論文の内容の要旨

論文題目　Chromatin Modifications in B-Cell Acute Lymphoblastic Leukemia Carrying DUX4 Fusions
（ＤＵＸ４融合遺伝子陽性Ｂ細胞性急性リンパ性白血病におけるクロマチン修飾の解析）

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DUX4 fusions are novel oncogenes encoding truncated forms of the transcription factor DUX4. They are preferentially found in B-cell acute lymphoblastic leukemia (B-ALL) in children and young adults, shaping a new clinical entity. *DUX4* fusions are generated from insertions of wild-type (WT) *DUX4*, mainly into the *IGH* locus. The translocation replaces the 3′ end of the WT *DUX4* coding region with a fragment of *IGH* or another gene, producing DUX4 out-of-frame fusion proteins devoid of the C terminus of WT DUX4. Usually, expression of WT DUX4 is epigenetically repressed in somatic tissues, as expression of WT DUX4 in somatic cells leads to cell death. Recently, WT DUX4 is identified to play a critical role in transcriptional programs at the cleavage stage of human fertilized egg. In B-ALL, DUX4-IGH is shown to be essential for leukemic transformation, however, the mechanism of leukemogenesis is not completely understood. I thus explored the transcriptional regulation and chromatin modification by *DUX4* fusions in B-ALL through RNA-seq, chromatin immunoprecipitation assay coupled with sequencing (ChIP-seq), and Assay for Transposase-Accessible Chromatin with sequencing (ATAC-seq).

I first analyzed the DNA binding capacity and histone modification ability of DUX4-IGH, using NALM6, a B-ALL cell line carrying *DUX4-IGH*. ChIP-seq analysis indicated that the genomic binding sites and the binding motif of DUX4-IGH were similar to those of the WT DUX4. DUX4 bound loci are mainly located in the intergenic or intron regions, and tri-methylation of histone H3 lysine 4 (H3K4me3) and acetylation of histone H3 lysine 27 (H3K27ac) were enriched in the DUX4 bound loci in NALM6 cells. Ectopic expression of DUX4-IGH in Reh cell, a B-ALL cell line carrying the other oncogenic fusion, increased H3K27ac along the DUX4 bound loci.

DUX4-IGH knockdown and ChIP-seq in NALM6 cells indicated that the transcription of genes characteristic to B-ALL carrying *DUX4* fusions, such as *STAP1*, *CCNJ*, and *ITGA6*, were positively regulated by DUX4-IGH directly or indirectly. In Reh cells, ectopic expression of DUX4-IGH induced expression of these direct and indirect target genes. In the RNA-seq from B-ALL clinical samples, I observed significantly higher expression of these genes in B-ALL cases with *DUX4* fusion, compared with other types of B-ALL cases, further validating the specific transcriptional activity of DUX4-IGH. Single knockdown of the representative target genes positively regulated by DUX4-IgH did not affect the number of NALM6 cells.

Transcriptome analysis comparing the status between ectopic expression of DUX4-IGH

and WT DUX4 in NALM6 cells suggested that the transcriptional target genes of DUX4 fusions and WT DUX4 are different. When I focused on the target genes of WT DUX4 including *ZSCAN4*, *DUXA, REFPL4A*, and Human Endogenous Retroviral elements, the transcriptional activity of DUX4-IGH was severely attenuated compared with that of WT DUX4. One possibility is that histone acetyl transferase cannot bind to DUX4-IGH due to the loss of C terminus of WT DUX4, and I confirmed that CBP is not the binding partner of DUX4-IGH through immunoprecipitation against DUX4 in NALM6 cells, that partially explained the attenuated transcriptional activity of DUX4-IGH.

Comparison of ATAC-seq of NALM6 cells between with or without knockdown of *DUX4-IGH* revealed that binding of DUX4-IGH opens the chromatin and regulates the transcription of the target genes. Motif analysis of the region with gained ATAC signals by knockdown of *DUX4-IGH* identified motifs of transcription factors related to hematopoietic differentiation, implying the association with the defective differentiation in leukemia carrying *DUX4* fusions. Comparing the expression data and ATAC-seq, I identified *BCL2* as a candidate gene for tumorigenesis driven by *DUX4-IGH*, and pharmacological inhibition of BCL2 caused apoptosis of NALM6 cells.

DUX4-IGH knockdown in NALM6 cells resulted in the increased expression of genes associated with hematopoietic cell differentiation, such as *TCF3* and *SPI1,* suggesting that these genes were negatively regulated by DUX4-IGH. Gene set enrichment analysis revealed that genes associated with pro-B cells, pre-B cells were enriched in the genes overrepresented in NALM6 cells with *DUX4-IGH* knockdown.

In conclusion, I revealed that DUX4-IGH exhibited transactivating activity related to leukemogenesis. DUX4-IGH also drives drastic chromatin changes both at DUX4-IGH bound genomic regions or other genomic regions. The latter genomic regions were associated with the defective differentiation of B-ALL carrying *DUX4* fusions. Further understanding of the mechanism of transcriptional network perturbation by DUX4-IGH should provide clues for a treatment strategy for B-ALL carrying *DUX4* fusions.