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ORAL ABSTRACTS

112.THALASSEMIA AND GLOBIN GENE REGULATION

Direct and Indirect Functions of TR4 in the Control of Fetal Hemoglobin Silencing

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Elevated levels of fetal hemoglobin (HbF) compensate for reduced normal β-globin chains and ameliorate clinical symptoms for patients with beta-globinopathies, such as sickle cell disease (SCD). The embryonic and fetal globin genes, but not the adult β-globin gene, have direct repeat (DR) elements in their promoters, which have been implicated in γ-globin gene repression (Tanabe et al., 2002). We previously purified a DR element-binding protein complex, which we named DRED, and was the first identified *HBG* gene repressor (Tanabe et al., 2002). DRED activity is conferred by a complex including LSD1, recruited by the nuclear receptors TR2/TR4 (Cui et al., 2011). BCL11A is a well-characterized transcription factor that independently represses the γ-globin genes through binding to their promoters (Liu et al., 2018). We have shown by co-immunoprecipitation that TR4 interacts with BCL11A in HUDEP2 cells.

To understand how TR4 acts to repress the γ-globin gene and the relationship of TR4 to BCL11A in γ-globin repression, we characterized the chromatin occupancy of TR4 and BCL11A within the β-globin locus by CUT&RUN (CNR) in HUDEP2 cells. We established HUDEP2 cells bearing TR4 tagged with 3FLAG+3Myc epitopes (FM-TR4) and in the same cells, BCL11A tagged with 3HA+3Myc (HM-BCL11A) epitopes by genome editing. In addition, another HUDEP2 cell line bearing BCL11A tagged with the 3HA+3Myc (HM-BCL11A) epitope as well a cell line bearing TR4 tagged with the 3FLAG+3Myc epitopes (FM-TR4) were generated. The tagged cell lines not only improved the efficiency of antibody targeting, but also facilitated direct comparison of TR4 vs BCL11A chromatin occupancy by binding of the common Myc epitope in FM-TR4 and HM-BCL11A cell lines. Unbiased motif discovery of TR4 CNR binding of these epitopes in all 3 cell lines yielded the motif of a direct repeat consisting of 2 AGGTCA repeat elements with a 1 nt spacer. The exact match of BCL11A motif with one of the direct repeat elements of TR4 motif suggested that BCL11A and TR4 may be compete for binding to the γ-globin promoter. Overall, TR4 and BCL11A showed a similar pattern of occupancy within the globin locus, although of lower intensity. TR4 presented strong interaction with the locus control regions, among which HS2 and HS3 were the highest, consistent with the model of long-distance interaction through chromosomal looping. However, TR4 was found to be significantly less occupied at the γ-globin promoters than BCL11A. To test whether TR4 and BCL11A can bind *in vitro* to the motifs found in the γ-globin promoters, we performed TR4 and BCL11A electrophoretic mobility shift assays (EMSAs). We showed that TR4 binds to the DR1 element within the γ-globin promoters, which contains the distal TGACCA motif that was bound by BCL11A. Notably, mutation of the TGACCA motif disrupted both BCL11A and TR4 binding, while the other direct motif is indispensable for TR4 binding, supporting overlapping binding of BCL11A and TR4. Interestingly, DNA-TR4 protein complexes were competitively depleted by high concentrations of BCL11A and vice versa, suggesting competitive binding between the two factors. Studies of this mechanism in the erythroid cells are underway.

In addition, unbiased motif discovery of TR4 CNR also enriched for KLF1, LRF and NF-Y motifs. Co-IP assays demonstrated interaction between TR4 and KLF1 and LRF, suggesting the presence of a large repressor complex. We also explore genome-wide occupancy of TR4 and discovered strong TR4 interactions at promoters of multiple HbF repressing transcription factors and corepressors, including NFIA and EHMT1, suggesting that the role of TR4 in HbF repression may be partially mediated by regulating expression of various HbF repressor genes.

In summary, these data suggest that the function of TR4 in HbF silencing is mediated in part by direct repression of *HBG* promoters in a competitive manner with BCL11A, and in part by regulating the expression of multiple HbF repressor genes.

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