BTB-ZF transcription factors, a growing family of regulators of early and late B cell development

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Abstract

The differentiation of early B cell precursors in the bone marrow into the variety of mature and effector B cell subsets of the periphery is a complex process that requires tight regulation at the transcriptional level. Different members of the BTB-ZF family of transcription factors have recently been shown to play key roles in many phases of B cell development, including early B cell development in the bone marrow, peripheral B cell maturation and specialization into effector cells during an immune response. This review highlights the critical functions mediated by BTB-ZF transcription factors within the B cell lineage and emphasises how the deregulation of these transcription factors can lead to B cell malignancies.

Introduction

B cells are critical for an effective immune response to infection, not only as the unique producers of immunoglobulin, but also as antigen presenting cells and cytokine producers[1]. However, B cells are also involved in life threatening pathologies such as autoimmune diseases and several types of lymphoma. To guarantee an efficient immune response while avoiding the risk of pathology, B cell development and differentiation are tightly regulated. Early B cell development takes place in the bone marrow and is designed to give rise to a pool of mature B cells able to respond to virtually any kind of foreign antigens while remaining non-reactive, or tolerant, to self-antigens[2].

Briefly, B cells differentiate in a stepwise process from the haematopoietic stem cells (HSCs) in the bone marrow. These first differentiate into multipotent progenitors (MPPs) containing a lymphoid primed MPP (LMPP) subset. This subpopulation contains the early lymphoid progenitors (ELPs), a population characterized by the expression of Rag1, and gives rise to the common lymphoid progenitors (CLPs). CLPs will subsequently differentiate into pre-pro-B cells, then pro-B cells and eventually pre-B cells only if they have successfully rearranged their B cell receptor (BCR) heavy chain genes. Once successful immunoglobulin (Ig) gene rearrangement and expression is accomplished, the cells leave the bone marrow (or liver, during foetal life) as immature BCR-positive cells for the periphery, where they further mature through transitional stages to become B1 cells, marginal zone B cells or follicular B cells.

The later, antigen dependent phase of B cell development occurs in the periphery when mature B cells encounter a specific antigen and differentiate into effector B cells. Depending on the type of B cell, the affinity of the antigen, and the presence or absence of T cell help, these mature B cells will rapidly differentiate into plasmablasts through the extra-follicular pathway[3] or give rise to germinal centers (GCs)[4]. In these dynamic structures, B cells undergo affinity maturation and isotype-switching under the influence of antigens and T helper cells. Consequently, only cells able to secrete high affinity antibodies are selected to become either memory B cells or long-lived plasma cells[5].

Every step of the early B cell development in the bone marrow and the late, antigen-dependent B cell differentiation in the periphery is tightly regulated at the transcriptional level. This regulation has attracted much attention over the past two decades, leading to the identification of numerous transcription factors that appear to be critical at different stages of the differentiation process (see[6-9]). In this review we will highlight the involvement of a particular family of transcription factors, known as Broad complex, Tramtrack, Bric-à-brac and Zinc Finger (BTB-ZF) proteins, which have recently emerged as key regulators of the B cell transcriptional network.

The BTB-ZF transcription factor family

BTB-ZF transcription factors (TFs) are a large and diverse family of regulators including 49 known members[10]. These TFs are involved in key biological processes such as development, malignant transformation and lymphocyte differentiation[11-15] (See table 1). They are characterized by varying numbers (from 3 to ~13) of C_2H_2 krüppel-like Zinc Fingers (ZF) on their carboxyl termini that are responsible for DNA binding (Figure 1a). However, what makes them such versatile and important proteins evolutionarily is the presence of the Broad complex, Tramtrack, and Bric-à-brac (BTB) domain near the amino terminus of each protein[16].

The BTB domain is present in many other types of protein, including ion transporters, signal transducers and proteases, and its function in TFs is a rather later evolutionary acquisition in higher organisms[16]. The BTB domain is 95 amino acids long, with only a dozen being conserved within the BTB-ZF family. Thus, the sequence diversity is high, even between proteins that are otherwise fairly closely related. The BTB domain is used as a protein-protein interaction

interface, and is important for homo- and hetero-dimerisation. Dimerisation appears to be important to regulate the activity of BTB-ZF TFs, by raising the affinity and specificity of DNA recognition or by rendering the dimers inactive below a certain binding threshold[17]. Thus BTB-domain mediated hetero-dimerisation is a critical process in gene regulation. The exchange of a component of the dimer can transform the complex from one that represses into one that activates transcription[17].

Another function of the BTB domain is to recruit transcriptional coregulators, which are particularly important to mediate rapid epigenetic changes in the chromatin structure through histone methylation or acetylation. Interestingly, some BTB-ZF TFs have been shown to direct the E3 ubiquitin ligase cullin 3 to the nucleus to modulate the ubiquitination pattern of their associated chromatin-modifying complexes, identifying a new mechanism by which epigenetic regulation can be mediated[18]. BTB-ZF TFs have also been shown to serve structural and organizational roles, in particular in facilitating DNA loop formation to mediate enhancer-promoter interactions[19, 20]. A new mechanism of chromatin compartmentalization involving BTB-ZF TFs has also been described recently. Zbtb7b, also known as Th-Pox, targets specific DNA sequences to the inner nuclear membrane through the interaction with HDAC3 and Lap2β, leading to the transcriptional silencing of particular regions of the genome[21].

BTB-ZF proteins have emerged as a family of TFs that are indispensable for different aspects of immune system development[13-15], in particular in the T cell[12] and dendritic cell lineages[22, 23]. Within the B cell lineage, six members of this family (Bcl6, LRF, Miz-1, Zbtb1, Zbtb20, Zbtb32), whose structures are depicted in Figure 1a, have been identified as critical players in the regulation of different steps of B cell biology, from the initial commitment of cells into the B cell lineage through to the survival of terminally differentiated plasma cells in the bone marrow (Figure 1b).

BTB-ZF TFs in early B cell development

B cell development starts when ELP or CLP commit towards the B cell rather than the T cell lineage. Recent work demonstrated that the BTB-ZF transcription factor Leukemia/Lymphoma related factor (LRF), encoded by the Zbtb7a gene (and also known as Pokemon, FBI-1 and OCZF) is a master regulator of the B versus T lineage fate decision[24]. Zbtb7a KO mice die at embryonic

day 16.5. Analysis of cultured foetal liver cells revealed a strong reduction in the $CD19^+$ $B220^+$ population due to a block at the earliest B cell developmental stage. Adult lymphopoiesis analysis, performed with foetal liver chimeras and by conditional deletion of LRF in HSCs, consistently revealed a block at the pre-pro-B cell stage. Conditional deletion of LRF in HSCs showed a simultaneous accumulation of extrathymic T cells in the BM. Quantitative gene expression studies demonstrated that the pre-pro B cells that accumulate upon LRF loss failed to induce B cell specific genes such as E2A, Ebf1 and Pax5, while mRNA encoding T cell specific genes such as Notch1, Notch3 and their targets were induced. This suggested that in the absence of LRF, pre-pro-B cells are aberrantly committed to the T cell lineage. Furthermore, when triggered in culture with the Notch ligand Delta, LRF deficient cells differentiate into more mature T cells. Hence, LRF is a critical TF promoting the commitment of lymphoid progenitors into the B cell lineage (Figure 2a).

Once committed to the B cell lineage, lymphocytes face a new challenge in generating a functional surface immunoglobulin, or antigen receptor. This stepwise process, which requires the successive rearrangement of the D-J and the V-DJ regions of the heavy chain locus, induces DNA damage that will lead to apoptosis in the absence of pro-survival molecules. In this context, the IL-7 signalling pathway is critical in mice in promoting the survival of these developing cells[25]. Interestingly, the BTB-ZF TF Miz-1 (Zbtb17) was found to play a role in the regulation of IL-7 signalling by repressing the transcription of SOCS1, a potent inhibitor of the JAK/STAT pathway by which IL-7 signals, and thus indirectly regulating precursor B cell development and survival[26]. Moreover, Miz-1 binds directly to the promoter of Bcl2 and induces the expression of this pro-survival factor. In the absence of Miz-1, mice exhibit a phenotype similar to IL-7 or IL-7 receptor (IL-7R) deficient mice, with loss of follicular B cells and a blockade at the pre-pro-B cell stage. The few cells able to differentiate into pro-B cells were highly apoptotic. Repression of SOCS1 in the cells restored their survival, but not their differentiation. The generation of $CD19^+$ B cells was restored only when both Bcl2 and Ebf1 were re-expressed, suggesting that Miz-1 is also required for the proper expression of Ebf1, and thus is important for both the survival and the differentiation of early B cell precursors (Figure 2b).

When pro-B cells have rearranged their *Igh* genes, the newly synthesised heavy chains interact with the surrogate light chains to form the pre-BCR. Upon successful assembly, the pre-BCR is expressed at the surface of these early pre-B cells, coinciding with a rapid expansion of the cells.

At the late pre-B cell stage, the cells stop dividing and start to rearrange the light chain Ig locus. The involvement of the BTB-ZF family at the transition between the early, large cycling pre-B cells and the late, small resting pre-B cells was revealed by Duy and colleagues, who showed that signalling through the pre-BCR induces the expression of Bcl6[27]. Based on their observations, they proposed a model in which Bcl6 and Myc control different features of early and late pre-B cell development in a mutually exclusive way. In early pre-B cells, signalling via the IL-7R/STAT5 pathway inhibits the induction of Bcl6[28] while promoting the Myc-CCND2 pathway. In late pre-B cells, signalling through the pre-BCR reduces the expression of IL-7R, which leads to the down-regulation of the Myc-CCND2 pathway and allows the transcriptional activation of Bcl6 through Pten/FoxO1[29]. Bcl6 subsequently directly represses Myc and CCND2 and therefore is critical for the cell cycle exit that is characteristic of late pre-B cells[29]. Interestingly, the induction of Bcl6 in late pre-B cells coincides with the stage where cells need to rearrange Ig light chain genes, an event that triggers extensive DNA damage and can lead to cell death, unless survival pathways are expressed. In this context, Bcl6 was found to directly repress ARF, p21 and p27, which blocked DNA damage induced apoptosis[27, 29]. As a consequence of these early Bcl6-mediated functions, Bcl6 deficient mice were found to harbour an increased population of pro-B cells, while the immature B cell compartment was significantly reduced[27]. Importantly, the absence of Bcl6 also lead to an oligoclonal B cell repertoire that could be explained by the fact that fewer cells could rearrange successfully the light chain genes and avoid apoptosis[27]. Therefore Bcl6 is important at the pre-B cell stage for the generation of a diverse primary B cell repertoire (Figure 2c).

A recent report by Punwani and colleagues demonstrated that pre-B cells expressed the highest level of Zbtb1 among the lymphoid lineages[30]. Consistent with a previous report, the absence of Zbtb1 affected primarily the T cell lineage while the effects on the B cell compartment were relatively mild[30, 31]. Although the pre-B cell population was significantly reduced in the absence of Zbtb1, both marginal zone (MZ) and follicular B cell subsets were surprisingly enlarged in the periphery and they both responded normally to in vitro stimuli. This suggests that Zbtb1 does not play a critical role in mature B cells, but more detailed analyses are required to fully understand the role Zbtb1 might play in early B cell development.

Involvement in mature B cell commitment

Upon successful rearrangement of the Ig light chain genes, non-autoreactive pre-B cells are selected to further differentiate into immature transitional B cells. At this time, different signals are required to trigger the differentiation of B cells into the two mature conventional B cell subsets, MZ B cells and follicular B cells. The origin of a third B cell subset, B1 cells, containing both the B1a and B1b cell types, remained controversial for a long time. Mounting evidence demonstrates that these cells are generated through a developmental pathway from foetal precursors that occurs essentially during perinatal life[32].

At least two members of the BTB-ZF transcription factor family, Zbtb20 and Zbtb32, are expressed in B1 cells[33] (unpublished observations). While the impact of Zbtb32 on the generation of the different B1a and B1b cell subsets remains to be assessed, Zbtb20 was shown to be dispensable for B1 cell generation[33] (Figure 2d). Regarding the conventional B cell subsets, different signalling pathways, such as BCR signalling, BAFF-R activation and the NFκB pathway, have been shown to influence the cell fate decision at the branch point between MZ B cells and follicular B cells[34]. The Notch signalling pathway is also critical for MZ B cell differentiation, as in the absence of Notch 2 or its ligand Delta-like 1 (Dll1), a complete disappearance of splenic MZ B cells is observed[35, 36]. Interestingly, LRF, which was shown to be a potent inhibitor of the Notch signalling pathway in early B cell precursors, also regulates the function of this signalling pathway during the maturation of MZ B cells and follicular B cells[37]. Mice in which LRF was deleted at the pro-B cell stage (Lrf $^{Flox/Flox}$/Mb-1 Cre^{+}) showed a strong increase of splenic MZ B cells that was due to aberrant activation of the Notch signalling pathway. Indeed, in the absence of Notch 2, transitional B cells deficient for LRF were unable to differentiate into MZ B cells[37]. Furthermore, Hes1, a Notch target gene, was up-regulated in LRF deficient MZ B cells[37]. Together, these observations strongly argue in favour of a model in which LRF counteracts Notch 2 signalling and therefore regulates the balance between MZ and follicular B cells at the transitional stage (Figure 2e).

Involvement in antigen activated B cells

Upon encounter with their cognate antigens, mature B cells in the periphery can differentiate into antibody secreting cells (ASCs). This interaction is sufficient to trigger the T cell independent differentiation of B1 and MZ B cells into immunoglobulin secreting plasmablasts[38]. Interestingly,

the absence of Zbtb20 resulted in a less efficient differentiation of those cells into ASC upon activation with either LPS or CpG in vitro, suggesting that this TF facilitates the rapid differentiation of B1 cells into ASC that has been described[33, 38].

Depending on the type of signals exchanged and the affinity of the BCR, some follicular B cells will quickly differentiate in an early wave of extrafollicular plasmablasts while others cells may require the help of T cells to be successfully driven to ASC or memory cell differentiation; these enter the GC reaction[4, 39]. GCs are structures commonly composed of a dark zone, where centroblasts strongly proliferate and undergo somatic hypermutation (SHM) and class switch recombination (CSR) in order to increase the affinity and efficacy of their immunoglobulins, and the light zone, where resting centrocytes compete for antigen presented by follicular dendritic cells. Only cells able to express a high affinity antigen receptor are selected by follicular helper T cells to differentiate into either plasma cells or memory B cells. The BTB-ZF TF Bcl6 appears to be the master regulator of GC[40]. In the absence of Bcl6, the development of GC is completely abrogated[41-43].

Bcl6 mediates pleiotropic effects in GCs and modulates in particular the activation status of the cells, their differentiation, proliferation and survival[40]. These functions are mediated through the transcriptional repressor activity of Bcl6, and numerous genes have been found to be directly or indirectly modulated by Bcl6[44]. The first key role of Bcl6 is to prevent premature activation of GC B cells. To this end, Bcl6 regulates different molecules involved in the BCR and CD40 signalling pathways. It also represses molecules involved in T-B interactions, such as CD80 and CD274 (B7-H1, PD-L1). Bcl6 blocks the differentiation of GC into plasma cells through the direct repression of the *Blimp-1/Prdm1* and *Irf4* genes which encode two transcription factors essential for ASC differentiation. This allows B cells to remain in the GC long enough to increase their affinity through somatic hypermutation (SHM) and selection, and for class switch recombination (CSR). Bcl6 is actively involved in these processes since it represses microRNAs miR-155 and miR-361, leading to the upregulation of AID, a key factor for both CSR and SHM[40].

Bcl6 is also required to promote cell proliferation and survival in a context where CSR and SHM trigger a strong DNA damage response, which would otherwise lead to cell cycle arrest and apoptosis. Bcl6 represses p53[45] and other genes involved in the DNA damage response, such as CDKN1A, ATR, and CHEK 1. As a consequence, GC B cells can undergo division despite the fact that they carry numerous mutations. However, Bcl6 is also able to repress genes encoding pro-

survival molecules such as Bcl2, and to promote the expression of pro-apoptotic genes[46]. This is thought to maintain the cells in a state that is susceptible to apoptosis, so they may be readily eliminated unless rescued by strong BCR signalling from a high affinity antigen receptor. Bcl6 mediates most of its functions by directly binding to the promoters of its target genes. However, in some cases Bcl6 indirectly binds to target gene promoters through an intermediate, the BTB-ZF TF Miz-1. This has been shown for the repression of both CDNK1a and Bcl2[46, 47].

Besides Bcl6, LRF also plays a pivotal role in GC biology. Indeed, in its absence the number of GC cells is strongly reduced[37]. Using conditional knockouts in which LRF was specifically deleted in GC, Sakurai and colleagues showed that LRF is required for the maintenance, rather than the initiation, of GC. In the absence of LRF, the percentage of highly proliferative centroblasts in the dark zone was decreased, while the percentage of apoptotic cells was increased. Similarly to Bcl6, LRF was shown to regulate the p53 pathway by inhibiting p19/Arf and p21[37]. Therefore, LRF plays a partially redundant role with Bcl6 to promote the proliferation and the survival of GC B cells. Zbtb20 is another member of the BTB-ZF family that is expressed in GCs[33, 48]. However, in vivo studies revealed that the generation and the maintenance of GCs were not affected in Zbtb20-null animals. In primary B cells, Zbtb20 promotes the induction of the transcription factor Blimp-1, suggesting that Zbtb20 may promote GC exit and further differentiation. Clearly, the function played by Zbtb20 in GCs needs further exploration.

At the end of the GC reaction, cells that have successfully increased their affinity for antigen will leave this structure to become memory cells that still express surface BCR and can quickly differentiate into ASCs upon antigen encounter. Alternatively cells will differentiate into isotype-switched, high affinity plasmablasts that will leave the spleen to find a survival niche in the bone marrow, and there to become long-lived plasma cells[49, 50]. Although no BTB-ZF TF has yet been implicated in memory B cell development, recent reports have highlighted the function of different members of this family in the differentiation into plasmablasts and plasma cells[33, 51 48]. The first member of the BTB-ZF family reported to be involved in plasma cell differentiation was Zbtb32, also known as repressor of GATA 3 (ROG), PLZF-like zinc finger protein (PLZP), testis zinc finger protein (TZFP), and fanconi anemia zinc finger (FAZF)[51]. This factor was identified in a screen designed to uncover potential CIITA repressor genes in *in vitro* activated B-cells. The silencing of CIITA and MHCII expression is indeed a key event in plasma cell differentiation[52, 53], and Blimp-1 only accounts partially for this silencing[54, 55]. After *in vitro* B cell activation, the rapid

induction of Zbtb32 correlated with the early repression of CIITA and MHCII molecules. When ectopically expressed in a human B cell line, Zbtb32 efficiently repressed CIITA mRNA[51]. Chromatin immunoprecipitation showed a binding site for Zbtb32 in the pIII promoter of CIITA and repression of MHCII was significantly impaired in Zbtb32 KO activated B cells. The requirement for Zbtb32 for an efficient humoral response *in vivo* is more elusive. Although Zbtb32 expression could be observed in plasma cells generated after LCMV infection, the Zbtb32 deficient mice showed no defect in mounting an effective humoral immune response[51]. Interestingly, we did not find high levels of Zbtb32 mRNA in plasma cells of unimmunised animals, but found the factor mainly specifically expressed in the peritoneal B1 cell populations (unpublished observations).

Besides its expression in GCs and B1 cells, Zbtb20 is strongly expressed in plasma cells[33, 48]. At the protein level, Zbtb20 expression correlated with plasma cell differentiation state, reaching its highest level in long-lived plasma cells found in the bone marrow. Ectopic expression of Zbtb20 in activated B cells *in vitro* strongly promotes plasma cell differentiation. Ectopic expression in a plasmacytoma cell line revealed an effect of Zbtb20 in cell survival and cell cycle arrest[33]. This was further confirmed *in vivo*, where reduced Zbtb20 levels caused decreased antibody responses upon alum adjuvanted immunization[33, 48]. In different systems, Zbtb20 was found to modulate key genes involved in cell survival, such as Bcl2, BCMA or Mcl1, and ectopic expression of Bcl2 could rescue the long lasting antibody response, suggesting that Zbtb20 is directly involved in ASC survival[33, 48]. Bhattacharya and Wang further reported that the decreased ASC survival could be restored using adjuvant activating TLR2 and TLR4 signalling, providing evidences that ASCs can engage different survival pathways [48]. Finally, the expression of Zbtb20 in B1 and ASCs is dependent on the TF Irf4, which binds directly to the promoter of Zbtb20. In absence of Irf4, B1 cells and in vitro activated B cells fail to express Zbtb20 while overexpression of Irf4 in a mature B cell line led to the induction of Zbtb20[33].

Involvement in tumorogenesis

Numerous members of the BTB-ZF family of transcription factors involved in B cell development have been associated with cell cycle and cell survival control. In most cases, BTB-ZF TFs regulate cellular proliferation through the inhibition or induction of cyclin kinase inhibitors. Some of those TFs were also shown to regulate members of the Bcl2 pro-survival family to promote or

inhibit cell survival. As a consequence, their deregulation is often correlated with tumorigenesis. Bcl6 is probably the BTB-ZF TF whose function as a proto-oncogene is best characterized[40]. Bcl6 chromosomal translocations occur in 40% of diffuse large B cell lymphoma (DLBCL) and 5-10% of follicular B cell lymphoma (FL)[56-59]. As a consequence, the downregulation of Bcl6 in post-GC cells is impaired[60, 61]. Alternative mechanisms leading to the deregulation of Bcl6 activity have also been linked to lymphoma development[62]. Although the exact role of Bcl6 in the tumour development remain to be fully understood, it is likely due to the tolerance to DNA damage induced by Bcl6[40].

LRF has been identified as a proto-oncogene in many different types of cancers and expression of LRF is found in 60-80% of DLBCL and FL[63]. To promote tumour development, LRF has been shown to block the transcription of the tumour suppressor p19-ARF[63]. However, LRF has also been shown to promote tumour development through alternative mechanisms[64-66].

Miz-1 has the capacity to activate or repress cell cycle and cell survival depending on the co-factors to which it binds[67]. Importantly, Miz-1 was initially identified as a c-Myc binding partner[68] and the presence of the Miz-1/c-Myc complex is critical for the formation and maintenance of c-Myc induced tumours[69]. Furthermore, Miz-1 itself is thought to promote tumour formation in some DLBCL cases, through the direct induction of Bcl2[46].

Recently, a cell of origin classification tool for DLBCL revealed that Zbtb32 was ranked as the best class-associated gene, being the most consistent and differentially expressed gene in activated B-cell DLBCL[70]. Its function in tumour formation remains, however, elusive. Similarly, in B cell chronic lymphocytic leukaemia (B-CLL), Zbtb20 was found in different gene expression profiling studies to be one of the top down-regulated genes in V(H) unmutated B-CLL relative to mutated B-CLL[34, 71]. Therefore, Zbtb20 expression was thought to correlate with the less aggressive form of B-CLL. However, in another study, Zbtb20 levels was shown to have no prognostic value for the patient survival in B-CLL[72]. While early work on Zbtb20 revealed that this gene is strongly expressed in lymphoid neoplasms, especially B cell lymphoma[73], more works remain to be done to understand how Zbtb20 may affect tumour formation. However its role in promoting cell survival may be important[33, 48].

Concluding remarks

BTB-ZF proteins constitute a broad class of transcriptional regulators, whose characterisation has revealed new mechanisms of gene regulation. The modular nature of these proteins allows them to interact with different partners in different contexts, leading to distinct cellular outcomes. Currently, six members of this family have been associated with B cell development, from early precursors to fully mature plasma cells. At each step, these TFs are critical for cell differentiation, proliferation, and survival, and as a consequence, their deregulation is often linked with tumorogenesis. Given the large number of BTB-ZF TFs, their capacity for interaction and the complexity of B cell development, it is likely that the proteins discussed here and other members of this family will be implicated in B cell development and transformation. Further studies are required to understand the full involvement of the BTB-ZF TFs in B cell biology.

Legends

Figure 1.

Structure and expression of the BTB-ZF TFs involved in the B cell lineage. (**a**) Schematic representation of the different members of the BTB-ZF TFs family with known functions in the B cell lineage. Each of them is characterized by one BTB domain and variable numbers of ZF domains. The length and the stages at which the different BTB-ZF TFs are involved are indicated. *BTB, Broad complex, Tramtrack, Bric-à-brac; ZF, Zinc Finger; aa, amino acid.* (**b**) Expression of the different BTB-ZF TFs presented in (a) in the indicated B cell populations. Expression is given as the number of reads per million per kb pair as measured by RNA sequencing. *PerC, peritoneal cavity; FO, follicular B cell; GC, germinal center; PC, plasma cell; SP, spleen; BM, bone marrow*.

Figure 2.

Role of BTB-ZF transcription factors in early B cell development and during B cell maturation in the periphery. (**a**) LRF requirement in T vs B cell differentiation. (b) Involvement of Miz-1 at the pre-pro B cell stage. (**c**) Induction of Bcl6 by pre-BCR signalling at the pre-B cell stage to promote cell cycle and cell survival. Zbtb1 is highly expressed at this stage, but its function remains to be assessed. (**d**) The function of Zbtb32 in B1 cell generation remains to be investigated. (**e**) LRF is required to modulate the balance between marginal zone and follicular B cell development.

HSC, hematopoietic stem cells; MPP, multipotent progenitor; LMPP, lymphoid primed MPP; ELP, early lymphoid progenitor; CLP, common lymphoid progenitor; T, T cell; Pre Pro, pre-pro-B cell; Pro, pro-B cell; Pre I, early pre-B cell; Pre II, late pre-B cell; Imm, immature B cell; MZ, marginal zone B cell; FO, follicular B cell.

Figure 3.

Role of BTB-ZF transcription factors in antigen dependent B cell activation. (**a**) Role of Zbtb32 and Zbtb20 in short-lived plasma cell development from B1, marginal zone and follicular B cells. (**b**) Bcl6 and LRF are critical to regulate germinal center biology. (**c**) Involvement of Zbtb32 and Zbtb20 in post-germinal center short-lived plasma cells. (**d**) Regulation of cell cycle arrest and promotion of cell survival in long-lived plasma cells mediated by Zbtb20. *MZ, marginal zone B cell; FO, follicular B cell; SLPC, short-lived plasma cell; GC, germinal center B cell; DZ, dark zone; LZ, Light zone; Mem; memory B cell; LLPC, long-lived plasma cell.*

Competing financial interests

The authors have no conflicting financial interests.

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Figure 1

a

Gene	Structure (BTB, ZF)	Length	Development	Maturation	Activation	Tumorogenesis
Bcl6		707 aa	✓		✓	✓
LRF		569 aa	✓	✓	✓	✓
Miz-1		794 aa	✓		✓	✓
Zbtb1		713 aa	✓			
Zbtb20		741 aa			✓	✓
Zbtb32		465 aa			✓	✓

b

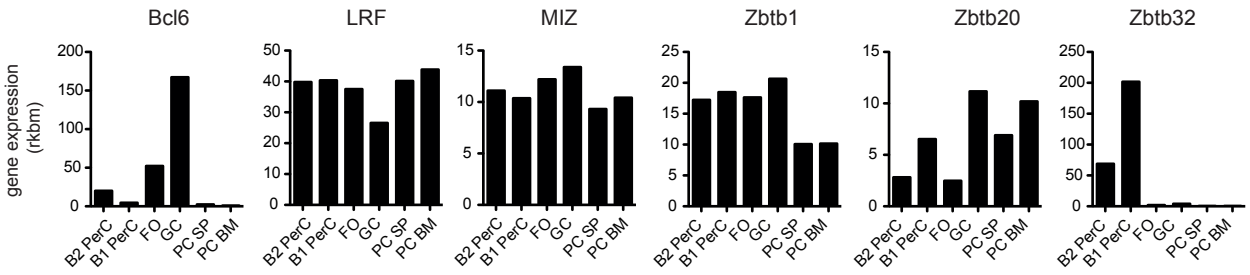


Figure 2

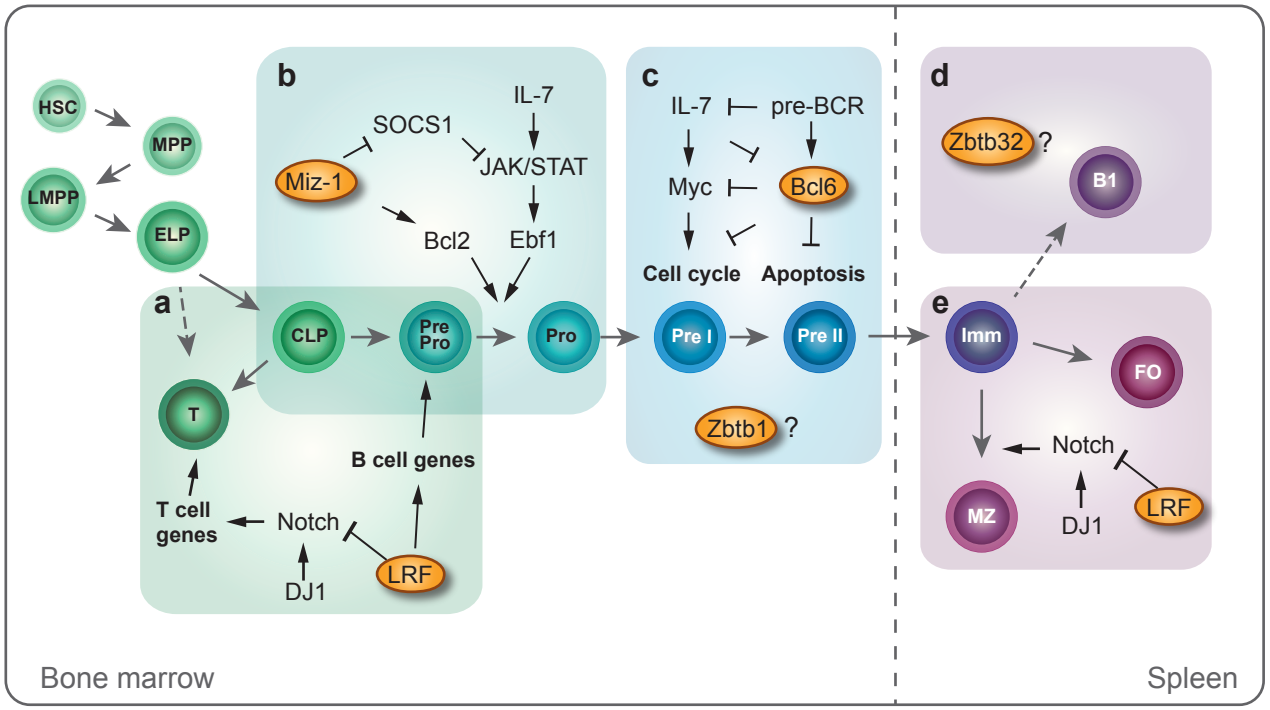
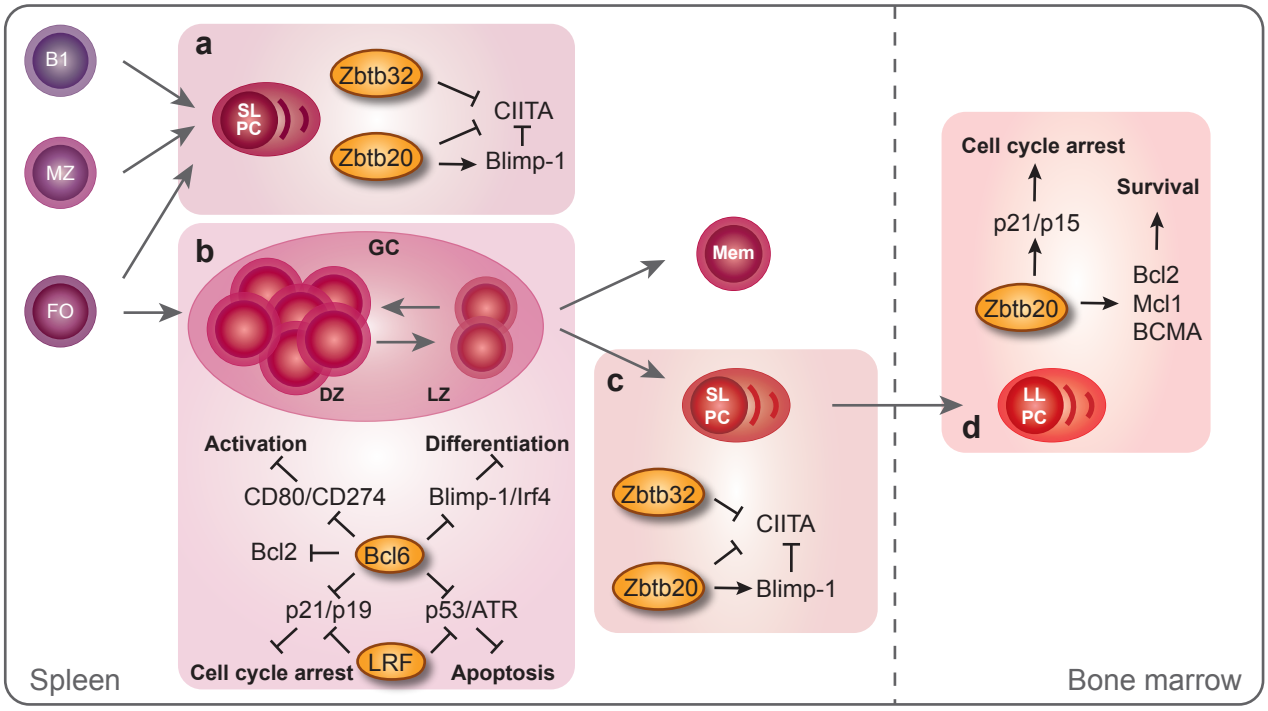

HSC
MPP
LMPP
ELP
a
CLP
Pre Pro
Pro
T
T cell genes
Notch
DJ1
LRF
B cell genes
b
SOCS1
IL-7
Miz-1
JAK/STAT
Bcl2
Ebf1
c
IL-7
pre-BCR
Myc
Bcl6
Cell cycle
Apoptosis
Pre I
Pre II
Zbtb1 ?
d
Zbtb32 ?
B1
e
Imm
FO
Notch
MZ
DJ1
LRF
Bone marrow
Spleen

Figure 3


B1
MZ
FO
a
SL PC
Zbtb32
Zbtb20
CIITA
Blimp-1
b
GC
DZ
LZ
Activation
Differentiation
CD80/CD274
Blimp-1/Irf4
Bcl2
Bcl6
p21/p19
p53/ATR
Cell cycle arrest
LRF
Apoptosis
Mem
c
SL PC
Zbtb32
Zbtb20
CIITA
Blimp-1
d
LL PC
Cell cycle arrest
Survival
p21/p15
Zbtb20
Bcl2
Mcl1
BCMA
Spleen
Bone marrow