

Regulation of MYB by long-range enhancer elements in human leukemia

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Abstract: The transcription factor MYB is a key regulator of hematopoiesis and plays important roles in cell proliferation, differentiation, apoptosis and other important life processes as well as a variety of diseases. Studies have shown that dysregulation or mutation of MYB can induce carcinogenesis, mainly leukemia. Although MYB has been well studied, its detailed underlying regulatory mechanisms still remain elusive. Here, we detected the long-range interaction between the upstream regions, -34k and -88k, and the MYB promoter in K562, U937, and HL-60 cells using circularized chromosome conformation capture (4C) assay, which declined when MYB was downregulated during chemical-induced differentiation. The enrichment of H3K4me1 and H3K27ac in these regions and luciferase assay further confirmed enhancer activity in the -34k and -88k regions. ChIP-qPCR showed the dynamic binding of GATA1, TAL1, and CCAAT/enhancer-binding protein (C/EBP β) at -34k and -88k during differentiation of K562 cells. Epigenome editing by a CRISPR-Cas9-based method showed that H3K27ac at -34k enhanced TF binding and MYB expression, while DNA methylation inhibited MYB expression. Taken together, we identified enhancer element at the -34k and -88k regions, which play roles in regulation of MYB in human leukemia cells. Subsequently, we found MYB 34kb upstream enhancer encodes two enhancer RNAs (eRNAs). Quantitative PCR (qPCR) revealed that both were more highly expressed in K562, U937, and HL-60 cells than in Hela cells. CCK8 proliferation and transwell assays confirmed that eRNAs promoted the proliferation and migration of leukemia cells, suggesting that eRNAs may have a critical role in promoting the development of leukemia. Our data revealed that enhancer element at -34k are required for MYB expression, and was transcribed to produce eRNAs, providing insights into the critical roles of enhancer in leukemia initiation and progression.

Keywords: MYB, enhancer, 4C, chromatin structure, enhancer RNA