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High glucose exposure promotes epigenetic activation of pro-inflammatory RELA/p65 gene in cord blood-derived CD34+ stem cells

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Background: Diabetes profoundly affects multiple signaling pathways and key transcription factors that account for the systemic pro-inflammatory state and increased propensity for the patients to develop atherosclerosis. In addition, as a consequence of hyperglycemia, diabetic patients have decreased number and dysfunctional circulating CD34+ cells that under normal physiological conditions contribute to the maintenance of vascular homeostasis and regeneration. Emerging evidence suggests that epigenetic mechanisms, such as DNA methylation and histone modifications, are involved in the regulation of inflammatory genes in vascular cells under diabetic conditions. Since CD34+ cells are also important precursors of immune cells, we hypothesized that uncontrolled hyperglycemia might epigenetically skew CD34+ cells towards inflammatory cells.

Purpose: We sought to evaluate epigenetic priming of inflammatory response genes by high glucose conditions in CD34+ stem cells.

Methods: CD34+ cells were purified from cord blood of healthy donors and expanded in normal-glucose (NG; with 30 mM mannitol for osmotic control) or high-glucose (HG; 30 mM) serum-free medium plus cytokines (FLT3, SCF, IL3 and IL6) for up to 20 days. The expression of RELA/p65, KAT2B/PCAF, and TNFa genes was assessed by qPCR. Western Blot was used to evaluate acetylation of NFkB p65 at lysine-310. H3K9me3 and RNA polymerase II recruitment to the RELA/p65 gene promoter were evaluated by ChIP-qPCR assay.

Results: Increasing evidence links glucose metabolism to changes in chromatin. We therefore examined H3K9me3 status of RELA gene promoter encoding for the p65 subunit of inflammatory transcription factor NF?B in CD34+ cells after high glucose exposure. ChIP-qPCR data showed significant reduction of H3K9me3 levels in HG-CD34+ cells (n=5; FC 1±0.16 SE vs 0.4 ± 0.1 SE; p=0.0327). The lowering of this repressive epigenetic modification coincided with increased recruitment of RNA polymerase II to the RELA/p65 gene promoter and a significant up-regulation of p65 gene expression (n=8; FC 1±0.27 SE vs 1.41±0.3 SE; p=0.0034). Interestingly, KAT2B/PCAF gene, a histone acetyltransferase implicated in NFkB p65 acetylation and co-activation was also overexpressed (n=12; FC 1±0.14 SE vs 1.4±0.19 SE; p=0.0225). This post-translational modification is critical for nuclear stabilization and full transcriptional activity of NF?B, responsible for the expression of inflammatory genes. Hence, we assessed the acetylation of NF?B p65 at lysine-310 and the expression of NF?B p65 target genes such as TNFa. The analysis revealed an increased acetylation at lysine-310 in HG-CD34+ cells and significant up-regulation of TNFa gene expression (n=9; FC 1±0.15 SE vs 1.38±0.28 SE; p=0.0408).

Conclusions: These results suggest that elevated glucose exposure might epigenetically prime CD34+ cells for a pro-inflammatory response and/or skew CD34+ cell differentiation into cell lineages with deleterious properties.