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The Endless Complexity of Lymphocyte Differentiation and Lymphomagenesis: IRF-4 Downregulates BCL6 Expression

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The *BCL6* gene is a key factor necessary for formation of germinal centers and is implicated in pathogenesis of diffuse large B cell lymphoma (DLBCL). In this issue of *Cancer Cell*, Saito and colleagues explore regulation of *BCL6* gene expression by CD40-NF-κB signaling pathway and show that the IRF4 transcriptional factor, induced by the NF-κB canonical pathway, directly downregulates *BCL6* expression. The authors further demonstrate that this negative regulatory mechanism may be disturbed in DLBCLs harboring *BCL6* gene translocations or mutations. These finding suggest that IRF4 may function as a key regulator of germinal center reaction and a guardian of lymphomagenesis.

In response to antigen encounter, uncommitted naive B cells are activated and undergo a complex maturational process yielding phenotypically distinct subpopulations, which form highly organized germinal centers (GC) in lymphoid organs. Within the GC, B cells undergo high rate proliferation and affinity maturation, are selected by antigen, switch toward advanced isotypes, and finally differentiate into either memory B cells or plasma cells. The maturation process leading to the generation of GC lymphocytes, and their subsequent differentiation to memory and plasma cells is characterized by tightly regulated suppression or increased expression of specific genes, resulting in distinctive gene expression signatures characterizing individual ontogeny stages of the lymphocytes. Currently, known key players at the GC stage include *BCL6* and activation-induced cytidine deaminase (*AID*), the former necessary for GC formation while the latter is essential for class switch recombination (CSR) and somatic mutations of the immunoglobulin genes. Toward the completion of GC reaction and terminal differentiation, the expression of these genes is downregulated, while genes necessary for plasma cell formation, such as *IRF4* (also known as MUM-1), *Prdm1*, encoding Blimp-1, and *XBP-1* are expressed. Aberrations in this orchestrated process may cause deregulated gene expression, leading to cell transformation and lymphomagenesis.

BCL6 is a proto-oncogene encoding a POZ/Zinc finger sequence-specific transcriptional repressor, which is distinctively expressed in GC B cells. In the GC, BCL6 exerts antiapoptotic effects and favors sustained proliferation of B cells by modulating the transcription of genes involved in cell cycle regulation, proliferation, activation, CSR, and differentiation (Shaffer et al., 2000). The expression of *BCL6* in the GC lymphocytes is tightly regulated at both the transcriptional and the protein levels. *BCL6* gene is negatively self-regulated by binding of BCL6 to specific binding sequences located in its first exon (Wang et al., 2002). Activation of STAT5 may either upregulate or suppress *BCL6* expression, and p53 may increase *BCL6* expression

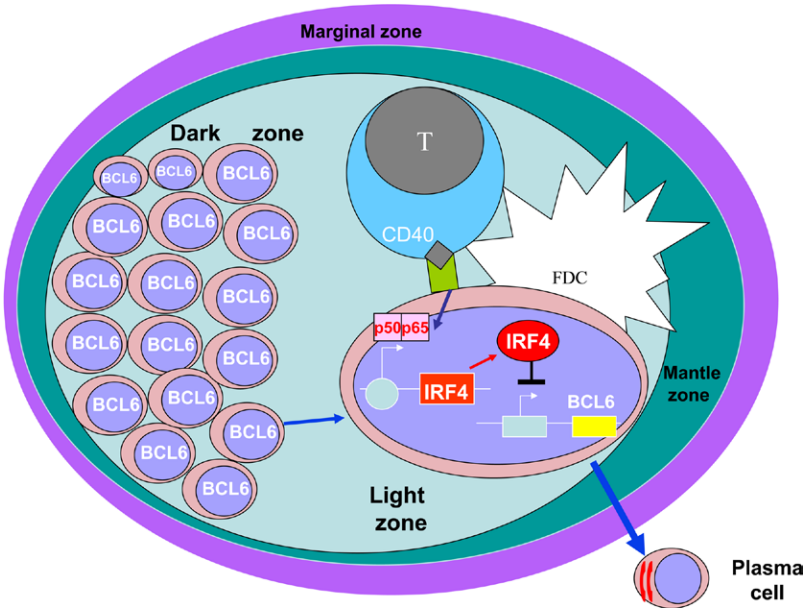

Figure 1. IRF4 Transcription Factor Downregulates BCL6 Expression
The germinal center (GC) reaction starts when naive B cells are activated by antigen in the presence of costimulatory signals from T cells. These events transform B cells into centroblasts that proliferate and undergo somatic mutation of immunoglobulin genes within a histologically defined "dark zone." Centroblasts then develop into noncycling centrocytes, which are selected in the "light zone" based on their ability to bind their cognate antigen with the help of follicular dendritic cells (FDC) and T cells. In the light zone of GC, engagement of CD40 on B cells by CD40 ligand on the T cells activates NF-κB canonical pathways and induces transcription of IRF-4 transcriptional factor. IRF4 can bind to the *BCL6* promoter region and downregulates its transcription, thus allowing terminal differentiation to post-GC lymphocytes.

(Margalit et al., 2006). Stimulation of B cell receptor induces MAP-kinase-mediated phosphorylation of BCL6, leading to its degradation by proteasome pathway (Niu et al., 1998). p300 mediates BCL6 acetylation, leading to inhibition of its transrepressive function (Bereshchenko et al., 2002). The tight regulation of *BCL6* is not only imperative for normal lymphocyte maturation, but is also implicated in lymphomagenesis. The *BCL6* gene located at 3q37 is involved in recurrent chromosomal translocations present in approximately 30%–40% of diffuse large B cell lymphomas (DLBCL) and 6%–15% of follicular lymphomas. The translocations deregulate *BCL6* expression by placing its intact coding sequence under the influence of a potent heterologous promoter. Such translocations lead to deregulated expression of the *BCL6* from the translocated allele (Lossos et al., 2003). In addition, the 5′ noncoding regulatory region of the *BCL6* is targeted by deletions and somatic mutation, some of which result in deregulated BCL6 expression. Recently, unequivocal proof for *BCL6*'s role in lymphomagenesis was provided by demonstration that constitutive expression of BCL6 in mice lymphocytes recapitulates the pathogenesis of human DLBCL (Cattoretti et al., 2005).

In this issue of *Cancer Cell*, Saito et al. achieve an additional milestone in elucidation of *BCL6* regulation, further illuminating the endless complexity of the GC reaction regulation (Saito et al., 2007). The authors demonstrate that CD40 receptor engagement, usually caused in vivo by the interaction of GC lymphocytes with surrounding T cells at the terminal stages of GC reaction, leads to NF-κB-mediated transcriptional activation of the *IRF4* gene (Figure 1). Involvements of the p50 and p65 subunits, which bind the *IRF4* promoter, suggest that the "canonical," as opposed to the "alternative" NF-κB pathway mediates this process. Furthermore, the authors demonstrate that IRF4 transcriptional factor binds to the promoter region of the *BCL6* gene and directly represses its transcription (Figure 1). Taken with the current knowledge of specifically downregulated activity of the NF-κB pathway in normal GC B cells and upregulation of its activity upon maturation to post-GC stages, this finding is of paramount importance for the explanation of gene expression changes governing this differentiation process. Furthermore, since BCL6 was previously reported to negatively regulate expression of the NF-κB p105/p50 subunit (Li et al., 2005), this finding suggests an existence of a double-negative circuit regulating this differentiation step. This adds an additional dimension of complexity to the regulation of GC reaction in general and *BCL6* expression in particular. While previously attributed at least partially to the double-negative circuit involving BCL6 and Blimp-1 proteins, in which BCL6 binds to the *Prdm1* gene and inhibits its transcription, whereas Blimp-1 protein represses the *BCL6* gene along with virtually every other GC signature gene (Shaffer et al., 2002), the demonstration of NF-κB-mediated regulation of BCL6 expression raises the possibility that multiple factors activating the canonical NF-κB pathways may also be implicated in the regulation of *BCL6* expression. Since IRF4-induced transcriptional activity is usually determined by cofactors with which it interacts, future studies examining the nature of these cofactors in the GC microenvironment are necessary. Overall, the work presented here by Saito et al. is likely to open the way toward a better understanding of B cell differentiation and maturation.

These findings may also have important implications for our understanding of lymphoma pathogenesis. Saito et al. demonstrate that the major IRF4-binding domain in *BCL6* gene overlap with the chromosomal breakpoints and mutations clusters in DLBCL and thus frequently is removed or altered by the translocations and mutations. The authors show that in DLBCL cell

lines and tumors in which the IRF4-binding domain in the *BCL6* gene is removed or altered, activation of the CD40-NF-κB-IRF4 pathway fails to downregulate *BCL6* gene expression. While previously translocations and mutations were implicated in deregulated expression of the *BCL6* gene by promoter substitution and elimination of the *BCL6* autoregulatory loop, the findings reported by Saito et al. suggest that the resistance to IRF4-mediated downregulation may also represent an essential mechanism of *BCL6* deregulation in tumors harboring translocated and mutated *BCL6* alleles within the IRF-4-responsive domain. The relative contribution of individual mechanisms to deregulated expression of *BCL6* will need to be evaluated in the future studies.

These new findings might also find their way to the clinic. DLBCL is the most prevalent subtype of lymphoma, accounting for 30%–40% of non-Hodgkin's lymphomas (NHL). At least two molecularly distinct forms of the disease, defined by specific gene expression signatures, are recognized: GC-like DLBCL, characterized by expression of genes normally expressed in GC B cells (including BCL6), and having a significantly better overall survival, and the activated B cell (ABC)-like DLBCL, characterized by high expression of NF-κB target genes (including IRF-4) (Alizadeh et al., 2000). Manipulating the BCL6-NF-κB-IRF-4 proteins might be useful in treating these lymphomas, given that inhibiting BCL6 in GC-like DLBCL leads to cell death, while inhibition of NF-κB pathways leads to ABC-like DLBCL cell death. It is possible that the plasticity inherent in the BCL6-IRF-4 double-negative regulatory circuit may be exploited for therapeutic benefit.

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