Diabetic retinopathy (DR) is a leading cause of blindness commonly affecting diabetic patients with variable severity. One of the earliest events occurring in diabetic retinas is hyperglycemia-induced functional impairment of microvascular cells (pericytes and endothelial cells). Although oxidative stress and the activation of inflammatory pathways have been reported as key contributors to DR pathogenesis, the molecular mechanisms underlying DR remain to be elucidated. Alterations in the methylation status of DNA, chemical modifications of histones and the activity of regulatory non-coding RNAs have been proposed as pathogenic in DR (Lee et al., 2017; Lin et al., 2016; Mortuza et al., 2014; Zhong and Kowluru, 2013; Tewari et al., 2012). A pathogenic role of methylglyoxal (MG) as mediator of hyperglycemia-induced alterations and metabolic memory (i.e. persistence of hyperglycemia induced damage even after glycemic normalization) has been proposed in macrovascular endothelial cells (Reddy et al., 2015; Turkseven et al., 2014; Mukohda et al., 2013; Brouwers et al., 2010), but its effects on human retinal microvascular cells have not been clarified.

Thus, this Project aims to clarify the MG-mediated damage in human retinal endothelial and pericyte cells in terms of impairment of cell functional properties and intracellular damage, by combining functional assays and advanced omic approaches. Moreover, this Project could contribute to assess the contribution of MG to hyperglycemia irreversible effects and thus to metabolic memory phenomenon.

To date, we reveal that the exposure of human retinal endothelial cells to MG (500μM for 72h) does not compromise cell viability but impairs both cell migration and tube formation capacity by ~30% (p≤0.05) and ~70% (p≤0.05), respectively. Furthermore, by combining two "omic" technologies (RNA-Seq and smallRNA-Seq) the entire network of altered genes in human retinal endothelial cells following exposure to MG was defined. Particularly, transcriptome analysis revealed that MG exposure increases expression of ~1200 genes encoding mainly transcriptional regulators (25%, pval=9.1^{-46}) and apoptosis-related genes (7%, pval=3.6^{-6}). Conversely, MGO exposure causes downregulation of ~500 genes encoding membrane glycoproteins (36.5%, pval=4.2^{-12}), cell cycle control factors (13%, pval=1.1^{-19}) and cell adhesion molecules (11%, pval=2.2^{-9}), including integrins (5%, p=1.5^{-6}). Thus, the analysis reveals a dual role of MG in the transcriptional perturbation of retinal microcirculation cells, i.e. by promoting the expression of molecules responsible for gene regulation and inhibiting cell cycle and cell interaction. Moreover, ChIP-Seq (ENCODE) data integration allowed us to predict a network of structural proteins regulated by MG-altered transcriptional factors - including NRSF, CTCF and ZNF263 - selected as potential hubs of MG-triggered transcriptional disruption. Finally, MiRNome analysis revealed ~70 DE miRNAs following MGO treatment. Interestingly, most of the DE genes involved in cell cycle, insulin, PI3K-Akt, FOXO, P53 (~60%) are predicted targets of DE miRNAs that potentially represent putative mediators of MGO-induced transcriptional perturbation. Experimental assays to address whether selected protein-coding genes and miRNAs mediate MGO-induced glucotoxicity in hRECs are in progress and whether their modulation can retain the pathogenic effects of MG in the cells of the retinal microcirculation, thus offering new tools for a more targeted and effective therapy of DR.
References:


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