

Hit-and-run lymphomagenesis by the *Bcl6* oncogene

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Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in humans, accounting for nearly 40% of all non-Hodgkin lymphomas.¹ In spite of recent advances in monoclonal antibody therapy, up to 40% of patients eventually relapse and die of their disease after treatment with chemo-immunotherapy regimens. Given the inability to cure many patients with DLBCL, and the significant toxicity of current therapies, better treatment strategies are needed. Functional genomics studies have allowed DLBCLs to be subdivided into biologically and clinically relevant disease subtypes, which are called germinal center B-cell (GCB-like) type or activated B-cell (ABC-like) type, based on their transcriptional similarity to the respective populations of primary B cells.² The ABC-like DLBCLs are generally more refractory to treatment than the GCB-like subtype. In spite of these advances, much remains unknown regarding the genetic basis and pathophysiology of this aggressive and highly heterogeneous B-cell malignancy.

In this regard, we have recently identified gain of 3q27.2 as being significantly associated with adverse outcome in DLBCL and linked with the ABC-like subtype.³ This lesion includes the *BCL6* oncogene, but, surprisingly, using integrative analysis of paired DNA copy number and gene expression microarray data, we found no significant overexpression of *BCL6* transcript in tumors with 3q27 gain, nor did we observe coordinate repression of previously identified *BCL6* target genes in these tumors.³ Furthermore,

transgenic mice that express *Bcl6* specifically in mature B cells develop lymphomas in only a subset of mice,⁴ and these tumors align most significantly with the GCB-like stage of murine B-cell development—a very different phenotype than aggressive human ABC-like DLBCL, in which genetic alterations of *BCL6* are most frequently observed. This observation led us to hypothesize that *BCL6* may function as a “hit-and-run” oncogene that acts at an early stage of B-cell development to reprogram hematopoietic stem/progenitor cells (HS/PCs) for malignancy. To test this hypothesis, we generated strains of mice with restricted expression of this oncogene within HS/PCs.³ Despite lack of *Bcl6* protein expression, mature non-malignant B cells from these mice show coordinate repression of *Bcl6* target genes, suggesting a lasting imprint from *Bcl6*-mediated reprogramming within the stem/progenitor compartment. These mice go on to develop aggressive and clonal B-cell lymphomas that accumulate at a post-germinal center stage of differentiation, in line with human lymphomas in which *BCL6* alterations are most frequently observed.³ These results therefore confirm that activity of the *Bcl6* oncogene restricted to HS/PCs can induce malignancies in mice that are of a post-germinal center stage of differentiation.

Bcl6 mediates suppression of target genes via recruitment of factors that epigenetically modify chromatin. We hypothesized that this may elicit changes in DNA methylation, a mark that is capable acting in gene silencing memory,

and therefore investigated whether this may be a potential mechanism for *Bcl6*-mediated “hit-and-run” oncogenesis. Using genome-wide DNA methylation profiling of populations of HS/PCs and mature B-cells from wild type and *Sca1-Bcl6* mice, we identified broad epigenetic changes associated with the expression of *Bcl6* in HS/PCs. Importantly, a significant subset of these changes were found to be maintained from HS/PCs to mature B cells in *Sca1-Bcl6* mice, resulting in these populations being epigenetically more similar to each other than to their comparative population from wild-type mice.³ Together these results suggest that the “hit-and-run” role of *Bcl6* activity may be manifested via epigenetic alterations. This epigenetic memory would maintain gene expression states through cell generations without a change in DNA sequence and in the absence of initiating signals.

In conclusion, we have provided the first evidence of a “hit-and-run” role for *Bcl6* in the oncogenesis of lymphoma. The implication of these findings is that, if ABC-like DLBCL occurs via a reprogramming-like mechanism, oncogenes that initiate tumor formation might be dispensable for tumor cell survival and/or tumor progression. In this context, mutations that activate oncogenes would have a driving role in the reprogramming process, but may act as passenger mutations thereafter, or may have a secondary role in evolved tumor cell clones.^{5,6} This may provide an explanation for the failure of some modern targeted therapies to clear tumor stem cells, despite being effective agents

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Submitted: 05/09/2014; Accepted: 05/12/2014; Published Online: 05/27/2014
<http://dx.doi.org/10.4161/cc.29326>
Comment on: Green MR, et al. Nat Commun 2014; 5:3904; PMID:24887457; <http://dx.doi.org/10.1038/hcomms4904>

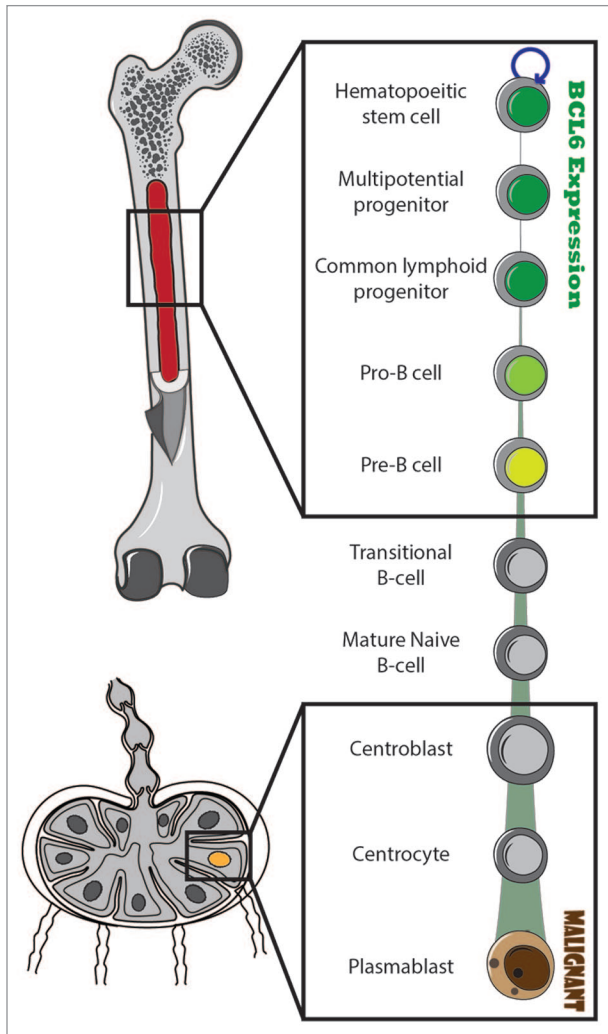


Figure 1. Bcl6 hit-and-run oncogenesis in lymphoma. Transient introduction of *Bcl6* into HS/PCs was sufficient to induce ABC-DLBCL lymphoma through specific genetic/epigenetic changes in HS/PCs that do not interfere with normal B-cell development. This model initially applies to the mouse and the role of BCL6 in human DLBCL progenitors is still unknown and an area of future investigation.

against evolved tumor cells. For instance, BCL6 has emerged as a potentially important therapeutic target in recent years

given its frequent involvement in lymphomagenesis and the fact that specific BCL6 inhibitors can kill lymphoma cells

in vitro and in vivo.⁷ If BCL6 was required to maintain lymphoma-repopulating stem cells, then such a BCL6-targeted therapy might indeed prove effective in eradicating residual tumor cells when translated to the human setting. On the other hand, the fact that BCL6 can also contribute to the malignant phenotype through a hit-and-run mechanism dependent on aberrant epigenetic programming of HS/PCs raises the possibility that tumor cells could emerge that become BCL6-independent. This prediction is supported by the fact that certain BCL6-positive DLBCL cell lines such as OCI-Ly8 (which contains a BCL6 translocation) and Karpas 422 are not killed by BCL6 shRNA or BCL6-targeted therapy. From the therapeutic standpoint, this would indicate the need to combine BCL6 inhibitors with drugs that can kill these BCL6-independent cells. These concepts are therefore central to the full understanding of disease genesis and critical for the development of modern therapies aimed at directly addressing such genetic lesions.

References

1. Armitage JO, et al. *J Clin Oncol* 1998; 16:2780-95; PMID:9704731
2. Alizadeh AA, et al. *Nature* 2000; 403:503-11; PMID:10676951; <http://dx.doi.org/10.1038/35000501>
3. Green MR, et al. *Nat Commun* 2014; 5:3904; PMID:24887457; <http://dx.doi.org/10.1038/ncomms4904>
4. Cattoretti G, et al. *Cancer Cell* 2005; 7:445-55; PMID:15894265; <http://dx.doi.org/10.1016/j.ccr.2005.03.037>
5. Vicente-Dueñas C, et al. *EMBO J* 2013; 32:1502-13; PMID:23632857; <http://dx.doi.org/10.1038/emboj.2013.97>
6. Shin K, et al. *Nat Cell Biol* 2014; 16:469-78; PMID:24747439; <http://dx.doi.org/10.1038/ncb2956>
7. Cerchetti LC, et al. *Blood* 2009; 113:3397-405; PMID:18927431; <http://dx.doi.org/10.1182/blood-2008-07-168773>