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TITLE
CTCF boundary remodels chromatin domain and drives aberrant HOX gene transcription in acute myeloid leukemia

HYPOTHESIS:
1. CTCF binding site located between HOXA7 and HOXA9 genes (CBS7/9) acts as a chromatin boundary to establish and maintain AML specific aberrant HOX gene expression patterns and is essential for pathogenesis of myeloid malignancies.
2. Alteration of CTCF mediated topological boundaries in the oncogene loci provides therapeutic approaches for targeting oncogenic expression program and leukemogenesis.

BACKGROUND/AIMS:
Background: The organization of the mammalian genome into separate domains either facilitates or restricts interactions between regulatory elements, e.g. enhancers and promoters, for normal gene regulation. CTCF insulator constrains the temporal HOX gene expression patterns within the confined chromatin domain for normal development. However, dysregulation of HOX genes is also a common feature of acute myeloid leukemia (AML). The molecular mechanisms of aberrant HOX expression and associated AML pathogenesis remain unclear.

Aims:
1. To identify which CTCF binding sites located in HOXA is critical for maintaining aberrant HOX gene expression in AML.
2. Alteration of CTCF mediated topological boundaries in the oncogene loci provides therapeutic approaches for targeting oncogenic expression program and leukemogenesis.

METHODS:
1. We employed a pooled CRISPR-Cas9 genetic knockout library screening to interrogate intergenic regulatory and chromatin boundary elements in all 4 HOX gene loci (HOXA,HOXB,HOXC and HOXD);
2. Two single-guide RNAs (sgRNAs of the core CTCF motif located between HOXA7 and HOXA9 genes were employed to delete the CBS7/9 boundary in AML cells;
3. Genome wide ChIP-seq, ATAC-seq, 4C-seq, and RNA-seq analysis to examine the changes in chromatin domain organization and corresponding gene expression patterns compared wild-type control and the CBS7/9 KO AML cells, including epigenetic markers H3K4me3 and H3K27me3 ChIP-seq;
4. CBS7/9 KO AML cells were transplanted into NSG mice to test its proliferation and mice survival;
5. Cytarabine has been used to determine chemo-sensitivity of AML cells through modulating HOXA9 levels.

RESULTS & CONCLUSIONS
1. we employed a pooled CRISPR-Cas9 genetic knockout library screening to find CBS7/9 chromatin boundary located between HOXA7 and HOXA9 genes that is critical for maintaining aberrant expression of posterior HOXA genes including oncogene HOXA9 and HOXA10 in primary AML patients and cells;
2. The defected CBS7/9 resulted in a decrease in CTCF binding to the caudal HOXA domain and an inhibition of posterior HOX gene expression;
3. Disruption of CBS7/9 boundary resulted in a spreading of repressive H3K27me3 domain into the caudal HOXA domain that subsequently impaired AML associated active TAD domain by altering enhancer/promoter chromatin accessibility and reducing ectopic interactions among the posterior HOXA genes;
4. CBS7/9 boundary depletion will increase chemo-sensitivity of AML cells to cytarabine by modulating HOXA9 levels, and thus this research provides a novel therapeutic target for treatment of myeloid malignancies.