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Dissertations-Kurzfassung

Epigenetic regulation of S100A9 and S100A12 expression in monocytes-macrophage system in hyperglycemic conditions

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The number of diabetic patients in Europe and world-wide is growing. Diabetes confers to about two-fold higher risk for a wide range of vascular diseases independently from common risk factors such as age, sex, smoking, high blood pressure, and BMI. Lack of insulin (T1D) or lack of insulin responsiveness (T2D) causes metabolic changes such as hyperglycemia (HG) which contributes to the pathology of diabetes. Macrophages are essential regulators of inflammation and play a critical role in diabetic macro- and microvascular complications. Previous work in our laboratory using Affymetrix chip profiling identified that hyperglycemia enhances the expression of several members of the S100 protein family in macrophages. S100A9 and S100A12 are pro-inflammatory molecules that activate endothelial cells. Their elevated levels in the circulation positively correlate with diabetes pathology. However the role of hyperglycemia in the production of *S100A9* and *S100A12* by macrophages was not investigated to date. The aims of the study were to investigate the regulation of S100A9 and S100A12 expression during macrophage differentiation in normal and hyperglycemic conditions and diabetic patients; to examine the effect of hyperglycemia on the histone code on the promoters of *S100A9* and *S100A12* and the involvement of specific histone modifying enzymes; to examine potential hyperglycemic memory for the expression of *S100A9* and *S100A12* in macrophages.
Human primary monocytes-derived macrophages were used and differentiated for 6 day in the presence of IFNγ or IL-4 to generate M1 and M2 macrophages respectively and without cytokines to generate M0 macrophages. Using RT-PCR it was demonstrated that S100A9 and –A12 are highly expressed in M1 macrophages compared to M2. Hyperglycemia increased the expression of S100A9 and S100A12 in M0 but mostly in M1 macrophages, up to 4.4 fold and 9.8 fold for individual donors respectively. Association for activating histone marks with the promoters of these genes was analysed by chromatin immunoprecipitation (ChIP). Hyperglycemia induced the increased association of activating histone marks; H3K4me1, H3K4me3 and general H3Ace with promoters of *S100A9* and *S100A12*. Association correlated negatively with the increase in gene expression. The total H3, representing the nucleosome density, was reduced under HG conditions. The increase in total endogenous H3 positively correlated to the fold change increases in gene expression of *S100A9* and *S100A12*. Histone methyltransferases regulate gene expression in differentiated M1 macrophages. Application of inhibitors of the MLL complex increased S100 gene expression synergistically with glucose. Inhibition of SMYD3 93 specifically down regulated S100A12 expression. Inhibition of SET7, the key histone methyltransferase that writes H3K4me1, down regulated both S100A9 and S100A12 gene expression. Expression of SET7 and its translocation to the nuclei was increased in M1 macrophages and under high glucose conditions. In a macrophage model were glucose concentrations were changed to normal glucose concentration after 6 days, and gene expression was measured on day 12, memorable changes of S100 genes expression in M1 macrophages were observed. In another model, where macrophages without polarizing factors are cultured in high glucose conditions for 6 days and subsequently stimulated overnight with TLR-ligands, increased expression of S10 proteins was identified. *S100A9* had increased in response to stimulation with PA (9.9-fold). *S100A12* was up regulated in LPS stimulation of macrophages (5.4-fold).
Therefore increased expression of S100 proteins may be indicative for the long-term proinflammatory effects of hyperglycemia. S100 proteins are expressed in M1 macrophage and their expression is up regulated in hyperglycemic conditions, whereas histone modifying enzymes SMYD3 and SET7 are involved. Also, histone code might regulate transcription independently from nucleosome density in our study and histone content constitutes of an extra layer of epigenetic regulation. At last, stimulation

of hyperglycemia-exposed macrophages by TLR-ligands revealed that S100 proteins are sensitive to glucose macrophages programming.

Individual differences in S100 protein expression in response to hyperglycemia and proinflammatory stimuli suggest that S100 proteins can be used to distinguish between responders and non-responders towards hyperglycemia indicating the risks for later vascular complications in diabetes patients. S100 proteins can be considered as a target in chronic inflammatory conditions, and macrophage directed treatments that aim to reprogram M1 macrophages, should take into account the level of induced epigenetic changes.