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ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

BTG2 Super-Enhancer Mutations Disrupt TFAP4 Binding and Deregulate BTG2 Expression in Diffuse Large B-Cell Lymphoma

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Diffuse Large B-cell Lymphoma (DLBCL), the most frequent lymphoid malignancy, remains incurable in ~40% of patients. Coding-genome sequencing efforts identified several genes/pathways altered in this disease, as well as genetic subgroups of prognostic and therapeutic significance. However, the large non-coding portion of the genome remained largely unexplored. We recently identified a pervasive hypermutation mechanism targeting active super-enhancers (SEs) in >90% of DLBCLs and leading to dysregulation of multiple genes, including well-known lymphoma oncogenes (Bal et al., Nature 2022). This pervasive phenomenon exhibits hallmarks of AID activity, and we provided evidence of its oncogenic relevance by demonstrating that mutational hotspots in the *BCL6*, *BCL2* and *CXCR4* SEs impair the binding of specific transcriptional repressors, preventing their negative regulation and creating oncogenic dependencies in DLBCL cells. Here we aimed to define the pathogenic role of non-coding mutations targeting the intragenic SE (iSE) of the *BTG2* gene, the second most commonly mutated in DLBCL (39% of samples analyzed, including 36/93 primary cases and 7/29 cell lines).

BTG2 encodes a member of the B-cell translocation gene (BTG)/ Transducer of ErbB2.1(TOB) family involved in transcriptional co-activation and modulation of mRNA abundance. *BTG2* is also targeted by somatic missense mutations in 6-11% of DLBCL samples, suggesting a major role in the pathogenesis of this disease, which has not been explored.

In order to identify functionally relevant *BTG2* SE mutations, we first screened an extended panel of 286 DLBCL samples for the presence of mutational clusters targeting predicted transcription factor binding motifs. We identified a 20bp sequence stretch that was significantly hypermutated in these tumors, as compared to other lymphoma types or to non-lymphoid tumors (n=64/286, 22%; vs 0/17 Burkitt Lymphomas; 18/284 Follicular Lymphomas; 1/240 Chronic Lymphocytic Leukemias, and 0/1596 other tumors; $p<0.0001$, Fisher's exact test). In particular, mutations of the C at position +904 to G or T were exclusively detected in 11% DLBCL samples (32/286, $p<0.0001$, Fisher's exact test).

To define the consequences of *BTG2* C904 mutations, we then investigated the impact of reverting the mutation to the wild type nucleotide in 2 mutant DLBCL cell lines, using the CRISPR-Cas9 technology. Correction of the mutation led to a significant counter selection of the corrected clones (~85% reduction in a clone recovery assay, compared to control; $p<0.001$, Fisher's exact test) and to reduced BTG2 mRNA expression ($p<0.01$, one-way ANOVA), suggesting oncogenic addiction.

In silico motif prediction and *in vitro* DNA-binding assays using mutant vs wild type oligonucleotides in two different DLBCL cell lines, followed by mass spectrometry, identified the Transcription Factor AP4 (TFAP4) as a protein specifically bound to the wild type, but not to the mutant sequences. TFAP4 is an important regulator of B-cell proliferation and cell fate decisions, which can function as a transcriptional activator or repressor in germinal center B-cells and acts downstream of/in parallel with c-MYC. We confirmed that the C904G and C904T SE mutation impaired TFAP4 binding in isogenic DLBCL cell lines by ChIP-qPCR and led to increased *BTG2* expression ($p<0.001$, one-way ANOVA). Moreover, loss of TFAP4 in CRISPR-Cas9 edited DLBCL cells led to higher BTG2 expression levels, documenting TFAP4 as a direct negative regulator of BTG2 expression ($p<0.001$, one-way ANOVA). Consistently, single cell analysis of normal GC B cells revealed an inverse correlation between the expression pattern of TFAP4 and BTG2 in cells primed to undergo plasma cell differentiation vs cells committed to dark zone re-entry, suggesting that these two genes may function as a molecular switch in the cell fate decision of GC B cells.

The programs modulated by BTG2 and TFAP4 in the GC, and disrupted in tumors carrying deregulated BTG2 alleles, will be presented, as defined by RNA-seq analysis of 3 isogenic GC-derived cell lines followed by interrogation of over 100 clinically annotated primary DLBCL biopsies.
Together, these data identify TFAP4 as a novel regulator of BTG2 and reveal a previously unappreciated mechanism by which mutations in the *BTG2* SE disrupt this circuit and contribute to lymphomagenesis through deregulating BTG2 expression.

Disclosures No relevant conflicts of interest to declare.

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