Hyperglycemia epigenetically propels senescence-associated secretory phenotype of CD34 hematopoietic stem cells and their myeloid expansion into inflammatory monocyte subpopulations

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Background: The "leitmotif" underlying the cardiovascular disease initiation and progression in diabetic patients is the chronic inflammation in which monocytes/macrophages are the key players [1]. Diabetes mellitus (DM) showed to alter hematopoiesis, by promoting myelopoiesis and the generation of monocyte subsets with inflammatory and atherogenic phenotype [2-4]. Epigenetic modifications have been described in the inflammation and in control of monocyte-derived macrophage polarization [5,6]. However, there is no notion of when these epigenetic modifications take place and whether can be transferred to more differentiated cells.

Purpose: The aims of the study were 1) to assess in vitro if high glucose (HG) exposure promotes epigenetic changes and alteration in myeloid differentiation of hematopoietic stem/progenitor cells HSPCs; 2) if such epigenetic modifications are transferred to cell progeny and 3) if similar mechanisms are at work in bone marrow (BM)-derived HSPCs of DM patients.

Methods: HSPCs were isolated form the cord blood (CB) and from BM of DM patients. CB-HSPCs were grown in normal-glucose (NG; 30 mM mannitol) or HG (30 mM) conditions and counted after 10, 20 and 30 days. The expression of p27, p21, IL6, TNFα, RELA/p65, KAT2B/PCAF, SetD7 genes and telomere length was assessed by qPCR; cytokines by ELISA. Western Blot was used to evaluate NFkB-p65 expression and acetylation at lysine-310. H3K9me3, H3K4me1 modifications and SetD7, RNA polymerase II at RELA/p65 gene promoter were assessed by ChIP-qPCR assay. Flow cytometry was used to assess ROS, CD14/CD16 monocyte subpopulations and NFkB-p65 nuclear translocation.

Results: HG-exposure of CB-HSPCs induced a senescent-associated secretory phenotype characterized by cell proliferation lowering, ROS production, telomere shortening, up-regulation of p21 and p27 genes, increased secretion of TNFα and IL6 cytokines, and upregulation of NFkB-p65 transcription factor (Fig.1). NFkB-p65 gene promoter analysis showed H3K9me3 and H3K4me1 histone mark alterations associated with increased SetD7 and RNA polymerase II recruitment. Importantly, the upregulation of KAT2B gene linked with increased lysine-310 acetylation, nuclear translocation and binding activity of NFkB-p65 (Fig.1). HG-HSPCs, once differentiated into myeloid lineage, generated higher level of intermediate (CD14++CD16+) proinflammatory monocyte subpopulations (Fig.2). Similarly, BM-HSPCs from DM patients displayed a senescent-associated secretory phenotype and abnormal intermediate (CD14++CD16+) monocyte output when differentiated into myeloid lineage. Importantly, epigenetic changes at the level of NFkB-p65 gene promoter persisted in both HG-HSPC- and DM-HSPC-derived monocytes populations (Fig.2).

Conclusions: Overall, our data show that hyperglycemia elicits epigenetic changes in HSPCs that persist in monocyte progeny and are potentially involved in their inflammatory phenotype.