The Role of DOT1L in the Regulation of the GC Reaction

L. Kealy[1,2], A. Di Pietro[1,2], L. Dalit[3,4], J.R. Groom[3,4], C. Zaph[1,2] and K.L. Good-Jacobson[1,2]

[1]Department of Biochemistry & Molecular Biology, Monash University, Australia
[2]Immunity Program, Biomedicine Discovery Institute, Monash University, Australia.
[3]Divisions of Immunology and Molecular Immunology, Walter and Eliza Hall Institute of Medical Research, Australia.
[4]Department of Medical Biology, University of Melbourne, Australia.

Abstract

DOT1L elicits mitotically heritable changes to gene expression via the methylation of lysine 79 on Histone 3 and is a major driver of malignant gene expression in MLL-rearranged B cell Acute Lymphoblastic Leukemia (MLLr B-ALL); an aggressive cancer with a particularly poor prognosis in the children and adults affected by it. Consequentially, phase I clinical trials have begun testing DOT1L-inhibitors in MLLr-ALL patients. Despite this, the role of DOT1L in normal lymphocyte biology remains largely unknown. Herein, we sought to characterize the role of DOT1L in the regulation of the humoral immune response.

Dot1l$^{f/f}$*Cd23*$^{Cre/+}$ mice were immunized with NP-KLH and their B cell responses were compared against wildtype controls. The conditional deletion of *Dot1l* resulted in the depletion of memory B cells, class-switched PCs and GCs produced in response to the antigen. Using RNA-seq analysis we were able to show that genes associated with B cell migration and signaling, such *S1pr2, Ackr2* and *Ackr4* were upregulated upon activation in control B cells, but not in *Dot1l* deficient B cells. Migration assays and histological analysis revealed that *Dot1l*-deficient activated B cells failed to migrate into the B cell follicle to form GCs and instead accumulated at follicular borders. H3K79me2 ChIP-seq performed on naive and GC B cells confirmed that most of the genes downregulated in *Dot1l*-deficient activated B cells were direct gene targets of DOT1L. Moreover, DOT1L was found to be targeting genes associated with biological processes that are central to the operation and maintenance of a fully formed GC, such as V(D)J recombination, DNA repair and cell division. We further showed that DOT1L was acting independently of other histone modifiers and in concert with several key transcription factors. Together, these results reveal a vital role for DOT1L in regulating the GC reaction and in establishing effective and lasting humoral immunity.