Review

B cell receptor signaling in chronic lymphocytic leukemia

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B cell receptor (BCR) signaling plays an important pathogenic role in chronic lymphocytic leukemia (CLL) and B cell lymphomas, based on structural restrictions of the BCR, and BCR-dependent survival and growth of the malignant B cells. In CLL and lymphoma subtypes, ligand-independent ('tonic') and ligand-dependent BCR signaling have been characterized, which can involve mutations of BCR pathway components or be triggered by (auto)antigens present in the tissue microenvironment. In CLL, based on high response rates and durable remissions in early-stage clinical trials, there is rapid clinical development of inhibitors targeting BCR-associated kinases [Bruton's tyrosine kinase (BTK), phosphoinositide 3-kinase (PI3K)δ], which will change treatment paradigms in CLL and other B cell malignancies. Here, we discuss the evolution of this field, from BCR-related prognostic markers, to mechanisms of BCR activation, and targeting of BCR-associated kinases, the emerging Achilles' heel in CLL pathogenesis.

Lymphatic tissue microenvironment stages BCR activation in CLL

CLL cells proliferate in distinct microanatomical tissue sites called proliferation centers or pseudofollicles; a hallmark finding in CLL histopathology [1]. In these areas, CLL cells are in intimate contact with accessory cells, such as monocyte-derived nurse-like cells (NLCs) [2,3], T cells [4], and mesenchymal stromal cells [5], which, together with matrix factors, constitute the CLL microenvironment [6]. Proliferation of CLL cells in tissues accounts for a daily birth rate of 0.1–2% of the entire clone, as demonstrated by deuterated heavy water (^2H_2O) labeling in patients [7]. The lymphatic tissues are the apparent principal site of BCR activation for normal and malignant B cells. BCR activation can be induced by antigen or can be ligand-independent ('tonic' BCR signaling), and triggers a cascade of signaling events that normally cause B cell selection, proliferation, differentiation, and antibody production [8]. Thereby, BCR signaling allows for the expansion of selected, foreign antigen-specific B cells, and deletion of unwanted, self-reactive B cells [9]. In B cell malignancies, such as CLL [10–13] and diffuse large B cell lymphoma (DLBCL) [14], BCR signaling plays a critical role in pathogenesis, even though the mechanisms of BCR stimulation are heterogeneous and to some degree controversial [15–19]. Antigen-dependent and -independent BCR activation are two fundamentally different mechanisms of BCR pathway activation that exist in B cell lymphomas. For example, there can be activating mutations in the BCR pathways, such as mutations in the coiled-coil (CC) domain of caspase recruitment domain 11 (CARD11) in DLBCL, gastric lymphoma, and primary central nervous system lymphoma, or activating mutations of the immunoreceptor tyrosine-based activation motifs (ITAMs) within the CD79B and CD79A signaling modules of the BCR, as in ABC DLBCL [14,16]. In CLL, however, BCR pathway activation does not appear to involve activating pathway mutation and therefore can be viewed as antigen-dependent, resulting from BCR ligation via antigens (autoantigens and/or microbial antigens) that are present in the microenvironment. Recent studies raise an additional target of BCR recognition, that is, the binding of an intrinsic IGHV motif [15,20]. There are several sets of indirect data suggesting the importance of BCR signaling in CLL; the most compelling are comparative gene expression profiling (GEP) data that have revealed BCR signaling as the most prominent pathway activated in CLL cells isolated from areas of proliferation within the lymphatic tissues [21]. In this review we discuss mechanisms of BCR activation in CLL and the potential for targeting BCR signaling with novel inhibitors of BCR-associated kinases BTK, spleen tyrosine kinase (SYK), and PI3Kδ.

BCR activation in the CLL: mechanism and relation with prognostic markers

The BCR is composed of a ligand binding moiety, the antigen-specific surface membrane immunoglobulin (smIg), paired with the signal transduction moiety, Ig-α/Ig-β heterodimers (CD79A, CD79B; Figure 1). Engagement of the BCR by antigen induces membrane movement and aggregation of BCR components that lead to phosphorylation of ITAMs in the cytoplasmic tails of CD79A and CD79B. The latter is accomplished by Lyn and other Src family kinases (Fyn and Blk), which in turn activate SYK, BTK, and PI3Ks (Figure 1). BCR oligomerization and BCR microcluster growth [22] activate downstream pathways, including calcium mobilization, mitogen-activated protein (MAP) kinases and RAS activation, activation of phospholipase Cγ, protein kinase C (PKC)β, and CARD11, causing recruitment of B cell lymphoma/leukemia 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation protein

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Keywords: chronic lymphocytic leukemia; B cell receptor; microenvironment; Bruton's tyrosine kinase; spleen tyrosine kinase; phosphoinositide 3-kinase δ.

1471-4906/$ – see front matter
 http://dx.doi.org/10.1016/j.it.2013.07.002

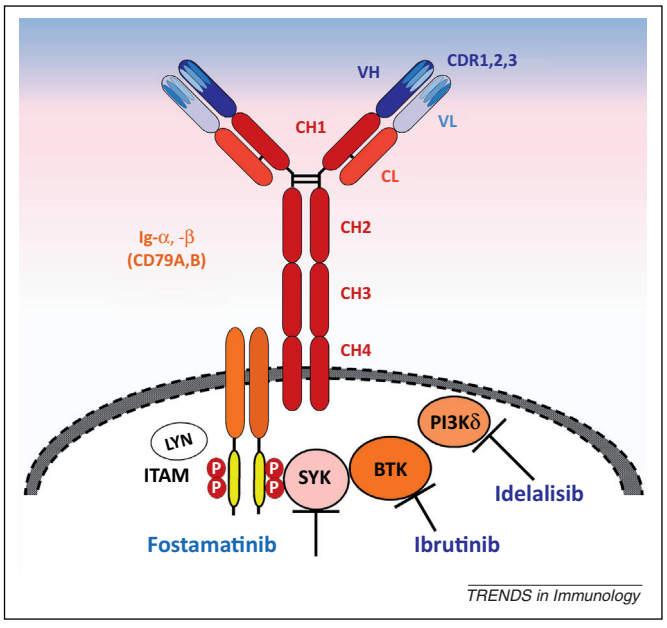

Figure 1. B cell receptor (BCR) components and upstream signaling. The BCR consists of the antigen-binding heavy chains IgH (VH and CH1–4) and light chains IgL (VL and CL) that are noncovalently coupled to the Ig-α (CD79A) and Ig-β (CD79B) signaling subunits. Antigen encounter and BCR clustering promotes tyrosine phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAMs) by Src-family kinases LYN, FYN, and BLK. Phosphorylated ITAMs then recruit spleen tyrosine kinase (SYK) through interactions with its SH2 domains. SYK activation triggers activation of a signaling cascade that engages Bruton's tyrosine kinase (BTK), phosphoinositide 3-kinases (PI3Ks; including PI3Kδ), nuclear factor (NF)-κB, PI3K, nuclear factor of activated T cells (NF-AT), mitogen-activated protein (MAP) kinase, and RAS signaling pathways, leading to cell survival and proliferation. Upstream BCR signaling kinases can be targeted by the small molecule SYK inhibitor fostamatinib [90], the BTK inhibitor ibrutinib [91], and the PI3Kδ inhibitor idelalisib [117].

1 (MALT1) into a multiprotein CBM complex that activates IκB kinase (IKK), thereby initiating nuclear factor (NF)-κB signaling [14,16]. Collectively, these signaling events promote B cell survival and proliferation [16].

Several prognostic markers in CLL, such as IgV_H mutational status [23,24], zeta-chain-associated protein kinase 70 (ZAP-70) [25,26] and chemokine CC ligand (CCL)3 [27] are associated with (auto)antigen binding and function of the BCR, suggesting a relation between enhanced BCR signaling and worse prognosis. Also, BCRs in CLL patients are characterized by a biased usage of *IGHV* and *IGLV*κ/λ genes, which differ from those of normal B cells. Oftentimes, specific *IGHV*s partner with specific *IGHD-J*s and specific *IGLV*s with specific *IGLJ*s, leading to remarkably similar, stereotyped heavy chain complementarity determining region 3 (HCDR3) and smIgs [28,29]. These data support the concept of antigen-driven selection and expansion of CLL clones, and they suggest that recurrent binding of restricted sets of antigenic epitopes are linked to the selection of those normal B cell clones that enter the CLL pathogenetic process [11,30–32]. More evidence for the importance of BCR signaling in CLL comes from comparative GEP data that revealed BCR signaling as the most prominent pathway activated in CLL cells isolated from lymphatic tissues [21]. Along the same lines, cells from those patients with the worse outcome (e.g., unmutated CLL (U-CLL)) display GEPs identifying activation of genes downstream of the BCR [33].

IGHV mutational status

The antigen-binding sites of Igs derive from recombination of V, D, J gene segments for the heavy chains and V and J segments for the light chains, which subsequently can be modified by somatic mutation to higher affinity after antigen engagement, with or without T cell help. Based on the degree of somatic hypermutation of the Ig heavy chain variable gene segments (*IGHV*s), patients can be classified as unmutated (U-CLL), if they have ≥98% sequence homology with the germline sequence, or mutated CLL (M-CLL) cases, if they have <98% sequence homology (Table 1) [34]. U-CLL cases have a more unfavorable clinical course and shorter survival, whereas M-CLL typically has slower disease progression and longer survival [23,24]. These findings supported the concept that CLL may consist of two distinct diseases with different clinical presentation, disease biology, and cellular origin [30,35].

Table 1. BCR-related risk factors in CLL.

	Cut off	Method of detection	Pros and cons	Prognostic value in CLL
IGHV mutational status	98% deviation from germline sequence	PCR amplification and sequencing	Does not change over the course of the disease, expensive, time-consuming	'unmutated' (U-CLL, ≥ 98% sequence homology = shorter PFS and OS; ''mutated'' cases (M-CLL, <98% sequence homology) = longer PFS and OS
CD38	Threshold is controversial (5%, 7%, 20% and 30% cut-offs)	FACS	Relatively inexpensive, now part of routine immunophenotyping, CD38 expression may vary over time	Not standardized and validated in prospective trials
ZAP-70	Threshold is controversial, 20% positive CLL cells by FACS is commonly used	Western blotting, quantitative RT-PCR, immunohistochemistry, and FACS	Relatively inexpensive, stable over time, difficult to standardize (quantification of cytoplasmic staining)	Not standardized and validated in prospective trials
CCL3, CCL4 (MIP-1α,β) plasma levels	10 pg/mL threshold for CCL3; 60 pg/mL threshold for CCL4	ELISA	Inexpensive, reliable and reproducible. Levels can change over time and can be influenced by non-CLL related events, such as inflammation	High levels correlate with shorter PFS, CCL3 levels rapidly normalize during therapy with inhibitors targeting BCR-associated kinases (BTK[69], PI3Kδ[70]), not validated in prospective trials