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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Integrated Genomic Analysis Identifies a Downstream Regulatory Element Oncogenically Activating *Hematopoietically Expressed Homeobox* (*HHEX*) in ETP-ALL

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Hematopoietically-expressed Homeobox (*HHEX*) is required for the maintenance of hematopoietic stem cells (HSPCs) and common lymphoid progenitor cells. *HHEX* is also the second most frequent integration site in retroviral insertional mutagenesis screens of leukemias and lymphomas arising in AKXD recombinant inbred mouse strains, implying that it is an important oncogene. Enforced expression of *HHEX* induces T-cell acute lymphoblastic leukemias (T-ALL) in murine bone marrow transplantation models. In human studies, *HHEX*mRNA expression is upregulated in human T-ALL studies, especially in Early T-cell Precursor (ETP-) ALL subtypes where it is concordantly expressed with *LMO2*. The *HHEX* locus is not rearranged in ETP-ALL where it is upregulated so the mechanism of *HHEX* activation is not clear.

We analyzed T-ALL induced by retroviral mutagenesis and found an intergenic site of frequent integration, 65kb 3′ of the *Hhex* coding exons. Integrations clustered within a 3 kb genomic area that is also an open chromatin region (OCR) by ATAC-seq analysis and that induced *Hhex* upregulation. The syntenic human region was highly enriched for H3K27 acetylation and for occupancy by multiple transcription factors, notably GATA1 and TAL1, per ENCODE. Our prior data had shown that *HHEX* was a downstream target of the LMO2 complex comprised of GATA1/2, TAL1 or LYL1, and nucleated by the scaffolding protein, LIM domain binding protein 1 (LDB1). We had previously shown by ChIP-PCR that the LMO2 complex was bound to the intron 1 enhancer. Thus, we performed ChIP-exonuclease analysis of LDB1 in the human ETP-ALL model cell line, LOUCY. We confirmed its occupancy at the intron 1 enhancer but we also observed LDB1 occupancy 68kb 3′ of the *HHEX* coding exons, in the exact region syntenic to the murine common insertion sites. ChIP-exo analysis allowed us to pinpoint a core element with composite GATA/E box sites that was highly conserved across multiple mammalian species.

LDB1 occupancy at intron 1 and at +68kb of the *HHEX* locus was reminiscent of LDB1's occupancy at the beta globin locus where LDB1's occupancy and homodimerization mediates chromatin looping between composite E box/GATA sites in the locus control region (LCR) and the beta globin proximal promoters. To test whether a similar interaction was occurring at the *HHEX* locus, we analyzed virtual 4C (chromatin conformation capture) data. We confirmed looping between the +68kb element and the proximal promoter of *HHEX* in human ETP-ALL primary samples and in human HSPCs. The experiments showed that looping occurs in certain developmental contexts in HSPCs and is recapitulated in ETP-ALL and mediated by the LMO2/LDB1 protein complex. *HHEX* is a downstream oncogene of the LMO2/LDB1 complex and is activated through this distal +68kb regulatory element. Our studies raise the possibility that dissection of this regulatory element and interference with chromatin looping are potential therapeutic mechanisms that could disrupt oncogene expression.

Disclosures No relevant conflicts of interest to declare.

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