Published in final edited form as:

Trends Mol Med. 2014 June ; 20(6): 343–352. doi:10.1016/j.molmed.2014.03.001.

Breaking bad in the germinal center: how deregulation of BCL6 contributes to lymphomagenesis

Katerina Hatzi^{1,2} and Ari Melnick^{1,2}

¹Division of Hematology and Medical Oncology, Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA

²Department of Pharmacology, Weill Cornell Medical College, New York, NY 10065, USA

Abstract

The B cell lymphoma 6 (BCL6) transcriptional repressor is a master regulator of the germinal center (GC) B cell phenotype, required for their unique proliferative and stress tolerant phenotype. Most B cell lymphomas arise from GC B cells and are dependent on the continued or deregulated expression of BCL6 to maintain their survival. The actions of BCL6 in B cells involve formation of distinct chromatin modifying complexes that silence specific promoter and enhancer networks, respectively. The same biochemical mechanisms are maintained in malignant lymphoma cells. Targeted inhibition of these BCL6 functions has emerged as the basis for rational design of lymphoma therapies and combinatorial regimens. In this review, we summarize recent advances on BCL6 mechanisms of action and the deregulation of its target gene networks in lymphoma.

Keywords

transcriptional repression; lymphomagenesis; gene enhancers; epigenetic regulation; BCL6; transcription factor targeted therapy

BCL6 in normal germinal center B cells and lymphoma

B cell lymphoma 6 (BCL6; see Glossary) is a transcription repressor with many critical roles in cell types that contribute to the innate and adaptive immune response. During the humoral response, BCL6 functions as a master regulator of the germinal center (GC) B cell phenotype. GCs emerge in the secondary lymphoid organs upon B cell activation and provide the setting for massive clonal expansion, immunoglobulin somatic hypermutation, and class switch recombination leading to antibody affinity maturation. B cells that generate high-affinity antibodies are then selected to undergo terminal differentiation to memory cells or long-lived antibody-secreting plasma cells [1]. BCL6 is essential for GC formation as BCL6 knockout mice fail to develop GCs and cannot undergo T cell dependent immunoglobulin affinity maturation [2–4].

BCL6 is upregulated in the interfollicular zone following long-lived interactions between B cells activated by their cognate antigen and T cells [5,6]. To orchestrate the GC B cell reaction, BCL6 attenuates DNA damage response and inhibits cell cycle checkpoint genes despite increased replicative stress, thus maintaining GC B cell proliferation and survival. However, by dampening these physiological cell responses BCL6 can tip the balance towards malignant transformation. Hence, BCL6 acts as an oncogene in GC-derived B cell lymphomas, which are often characterized by deregulated BCL6 expression, or feature genetic alterations in pathways normally regulated by BCL6. Such mechanisms indicate a propensity for disruption of the BCL6 physiological networks in lymphoma. Recently, the BCL6 mechanism of action in GC B cells has been described showing that BCL6 forms distinct complexes at promoters versus enhancers acting through different biochemical mechanisms [7]. Moreover, a new mouse model designed to dissect the functions of BCL6 in GC B cells suggests that mutations specifically blocking recruitment of broad complex/tramtrack/bric-a-brac (BTB) domain corepressors affect GC B cell survival, but spare BCL6 functions in other cell types [8]. These results raise new questions on how BCL6 mediates its effects in the various immune cell types where it is involved and what cofactors are involved. Notably, these new discoveries indicate that the BCL6 mechanisms in normal GC B cells are maintained in lymphoma cells, highlighting the therapeutic potential of targeting these BCL6 functions.

BCL6 and its target genes: a precarious balance between normal and malignant phenotypes

GC B cells uniquely manifest under physiological conditions some of the characteristic hallmarks of tumor cells: they proliferate rapidly, evade growth checkpoint controls, and tolerate ongoing genomic instability occurring as a byproduct of somatic hypermutation. BCL6 enables and maintains the GC phenotype by repressing genes that control the cell cycle, cell death, terminal plasma cell differentiation, and DNA damage response [9–13]. For example, BCL6 represses *TP53* (*tumor protein p53*), *CDKN1A* (*cyclin-dependent kinase inhibitor 1A*), *ATR* (*ataxia telangiectasia and Rad3 related*), and *CHEK1* (*checkpoint kinase 1*) [9–12], allowing cells to sustain proliferation while rendering them resistant to DNA-damage induced apoptosis. BCL6 binds and represses numerous other tumor suppressors including *CDKN1B* (*cyclin-dependent kinase inhibitor 1B*), *CDKN2A* (*cyclin-dependent kinase inhibitor 2A*), *CDKN2B* (*cyclin-dependent kinase inhibitor 2B*), *PTEN* (*phosphatase and tensin homolog*), and others [14]. BCL6 also regulates genes involved in B cell signaling, thereby preventing premature termination of affinity maturation by T cells such as *CD69*, *CD44*, *CD23b*, and *NF-KB1* (*nuclear factor NF-kappa-B p105 subunit*) [13,15,16], and silences genes that mediate terminal differentiation downstream of these signaling pathways such as *PRDM1* (*PR domain containing 1, with ZNF domain*) and *IRF4* (*interferon regulatory factor 4*) [13,17].

The prosurvival and proliferation functions of BCL6 are potentially dangerous and act as a double-edged sword. Thus, although these actions are essential to permit immunoglobulin mutagenesis and maturation, they also make GC B cells prone to malignant transformation. Accordingly, a majority of B cell lymphomas arise from GC B cells and express BCL6

constitutively, including the diffuse large B cell lymphomas (DLBCLs) and follicular lymphomas (FLs) [18]. Mice constitutively expressing BCL6 in GC B cells develop tumors resembling human DLBCL [19,20]. BCL6 may compensate for its potentially oncogenic actions in silencing tumor suppressor genes [14], by also binding and repressing a large set of genes that can contribute to tumorigenesis when deregulated in normal GC B cells including *BCL2* (*B cell CLL/lymphoma 2*), *c-MYC*, *CCND1* (*cyclin D1*), and *BM11* (*B lymphoma Mo-MLV insertion region 1 homolog*) [14] (Figure 1). It is plausible that BCL6 repression of these genes evolved as a mechanism to counterbalance its tumorigenic potential in GC B cells. Along these lines many of the commonly occurring somatic mutations in DLBCL and FL enable the escape of these key proto-oncogenes from their silencing by BCL6. For example, *BCL2*, a gene involved in suppressing the intrinsic apoptosis pathway is frequently translocated in FL and DLBCL [21]. *BCL2* is normally silenced by BCL6, which may cooperate with transcription factor MIZ1 (Myc-interacting zinc finger protein 1) to repress this locus [22]. However, chromosomal translocations and promoter mutations in DLBCL and FL enable the *BCL2* locus to escape the silencing effects of BCL6 [14,22]. Similar mechanisms pertain to the deregulation of *c-MYC*, a key cellular proliferation regulator. BCL6 downregulates *c-MYC* after the first few cellular divisions that follow B cell activation [23]. After undergoing somatic hypermutation, GC B cells undergo selection through interactions with T cells and follicular dendritic cells (FDCs). At this time, repression of *c-MYC* may be temporarily released to enable a subset of B cells to return to the GC for additional rounds of affinity maturation [23]. *c-MYC* translocations occurring in DLBCL enable release from BCL6-mediated repression and are also associated with poor prognosis [24].

BCL6 represses several components of the B cell receptor (BCR) and CD40 signaling pathways and counteracts NF- κ B activation [15] (Figure 2). Constitutive NF- κ B signaling is essential for survival of the activated B cell subtype of DLBCL (ABC-DLBCL) downstream of chronic active BCR or Toll-like receptor (TLR) signaling [25]. ABC-DLBCLs are believed to derive from plasmablastic GC B cells that are in the process of exiting the GC reaction [26]. Recurrent somatic mutations in genes including *CD79A/B* (BCR coreceptor), *CARD11* (*caspase recruitment domain family, member 11*), *TNFAIP3* (*tumor necrosis factor, alpha-induced protein 3*; a negative NF- κ B regulator), and *MYD88* (*myeloid differentiation primary response 88*; a component of TLR signaling) are detected in a substantial proportion of ABC-DLBCLs and result in aberrant activation of these pathways and survival of lymphoma cells [27–30]. Activating mutations in these pathways might provide a mechanism through which transformed B cells can escape BCL6-mediated counterregulation. Indeed, *BCL6* translocations are more frequently detected in ABC-DLBCLs [31], raising the possibility that these cells may require these somatic mutations in BCR and TLR pathway genes to escape from the BCR and NF- κ B attenuating effects of constitutive BCL6 expression. However, it is also possible that pathways downstream of BCR signaling, such as calcium signaling, could induce BCL6, which could be relevant to the initiation of the GC reaction or normal GC B cell survival during clonal selection [32]. Finally, BCL6 blocks terminal differentiation of GC B cells into memory or antibody-secreting plasma cells [13,17]. This effect is due at least in part through repression of the *IRF4* and *PRDM1* transcription factors, which are master regulators of GC exit and terminal

differentiation. PRDM1 is also a tumor suppressor that is mutated or silenced in approximately 25% of ABC-DLBCLs [33,34]. PRDM1 inactivation is an alternative mechanism through which B cell differentiation can be blocked and lead to lymphomagenesis. Finally, BCL6 represses microRNAs including *mir155* and *mir136* in GC B cells which downregulate AID (activation-induced cytidine deaminase) and other key GC B cell transcription factors [35]. Given that AID plays a central role in inducing DNA damage and mutagenesis in GC B cells and is required for lymphomagenesis [35,36], it is feasible that BCL6 suppression of these microRNAs could contribute to malignant transformation of GC B cells.

Loss of control of BCL6 expression can lead to a B cell transformation 'chain reaction'

BCL6 transcript and protein expression is under tight control during the GC reaction (Figure 3). Both BCL6 mRNA and protein are highly upregulated upon B cell activation. IL21R (interleukin 21 receptor) signaling is one of the pathways that induces BCL6 during B cell activation [37]. Although interferon regulatory factor 8 (IRF8) and activated signal transducer and activator of transcription 5B (STAT5B) may also induce BCL6 upregulation [38,39], which transcription factors are most responsible for driving BCL6 expression upon B cell activation remains unclear. BCL6 mRNA stability is enhanced in GC B cells due to the actions of the AUF1 (AU-rich element RNA-binding protein 1) mRNA chaperone and HSP90 (heat shock protein 90), which may be induced by replicative and other sources of stress [40]. Nuclear export of BCL6 mRNA is augmented by eIF4e (eukaryotic translation initiation factor 4E), and BCL6 protein stability is also maintained by HSP90 [40,41]. Other mechanisms ensure that BCL6 is silenced once affinity maturation is achieved to enable terminal differentiation to antibody-secreting and memory B cells. For example, antigen engagement stimulates BCR signaling, which triggers BCL6 phosphorylation by mitogen-activated protein kinases (MAPKs), leading to ubiquitin-mediated proteasomal degradation [42,43]. Moreover, CD40 stimulation leads to NF- κ B-mediated activation of IRF4, which in turn represses the BCL6 promoter [44]. Accordingly, the Epstein–Barr virus oncoprotein LMP1 (latent membrane protein 1), which mimics an activated CD40 receptor, also suppresses BCL6 [45]. Failure of BCL6-suppressing mechanisms is a common underlying cause of malignant transformation of B cells. Indeed constitutive expression of *BCL6* in genetically engineered mice induces GC hyperplasia and is sufficient to drive formation of DLBCLs [20]. DLBCLs often feature chromosomal translocations fusing the *BCL6* coding regions to strong heterologous promoters such as the immunoglobulin loci, resulting in *BCL6* promoter substitution and deregulated expression [46,47]. Alternatively, the binding sites for transcription factors such as IRF4, STAT5, and BCL6 itself, which normally repress *BCL6* expression, may be disrupted through somatic hypermutation [44]. Somatic mutations of MEF2B (myocyte enhancer factor 2B) disrupt its interaction with corepressor CABIN1 (calcineurin-binding protein cabin-1) or reduce MEF2B responsiveness to upstream regulatory signals, resulting in sustained BCL6 expression in lymphoma cells [48]. Hypermethylation of CpG islands located in intron 1 of *BCL6* is proposed to block binding of CTCF (CCCTC-binding factor), a negative regulator of *BCL6* [49]. Primary lymphoma specimens were hypermethylated in this region, suggesting possible relevance to disease

pathogenesis [49]. Finally, *BCL6* expression is also controlled through a cluster of enhancer elements that form a locus control region (LCR) located far upstream of its transcriptional start site [7,50]. This *BCL6* LCR encompasses a frequent translocation breakpoint region, a noncoding RNA and a genetic variant that confers lymphoma susceptibility [51], and can loop to the *BCL6* promoter, suggesting a regulatory role on *BCL6* expression [50]. Interestingly, the BCL6 protein can bind to this LCR, suggesting that BCL6 autoregulation might extend beyond repression of its own promoter [52] and involve regulation of upstream elements. The *BCL6* LCR also features binding of the BRD4 (bromodomain-containing protein 4) bromodomain protein, and inhibitors of BRD4 can result in a partial reduction of BCL6 expression in DLBCL cells, perhaps by reducing the functionality of the *BCL6* LCR region [53]. How BRD4 inhibitors might affect looping of the LCR to the BCL6 promoter still remains unknown.

Post-transcriptional mechanisms may also sustain BCL6 expression in B cell lymphomas. Duan *et al.* recently showed that BCL6 is targeted for degradation by a ubiquitin ligase complex containing the F-box protein FBXO11 (F-box only protein 11) [54]. Monoallelic *FBXO11* deletions and mutations are observed in DLBCL cases (~9%), suggesting that loss of FBXO11 function might result in augmented BCL6 protein contributing to lymphomagenesis [54]. Nuclear-localized HSP90 is observed in a majority of DLBCLs and maintains the stability of BCL6 mRNA and protein. HSP90 also forms a complex with BCL6 at its target genes enabling its transcriptional repressor function [40]. Furthermore, BCL6 forms a critical positive feedback loop with HSP90 to maintain stress signaling in DLBCL [55]. Specifically, BCL6 repression of the *EP300* (*E1A binding protein p300*) locus, which encodes a lysine acetyltransferase, prevents the acetylation and inactivation of HSP90, which in turn continues to maintain BCL6 repression of *EP300* [55]. Suppression of BCL6 or of HSP90 breaks this loop and kills DLBCL cells. Finally, certain BCL6 repressor functions are suppressed through acetylation of a KKYK motif within the middle region of the protein by EP300 and the closely related protein CREBBP (CREB-binding protein) [54,55]. Recurrent somatic inactivating mutations in the acetyltransferase domain of *EP300* and *CREBBP* occurring in DLBCL and FL [54,55] might thus result in deregulated activity of BCL6.

BCL6 transcriptional mechanism varies in a cell type-specific manner

Ultimately, BCL6 mediates its crucial functions as a master regulator of GC B cells and lymphomas by coordinating the assembly of transcriptional repression complexes on chromatin. From a structural standpoint, BCL6 contains an evolutionarily conserved N terminal BTB/POZ domain, a middle unstructured region (often referred to as ‘second repression domain’ or ‘RD2’) and binds to a consensus DNA motif through six C2H2 *Krüppel*-type zinc fingers at its C terminus [56]. Both the BTB and RD2 domain have autonomous repressor activity [57,58] and recruit distinct sets of corepressor partner proteins. The zinc finger domain may recruit additional corepressors, but may also repress transcription by competing for binding with STAT family transcriptional activators, which bind to overlapping DNA-binding elements [59,60]. Pharmacological blockade of the BCL6 BTB domain is sufficient to prevent GC formation in immunized mice, kill DLBCL cells *in vitro*, and eradicate DLBCLs in mice, pointing towards this specific motif as driving

fundamental actions of BCL6 in lymphomagenesis. Hence, understanding how the BTB domain functions is key to understanding how transcriptional regulation works in normal and malignant B cells [61–63].

The BCL6 BTB domain forms an obligate homodimer. Dimerization generates two identical and symmetrical extended lateral grooves at the interface between BTB monomers. This interface serves as a docking site for recruitment of three corepressors: SMRT (silencing mediator of retinoid and thyroid receptor), NCOR (nuclear receptor corepressor), and BCOR corepressors (BCL6 corepressor) [64,65]. These in turn serve as scaffolds for various chromatin-modifying functions, forming biochemically and functionally distinct corepressor complexes. SMRT, NCOR or BCOR interact with the lateral grooves of BCL6 through an 18 residue unstructured peptide (BCL6-binding peptide, BBP). SMRT and NCOR are highly related and bind to the BCL6 BTB groove through an identical BBP sequence. Both proteins form similar higher order complexes with HDAC3 (histone deacetylase 3) and allosterically facilitate histone deacetylation [66]. By contrast, BCOR shares no sequence or structure similarity with SMRT and NCOR, and binds to BCL6 using a completely different BBP sequence [57,67]. Hence, to accommodate the SMRT, NCOR or BCOR BBPs the BCL6 groove displays a surprising degree of ligand flexibility, given its length and complexity. BCOR forms an entirely different type of complex, closely related to PRC1 (polycomb repressor complex 1), containing the histone demethylase KDM2B [lysine (K)-specific demethylase 2B], ubiquitin ligases RING1A and RING1B, and other PRC1-related proteins [68,69]. BCL6 BTB domains contain two corepressor binding sites, and hence are uniquely able to form hybrid complexes containing both SMRT/NCOR and BCOR [57,67]. This enables the BCL6 BTB domain to function as a virtual ‘Swiss army knife’, bringing multiple different functionalities to its target genes.

Remarkably, although BCL6 has pleiotropic functions in various cell lineages, the BTB domain corepressor complex seems largely restricted to BCL6 functions in B cells under physiological conditions. A genetically engineered strain of mice was generated to express a mutant form of BCL6 that is unable to bind SMRT, NCOR, and BCOR, but is otherwise functionally intact [8]. These mice failed to form functional GCs due to failure of activated B cells to proliferate and survive [8]. Indeed ChIP-seq studies in primary human GC B cells showed that BCL6 repressed key checkpoint genes *ATR*, *TP53*, and *CDKN1A* [8], through recruitment of SMRT or BCOR. In contrast to BCL6 knockout mice, which display a lethal inflammatory phenotype, *Bcl6^{BTBmut}* mice lived normal healthy lives [8]. BCL6 actions in T cells and macrophages were not significantly impaired in *Bcl6^{BTBmut}* mice [8]. A similar, B cell specific phenotype was elicited *in vivo* through the use of peptides or small molecules that specifically disrupt the recruitment of SMRT, NCOR, and BCOR to BCL6. These inhibitors also cause derepression of *ATR*, *TP53*, and *CDKN1A*, and induce proliferation arrest and apoptosis in DLBCL cells [61–63]. Hence, the role of BCL6 as a checkpoint suppressor is principally mediated through the BTB domain and is mostly relevant to its physiological actions in T cell dependent B cell activation and maintenance of lymphoma cell proliferation and survival. The functions of BCL6 in other cell types must be mediated by distinct mechanisms. Along these lines, the role of BCL6 in suppressing macrophage inflammatory signaling was associated with direct competition with STAT5 for DNA-

binding sites through BCL6 zinc fingers [8]. The RD2 domain of BCL6 was reported to interact with CTBP (C terminal binding protein 1) and MTA3 (metastasis associated 1 family, member 3) proteins, and may play a role in helping BCL6 repress the *PRDM1* locus and suppress terminal differentiation [52,58,70].

BCL6 controls transcription through distinct promoter and enhancer specific mechanisms

Further dissection of BCL6 BTB domain-dependent repression identified two simultaneous but functionally independent mechanisms of transcriptional repression [7] (Figure 4). Firstly, although BCL6 binds thousands of promoters, it only potently repressed the subset (~20% of target promoters) where it formed a unique ‘ternary complex’ with simultaneous recruitment of BCOR and SMRT/NCOR complexes [7]. Ternary BCL6 complex formation was linked to specific epigenetic chromatin marks that constitute a biochemical ‘BCL6 repression fingerprint’. This BCL6 promoter repression fingerprint consists of depletion of the activating histone modifications (H3K4me3, H3K9Ac, H3K79me2, and H3K36me3) and enrichment in repressive marks H3K27me3 and DNA methylation. Many of these marks are directly mediated by BCL6 ternary complexes and distinguish target promoters that are regulated by BCL6 from those that are not [7]. Notably, BCL6 does not disrupt association of RNA polymerase II with gene promoters or mediate formation of heterochromatin. Instead, BCL6 seems to suppress RNA polymerase II transcriptional elongation, and hence is more of an ‘elongation suppressor’ than blocker of polymerase assembly [7].

A second, independent mechanism involves BCL6 regulation of enhancers linked to a different set of genes than those regulated through ternary promoter complexes. Enhancers exist in at least two configurations: ‘active’ enhancers are associated with transcriptionally activated genes and are marked with H3K4 monomethylation or dimethylation plus H3K27 acetylation; and ‘poised’ enhancers feature H3K4 mono/dimethylation but no H3K27 acetylation, and are associated with repressed genes. BCL6 is the first example of a transcription factor shown to switch, or ‘toggle’, enhancers from the active to poised configuration. This action is mediated by recruitment of SMRT–HDAC3 complexes to deacetylate H3K27, opposing the H3K27 acetylating activity of EP300. This mechanism might be essential for B cells to undergo rapid phenotypic changes in response to environmental cues, such as recycling between the proliferative dark zone and the T cell rich light zone of the GC, where GC B cells are selected for terminal differentiation [7]. Consistent with this notion, enhancers occupied by BCL6–SMRT complexes are linked to light zone upregulated genes and enriched in CD40 (CD40 ligand), IL10 (interleukin 10), and STAT3-induced transcriptional signatures [7]. In further support of this physiological enhancer toggling mechanism, CD40 signaling causes ERK-mediated phosphorylation of SMRT in GC B cells, resulting in its cytoplasmic localization and derepression of BCL6 target genes. This effect is rapidly reversible upon washout of the CD40 ligand [71].

BCL6 as a therapeutic target for B cell lymphomas

The fact that BCL6 is expressed in a majority of DLBCLs and FLs, and is required to maintain the survival of established lymphoma cells, points towards its significance as a

therapeutic target. Targeting transcription factors has long been considered to be an insurmountable challenge of cancer therapy. However, in-depth structural studies of the BCL6 BTB domain corepressor interface have provided a path forward for the rational design of such inhibitors. Features that make the BCL6 BTB domain an attractive target include: (i) residues through which BCL6 interacts with the SMRT, NCOR, and BCOR are unique to BCL6 and not conserved in other BTB domains, raising the possibility of developing specific inhibitors less likely to disrupt other related transcription factors; (ii) the BCL6 BTB domain corepressor interface is not involved in the lethal inflammatory phenotype that is caused by total loss of the BCL6 protein, thus reducing the likelihood of on-target toxicity; and (iii) the structure of the BTB corepressor interface contains hotspots with key intermolecular interactions susceptible to design of competitive inhibitors. Proof-of-principle studies using recombinant SMRT peptides fused to TAT (trans-activator of transcription) cell penetrating domains show that these BCL6 peptide inhibitors (BPIs) can disrupt the formation of BCL6 repression complexes, derepress BCL6 target genes, and rapidly kill DLBCL cells. Drug design efforts led to the development of truncated, retro-inverso BCL6 peptidomimetic inhibitors (RI-BPIs), which display favorable pharmacokinetic properties and can fully eradicate established lymphomas in mouse models [7,62,63]. RI-BPIs and other BCL6 small molecule inhibitors are currently being translated for use in humans. Computer-aided drug design enabled identification of a small molecule lead compound called 79-6 with similar anti-lymphoma activity. RI-BPIs and 79-6 do not induce any toxicity in animals, even when administered for up to 1 year in a continuous manner [62], which is consistent with the *Bcl6^{BTBmut}* mouse model.

The transcriptional signatures induced by treatment with RI-BPIs are significantly enriched both in genes repressed by BCL6–ternary promoter complexes as well as genes linked to BCL6–SMRT silenced enhancers [7]. Moreover, BCL6 BTB domain small molecule inhibitors induce enhancer-associated H3K27 acetylation, pointing to pharmacological reactivation of the BCL6 enhancer network [7]. Constitutive toggling of enhancers to the poised position may play a critical role in lymphomagenesis, and hence BCL6 inhibitors could have therapeutic value, particularly in DLBCL tumors with inactivating *EP300/CBP* mutations [54,55] where the BCL6 enhancer network maybe locked in a silenced state. In this and other ways, understanding the biochemical mechanisms of the BCL6 BTB domain function could inform patient selection in future trials testing BCL6 inhibitors in the clinic. For example, given the variable clinical outcome of DLBCLs, improved classification of patients based on updated transcriptional signatures derived from both BCL6-targeted promoter and enhancer networks could point to the BCL6 BTB-dependent tumors that would benefit from BCL6 inhibitors. Indeed, earlier studies used BCL6 target genes defined by ChIP-on-chip to classify DLBCLs into BCL6-dependent and -independent subtypes [72]. BCL6-dependent tumors include both GCB type and ABC-DLBCLs. Mechanisms of resistance are not yet defined.

Delineating BCL6-repressed networks in lymphoma cells, as well as its biochemical mechanisms of action, can facilitate the design of rational combinatorial treatments of BCL6 inhibitors with other drugs. Derepression of *TP53* and apoptosis pathways results in synergistic killing of lymphoma cells when BCL6 inhibitors are combined with

chemotherapy or p53-activating compounds [73]. Therapeutic strategies combining BCL6 blockade with HSP90 or HDAC inhibitors synergistically kill DLBCL cells by disrupting the positive feedback loop among BCL6, *EP300* repression, and HSP90. BCL6 inhibition also induces expression of antiapoptotic genes such as *BCL2* and *BCL2A1* [14]. These may represent a type of feedback mechanism that could enable the survival of DLBCL cells exposed to BCL6 inhibitors. Along these lines, combinatorial therapy using BH3 mimetic drugs such as ABT-737 yielded potent synergy with BCL6 inhibitors [74]. BCL6 regulation of B cell receptor and NF- κ B signaling could also be harnessed to design combined targeted treatments. For example, BCL6 was shown to maintain BCR signaling through repression of the SYK (spleen tyrosine kinase) inactivating phosphatase PTRPRO (protein tyrosine phosphatase, receptor type, O) [75]. Therefore, BCL6 inhibition combined with compounds targeting BCR signaling such as SYK and BTK inhibitors might provide synergistic killing of lymphoma cells. Collectively, the potent anti-lymphoma activity and favorable pharmacological properties of BCL6 inhibitors hold great promise for the development of definitive therapeutic regimens to eradicate lymphomas with less toxicity compared with current options. Inhibitors targeting the BCL6 BTB domain are likely to be superior to targeting DNA binding by BCL6 zinc fingers or antisense type strategies that downregulate BCL6 protein. BCL6 knockout mice lacking the zinc finger domain manifest a rapidly fatal phenotype due to systemic inflammation mediated by macrophages and T cells [3,4]. Hence, eliminating BCL6 binding to chromatin would very likely cause significant toxic side effects. By contrast, targeting the BTB domain corepressor-binding site, which is not involved in the macrophage inflammatory response or T cell functions, does not elicit these undesired biological effects.

Concluding remarks and future perspectives

Recent studies on the BCL6 mechanism of action indicate surprising biochemical diversification of its functions in B cells and other cell types. Distinct BCL6 transcription complexes form within the same cells to mediate silencing of promoters and enhancers, respectively. How specific sites in the genome assemble distinct types of functional BCL6 complexes remains an unanswered question (Box 1). Presumably, this involves various extracellular signals, interactions with other cell types, sources of stress, and metabolic sensors to direct site-specific formation of BCL6 complexes. BCL6 plays distinct biological roles in different cell types. Emerging data indicate that this is not only due to binding to distinct target gene sets but is also due to BCL6 functioning through biochemically distinct mechanisms in a cell context-dependent manner. Whereas normal and malignant B cells are dependent on transcriptional complexes assembled through the BCL6 BTB domain, actions in T cells and other cell types remain to be discovered. BCL6 poises B cells in a precarious position between a transformed and nontransformed state. Loss of its tumor suppressor activities results in lymphomagenesis. Although its oncogene functions are known to involve suppression of proliferation and differentiation, as well as maintenance of the stress positive feedback loop, it is clear from target gene studies that BCL6 must have a number of other functions in lymphomas that require further study. The fact that the oncogenic function of BCL6 is distinct from its actions in inflammation and T cell biology raise the possibility of specific therapeutic targeting of this oncogene without harming normal tissues. Recently

developed BCL6 inhibitors can accordingly eradicate lymphomas without evident toxicity to animals. Although BCL6 inhibitors are not yet FDA approved for use in humans, their translation to the clinic could provide the basis for powerful therapeutic regimens for a variety of B cell lymphomas. Elucidation of other BCL6 mechanisms may enable development of therapies specific to functions of BCL6 in other cell types.

References

1. Allen CD, et al. Germinal-center organization and cellular dynamics. *Immunity*. 2007; 27:190–202. [PubMed: 17723214]
2. Cattoretti G, et al. BCL-6 protein is expressed in germinal-center B cells. *Blood*. 1995; 86:45–53. [PubMed: 7795255]
3. Dent AL, et al. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science*. 1997; 276:589–592. [PubMed: 9110977]
4. Ye BH, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. *Nat Genet*. 1997; 16:161–170. [PubMed: 9171827]
5. Kerfoot SM, et al. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity*. 2011; 34:947–960. [PubMed: 21636295]
6. Kitano M, et al. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity*. 2011; 34:961–972. [PubMed: 21636294]
7. Hatzi K, et al. A hybrid mechanism of action for BCL6 in B cells defined by formation of functionally distinct complexes at enhancers and promoters. *Cell Rep*. 2013; 4:578–588. [PubMed: 23911289]
8. Huang C, et al. Lineage-specific functions of Bcl-6 in immunity and inflammation are mediated by distinct biochemical mechanisms. *Nat Immunol*. 2013; 14:380–388. [PubMed: 23455674]
9. Phan RT, Dalla-Favera R. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. *Nature*. 2004; 432:635–639. [PubMed: 15577913]
10. Phan RT, et al. BCL6 interacts with the transcription factor Miz-1 to suppress the cyclin-dependent kinase inhibitor p21 and cell cycle arrest in germinal center B cells. *Nat Immunol*. 2005; 6:1054–1060. [PubMed: 16142238]
11. Ranuncolo SM, et al. Bcl-6 mediates the germinal center B cell phenotype and lymphomagenesis through transcriptional repression of the DNA-damage sensor ATR. *Nat Immunol*. 2007; 8:705–714. [PubMed: 17558410]
12. Ranuncolo SM, et al. BCL6 represses CHEK1 and suppresses DNA damage pathways in normal and malignant B cells. *Blood Cells Mol Dis*. 2008; 41:95–99. [PubMed: 18346918]
13. Shaffer AL, et al. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity*. 2000; 13:199–212. [PubMed: 10981963]
14. Ci W, et al. The BCL6 transcriptional program features repression of multiple oncogenes in primary B cells and is deregulated in DLBCL. *Blood*. 2009; 113:5536–5548. [PubMed: 19307668]
15. Li Z, et al. BCL-6 negatively regulates expression of the NF-kappaB1 p105/p50 subunit. *J Immunol*. 2005; 174:205–214. [PubMed: 15611242]
16. Harris MB, et al. Transcriptional repression of Stat6-dependent interleukin-4-induced genes by BCL-6: specific regulation of iepsilon transcription and immunoglobulin E switching. *Mol Cell Biol*. 1999; 19:7264–7275. [PubMed: 10490661]
17. Tunyaplin C, et al. Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. *J Immunol*. 2004; 173:1158–1165. [PubMed: 15240705]
18. Lenz G, Staudt LM. Aggressive lymphomas. *N Engl J Med*. 2010; 362:1417–1429. [PubMed: 20393178]
19. Baron BW, et al. The human BCL6 transgene promotes the development of lymphomas in the mouse. *Proc Natl Acad Sci USA*. 2004; 101:14198–14203. [PubMed: 15375218]
20. Cattoretti G, et al. Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. *Cancer Cell*. 2005; 7:445–455. [PubMed: 15894265]

21. Weiss LM, et al. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. *N Engl J Med.* 1987; 317:1185–1189. [PubMed: 3657890]
22. Saito M, et al. BCL6 suppression of BCL2 via Miz1 and its disruption in diffuse large B cell lymphoma. *Proc Natl Acad Sci USA.* 2009; 106:11294–11299. [PubMed: 19549844]
23. Dominguez-Sola D, et al. The proto-oncogene MYC is required for selection in the germinal center and cyclic reentry. *Nat Immunol.* 2012; 13:1083–1091. [PubMed: 23001145]
24. Savage KJ, et al. MYC gene rearrangements are associated with a poor prognosis in diffuse large B cell lymphoma patients treated with R-CHOP chemotherapy. *Blood.* 2009; 114:3533–3537. [PubMed: 19704118]
25. Davis RE, et al. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med.* 2001; 194:1861–1874. [PubMed: 11748286]
26. Wright G, et al. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. *Proc Natl Acad Sci USA.* 2003; 100:9991–9996. [PubMed: 12900505]
27. Compagno M, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B cell lymphoma. *Nature.* 2009; 459:717–721. [PubMed: 19412164]
28. Ngo VN, et al. Oncogenically active MYD88 mutations in human lymphoma. *Nature.* 2010; 470:115–119. [PubMed: 21179087]
29. Kato M, et al. Frequent inactivation of A20 in B cell lymphomas. *Nature.* 2009; 459:712–716. [PubMed: 19412163]
30. Davis RE, et al. Chronic active B cell-receptor signalling in diffuse large B cell lymphoma. *Nature.* 2010; 463:88–92. [PubMed: 20054396]
31. Iqbal J, et al. Distinctive patterns of BCL6 molecular alterations and their functional consequences in different subgroups of diffuse large B cell lymphoma. *Leukemia.* 2007; 21:2332–2343. [PubMed: 17625604]
32. Kim J, et al. Wnt5a is secreted by follicular dendritic cells to protect germinal center B cells via Wnt/Ca²⁺/NFAT/NF-kappaB-B cell lymphoma 6 signaling. *J Immunol.* 2011; 188:182–189. [PubMed: 22124122]
33. Pasqualucci L, et al. Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. *J Exp Med.* 2006; 203:311–317. [PubMed: 16492805]
34. Tam W, et al. Mutational analysis of PRDM1 indicates a tumor-suppressor role in diffuse large B cell lymphomas. *Blood.* 2006; 107:4090–4100. [PubMed: 16424392]
35. Basso K, et al. BCL6 positively regulates AID and germinal center gene expression via repression of miR-155. *J Exp Med.* 2012; 209:2455–2465. [PubMed: 23166356]
36. Pasqualucci L, et al. AID is required for germinal center-derived lymphomagenesis. *Nat Genet.* 2008; 40:108–112. [PubMed: 18066064]
37. Linterman MA, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J Exp Med.* 2010; 207:353–363. [PubMed: 20142429]
38. Lee CH, et al. Regulation of the germinal center gene program by interferon (IFN) regulatory factor 8/IFN consensus sequence-binding protein. *J Exp Med.* 2006; 203:63–72. [PubMed: 16380510]
39. Scheeren FA, et al. STAT5 regulates the self-renewal capacity and differentiation of human memory B cells and controls Bcl-6 expression. *Nat Immunol.* 2005; 6:303–313. [PubMed: 15711548]
40. Cerchiotti LC, et al. A purine scaffold Hsp90 inhibitor destabilizes BCL-6 and has specific antitumor activity in BCL-6-dependent B cell lymphomas. *Nat Med.* 2009; 15:1369–1376. [PubMed: 19966776]
41. Culjkovic B, et al. The eukaryotic translation initiation factor 4E (eIF4E) has oncogenic functions and may represent a new therapeutic target in diffuse large B cell lymphoma (DLBCL). *Blood.* 2013; 122:3785.
42. Moriyama M, et al. BCL-6 is phosphorylated at multiple sites in its serine- and proline-clustered region by mitogen-activated protein kinase (MAPK) in vivo. *Oncogene.* 1997; 14:2465–2474. [PubMed: 9188861]

43. Niu H, et al. Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. *Genes Dev.* 1998; 12:1953–1961. [PubMed: 9649500]
44. Saito M, et al. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. *Cancer Cell.* 2007; 12:280–292. [PubMed: 17785208]
45. Panagopoulos D, et al. Comparative analysis of signal transduction by CD40 and the Epstein–Barr virus oncoprotein LMP1 in vivo. *J Virol.* 2004; 78:13253–13261. [PubMed: 15542676]
46. Ye BH, et al. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large cell lymphoma. *Science.* 1993; 262:747–750. [PubMed: 8235596]
47. Ye BH, et al. Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B cell lymphoma. *Cancer Res.* 1993; 53:2732–2735. [PubMed: 8504412]
48. Ying CY, et al. MEF2B mutations lead to deregulated expression of the oncogene BCL6 in diffuse large B cell lymphoma. *Nat Immunol.* 2013; 14:1084–1092. [PubMed: 23974956]
49. Lai AY, et al. DNA methylation prevents CTCF-mediated silencing of the oncogene BCL6 in B cell lymphomas. *J Exp Med.* 2010; 207:1939–1950. [PubMed: 20733034]
50. Ramachandradeddy H, et al. BCL6 promoter interacts with far upstream sequences with greatly enhanced activating histone modifications in germinal center B cells. *Proc Natl Acad Sci USA.* 2010; 107:11930–11935. [PubMed: 20547840]
51. Tan DE, et al. Genome-wide association study of B cell non-Hodgkin lymphoma identifies 3q27 as a susceptibility locus in the Chinese population. *Nat Genet.* 2013; 45:804–807. [PubMed: 23749188]
52. Mendez LM, et al. CtBP is an essential corepressor for BCL6 autoregulation. *Mol Cell Biol.* 2008; 28:2175–2186. [PubMed: 18212045]
53. Chapuy B, et al. Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. *Cancer Cell.* 2013; 24:777–790. [PubMed: 24332044]
54. Duan S, et al. FBXO11 targets BCL6 for degradation and is inactivated in diffuse large B cell lymphomas. *Nature.* 2012; 481:90–93. [PubMed: 22113614]
55. Cerchietti LC, et al. BCL6 repression of EP300 in human diffuse large B cell lymphoma cells provides a basis for rational combinatorial therapy. *J Clin Invest.* 2010; 120:4569–4582.
56. Kawamata N, et al. Recognition DNA sequence of a novel putative transcription factor, BCL6. *Biochem Biophys Res Commun.* 1994; 204:366–374. [PubMed: 7945383]
57. Ahmad KF, et al. Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. *Mol Cell.* 2003; 12:1551–1564. [PubMed: 14690607]
58. Fujita N, et al. MTA3 and the Mi-2/NuRD complex regulate cell fate during B lymphocyte differentiation. *Cell.* 2004; 119:75–86. [PubMed: 15454082]
59. Chevallier N, et al. ETO protein of t(8;21) AML is a corepressor for Bcl-6 B cell lymphoma oncoprotein. *Blood.* 2004; 103:1454–1463. [PubMed: 14551142]
60. Vasanwala FH, et al. Repression of AP-1 function: a mechanism for the regulation of Blimp-1 expression and B lymphocyte differentiation by the B cell lymphoma-6 protooncogene. *J Immunol.* 2002; 169:1922–1929. [PubMed: 12165517]
61. Cerchietti LC, et al. A small-molecule inhibitor of BCL6 kills DLBCL cells in vitro and in vivo. *Cancer Cell.* 2010; 17:400–411. [PubMed: 20385364]
62. Cerchietti LC, et al. A peptomimetic inhibitor of BCL6 with potent antilymphoma effects in vitro and in vivo. *Blood.* 2009; 113:3397–3405. [PubMed: 18927431]
63. Polo JM, et al. Specific peptide interference reveals BCL6 transcriptional and oncogenic mechanisms in B cell lymphoma cells. *Nat Med.* 2004; 10:1329–1335. [PubMed: 15531890]
64. Huynh KD, Bardwell VJ. The BCL-6 POZ domain and other POZ domains interact with the co-repressors N-CoR and SMRT. *Oncogene.* 1998; 17:2473–2484. [PubMed: 9824158]
65. Huynh KD, et al. BCoR, a novel corepressor involved in BCL-6 repression. *Genes Dev.* 2000; 14:1810–1823. [PubMed: 10898795]
66. Karagianni P, Wong J. HDAC3: taking the SMRT-N-CoR road to repression. *Oncogene.* 2007; 26:5439–5449. [PubMed: 17694085]

67. Ghetu AF, et al. Structure of a BCOR corepressor peptide in complex with the BCL6 BTB domain dimer. *Mol Cell*. 2008; 29:384–391. [PubMed: 18280243]
68. Gearhart MD, et al. Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. *Mol Cell Biol*. 2006; 26:6880–6889. [PubMed: 16943429]
69. Sanchez C, et al. Proteomics analysis of Ring1B/Rnf2 interactors identifies a novel complex with the Fbxl10/Jhdml1B histone demethylase and the Bcl6 interacting corepressor. *Mol Cell Proteomics*. 2007; 6:820–834. [PubMed: 17296600]
70. Parekh S, et al. BCL6 programs lymphoma cells for survival and differentiation through distinct biochemical mechanisms. *Blood*. 2007; 110:2067–2074. [PubMed: 17545502]
71. Polo JM, et al. Reversible disruption of BCL6 repression complexes by CD40 signaling in normal and malignant B cells. *Blood*. 2008; 112:644–651. [PubMed: 18487509]
72. Polo JM, et al. Transcriptional signature with differential expression of BCL6 target genes accurately identifies BCL6-dependent diffuse large B cell lymphomas. *Proc Natl Acad Sci USA*. 2007; 104:3207–3212. [PubMed: 17360630]
73. Cerchiotti LC, et al. Sequential transcription factor targeting for diffuse large B cell lymphomas. *Cancer Res*. 2008; 68:3361–3369. [PubMed: 18451163]
74. Dupont, T., et al. Combinatorial targeting of BCL6 and anti-apoptotic proteins in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL). 54th ASH® Annual Meeting and Exposition; Atlanta, GA. December 8-11, 2012; American Society of Hematology; 2012. Abstract 64
75. Juszczynski P, et al. BCL6 modulates tonic BCR signaling in diffuse large B cell lymphomas by repressing the SYK phosphatase, PTPROT. *Blood*. 2009; 114:5315–5321. [PubMed: 19855081]

Glossary

B cell lymphoma 6 (BCL6)	a transcriptional repressor and member of the BTB/POZ zinc finger family of transcription factors. BCL6 was initially cloned from a translocation occurring on chromosome 3q27 in diffuse large B cell lymphoma (DLBCL)
BCL6 corepressor (BCOR)	a transcriptional corepressor that interacts with BCL6 in GC B cells and lymphoma. BCOR forms a polycomb repression complex 1 (PRC1)-like complex containing the histone demethylase KDM2B, ubiquitin ligases RING1A and RING1B and other PRC1-related proteins
Broad complex/tramtrack/bric-a-brac (BTB)	a structural domain located at the N terminus of BCL6 that mediates BCL6 dimerization and nuclear localization. BCL6 BTB dimerization generates two identical and symmetrical lateral grooves at the interface between BTB monomers. These serve as docking sites for corepressor recruitment
Centroblasts	GC B cells undergoing rapid division and somatic hypermutation of their immunoglobulin loci. Centroblasts form the dark zone of the GC
Centrocytes	nondividing activated GC B cells that derive from centroblasts and migrate to the light zone of the GC where they test the affinity of their antibody receptors with help from FDCs and follicular GC T cells

Germinal center (GC)

sites within secondary lymphoid organs that emerge upon B cell activation and mount the generation of long-lived, high-affinity T cell dependent B cell responses. Within the GCs, B cells undergo clonal expansion, immunoglobulin somatic hypermutation, and class switch recombination, leading to antibody affinity maturation

Retro-inverted BCL6 peptide inhibitors (RI-BPIs)

small peptides designed to mimic the SMRT peptide sequence that directly interacts with the BCL6 dimer. These peptides bind to the BCL6 BTB domain and result in disruption of the interaction of BCL6 with BTB domain corepressors, derepression of BCL6 target genes, and lymphoma cell death. RI-BPIs display favorable pharmacokinetic properties and can fully eradicate established lymphomas in mouse models

Silencing mediator for retinoid or thyroid hormone receptors/nuclear receptor corepressor 2 (SMRT/NCOR)

homologous corepressor proteins that interact with many repressive transcription factors. The core SMRT/NCOR repression complex contains HDAC3, TBL1 (and/or its homolog TBLR1), and GPS2

Box 1**Outstanding questions**

- BCL6 forms distinct complexes at GC B cell enhancers and promoters. How is the assembly of such complexes coordinated throughout the genome? What are the signals instructing site-specific formation of BCL6 complexes?
- BCL6 plays various biological roles in different cell types. What are the specific mechanisms of BCL6 action in these cells types? What functional BCL6 complexes assemble in these cells?
- BCL6 targets thousands of genes in GC B cells and lymphoma. What are other currently unknown functions central to the role of BCL6 in these cells? Are any of these essential for lymphoma cell survival?
- Most BCL6 binding sites do not contain the BCL6 consensus DNA motif. How is BCL6 recruited to the chromatin? What are the stepwise biochemical mechanisms by which it can establish transcriptional repression?
- BCL6 inhibitors can eradicate lymphomas without evident toxicity to animals. These inhibitors could provide a basis for efficient therapy of B cell lymphomas. Could BCL6 inhibitors enable therapeutic targeting of BCL6 functions in other cell types?

Figure 1.

BCL6 maintains a precarious balance between normal and malignant phenotypes in GC B cells. **(A)** During the GC response, B cells undergo somatic hypermutation and rapid proliferation that leads to accumulation of genotoxic stress. Attenuated DNA damage sensing in these cells allows their survival and upon completion of clonal selection GC B cells either differentiate to plasmacytes or die through apoptosis. However, the burden of attenuated DNA damage sensing and error prone division can drive the cells towards lymphomagenesis. **(B)** In normal B cells, BCL6 maintains a delicate balance between survival and apoptosis. This is achieved by simultaneously repressing tumor suppressors and oncogenes. **(C)** Promoter mutations or translocations of BCL6-repressed oncogenes often characterize lymphoma cells, suggesting that loss of the BCL6 tumor suppressor function can tip the balance towards malignant transformation. Abbreviations: BCL6, B cell lymphoma 6; GC, germinal center.

Figure 2.

Repression of BCL6 target gene networks enable certain hallmarks associated with transformation. To sustain proliferation and survival during affinity maturation, BCL6 represses key genes involved in sensing DNA damage, error prone division, and cell cycle checkpoints. BCL6 also represses *EP300*, which negatively regulates HSP90. In turn, HSP90 sustains BCL6 expression forming a feed-forward loop, causing GC B cells to adapt to and become dependent on stress signaling. To maintain the GC B cell phenotype and prevent termination of the GC transcriptional program, BCL6 suppresses certain aspects of BCR and CD40 signaling pathways that together with TLR signaling converge to activate NF- κ B and promote GC exit. Moreover, by repressing terminal differentiation genes, such as *PRDM1*, BCL6 can maintain the GC phenotype and prevent premature terminal differentiation into memory or plasma cells. Critical BCL6 target genes are recurrently mutated in lymphoma (yellow), thus escaping BCL6 control. Green arrows indicate activation and red lines repression. Abbreviations: BCL6, B cell lymphoma 6; BCR, B cell receptor; GC, germinal center; TLR, Toll-like receptor; NF- κ B, nuclear factor- κ B; *PRDM1*, *PR* domain containing 1, with ZNF domain; *EP300*, *E1A* binding protein p300; HSP90, heat shock protein 90. Note: symbols denoting both gene and protein are not italicized. Symbols referring to genes only are italicized.

Figure 3.

Mechanisms of *BCL6* transcript and protein regulation in normal GC B cells versus lymphoma cells. **(A)** Normal B cells. *BCL6* transcript and protein levels are highly upregulated upon B cell activation. Transcription factors activated by extracellular signals such as IRF8 and MEF2B induce *BCL6* transcription. *BCL6* expression is normally shut down after affinity maturation is accomplished to allow B cell differentiation. At this point, *BCL6* repression is mediated by IRF4. *BCL6* represses its own expression by binding to its own promoter and might also regulate an upstream LCR. It is plausible that looping of the LCR to the *BCL6* promoter could regulate *BCL6* expression in normal GC B cells. HSP90 and AUF1 stabilize *BCL6* transcripts. Furthermore, eIF4e augments *BCL6* mRNA nuclear export and translation. *BCL6* protein is also stabilized by the HSP90 chaperone, which might be induced by replicative and other sources of stress. At the post-transcriptional level, the *BCL6* protein is acetylated and inactivated by P300/CREBBP. Moreover, *BCL6* is targeted for ubiquitination and proteasomal degradation by ubiquitin ligase complex SCF (SKP1CUL1F-box protein) that contains the FBXO11. **(B)** *BCL6* is constitutively expressed and deregulated in B cell lymphomas. Sustained *BCL6* transcript levels can result from *BCL6* promoter mutations or translocations (red asterisks) that abolish DNA binding of negative regulators of *BCL6*, such as IRF4 and *BCL6* itself. Alternatively, hypermethylation (black peps) of *BCL6* regulatory regions may abrogate CTCF-mediated *BCL6* downregulation. Somatic mutations affecting P300/CREBBP result in failure to acetylate and inactivate *BCL6* protein. FBXO11 mutations result in failure to ubiquitinate and degrade *BCL6* (red x). Small molecule inhibitors (gold star) of factors that maintain *BCL6* transcript and/or protein stability (such as HSP90 and BRD4) could block *BCL6* expression

and exhibit anti-lymphoma activity. Abbreviations: BCL6, B cell lymphoma 6; LCR, locus control region; GC, germinal center; Ac, acetylation; P, phosphorylation; Ub, ubiquitination; HSP90, heat shock protein 90; IRF8, interferon regulatory factor 8; MEF2B, myocyte enhancer factor 2B; AUF1, AU-rich element RNA-binding protein 1; eIF4e, eukaryotic translation initiation factor 4E; CREBBP, CREB-binding protein; FBXO11, F-box only protein 11; CTCF, CCCTC-binding factor; BRD4, bromodomain-containing protein 4.

Figure 4.

BCL6 mediates its GC B cell functions through distinct biochemical mechanisms. GCs form in the secondary lymphoid organs by activated B cells. After forming long interactions with T cells within the interfollicular regions, activated B cells upregulate BCL6 and enter the follicles where they rapidly proliferate while undergoing somatic hypermutation. These large mitotically active B cells are called centroblasts and are located in the dark zone of the GC. Centroblasts then differentiate into centrocytes and migrate to the light zone of the GC where they test the affinity of their Ig receptors with help from GC T_{FH} cells and FDCs. Centrocytes with high affinity BCRs are selected to differentiate into plasmacytes or memory B cells but those with low affinity die through apoptosis. Recent findings suggest that the BCL6 BTB domain is essential for sustaining GC B cell proliferation. BCL6 BTB mutations disrupting the ability of BCL6 to recruit SMRT/NCOR and BCOR corepressors impair GC formation and affinity maturation. BCL6 mediates transcriptional repression through two biochemically distinct mechanisms acting at key B cell promoters and enhancers. At promoters, BCL6 dimers can simultaneously recruit PRC1-like BCOR

complexes and HDAC3-containing SMRT/NCOR complexes to effectively repress transcription in a repressed chromatin environment. At enhancers, BCL6 selectively recruits HDAC3-containing SMRT/NCOR complexes to functionally inactivate these elements through H3K27 deacetylation. In this model, interaction of GC B and GC T_{FH} cells in the light zone activates CD40 signaling in centrocytes and causes ERK-mediated phosphorylation of SMRT, which leads to cytoplasmic localization. Eviction of the SMRT corepressor from the BCL6 complexes leads to reactivation of BCL6-targeted gene programs during clonal selection. Switching these networks ‘on’ might be reversible allowing cycling back to the dark zone for additional rounds of affinity maturation. Abbreviations: BCL6, B cell lymphoma 6; BCR, B cell receptor; GC, germinal center; T_{FH}, follicular B helper T cells; BTB, broad complex/tramtrack/bric-a-brac; FDC, follicular dendritic cell; TSS, transcription start site; SMRT, silencing mediator of retinoid and thyroid receptor; NCOR, nuclear receptor corepressor; BCOR, BCL6 corepressor; PRC1, polycomb repressor complex 1; HDAC3, histone deacetylase 3.