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Mass spectrometry-based approaches to quantify retinal proteome changes in spontaneous auto-immune uveitis

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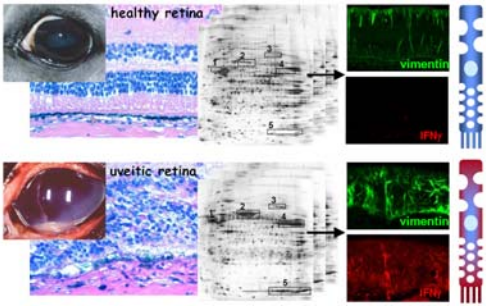
Background

Spontaneous equine recurrent uveitis (ERU) is an incurable autoimmune disease affecting the eye. Although retinal-autoantigen specific T-helper 1 cells have been demonstrated to trigger disease progression and relapses, the molecular processes leading to retinal degeneration and consequent blindness remain unknown. We have previously applied conventional 2D electrophoretic separations in combination with MALDI mass spectrometry to study the disease related changes in the retinal proteome of ERU-diseased horses compared to healthy controls (Hauck et al., J. Prot. Res., 2007) and identified and validated 12 differentially expressed proteins, indicating the essential role of retinal Mueller glial cells for the onset of disease.



Aim

We aimed at increasing overall detection sensitivity and at including the detection of differentially expressed membrane proteins by alternative analytical strategies: separation of membrane proteins from soluble proteins and mass spectrometry-based quantification techniques.

Methods

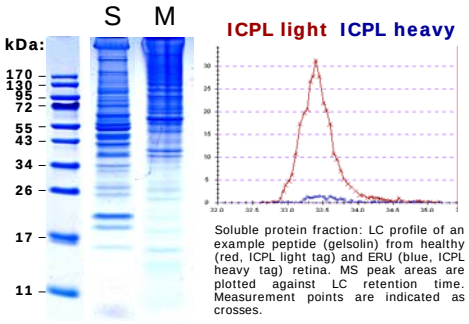
Retinal tissue from healthy and spontaneous uveitis cases (horses) were separated into soluble and membrane proteins by tissue disruption (IKA blender) with increasing buffer stringency (adapted from Nagaraj et al., 2008). Specifically, 40mg of each tissue type were extracted first with buffer containing 2M NaCl followed by two extractions with carbonate buffer and one extraction by 4M urea containing buffer. Non-soluble membrane proteins were collected after eauch extraction step by centrifugation, soluble proteins were combined and dialysed. After dialysis, soluble proteins were precipitated with acetone, resolved and labelled with ICPL followed by 1D PAGE. Membrane fractions remained unlabelled and were directly digested with trypsin and applied to LC-MSMS measurement (OrbiTrap).

Quantification of the ICPL labelled soluble proteins was performed by using the **MSQuant software** with 2 or more ICPL lebelled peptides. Membrane proteins were quantified with the **Progenesis software** (Nonlinear) on the de-isotoped cumulative TIC intensities in the aligned LC chromatograms. After cross sample normalisation, p values were calculated (ANOVA) for every feature (=peptide) and FDR was set <2% (q value).

Results

1. Separation of proteins:

Separation of membrane proteins from soluble proteins by the applied extraction method resulted in distinct patterns as observed by 1D PAGE. Mass spectrometric identifications from the separated cellular compartmets confirmed good separations. In the soluble ICPL-labelled samples, solely non-membrane proteins were identified and among the diffentially detected proteins from the membrane fraction a large proportion (appr. 70%) were either membrane or membrane-associated proteins.



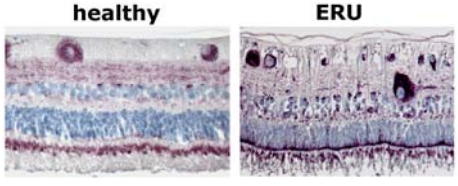
Soluble protein fraction: LC profile of an example peptide (gelsolin) from healthy (red, ICPL light tag) and ERU (blue, ICPL heavy tag) retina. MS peak areas are plotted against LC retention time. Measurement points are indicated as crosses.

3. Label-free quantification of membrane proteins:

Membrane proteins from healthy and ERU retinal tissue were directly digested and applied to LC-MSMS analysis. After alignment of the chromatograms in the Progenesis software, 2D profiles were compared and differentially abundant peptides were selected: total peptide ions detected: 26041; 11640 have a p <0.05 (Anova) and 8943 (77%) are with Power >0.8 with an FDR of <2% (q value). Differential peptides for which no MSMS spectra had been recorded, were accumulated as inclusion list for an additional measurement on the OrbiTrap. All MSMS spectra for differential peptides were then searched against UNIref100 database (mamalian sequences) and identifications were accepted for proteins covered by ≥2 different peptides, each with a significant score (≥30).

With this approach we identified a total of **577** differentially abundant proteins, of which 293 proteins are decreased in ERU and 284 are increased in ERU. Among the incrased proteins were many MHC class I and II membrane proteins as well as Antigen peptide transporter 1 (TAP1; 1200fold increase), a 10 transmembrane domain containing protein essential for peptide transport across ER membrane and assembly of MHC-peptide complexes in immune response.

Combining the two new experimental approaches (soluble and membrane proteins) allows us to suggest additional **819** proteins potentially differentially expressed in the context of uveitis, which are now validated by immunohistochemistry.



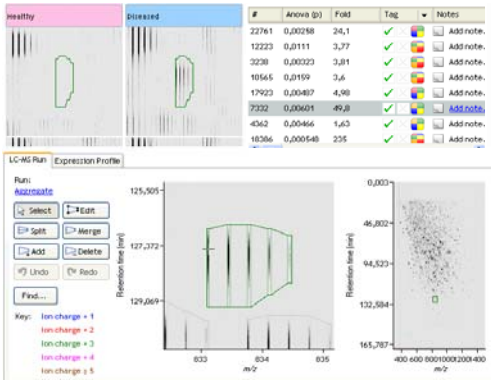
2. Quantification of soluble Proteins with ICPL labeling:

Soluble proteins from healthy and ERU retinal tissue were labelled with ICPL light and heavy tag, respectively. Labelled samples were combined, loaded onto 1D-SDS-PAGE, separated and cut into 8 bands. The gelbands were subjected to in-gel tryptic digestion and identified by LC-MSMS and database search (Mascot). Mascot files from 8 bands were combinded and imported into MSQuant software for quantification.

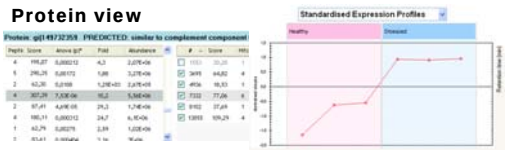
After filtering for proteins identified with at least 2 ICPL labelled peptides for quantification and a total ion score of >60, we could quantify a total of **254** proteins. 179 of them are increased in ERU ≥2 fold. Among them were all previously identified proteins, such as GFAP (40fold), serotransferrin (4fold), alpha-crystallin (4.5fold), apolipoprotein A1 (6.8fold), vimentin (12.3fold), hemoglobin (37fold) and albumin (8.7fold). We also could confirm the stable expression of S-antigen, one of the major targets of autoantibodies (Deeg et al., Mol. Immunol. 2007).

Label free quantification:

Peptide view



Protein view



Conclusion

By applying these quantitative, mass spectrometry-based methods, we could confirm all previously identified differentially expressed proteins (12). Additionally we could identify more than 463 proteins upregulated in ERU and around 368 proteins downregulated in ERU compared to healthy condition. More than 30% of the identified proteins were membrane or membrane-associated and some could readily be associated to the immunologic process underlying the disease. The majority of differentially expressed proteins were identified by label free analysis and this approach thus proofed very efficient.

References

Deeg et al., Mol. Immunol., 2007
Hauck et al., J. Prot Res., 2007
Nagaraj et al., J. Prot. Res., 2008