



Quantitative analysis of N-linked glycoproteins in tear fluid of climatic droplet keratopathy by glycopeptide capture and iTRAQ

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Resumen

Glycoproteins are potentially important biomarkers of disease and therapeutic targets. In particular, the N-linked glycoproteins are a focus of interest as they can be found in the extracellular environment and body fluids. In this study, we have sampled the tears, the extracellular fluid of the epithelial cells covering the surface of the eye, of patients with climatic droplet keratopathy (CDK) using tears of unaffected normal patients for comparison. Prefractionation of the tear sample used a hydrazide-resin capture method, and the previously N-glycosylated peptides were then subjected to two-dimensional nano-LC-nano-ESI-MS/MS analysis to obtain peptide fragmentation patterns for identification through protein database searches. We have identified a total of 43 unique N-glycoproteins, 19 of which have not previously been reported in tear fluid. In addition, we have quantitatively compared N-glycoprotein profiles in tear fluid of patients with CDK to tears of nondiseased controls using glycopeptide capture, iTRAQ labeling and 2D nano-LC-nano-ESI-MS/MS analysis. In tears of CDK patients, increased levels of four N-glycosylated proteins including haptoglobin (at sites N207, N211 and N241), polymeric immunoglobulin receptor (at sites N83, N90, N135, N186, N421, and N469), immunoglobulin J chain (at site N49) and an uncharacterized protein DKFZp686M08189 (at site N470), as well as a decrease in the N-glycosylation level of one N-glycosylated protein, lacritin (at site N119) were observed. However, the overall levels of these five proteins showed no appreciable changes between control and CDK samples. The findings could be clinically significant in terms of disease etiology and biomarkers.

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