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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

CTCF Is a Haploinsufficient Tumor Suppressor in B-Cell Lymphoma

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Alteration of epigenetic regulators is a hallmark of diffuse large B cell lymphoma (DLBCL), the most common type of non-Hodgkin B cell malignancy. It has been recently shown that haploinsufficiency of the cohesin complex ATPase subunit Smc3 cooperates with the oncogene IμBcl6 to induce lymphoma in mice, by a mechanism that involves decreased intra-topological associating domain (TAD) chromosomal interactions and downregulation of tumor suppressor genes. The transcription factor CTCF is an obligate partner of the cohesin complex in defining TADs and regulatory gene loops, acting as the boundaries of TADs and restricting the interaction of promoters and enhancers. Alterations in CTCF have been shown in several cancer types, including lymphomas. In this study, we aim to define the role of CTCF during B-cell lymphoma and lymphomagenesis. We generated germinal center (GC) B cell-specific conditional knockout mice for CTCF by crossing the CTCF floxed mice to the Ighg1-Cre (Cγ1-Cre) allele. In these experiments, we compared haploinsufficient CTCF conditional knockout mice-in which only one allele of CTCF was knocked out-versus wild-type mice expressing the Cγ1-Cre. Eight days after immunization with the T-cell-dependent antigen sheep red blood cells, we excised spleens and surveyed B cell populations by flow cytometry and immunohistochemistry. Remarkably, CTCF haploinsufficient mice contained a similar percentage of follicular GC B cells compared to wild-type mice (Live B220 $^{+}$CD95 $^{+}$GL7 $^{+}$ or live B220 $^{+}$CD95 $^{+}$CD38 ^{DIM} splenocytes). However, CTCF haploinsufficient GCs displayed a bias in the GC subpopulations, namely an increased percentage of light zone centrocytes (LZ: GC $^{+}$ markers CXCR4 ^{lo}CD86 ^{hi}) and decreased dark zone centroblasts (DZ: GC $^{+}$ markers CXCR4 ^{hi}CD86 ^{lo}).

Because biases in GCs were an early indication of malignant transformation in previous studies, we wondered whether CTCF haploinsufficiency would cooperate with lymphoma oncogenes to induce B-cell lymphoma. To address the lymphomagenic potential of CTCF haploinsufficiency, we crossed CTCF conditional knockout mice to the vavP-Bcl2 mice that develop GC-derived follicular-like lymphoma with long latency. We harvested bone marrow blood cell precursors from Cγ1-Cre ("WT"), Cγ1-Cre;CTCF $^{+/-}$ ("CTCF"), vavP-BCL2 $^{+}$;Cγ1-Cre ("BCL2"), and vavP-BCL2 $^{+}$;Cγ1-Cre;CTCF $^{+/-}$ ("BCL2/CTCF") and transplanted them into lethally irradiated C57BL6/J (30 per group). Mice that survived more than two weeks after irradiation and bone marrow transplant were considered stably engrafted and monitored for any signs of disease. Lethargic mice were sacrificed and macroscopically inspected for the presence of tumors. Survival analysis by Kaplan-Meier showed that BCL2/CTCF mice had the shortest survival from all four groups, having a median survival of 230 days, while survival for the BCL2 alone group did not fall below 50% up to day 370. Immunohistochemistry against B220 $^{+}$ was performed in tissue sampling from dying mice to confirm the cause of death. Curiously, CTCF haploinsufficiency accelerated BCL2-induced lymphomagenesis but did not affect the survival of IμBCL6-induced lymphoma.

To explore the mechanistic basis of CTCF haploinsufficiency-induced lymphoma in mice, we FACS sorted DZ and LZ GC B-cells from 8-day immunized mice and explored global transcriptomics by RNA-sequencing. While the transition from DZ to LZ involves the regulation of around ~1,500 genes, we failed to detect changes in gene expression between wild-type and CTCF haploinsufficiency cells. ATAC-seq assay on the same cells showed little to no change in chromatin accessibility between cells from CTCF haploinsufficient and wild-type mice. TOBIAS footprinting analysis, which identifies biases in Tn5 transposase activity due to protein binding, showed that CTCF haploinsufficient cells maintained a deep depletion in CTCF binding. Hi-C chromosomal conformation analysis identified an overlapping set of gene regulatory loops that lose interactions and showed decreased signal by gene set enrichment analysis.

In this study, we characterized the role of CTCF haploinsufficiency phenotypically and are currently exploring the molecular mechanisms that contribute to the acceleration of the lymphomagenesis induced by the BCL2 oncogene.

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