the PI3K pathway due to loss of PTEN expression has been described in 37% of the DLBCL and 19% of MCL [26,27]. Activating mutations in exons 9 and 20 of *PI3K* have also been reported [26].

Role of MYC In lymphomagenesis

The MYC family includes C-MYC, N-MYC, L-MYC and S-MYC, which are involved in the control of cell growth, differentiation, and apoptosis. MYC-MAX heterodimers bind DNA sequences called E-box motifs to activate transcription of a large number of targets, estimated at 10–15% of all human genes. Enhanced *MYC* expression is seen in up to 70% of human malignancies and usually requires co-operation with other genetic lesions for oncogenic transformation.

MYC plays a crucial role in the initial stages of formation of the germinal center, where it is rapidly suppressed by *BCL6* expression in

centroblasts. In the light zone, *MYC* is again expressed in a subset of centrocytes, accompanied by *BCL6* downregulation, NF- κ B activation, and *IRF4* expression. These cells later re-enter the dark zone for further antigen receptor maturation. *MYC*-negative centrocytes in the light zone will exit the germinal cell to differentiate into memory B cells or long-lived plasma cells [28].

BL is characterized by a translocation that places the *MYC* gene under the control of the immunoglobulin enhancer. *MYC* deregulation is frequent in DLBCL; *MYC* translocations are seen in 5–14% of DLBCL, and *MYC* gain and amplification in 21–38% of DLBCL. Concurrent translocations of *BCL2* or *BCL6* gene with *MYC*, which are designated ‘double-hit lymphomas’ impart a significantly worse clinical prognosis. High *MYC* expression without *MYC* gene abnormalities, presumably induced by other mechanisms, is observed in 28–41% of DLBCL cases. In addition, the *BCL2* protein is overexpressed concurrently in 60% of *MYC*-positive DLBCL cases, independent of the presence of gene rearrangement. These cases described as ‘double expressor lymphoma’ behave more aggressively than cases with single protein overexpression. *MYC* alterations are also seen in plasmablastic lymphoma (PBL), and anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma.

Role of p53 and DNA repair pathways

p53 is induced and activated by a number of stress signals, including DNA damage, oxidative stress, telomere shortening, hypoxia, and activated oncogenes leading to cell cycle arrest, cellular senescence, or apoptosis. Inactivation of p53 is seen in a variety of lymphomas such as BL, DLBCL, including those derived from transformation of FL, MCL, CLL, peripheral T-cell lymphoma (PTCL), and natural killer cell lymphoma/leukemia (NKCL). HDM2, the negative regulator of TP53, is often amplified in lymphoma.

DNA repair pathways preserve genomic integrity – there are at least six major DNA repair mechanisms in human cells. Somatic and germline mutations have been identified in various DNA repair pathways, mainly in DLBCLs [29]. Mutations in mismatch repair genes (*EXO1*, *MSH2*, and *MSH6*) were associated with microsatellite instability, increased

number of somatic insertions/deletions, and altered mutation signatures in tumors. Somatic mutations in non-homologous end-joining (NHEJ) genes (*DCLRE1C/ARTEMIS*, *PRKDC/DNA-PKcs*, *XRCC5/KU80*, and *XRCC6/KU70*) were almost exclusively associated with translocations involving the immunoglobulin-heavy chain locus [29]. *ATM* abnormalities are often seen and are particularly common in patients with MCL and CLL.

Regulation of apoptosis

Apoptosis is a genetically controlled mechanism of cell death involved in the regulation of tissue homeostasis controlled by enzymes called caspases. Caspases are cysteine proteases that cleave proteins at the Aspartate residues – at least 17 caspases have been described in mammals. Caspases include ‘executioner caspases’ (caspases 3, 6, and 7), which are activated by ‘initiator caspases’. There are two major pathways of apoptosis: the extrinsic pathway initiated by Fas and the other tumor necrosis factor receptor (TNFR) superfamily members leading to caspase-3 activation, and the intrinsic mitochondria-associated pathway, which is activated by apoptotic stimuli that induce mitochondrial outer membrane permeabilization (MOMP) followed by the release of molecules that facilitate the cell death program such as, cytochrome c, SMAC, and Omi. Cytochrome c interacts with Apaf-1 and caspase-9 to activate caspase-3. As a common final pathway, caspase-3 activation leads to the degradation of cellular proteins necessary for cell survival and maintenance of cellular integrity.

The BCL2 family of proteins, which include pro-apoptotic (BAX, BAK, BIM, and BID) and anti-apoptotic (BCL-2, BCL-XL, BCL-w, MCL-1, and BFL-1) members, regulate apoptosis by controlling MOMP. Translocations affecting the *BCL2* gene represent the genetic hallmark of FL and are seen in 80–90% cases, it is also detected in about 30% of GCB-DLCBL. Ectopic *BCL2* expression prevents apoptosis of germinal center B cells leading to accumulation of other genetic abnormalities. High expression of anti-apoptotic proteins such as MCL1 and inactivation of BIM and other pro-apoptotic proteins have also been reported.

Impairment of differentiation to plasma cells

BCL6 is the master regulator of the germinal center reaction. As a transcriptional repressor, BCL6 negatively regulates multiple target genes involved in BCR and CD40 signaling, T-cell mediated B-cell activation and induction of apoptosis in response to DNA damage. BCL6 therefore maintains the proliferative status of centroblasts and prevents terminal differentiation to plasma cells via suppression of PRDM1/BLIMP1, the master regulator of plasma cell differentiation. The *PRDM1/BLIMP1* gene on 6q21 is bi-allelically inactivated in ~25% of ABC-DLBCL cases [30] and represents another mechanism of impairing differentiation with the tumor cells arrested at the plasmablastic stage.

Epigenetic alterations

The epigenome is composed primarily of cytosine modifications, histone modifications, and expression of non-coding RNA molecules, which are particularly important in the germinal center reaction [31,32]. In a normal somatic cell, approximately 70–80% of cytosines that occur as a CpG dinucleotide are methylated whereas CpG islands are largely unmethylated [33] and this methylation status is mediated by DNA methylases and demethylases. Hemi-methylated DNA is the target of DNA methyl transferase 1 (DNMT1) and is needed to maintain methylation patterns during DNA replication. DNA methyl transferases 3A and 3B (DNMT3A and DNMT3B) target primarily unmethylated CpG's. A deeper understanding of the epigenetic modifications would enable rational use of epigenetic therapies.

Histone octamers composed of H2A, H2B, H3, and H4 proteins form the scaffold upon which DNA is wound to form nucleosomes. Histones can be chemically modified by acetylation, methylation, phosphorylation, sumoylation, or ubiquitylation. Trimethylation of H3 lysine 27 (H3K27me3) by the polycomb repressive complex 2 (PRC2), which includes EZH2, SUZ12, and EED is associated with transcriptional repression. EZH2 is upregulated in centroblasts and mediates H3K27 methylation and epigenetic silencing of genes required for memory and plasma cell differentiation. *EZH2* gain of function mutations have been observed in >20% of patients with FL and 23% of those with GCB-DLBCL but are

rare in ABC-DLBCL [5]. EZH2 inhibition in GCB-DLBCL alters the gene expression profile and induces proliferation arrest and plasma cell differentiation. In contrast to H3K27me₃, trimethylation of H3 lysine 4 (H3K4me₃) by the MLL family of histone methyltransferases is associated with active chromatin and gene expression. Loss of function mutations in *MLL* and *MLL2* are frequent in FL but seen less often in DLBCL patients. The outcome of epigenetic modifications is related to the ‘geography’ and ‘topology’ of the genome [34]. For example, cytosine methylation of transcription factor binding sites can result in either transcriptional activation or repression. Epigenetic modifications have different degrees of plasticity and may be reprogrammed based on environmental signals. In addition, epigenetic marks are combinatorial [35]. For example, the combination of H3K27me₃, which is a repressive mark and an activation mark H3K4me₃ in gene promoters leads to a bivalent chromatin domain that is transcriptionally poised but transiently repressed. Subsequent loss of an H3K27me or H3K4me₃ mark leads to more durable activation or repression, respectively. Such bivalent chromatin marks are common in genes associated with differentiation.

EP300 and *CREBBP* are histone acetyltransferases, which are commonly mutated in FL and DLBCL [32] and may alter the enhancer profile of the tumor. Such loss of function mutations also lead to defective acetylation of other proteins including BCL6 and TP53 that may promote germinal center B-cell lymphomagenesis [36]. Additional chromatin modifiers that are often mutated in lymphoma include components of the SNF/SWI complex, *ARID1A* and *B*, and *SMARC* family proteins.

Host/tumor interaction

Lymphomas typically arise in secondary lymphoid tissues, extranodal sites or uncommonly in the bone marrow. The microenvironment in lymphomas is composed of a mixture of stromal cells (follicular dendritic cells, fibroblastic reticular cells, and mesenchymal cells), immune cells (antigen-presenting cells, macrophages, T- and B-lymphoid cells, plasma cells, and mast cells), and extracellular matrix proteins as well as blood vessels [37]. Interactions between the neoplastic cells and the microenvironment promote tumor cell survival and malignant cells

constantly evolve to evade anti-tumor immunity. Mutations or loss of molecules important in immune response are common in tumor cells; these include *B2M*, *CD58*, *CIITA*, and *HLA* components. There may also be increased immunosuppressive cells or cytokines in the stroma, which could be orchestrated by STAT3 activation among others.

Recent advances in understanding the interactions between the host immune system and tumors has enabled therapeutic interventions designed to enhance the anti-tumor response. The adaptive immune response, mediated primarily by T cells, requires three kinds of signals for activation and differentiation. The primary signal is provided by engagement of the T-cell receptor with the peptide-major histocompatibility locus (MHC) complex and the second signal is provided by various costimulatory molecules, the most well-characterized of which are members of the B7 family (Figure 2.3) expressed on antigen-presenting cells. Signal 3 is delivered mostly by cytokines such as IL-6, IL-12, IL-4,

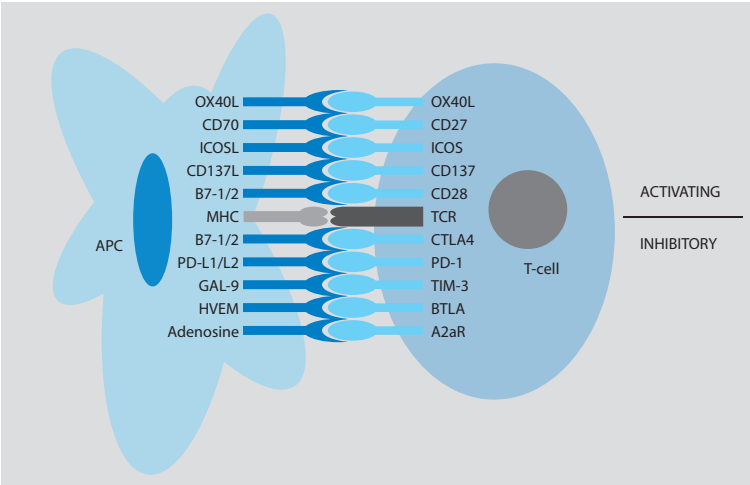


Figure 2.3 The role of PD-1, CTLA-4, and other immune checkpoint pathways is represented. A2aR, adenosine A2a receptor; APC, antigen-presenting cell; BTLA, B- and T-lymphocyte-associated; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; Gal-9, lectin, galactoside-binding, soluble 9; HVEM, tumor necrosis factor receptor superfamily, member 14; ICOS, inducible T-cell co-stimulator; ICOSL, inducible T-cell co-stimulator ligand; MHC, major histocompatibility locus; OX40, tumor necrosis factor receptor superfamily, member 4; PD-1, programmed-death 1; PD-L1/L2, programmed-death 1 ligand 1/2; TCR, T-cell receptor; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3. Adapted from © The American Society of Hematology, 2015. All rights reserved. Armand [38].

and transforming growth factor (TGF)- β and promotes T-cell differentiation into effector subsets. Activation of CD28 receptors on T cells by B7 molecules induces expression of IL-2 and the high affinity IL-2 receptor. T-cell co-stimulation is also modified by inhibitory molecules such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4), which binds B7 molecules with 20 times more avidity than CD28. Signaling through the programmed-death 1 (PD-1) protein leads to decreased T-cell proliferation, cytotoxicity, and cytokine production, and increased susceptibility to apoptosis. Other inhibitory signals are mediated by T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), B- and T-lymphocyte-associated (BTLA), and adenosine A2a receptor (A2aR) molecules on the T cells (Figure 2.3).

Tumors can selectively block anti-tumor immune responses through expression of the checkpoint receptor ligands such as CTLA4 and programmed death-1 ligand-1/2 (PD-L1/PD-L2). Immune checkpoint blockade using anti-CTLA-4, anti-PD1, and anti-PDL1 antibodies and their combinations have been shown to be effective in stimulating a cellular immune response in many hematological malignancies including classical Hodgkin lymphoma (cHL), FL, and DLBCL [38].

Pathogenesis of T-cell and natural killer cell lymphomas

PTCL represents about 10% of all non-Hodgkin lymphomas (NHLs), with angioimmunoblastic T-cell lymphoma (AITL) and anaplastic large cell lymphoma (ALCL) being the two most common entities. A large group of T-cell lymphomas cannot be classified further by current criteria and are designated as PTCL-not otherwise specified (PTCL-NOS). In comparison to B-cell lymphomas, much less is known about the pathogenetic mechanisms in T-cell lymphomas.

Next generation sequencing studies in AITL have shown frequent mutations of *TET2*, *DNMT3A*, *IDH2*, and *RHOA* with *IDH2* mutations exclusively involving R172 [39]. In contrast to the situation in AML, *IDH2* and *TET2* mutations are not exclusive and frequently occur together. Tet methylcytosine dioxygenase (TET) enzymes are α -ketoglutarate-dependent dioxygenases that catalyze demethylation of 5-methylcytosine. *IDH2* gain

of function mutations increase 2-hydroxyglutarate (2HG) levels leading to competitive inhibition of 2 ketoglutarate (2KG) dependent enzymes including TET enzymes and histone demethylases thus leading to aberrant histone lysine and DNA cytosine methylation and alteration in gene expression [40]. Other mutations are far less common and some mutations such as *CD28* are unique to PTCL. *CD28* mutations appear to enhance its signaling either through increased binding affinity to its ligand CD80/86 on antigen-presenting cells or its interaction with cytoplasmic adaptor molecules such as GRB2 eventually resulting in increased activation of the NF- κ B pathway [41]. Uncommon fusions involving the extracellular domain of *CTLA4* and the cytoplasmic domain of *CD28* are expected to markedly increase the affinity of binding to CD80/86 and thus CD28 signaling [41].

The genetic hallmark of ALK-positive ALCL is a chromosomal translocation involving the *ALK* gene. Translocations involving *DUSP2* and *TP63* have been described in some cases of ALK-negative ALCL with good and poor prognosis, respectively [42]. Additional translocations often involving a kinase and a tissue factor (TF) have recently been described [43].

Many cytokines signal through the Janus-associated kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, which includes four cytoplasmic JAK kinases and seven STAT proteins. Cytokine/receptor binding leads to activation of the associated JAK kinase, docking and phosphorylation of the cognate STAT protein, and translocation of the dimer to the nucleus where it functions as a transcription factor. A systematic genomic analysis in ALK-ALCLs identified activating mutations of *JAK1* and/or *STAT3* genes in 18% of ALK-ALCLs and 5% of cutaneous ALCLs. Of the positive cases, co-occurrence of *JAK1* and *STAT3* mutations were seen in 38% of systemic ALK-ALCLs but not in cutaneous ALCLs [43]. Recurrent chimeras combining a transcription factor (*NFkB2* or *NCOR2*) with a tyrosine kinase (*ROS1* or *TYK2*) that lead to the constitutive activation of the *JAK/STAT3* pathway were also observed [43]. *STAT3* is also one of the key downstream targets of activated ALK.

Two major subgroups have been described within the PTCL-NOS category by gene expression profiling. The first group is characterized by the

high expression of *GATA3* and some of its target genes and is associated with poor prognosis. The second group demonstrates high expression of *TBX21* and some of its target genes – increased expression of the cytotoxic gene signature in this group also showed poor clinical outcome [44]. The mutational landscape of PTCL-NOS is not well defined but some of the mutations seen in AITL and other lymphomas including epigenetic mediators (*MLL*, *TET2*, and *DNMT3A*), signaling pathway proteins (TNFAIP3, APC, and CHD8), and tumor suppressors (*TP53*, *FOXO1*, and *ATM*) have been detected [45,46]. Multiple studies on cutaneous T-cell lymphoma/Sezary syndrome have been reported recently [47–50]. Recurrent SNVs in *CD28*, *RHOA*, *PLCG1*, *TP53*, *DNMT3A*, and *FAS* were reported by Choi et al in cutaneous T-cell lymphomas. However, in this study copy number variations (CNVs) were much more frequent than single-nucleotide variations (SNVs) [50]. A large study detailing the mutations and CNVs in adult T-cell leukemia/lymphoma (ATLL) has also been published [51].

The gene expression profiles of NK cell-derived and $\gamma\delta$ T-cell-derived lymphomas have been shown to be alike indicating that lymphoma derived from cells of the innate immune system may be biologically similar [52]. Recent whole-exome sequencing studies of extranodal natural killer/T-cell lymphoma (ENKTCL) found frequent mutations involving the *DDX3X*, *TP53*, *STAT3*, and *STAT5B* genes. In addition, a number of mutations in epigenetic modifiers including *MLL2*, *ARID1A*, *EP300*, and *ASXL3* were also reported [53]. Targeted DNA sequencing from a larger group of cases confirmed the high frequency of *STAT3* and *STAT5B* mutations, especially the *N642H* mutation, in $\gamma\delta$ lineage lymphomas including cutaneous, intestinal, and hepatosplenic lymphomas [54,55]. Mutation results in increased binding affinity of the dimers between the SH2 domain and phosphotyrosine leading to increased persistence of the activated dimeric molecule and prolonged occupancy of the STAT binding site on the target genes.

Pathogenesis of Hodgkin lymphoma

Classical HL arises from post-germinal center B cells as evidenced by clonally rearranged and somatically mutated immunoglobulin genes. Surface Ig is not expressed indicating independence from antigen stimulation.

Other important pathogenetic features include loss of expression of many B-cell specific genes, activation of the NF- κ B pathway, Epstein-Barr virus infection in a subset of cases, and genetic changes that modulates the tumor environment to favor HL cell survival.

Constitutive activation of the NF- κ B pathway occurs through multiple mechanisms in cHL, including *REL* amplification [56], increased expression of the positive NF- κ B regulator BCL3, or inactivating mutations in negative regulators such as *NFKBIA* (20% of cases), *NFKBIE* (15%), and *TNFAIP3* (40%) [57]. Interestingly, TNFAIP3 loss and EBV positivity were mutually exclusive. A recent study revealed alterations in genes involved in antigen presentation, chromosome integrity, transcriptional regulation, and ubiquitination [58]. *Beta2-microglobulin* was the most commonly altered gene in Hodgkin and Reed/Stenberg (HRS) cells, with inactivating mutations leading to loss of class I MHC expression. Genomic studies have identified PD-L1 and PD-L2 as key targets of 9p24.1 amplification, which is a recurrent abnormality seen in cHL [59]. PD-L1 transcription is also enhanced by amplification of the Janus kinase 2 (JAK2) locus as part of the 9p24.1 amplification as well as by EBV infection. Recent clinical trials with PD-1 checkpoint blockade have validated the effectiveness of this approach. The novel highly recurrently mutated genes *DUSP2*, *SGK1*, and *JUNB* were recently described in nodular lymphocyte-predominant HL (NLPLHL) [60].

Conclusions

Most lymphomas arise from a combination of somatic mutations and aberrantly regulated genes that lead to activation of biochemical and signaling pathways promoting tumor growth. Panomic studies have mostly confirmed the validity of traditional histologic classification of lymphomas. Future diagnostic paradigms are expected to incorporate an integrated analysis of the mutations, copy number changes, and transcriptomic and epigenomic alterations in addition to traditional histology and immunophenotyping. These results interpreted in the context of the patient's clinical features and knowledge of available pharmacological interventions will likely enable multipoint targeting of tumor drivers.

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