Proteomic Analysis of Human Vitreous Humor

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Abstract.

Aim

The aim of this study was to investigate the proteome of the normal human vitreous humor.

Material and Methods

Ten samples of vitreous humor were collected from patients undergoing vitrectomy surgery for macular hole and cataract surgery with anterior vitrectomy and posterior capsulotomy. These samples were the closest we could get to normal samples as all of them had no clinically obvious vitreous abnormality. □The vitreous samples were pooled and concentrated by passing it through 3 kDa filter. Protein estimation was carried out for pooled concentrated sample using Lowry's assay. 1 mg of pooled vitreous sample was subjected to Agilent Multiple Affinity Removal System 14 (MARS 14) to deplete it of high abundant proteins. The depleted vitreous samples from four depletion runs were pooled and desalted with C18 macro-spin column. The pooled samples were divided into three aliquots and used for in-gel digestion, in-solution digestion followed by Strong Cation Exchange (SCX) chromatography and OFFGEL fractionation. MS/MS analysis was carried out for in-gel, SCX and OFFGEL fractions and the data were searched using three different search algorithms – Mascot, SEQUEST and *X*! Tandem against the NCBI RefSeq human protein database. Sub cellular localization, molecular function and biological process of identified proteins were analyzed using Human Protein Reference Database (HPRD: http://www.hprd.org) and Human Proteinpedia.

Results

We were able to identify 1051 proteins in the human vitreous humor. Of these 782 proteins were uniquely identified in this study. Majority of the proteins were localized to the extra cellular space (26%), cytoplasm (20%) or plasma membrane (16%). Classification based on molecular function showed that a significant number of proteins were involved in binding activity such as calcium ion binding, receptor binding and complement binding

(26%), catalytic activity (27%), structural molecule activity (11%) and transporter activity (7%). Further categorization for biological processes showed 34% of proteins participate in metabolism, 20% in signal transduction, 13% in cell growth and 7% in immune responses.

Conclusion

Many of the pathologic changes occurring in the retina are likely to be reflected in the vitreous because of its close proximity to the retina and also because of the breakdown of the blood retinal barrier. Hence, a proteomic study of the vitreous in related retinal diseases such as diabetic retinopathy, retinal detachment and central or branch retinal vein occlusions would provide valuable insights about the pathophysiology of these diseases.[1,2,3] The information from this study could serve as a baseline for future studies especially those aimed at identifying biomarkers for retinal disorders.

Keywords: Retina, SCX chromatography, Proteome Discoverer, Body fluid proteomics, Protein biomarkers, Secreted proteins.

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