The role of EZH2 and H3K27me3 epigenetic signature in modulating T-cell polarization in atherosclerosis

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Background: Atherosclerosis is a chronic inflammatory disease, characterized by the formation of luminal plaques in arteries. Atheroprogession is accompanied by strong immune activation, during which T cells play a pivotal role. Genes driving T-cell activation are regulated by the polycomb repressive complex 2 (PRC2), whose major component is the Enhancer of Zeste Homolog 2 (EZH2), responsible for the methylation of Histone 3 Lysine 27 (H3K27me3) epigenetic mark.

Purpose: We hypothesize that EZH2 mediates T-cell activation and modulates T-helper (Th) cell polarization, thus atherogenesis.

Methods: To study the effect of EZH2 in atherosclerosis, we backcrossed mice with Ezh2 flanked by loxP-sites sensitive to Cre-mediated inactivation in T cells to apolipoprotein E (Apoe-/-) mice and fed a high cholesterol diet to have hyperlipidemic Ezh2fl/fl Cd4CreApoe-/- mice and controls. Atherosclerotic plaque progression and immune cell phenotyping were assessed by histology, flow cytometry (FC), gene and protein expression analysis and single cell (sc)RNA-sequencing (Seq) as well as by functional in vitro assays.

Results: T cell-specific EZH2 deficiency resulted in a significant reduction in H3K27me3 in T-cells. Female Ezh2fl/fl Cd4Cre mice developed 34% less atherosclerotic plaques in the aortic root compared to wildtype (WT) littermates. FC analysis of spleen and lymph nodes of Ezh2fl/fl Cd4Cre mice revealed a shift from naive to effector CD4+ T cells, and the majority were Th2 cells (p<0.0001). Accordingly, plasma levels of IL4 and aortic IL4 transcripts were increased in Ezh2fl/fl Cd4Cre mice (p=0.05 and p=0.0079, respectively). Functional analysis revealed that splenic CD4+ T cells from Ezh2fl/fl Cd4Cre mice showed decreased migratory capacity towards the chemokines CXCL10, CCL17, CCL22, CCL19. To unravel mechanisms driving the type 2 immune response, bulk RNA-Seq of CD4+ T cells and scRNA-Seq of splenic CD3+ T cells from Ezh2fl/fl Cd4Cre and WT mice revealed that CD4+ T cell EZH2 deficiency caused upregulation of promyelocytic leukemia zinc finger (Plzf), a transcription factor of NKT cells of the NKT2 subtype, characterized by high IL4 production. Via FC we confirmed the increase in splenic NKT cells in Ezh2fl/fl Cd4Cre mice. Likewise, Plzf aortic mRNA levels were also significantly increased (p=0.002). To exploit the translational potential of T cell-specific EZH2 targeting, we treated Jurkat T cells with EZH2 inhibitors, the FDA-approved drug Tazemetostat and GSK126. Corroborating our in vivo data, EZH2 inhibitor-treated cells showed decreased H3K27me3 and 70% reduced migration towards the chemokine CXCL12.

Conclusions: Our study demonstrates that CD4+ T-cell specific EZH2 deficiency impairs T cell-migration and skews the immune response towards a type 2 response, resulting in protection against atherosclerosis. We aim at investigating whether the epigenetic mark H3K27me3 in T cells may be a promising therapeutic target for future cardiovascular immunotherapies.