BLIMP1 against Lymphoma: The Verdict Is Reached

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BLIMP1, a master regulator of plasma-cell differentiation, is implicated in the pathogenesis of Activated B cell (ABC)-like Diffuse Large B cell Lymphoma (DLBCL). In this issue of *Cancer Cell,* Mandelbaum and colleagues and Calado and colleagues unequivocally demonstrate that BLIMP1 functions as a tumor suppressor and guardian of ABC-like DLBCL lymphomagenesis.

Diffuse large B cell lymphoma (DLBCL), the most common subtype of non-Hodgkin's lymphoma (NHL), is a genetically and clinically heterogeneous disease. The standard treatment R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) cures about 50%–60% of patients, while the remaining DLBCL patients succumb to their disease. Better understanding of DLBCL pathogenesis is needed in order to identify new therapeutic approaches that, hopefully, will improve outcome and survival of these patients.

The pathogenesis of DLBCL represents a complex multistep process involving collaboration between biological programs of normal B cells that are utilized by transformed malignant cells and multiple acquired genetic and molecular lesions (Lenz and Staudt, 2010; Lossos, 2005). Therefore, transcriptional programs characteristic to the specific ontogenic stages of B cell differentiation from which the tumors originate, chromosomal translocations, somatic mutations, as well as gene amplifications and deletions all contribute to the complex process of the molecular pathogenesis of DLBCL. Marked advances in understanding the pathobiology of DLBCL have been made by applying gene expression arrays, leading to identification of previously unrecognized germinal center B cell-like (GCB) and activated B cell-like (ABC) DLBCL subtypes characterized by different outcomes (Alizadeh et al., 2000). This classification of the DLBCL tumors has also led to the appreciation that while some genetic and molecular lesions with known or predicted oncogenic potential occur in both GCB-like and ABC-like DLBCL subtypes, many oncogenic pathways are exclusively or predominantly used by only one subtype (Table 1). In recent years, there was marked progress in identifying genetic and molecular aberrations occurring in these tumors. However, the roles of some of the identified aberrations, targeting specific genes or signaling pathways, in the pathogenesis of DLBCL are still unknown. Moreover, identified pathobiologic events do not account for all the mechanisms underlying the pathogenesis of these tumors.

The B cell differentiation process leading to the generation of GC lymphocytes and their subsequent differentiation to memory and plasma cells is tightly regulated. PR domain-containing 1, with Zinc Finger Domain 1 (PRDM1), also known as B-lymphocyte-induced maturation protein-1 (BLIMP1), is a master regulator of plasma-cell differentiation. BLIMP1 is a zinc finger transcriptional repressor that binds directly to DNA and acts as a scaffold to recruit multiple corepressor proteins, including the histone H3 methyltransferase G9a, the histone deacetylase HDAC2, the arginine methyltransferase PRMT5, and the histone demethylase LSD1 (Bikoff et al., 2009), thus turning off the gene expression program of GC and mature B cells. Conditional knockout of *Blimp1* in the mouse B cell compartment leads to an accumulation of activated B cells and a loss of plasma cell differentiation (Shapiro-Shelef and Calame, 2005). Conversely, enforced expression of BLIMP1 in lymphoma cell lines promotes either partial differentiation or apoptosis induction (Messika et al., 1998). BLIMP1 is highly expressed at the RNA level in the ABC-like DLBCL (Alizadeh et al., 2000); however, most of these tumors lack BLIMP-1 protein expression. Inactivating mutations of BLIMP1 have been found in 24% of the ABC-like DLBCL, but these do not account for absence of BLIMP1 protein expression in 77% of these tumors (Pasqualucci et al., 2006). Furthermore, while BLIMP1 inactivation in the ABC-like DLBCL suggests that it may function as a tumor suppressor gene, the precise mechanism by which BLIMP1 aberrations contribute to lymphomagenesis is unknown.

In this issue of *Cancer Cell*, Mandelbaum et al. (2010) and Calado et al.(2010) independently and unequivocally demonstrate that BLIMP1 functions as a tumor suppressor gene in the pathogenesis of the ABC-like DLBCL. Furthermore, they establish the spectrum of the BLIMP1 lesions, show the mechanism by which mutations affect the BLIMP1 function, and illustrate collaboration between BLIMP1 and NF-κB pathway, another signaling pathway frequently activated in the ABC-like DLBCL.

Mandelbaum et al. (2010) demonstrate the presence of BLIMP1 mutations, including frame-shift insertions and deletions, splice site mutations, and nonsense mutations in 27% of ABC-like DLBCL, but none of the GCB-like tumors, again confirming the subtype specificity of the BLIMP1 mutations. Furthermore, in the majority of these mutated cases, the authors show the presence of biallelic inactivation of the gene by wild-type

Table 1. Pathogenesis Mechanisms of DLBCL

	GCB-like	ABC-like
Transcriptional program	GC B cells	activated B cells
Ongoing Ig somatic mutations	++	–
AID Expression	++	++
Aberrant Ig class switch recombination/translocation	–	++
Aberrant somatic mutations		
BCL6	++	++
EZH2	++	–
CD79B	–	++
PAX5	–	++
MYC	–	++
RhoH/TTF	–	++
PIM1	–	++
CARD11	+	++
TNFAIP3(A20)	+	++
TNFRSF11A(RANK)	+	++
TRAF5	++	++
TRAF	++	+
MAP3K7(TAK1)	–	++
Constitutive activation of NF-κB	–	++
Constitutive activation of STAT3	–	++
Aberrant IL-4 signaling	–	++
BCL2 translocations-t(14;18)	++	–
BCL6 translocations	++	++
MYC translocations	++	++
Chromosomal alterations	gain of 12q12(MDM2)	trisomy 3
	loss 10q (PTEN)	gain/amplification 3q(BCL6, FOXP1)
	amplification 2p(REL)	gain/amplification18q(BCL2)
	loss 13q(ING1)	loss 6q21-q22
	amplification mir17-92	
		loss 9p(INK4a/ARF)
		gain/amplification 19q(SPIB)
Mutations of TP53	++	++
BLIMP1 mutations/deletions	–	++

allele deletion, epigenetic silencing, or uniparental disomy of the mutated gene. They also demonstrate the presence of biallelic gene deletion and BLIMP1 missense mutations, each in 6% of the ABC-like DLBCL. The BLIMP1 missense mutations affect protein stability and/or its transrepression activity, as demonstrated experimentally in both 293T and B cells. In addition, 26% of the ABC-like DLBCL tumors harbor translocations of *BCL6* and had significantly lower BLIMP1 mRNA levels, implicating known transcriptional repression of *BLIMP1* by BCL6 protein (Tunyaplin et al., 2004). Indeed, knockdown of BCL6 expression in DLBCL cell lines harboring *BCL6* translocations promoted increases in BLIMP1 mRNA and protein expression, supporting the findings that high BCL6 expression in ABC-like tumors containing *BCL6* translocations may account for low or absent expression of BLIMP1. Overall, these findings are of paramount importance for elucidating the mechanisms underlying the lack of BLIMP1 protein expression in the ABC-like DLBCL tumors. To assess the role of BLIMP1 inactivation in pathogenesis of the ABC-like DLBCL in vivo, Mandelbaum et al. generated two types of mice lacking *Blimp1* specifically in B cells. In both mouse models, there was increased incidence of lymphoproliferative disease and DLBCL. The majority of the DLBCL tumors were IRF4$^+$, negative for GC differentiation markers, and demonstrated constitutive activation NF-κB, consistent with the ABC-like phenotype.

Calado et al. (2010) recapitulated in mice, using a sophisticated genetic approach, constitutive activation of NF-κB and *Blimp1* inactivation, frequently found in human ABC-like DLBCL tumors. Mice with constitutive activation of NF-κB alone showed similar life spans as control mice, while deletion of *Blimp1* in GC B cells led to shortened survival of the animals that was further shortened by concomitant constitutive activation of the canonical NF-κB pathways. Mice

with constitutive activation of the NF-κB pathway demonstrated B cell and plasma cell hyperplasia, with frequent presence of serum monoclonal M-spikes. Mice with *Blimp1* inactivation demonstrated presence of $CD138^-$, $IRF4^+$, and $BCL6^-$ monoclonal large B cell tumors with highly somatically mutated immunoglobulin genes, consistent with an ABC-like human DLBCL. Similarly, mice with combined constitutive activation of NF-κB and *BLIMP1* inactivation who showed shorter survival also developed B cell lymphomas similar to human ABC-like DLBCL.

These two manuscripts explicitly demonstrate that *BLIMP1* is a bona-fide tumor suppressor gene involved in the pathogenesis of ABC-like DLBCL. They also prove that blocking differentiation is an important step in the pathogenesis of the ABC-like subtype. However, they still do not resolve the enigma of the nature of the precursor cell in this subtype. Furthermore, the study by Calado et al. also clearly demonstrates the synergy in oncogenesis between lesions that block differentiation (BLIMP1) and promote proliferation/survival (NF-κB). However, the low DLBCL penetrance and long latency in these mice suggest that additional transformation events are most probably required for development of the ABC-like tumors. A search in mice tumors for mutations affecting proteins controlling activation of the NF-κB (e.g., A20), commonly found in human ABC-like DLBCL, was negative. Identifying additional pathophysiologic mechanisms contributing to the pathogenesis of DLBCL and generation of drugs specifically targeting the "culprits" will be next important steps.

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Partners in Crime: Genes within an Amplicon Collude to Globally Deregulate Chromatin in Lymphoma

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In this issue of *Cancer Cell*, Rui et al. identify *JAK2* and *JMJDC2* as two contiguous, coamplified oncogenes in primary mediastinal B cell and Hodgkin lymphoma. Together, JAK2 and JMJD2C induce major changes in chromatin structure and gene expression. Targeting these proteins with small molecules represents a new avenue for therapy.

The classification of lymphoma has moved beyond morphology to ever more refined molecular subtypes, with distinct therapeutic implications. Gene expression profiling led to the classification of diffuse large B cell lymphoma (DLBCL) into activated B cell, germinal center B cell, and primary mediastinal B cell lymphoma (PMBL) subtypes (Dave et al., 2006). Profiling also showed that PMBL and Hodgkin's lymphoma (HL) could be grouped together (Rosenwald et al., 2003). Genome-wide copy number analysis coupled with expression profiling reinforced the view that these subtypes are pathogenetically distinct diseases (Lenz et al., 2008). PMBL and HL, in particular, frequently display amplification of chromosomes 2p and 9p. Amplification of *REL* at 2p14-16, which encodes c-Rel (a subunit of NF-κB), induces the constitutive activation of that antiapoptotic pathway.

JAK2 and JMJDC2 were among the genes previously suspected but not proved to have pathogenic roles within the 9p23-24 amplicon. To define the critical genes within the 9p amplicon in