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B cell differentiation stimulated by physiologic DNA double strand breaks

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Abbreviations: AID, activation-induced cytadine deaminase; ATM, ataxia-telangiectasia mutated; BCR, B cell antigen receptor; CRTC2, CREB-regulated transcription coactivator 2; CSR, class switch recombination; DDR, DNA damage response; DSB, double strand break; GC, germinal center; Ig, immunoglobulin; LKB1, liver kinase B1; RAG, recombinase activating gene; SHM, somatic hypermutation

DNA double strand breaks (DSBs) induced by stage-specific endonucleases are required for the assembly and diversification of B cell antigen receptor (BCR) genes in developing vertebrate B lymphocytes. BCR genes encode for antibodies that contain immunoglobulin (Ig) heavy (H) and light (L) chains and are assembled by the somatic recombination of variable (V), diversity (D) and joining (J) gene segments. In the bone marrow, the recombinase activating gene (RAG) endonuclease generates DNA DSBs in developing B cells at the border of two *Ig* gene segments and their flanking recombination signal sequences.[1] RAG DSBs activate a DNA damage response (DDR) program orchestrated by damage-sensing kinases, such as ATM, which phosphorylates hundreds of proteins that participate in DNA repair, tumor suppression and cell cycle regulation.[2,3]

B cells with surface BCRs that survive bone marrow maturation emigrate into the circulation and home to lymphoid organs throughout the body. Here, naïve B cells in primary lymphoid follicles can be induced to form secondary follicles via T cell-dependent antigenic stimulation. Secondary follicles comprise a mantle zone of non-responding bystander B cells and a germinal center (GC) of antigen-responsive B cells. GC B cells undergo rapid expansion and a second round of *Ig* gene refinements mediated by another endonuclease, activation-induced cytidine deaminase (AID).[4] GCs are sites of class switch recombination (CSR) and somatic hypermutation (SHM), which are AID-initiated DNA strand breaking and repair processes that result in the expression of high affinity antibodies of different isotypes. The generation of mature antibodies is required for the differentiation of responsive memory B cells and for plasma cells that secrete effective antibodies during a humoral immune response.

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In general, three cellular outcomes are recognized for DDR signaling in response to DNA DSBs. These include transient cell cycle arrest coupled with DNA repair, apoptosis or senescence.[5] However, two recent studies suggest an additional, fourth outcome for developing B cells with DNA DSBs, which is for DDR programs to promote B cell differentiation.

Bredemeyer and colleagues showed that RAG DSBs induced during *IgL* gene rearrangements in pre-B cells regulates the ATM-dependent and ATM-independent expression of ~300 genes.[6] ATM-dependent intracellular signaling activated NFκB-dependent gene transcription, which was required for the migration and homing of pre-B cells. These results indicate that signaling from RAG DSBs through ATM and NFκB links a DDR program with early B cell differentiation and function, which is beyond the known DDR that regulates DNA repair, the cell cycle and apoptosis (**Fig. 1**). More work is needed to determine whether RAG DSBs induced during *IgH* gene rearrangements in pro-B cells also regulate genes that control B cell differentiation and function.

Sherman and colleagues studied the role of AID DSBs in B cells stimulated to undergo a GC-like reaction. AID DSBs activated an ATM-dependent signaling pathway that ended by phosphorylating and inactivating the transcriptional coactivator CRTC2.[7] CRTC2 was shown to activate 136 direct target genes, some of which are involved in processes that regulate GC B cell proliferation, self-renewal and inhibit plasma cell differentiation. During CSR, AID DSBs activated ATM which signaled successively through LKB1 and an unknown AMPK family member protein to inactivate CRTC2, resulting in the end of an ongoing GC reaction and differentiation of GC B cells into antibody-secreting plasma cells (**Fig. 1**). GC B cells with ATM or LKB1 knockdown continued to proliferate, were impaired for antibody secretion and failed to properly execute the plasma cell differentiation program. Molecular changes in the signaling pathway from ATM to CRTC2 inactivation were identified in human B cell lymphoma patient samples, including repressed ATM and LKB1 expression and sequence alterations in the CRTC2 kinase target domain, which suggested a potential role in malignant B cell transformation. More work is needed to determine whether defects in AID DSB-induced differentiation facilitate B lymphomagenesis.

Studies of pre-B and GC B cells also showed that chemical and physical agents

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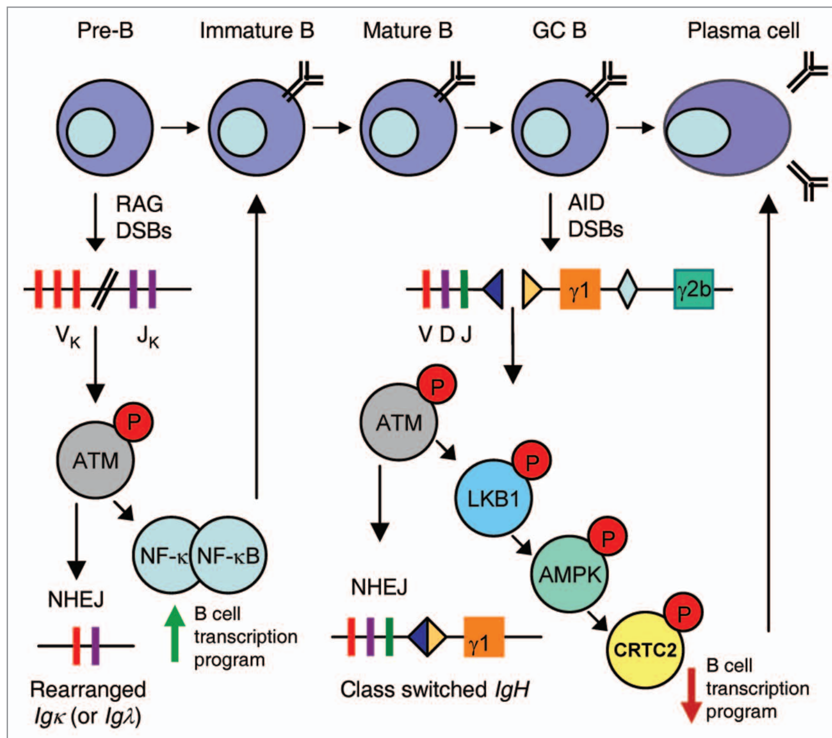

Figure 1. DNA DSBs promote B lymphocyte differentiation. RAG-initiated DNA DSBs during VJ recombination of *Ig*κ (or *Ig*λ) light chain loci in pre-B cells activates an ATM-dependent DDR program that includes the induction of NFκB target genes involved in regulating pre-B cell migration and homing. Later in B lineage development, AID-initiated DNA DSBs during class switch recombination of *Ig* heavy chain genes activates an ATM and LKB1-dependent DDR program that includes repression of CRTC2 target genes involved in regulating the physiology of germinal center B cells, resulting in the terminal differentiation of antibody-secreting plasma cells.

causing DSBs outside of antigen receptor loci regulate gene expression and differentiation changes that are similar to the changes induced by RAG and AID DSBs, respectively.[6,7] Findings from these non-endonuclease DSB studies also suggest that cell differentiation may be a general response to DNA DSBs beyond the lymphocyte lineage, which requires further investigations.

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