HelmholtzZentrum münchen
German Research Center for Environmental Health

Scientific Highlight October 2010

[X] co-ordinated with the Director of the Institute / Head of Department

Institute / Independent Department / Clinical Co-operation Group / Junior Research Group:
Department of Protein Science

PSP-Element:
G-505700-001, A-630070-001

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Title of the Highlight:
Deciphering membrane-associated molecular processes in target tissue of autoimmune uveitis by label-free quantitative mass spectrometry

Keywords:
Label-free quantitative proteomics, autoimmune disease, inflammation, membrane tissue fraction, retina, LC-MSMS,

Central statement of the Highlight in one sentence:
Quantitative label-free proteomics of disease tissue reveals novel pathways involved in autoimmune processes

Text of the Highlight:
Autoimmune uveitis is a blinding disease presenting with autoantibodies against eye-specific proteins as well as autoagressive T-cells invading and attacking the immune-privileged target tissue retina. The molecular events enabling T-cells to invade and attack the tissue have remained elusive. Disease associated changes in membrane protein expression patterns are likely to mark etiologically relevant initiating events of early onset of disease. Since disease progression is accompanied with a break-down of the blood-retinal barrier, serum-derived proteins mask the potential target tissue-related changes.

To overcome these limitations we established a novel membrane-centered label-free quantitative proteomics method to directly compare protein patterns from retinas derived from the only available spontaneous animal model for this autoimmune disease (equine recurrent uveitis, ERU) to healthy control samples. Pathway enrichment analyses of disease-specific patterns indicated an increase in proteins related to antigen processing and presentation, TNF receptor signaling, integrin cell surface interactions and focal adhesions. Additionally, loss of retina-specific proteins reflecting decrease of vision was observed as well as

an increase in Müller glial cell-specific proteins indicating glial reactivity. Selected protein candidates from the identified pathways were validated in situ and revealed a significant increase of these proteins at the level of the outer limiting membrane (OLM) which is part of the outer blood-retinal barrier. Taken together, the membrane enrichment in combination with LC-MSMS-based label-free quantification resulted in detection of novel molecular pathways related to ERU and revealed the OLM as major target structure for autoimmune processes.

The approach taken can generally advance molecular understanding of autoimmune diseases (e.g. Multiple Sclerosis, Diabetes Type 1, Idiopathic Dilated Cardiomyopathy). It also provides cues to understand pathological changes at the target site of immune attack and help to generate a rationale towards early diagnosis and therapy development, the final goal of our studies.

Publication:
stefanie M. Hauck, Johannes Dietter, Roxane L. Kramer, Florian Hofmaier, Johanna K. Zipplies, Barbara Amann, Annette Feuchtinger, Cornelia A. Deeg and Marius Ueffing “Deciphering membrane-associated molecular processes in target tissue of autoimmune uveitis by label-free quantitative mass spectrometry” MCP, 2010 Jul 4. [Epub ahead of print]

Taking account of the HMGU mission:
The development of a new quantitative label-free proteomics method to analyse tissue from spontaneous diseases in combination with bioinfomatic pathway analyses paves the road to analyse multi-factorial diseases on the proteome level and thus enables molecular understanding of underlying patho-mechanisms
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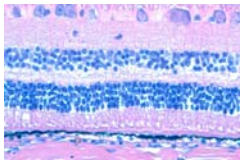
The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:
Institute of Pathology (G-500300-001)

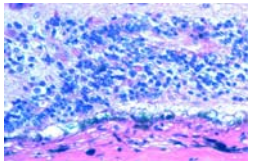
Deciphering membrane-associated molecular processes in target tissue of autoimmune uveitis by label-free quantitative mass spectrometry

PROT

Strategy to identify membrane-associated proteomic changes in autoimmune uveitis

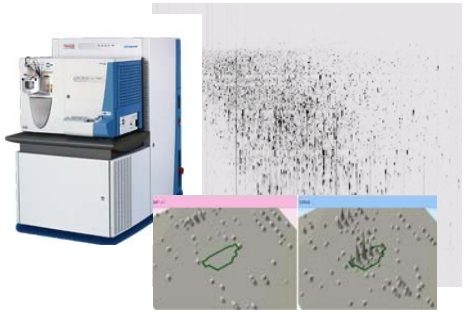


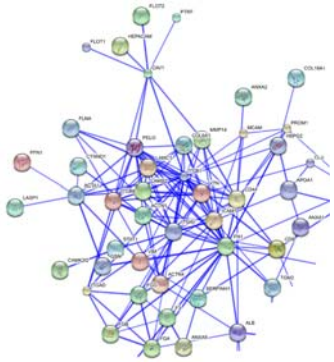
healthy retina → membrane-enriched sub-fractions → LC-MSMS



ERU retina → membrane-enriched sub-fractions → LC-MSMS

LC-MSMS → label-free quantification (Progenesis) → network/ pathway analyses





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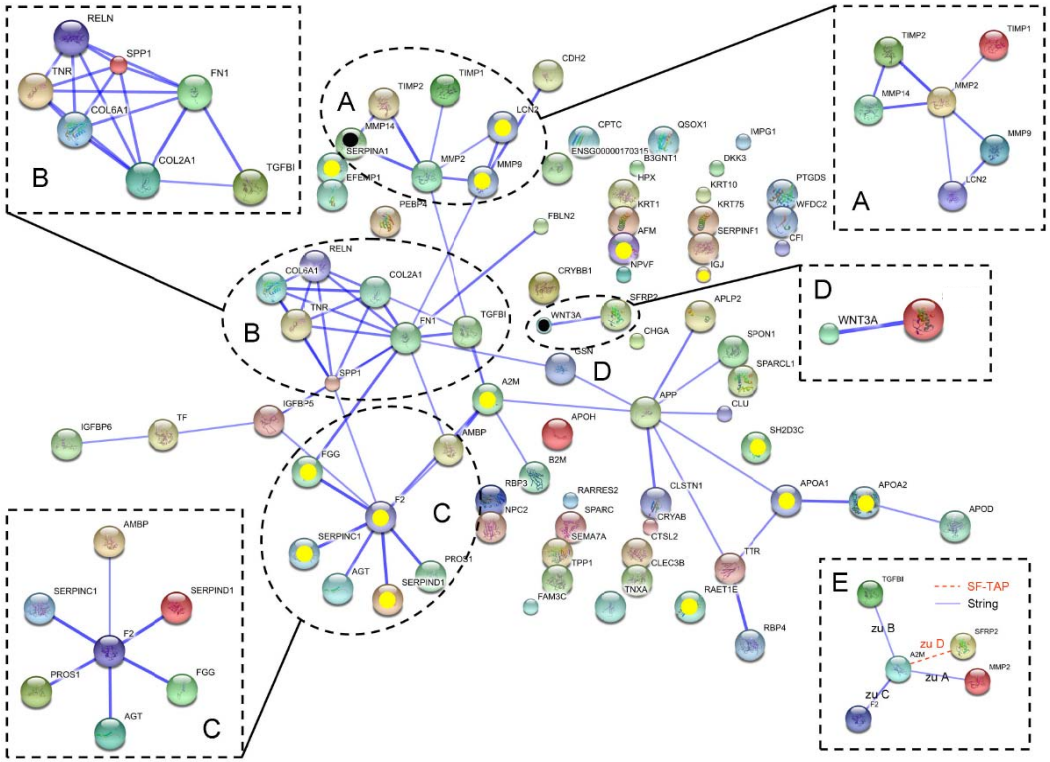
Interaction network analyses of differentially exressed proteins:



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Validation in situ: Quantitative Image Analyses (cooperation with PATH)