

Quantitative Mass Spectrometric Analysis of Proteins in Bruch's Membrane from Normal and AMD Donor Eyes

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Abstract

Purpose: To identify and quantify proteome changes in Bruch's membrane from donors with age-related macular degeneration (AMD).

Methods: Bruch's membrane was isolated from human normal and AMD donor eyes and 6 mm diameter trephined samples prepared from the central region. Detergent solubilized protein was quantified then digested with trypsin. Peptides were labeled with diagnostic, amine specific, isobaric ITRAQ tags, then the AMD and normal preparations were mixed together. The peptide mixtures were separated by strong cation exchange chromatography, fractions collected and analyzed by LC-MS/MS. Protein identifications utilized ProteinLynx™ Global Server and Mascot search engines and the Swiss-Prot and NCBI protein sequence databases. A macro written in visual basic was used for relative quantification.

Results: To date, the soluble proteome of Bruch's membrane from four AMD donors has been individually compared with that from five pooled, age-matched normal donor samples. Totally, 487 proteins were identified and quantified using ITRAQ technology.

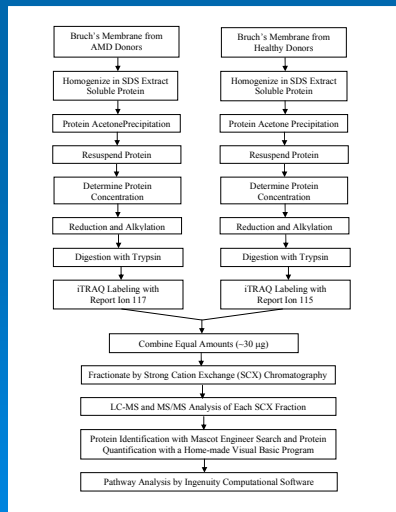
Conclusions: Quantitative mass spectrometric analysis of the proteome from Bruch's membrane offers promise for identifying disease-associated changes in the protein composition. Current observations are consistent with our previous qualitative analyses of drusen from AMD and normal donors.

Introduction

Age-related macular degeneration (AMD) is a major cause of visual impairment in the western countries. While both genetic and environmental factors impact AMD pathology, the etiology of AMD is unknown. Bruch's membrane is an extracellular matrix between the retinal pigment epithelium and choriocapillaris, influencing the hydraulic conductivity of waste products out of and nutrients into the neural retina. Age-related changes in Bruch's membrane lead to thickening and loss of permeability, and could be involved in the pathology of AMD. Therefore, the proteomes of Bruch's membranes from AMD donors were compared with those from health donors by combination of isotope labeling and mass spectrometry.

Methods

Bruch's membrane samples from four AMD donors were individually compared by quantitative mass spectrometry with a normal sample pooled from five healthy donors as outline in Scheme 1.



Scheme 1. Overall Methods

Results

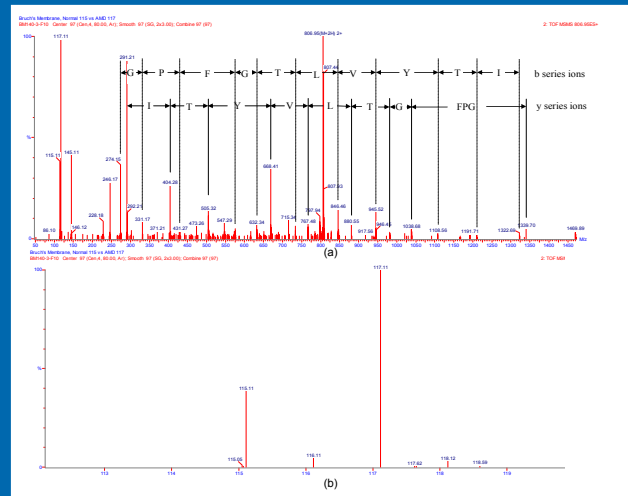


Figure 1 (a) A representative MS/MS spectrum of an ITRAQ labeled tryptic peptide; (b) Zoomed-in MS/MS spectrum of this peptide. This peptide was derived from metalloproteinase inhibitor 3. Its sequence is EGPFGTLVYTIK and its N-terminal and lysine were labeled with ITRAQ, m/z 117 represents the report ion of peptide from AMD and m/z 115 represents the report ion of peptide from Normal. The peak intensity ratio of 117-to-115 represents the amount ratio of peptide from AMD to peptide from Normal.

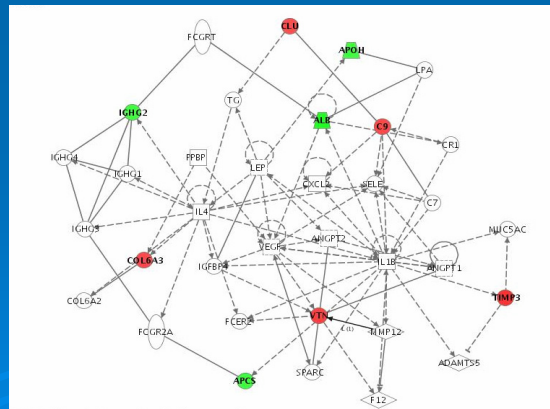


Figure 2 Ingenuity pathway network obtained for nine differentially expressed proteins in Bruch's membrane from AMD samples. Because Bruch's membrane is an extracellular matrix, 8 proteins locating in extracellular space and 2 proteins whose locations are unknown (Table 2) from Table 1 were used for overlapping network function analysis. Data were analyzed through Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). A network was obtained for nine out of ten uploaded proteins. The score for this network is 22 (Score >2 indicate a significant network). Proteins with colored background were detected in Bruch's membrane from AMD donors. ALB, C9, CLU, TIMP3 and VTN are involved in cell death, growth and proliferation. COL6A3 is also involved in cell growth and proliferation. APOH, C9, CLU and VTN are associated with tissue development. APCS and CLU are involved in inflammatory disease.

Table 1
Quantitative Proteome Analysis of Bruch's Membrane from AMD and Normal Donors

| Accession Code | Protein Name | Average Ratio AMD/Normal | Standard Deviation | |
|----------------|--------------|---|--------------------|------|
| 1 | Q14103 | Heterogeneous nuclear ribonucleoprotein D0 | 1.69 | 0.13 |
| 2 | P36625 | Metalloproteinase inhibitor 3 precursor | 1.66 | 0.36 |
| 3 | Q09536 | Synaptic vesicle membrane protein VAT-1 homolog | 1.61 | 0.41 |
| 4 | P04004 | Vitronectin precursor | 1.54 | 0.29 |
| 5 | P60903 | Calpactin-1 light chain | 1.53 | 0.19 |
| 6 | P00352 | Ratinal dehydrogenase 1 | 1.52 | 0.52 |
| 7 | P00265 | Maltase dehydrogenase | 1.51 | 0.65 |
| 8 | P51991 | Heterogeneous nuclear ribonucleoprotein A3 | 1.49 | 0.39 |
| 9 | P62805 | Histone H4 | 1.47 | 0.28 |
| 10 | Q0H429 | Adipocyte plasma membrane-associated protein | 1.47 | 0.46 |
| 11 | P17066 | Heat shock 70 kDa protein 6 | 1.47 | 0.33 |
| 12 | P02748 | Complement component C9 precursor | 1.47 | 0.53 |
| 13 | P39319 | 40S ribosomal protein S19 | 1.46 | 0.49 |
| 14 | P22628 | Heterogeneous nuclear ribonucleoproteins A2/B1 | 1.46 | 0.57 |
| 15 | P12111 | Collagen alpha-3(VI) chain precursor | 1.46 | 0.28 |
| 16 | P22743 | Carbonic anhydrase 4 precursor | 1.45 | 0.17 |
| 17 | P04792 | Heat shock protein beta-1 | 1.45 | 0.28 |
| 18 | P10909 | Clusterin precursor | 1.42 | 0.29 |
| 19 | Q14956 | Transmembrane glycoprotein NMB precursor | 1.42 | 0.47 |
| 20 | P02249 | 40S ribosomal protein S16 | 1.42 | 0.23 |
| 21 | P13073 | Cytochrome c oxidase subunit 4 isoform 1 | 1.41 | 0.23 |
| 22 | P68104 | Elongation factor 1-alpha 1 | 1.41 | 0.20 |
| 23 | P00403 | Cytochrome c oxidase subunit 2 | 1.39 | 0.31 |
| 24 | Q01995 | Transferrin | 1.36 | 0.20 |
| 25 | Q96VC6 | Transmembrane protein 109 precursor | 1.36 | 0.24 |
| 26 | P05565 | Integrin beta-1 precursor | 1.35 | 0.34 |
| 27 | Q75767 | Gore histone maturation-H2A.1 | 1.35 | 0.45 |
| 28 | P45880 | Voltage-dependent anion-selective channel protein 2 | 1.35 | 0.21 |
| 29 | Q14773 | Tripeptidyl-peptidase 1 precursor | 1.34 | 0.35 |
| 30 | P05072 | Transitional endoplasmic reticulum ATPase | 1.28 | 0.21 |
| 31 | P04859 | Gustainase nucleotide-binding protein G(i), alpha-2 subunit | 1.32 | 0.25 |
| 32 | P18206 | Vinculin | 1.31 | 0.18 |
| 33 | P02245 | Lamin-A/C | 1.31 | 0.34 |
| 34 | Q02511 | Alpha crystallin B chain | 1.30 | 0.44 |
| 35 | P63104 | 14-3-3 protein zeta/delta | 1.30 | 0.54 |
| 36 | Q14697 | Neutral alpha-glucosidase AB precursor | 1.30 | 0.29 |
| 37 | P05897 | 60S acidic ribosomal protein P2 | 1.30 | 0.57 |
| 38 | P21980 | Protein-glutamine gamma-glutamyltransferase 2 | 1.30 | 0.23 |
| 39 | P00387 | NADH-cytochrome b5 reductase | 1.30 | 0.24 |
| 40 | P01859 | Ig gamma-2 chain C region | 0.70 | 0.22 |
| 41 | P02749 | Beta-2-glycoprotein 1 precursor | 0.69 | 0.14 |
| 42 | P02743 | Serum amyloid P-component precursor | 0.69 | 0.16 |
| 43 | P02768 | Serum albumin precursor | 0.69 | 0.19 |

Select proteins are listed that were detected in at least 3 AMD samples. The AMD/normal ratios \pm 1.3 represent proteins elevated in AMD tissue; ratios \leq 0.7 represent decreased abundance.

Table 2
Proteins used in the pathway analysis

| Accession Code | Gene | Protein Name | Location | Average Ratio AMD/Normal | |
|----------------|--------|--------------|---|--------------------------|-------------|
| 1 | P36625 | TIMP3 | Metalloproteinase inhibitor 3 precursor | Extracellular Space | 1.66 ± 0.36 |
| 2 | P04004 | VTN | Vitronectin precursor | Extracellular Space | 1.54 ± 0.29 |
| 3 | P02748 | C9 | Complement component C9 precursor | Extracellular Space | 1.47 ± 0.53 |
| 4 | P17066 | HSPA6 | Heat shock 70kDa protein 6 | Unknown | 1.47 ± 0.33 |
| 5 | P12111 | COL6A3 | Collagen alpha-3(VI) chain precursor | Extracellular Space | 1.46 ± 0.28 |
| 6 | P10909 | CLU | Clusterin precursor | Extracellular Space | 1.42 ± 0.29 |
| 7 | P01859 | IGHG2 | Ig gamma-2 chain C region | Unknown | 0.70 ± 0.22 |
| 8 | P02743 | APCS | Serum amyloid P-component precursor | Extracellular Space | 0.69 ± 0.16 |
| 9 | P02749 | APOH | Beta-2-glycoprotein 1 precursor | Extracellular Space | 0.69 ± 0.14 |
| 10 | P02768 | ALB | Serum albumin precursor | Extracellular Space | 0.59 ± 0.19 |

Select proteins were quantified in four AMD Bruch's membrane samples.

Conclusion

To date, 487 proteins were identified and quantified from comparative analyses of four AMD Bruch's membrane samples. Among these 487 proteins, 110 proteins were quantified with 4 samples, 80 proteins were quantified with 3 samples, 89 proteins were quantified with 2 samples, and ~43% from only 1 sample. Additional analyses are in progress to determine statistically significant compositional differences between AMD and normal Bruch's membrane.

Some proteins such as tissue metalloproteinase inhibitor, clusterin, heat shock protein beta-1 and vitronectin were elevated in Bruch's membrane from AMD samples. These results are consistent with our previous qualitative analyses of drusen from AMD and normal donors (2002 Proc. Natl. Acad. Sci. USA 99, 14682).

Pathway analysis may provide insights regarding direct or indirect protein interactions and the mechanisms contributing to Bruch's membrane thickening and loss of permeability with age.

Reference

Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG, Hollyfield JG. Proc Natl Acad Sci U S A. 2002; 99:14682-7.