Session 22: Proteomics of Ageing and Diseases

Lectures

L22.1

Large scale proteomics for studying physiology and disease

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Information on concentrations of individual proteins is a prerequisite for understanding biological systems. In classical biochemistry this information is commonly achieved by immunoassays or staining techniques. Alternatively, abundance of proteins or enzymes can be assessed indirectly by studying ligand binding or measuring rates of substrate conversion by enzymes. Proteomics – with its fundamental mission to procure global information on individual proteins encompassed in a system – has the power to replace these techniques. By quantifying as many proteins as possible, functions in biological and physiological systems can more readily be elucidated; for instance by pathway analysis. The Total Protein Approach (TPA) and its relative the Proteomic Ruler offer quantification of thousands of proteins across datasets, providing protein concentrations and protein copy numbers. Furthermore, combining both latter measures allows discrimination between global and organelle specific alterations in cells. Since TPA and Proteomic Ruler quantifications are label free and standard free methods they can be applied to any comprehensive proteomic dataset.

L22.2

Proteomics of plasma fibrin clot in antiphospholipid syndrome

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Objective: The prothrombotic fibrin clot phenotype has been reported in patients with thrombotic antiphospholipid syndrome (APS) and venous thromboembolism (VTE). Protein composition of plasma fibrin clots in APS has not been studied.

Approach and Results: We evaluated 23 patients with thrombotic APS, 19 with VTE alone, and 20 well-matched controls. A proteomic analysis of fibrin clots generated from citrated plasma was based on liquid chromatography-mass spectrometry. Plasma levels of thrombospondin-1 (TSP1), apolipoprotein(a), A-I, and B-100, complement components (C)3a, C5b-C9, histidine-rich glycoprotein (HRG), and prothrombin were evaluated using immunoenzymatic tests. In plasma fibrin clots of APS patients, compared with VTE subjects and controls, we identified decreased amounts of (pro)thrombin (1.8-2.0-fold difference [FD]), antithrombin-III (1.5-FD), apolipoprotein A-I (1.5-FD), and HRG (1.4-FD) with no differences in plasma levels of antithrombin, prothrombin, along with lower plasma HRG and apolipoprotein AI. In APS patients, plasma HRG positively correlated with amounts of clot-bound HRG, while apolipoprotein A-I was inversely associated with clot-bound levels of this protein. The most predominant proteins within the clots of APS patients compared to VTE subjects and controls were bone marrow proteoglycan (23-39-FD), C5-C9 (1.6-5.5-FD), immunoglobulins (1.6-4.8-FD), apolipoprotein B-100 (2.0-FD), platelet-derived proteins (5.6-6.3-FD), and TSP1 (3.3-FD). Plasma C5b-C9 positively correlated with clot-bound C5b-C9 amounts.

Conclusion: Our study is the first to demonstrate differences in the protein composition of fibrin clots generated from plasma of thrombotic APS patients versus those with VTE alone. Our clot proteomic approach could be useful to identify plasma proteins with potential clinical utility as biomarkers in thrombotic diseases.

L22.3

Aging-associated changes in hippocampal glycogen metabolism. Evidence for and against astrocyte-neuron lactate shuttle

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Lactate derived from astrocytic glycogen has been shown to support memory formation in hippocampi of young animals, inhibiting it in old animals. Here we show, using quantitative mass spectrometry-based proteomics, immunofluorescence, and qPCR that aging is associated with an increase of glycogen metabolism enzymes concentration and shift in their localization from astrocytes to neurons. These changes are accompanied with reorganization of hippocampal energy metabolism which is manifested by elevated capacity of aging neurons to oxidize glucose in glycolysis and mitochondria, and decreased ability for fatty acids utilization. Our observations suggest that astrocyte-to-neuron lactate shuttle may operate in young hippocampi, however, during aging neurons become independent on astrocytic lactate and the metabolic crosstalk between the brain's cells is disrupted.

Posters

P22.1

Missense mutation of FBP2 gene causes reversible early childhood leukodystrophy

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A novel type of leukodystrophy causing severe symptoms in early childhood was identified. Contrary to most other leukodystrophies, patients recovered well in later life. The disease was linked with a heterozygous missense p.V115M mutation in the FBP2 gene. Analysis of recombinant mutated FBP2 protein revealed lower enzymatic activity and thermodynamic stability compared to the wild type. Investigation of fibroblast cultures from patients and healthy kin show abnormal cellular localization and increased ubiquitination of the FBP2 protein. This suggests that the p.V115M mutation prevents proper folding of FBP2 leading to its degradation and/or general unfolded protein response. Certainly, FBP2 plays a crucial role in the development of brain white matter. However, the mechanism linking p.V115M mutation effects to the demyelination observed in patients remains to be discovered. The cause of reversal of the symptoms remains unknown as well. Researching this topic further can help bring new treatments not only for this particular type of leukoencelopathy but for other similar diseases as well, in addition to giving new insight on post-natal development of the human brain.

P22.2

MicroLC-SWATH-MS proteome profiling of fresh-frozen normal and cancerous human breast tissue

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Quantitative proteome profiling of fresh frozen breast tissue and tumor samples may improve our understanding of the molecular processes leading to the breast cancer development and progression, contributing to further progress in the prevention, diagnosis and treatment of the disease. Liquid chromatography (LC)-tandem mass spectrometry (MS/MS) technique in data-independent SWATH acquisition mode is a powerful tool for such profiling, because it enables label-free identification and quantification of theoretically all proteins in the sample. In this study we evaluated applicability and effectiveness of microLC separation before SWATH-MS analysis, as microLC is still more sensitive than analytical LC, but more robust than nanoLC. This methodology enabled us to detect 761 proteins in all qualitative experiments, which served then as a spectral library for further label-free SWATH quantification of separate breast tumor and adjacent normal breast tissue samples of 8 patients. 299 of those proteins were successfully quantified. Levels of 188 of them varied significantly ($p<0.05$) between normal breast tissue and breast tumor samples. 14 proteins were down-regulated, while 31 were upreugulated at least two fold in breast tumors comparing to normal breast tissues. The presented study showed that the microLC-SWATH-MS technique can be efficiently applied for proteomic profiling of normal human breast tissue and tumor samples.

P22.3

Proteomic analysis red blood cell membrane from patients with polycythemia vera

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Red blood cells and their membranes are associated with osmotic hemolysis and its reversal. During past decade, red blood cell composition has been extensively studied especially in the term of diseases connected with the regulation of red cell production. But there is a significant need to develop the knowledge about erythrocyte membrane and its function, which can affect the mechanism of a disease. The term "white ghost" describes the pale membrane of a haemoglobin-depleted red cell, and is thus used to study erythrocyte membrane structure and function.

The aim of this study was to conduct a proteomic analysis of red blood cell membrane fraction (white ghosts) from patients who suffer from polycythemia vera (PV) and healthy donors. PV is a heterogenous disease of stem cells with preferential increase in the number of erythrocytes which, through resulting increased viscosity in combination with other pathogenic factors, may lead to thromboebolic complications. As far as the sample preparation is concerned, samples were processed using the multienzyme digestion filter-aided sample preparation (MED-FASP). To analyze samples we used a Q-Exactive mass spectrometer. Protein abundance were computed using the total protein approach (TPA). Our results provided a comprehensive map of proteins which are present in the erythrocyte membrane. Identification of the differences of "ghost" proteome between healthy donors and patients can improve knowledge about the mechanism of polycythemia vera and its diagnosis and therapy.

P22.4

Sex affects homocysteine modification at lysine residue 212 of albumin in mice

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Introduction: Homocysteine (Hcy) modification of protein lysine residues (KHcy) is linked to heart and brain diseases. However, factors affecting KHcy modifications are not fully understood.
Objectives: Our objective was to examine effects of sex, age, and cystathionine β-synthase (*Cbs*) genotype on K525Hcy and K212Hcy modifications in mouse albumin.
Methods: We developed a LC/MS targeted assay, based on MRM, for quantification of K525Hcy and K212Hcy sites in serum albumin. We studied 1 to 9-months-old *Tg-I278T* $Cbs^{-/-}$ mice (tHcy 272±50 μM) and their *Tg-I278T* $Cbs^{+/-}$ siblings (tHcy 5.0±2.6 μM).
Results: Female (n=20) and male (n=13) $Cbs^{-/-}$ mice had significantly elevated albumin K525Hcy and K212Hcy modifications relative to their $Cbs^{+/-}$ female (n=19) and male (n=17) siblings. Age and tHcy explained 1.8-4.6% and 3.8-7.5% of the variance in K525Hcy and K212Hcy, respectively. Male mice had more K212Hcy in albumin than females ($Cbs^{-/-}$ mice: 5.8±4.2 *vs.* 3.2±1.4 units, P=0.023; $Cbs^{+/-}$ mice: 2.7±0.8 *vs.* 1.9±1.1 units, P=0.008). In contrast, albumin K525Hcy level was similar in males and females, both in $Cbs^{-/-}$ (1.6±1.0 *vs.* 1.4±0.7 units, P=0.54) and $Cbs^{+/-}$ (0.92±0.41 *vs.* 0.87±0.50 units, P=0.514) mice.
Conclusion: These results suggest that the sex-specific K212Hcy modification in albumin plays an important biological function in mice. Other factors that affect KHcy modifications remain to be identified.

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P22.5

Methionine-induced hyperhomocysteinemia causes changes in the mouse kidney proteome associated with blood coagulation

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Homocysteine (Hcy) arises from the metabolism of methionine (Met). Inordinate consumption of Met causes hyperhomocysteinemia (HHcy), which is linked to pathologies in the cardiovascular system. However, underlying mechanisms are not fully understood.
We hypothesize that HHcy induces changes in gene expression that impair kidney homeostasis, which in turn can lead to increased blood coagulation and thrombotic complications.
We induced dietary HHcy by providing 3-month old C57BL/6J mice (n=8) with 1% Met in drinking water for 3-months. Control mice (n=8) received a plain water. Kidney proteomes were analyzed using label-free relative quantitative mass spectrometry. Proteins identified by at least 2 peptides and p values <0.05 for Met vs. control mice were considered as differentiating. Bioinformatic analyses were carried out using DAVID resources.
We identified 36 kidney proteins with expression significantly altered by HHcy: 19 up- (e.g., Ctsa, Acat1) and 17 down-regulated (e.g., Fga, Ces1c). One of the KEGG pathways overrepresented in kidneys of HHcy mice is complement and coagulation cascades (12,7-fold). The GO biological processes analysis revealed that the affected proteins participate in fibrin clot formation and fibrynolysis (e.g.,Fga, Fgb, Fgg) .
These findings show that Met-induced HHcy dysregulates kidney proteostasis and induces pro-thrombotic changes that are associated with cardiovascular disease.

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P22.6

LC-MS/MS analysis of the monocytes CD14$^+$ proteome in atherosclerosis related and non-related to chronic kidney disease

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The major cause of mortality in patients with chronic kidney disease (CKD) is atherosclerosis related to traditional and non-traditional risk factors. However, the molecular mechanisms that promote cardiovascular disease (CVD) in patients with CKD remain poorly understood. Atherosclerosis is a multifaceted, progressive and inflammatory disease. Monocytes and other leukocytes play important role in the development of atherogenesis. During the early stages of atherosclerosis, blood monocytes are recruited to the intima and subintima and differentiate into foam cells to form early plaques. Additionally, human monocytes produce pro- and anti-inflammatory cytokines which are responsible for subsequent leukocyte trafficking and migration related to atherosclerosis. The accumulation of inflammatory cells and extracellular lipids leads to formation of mature plaques and to the thickening of the vessels.
Mass spectrometry-based proteomic analysis is excellent, powerful tool for tracking molecular changes during progression of CKD and CVD. In this study we investigated the alterations in protein accumulation of CD14$^+$ cells in patients with CKD and classical CVD. Cells were collected using immunomagnetic techniques from patients in various stages of CKD, patients with CVD but without symptoms of kidney dysfunction and healthy volunteers (HVs). Obtained samples were analyzed by a label-free proteomic approach. All proteomic data were subjected to bioinformatic analysis for identification of specific for CKD and CVD pathways.
Mass spectrometric analysis of monocytes showed that differentially expressed proteins between CKD and CVD patients are involved in various physiological pathways among other RNA metabolic process, RNA splicing and RNA/DNA binding. Also proteins involved in cell adhesion and leukocyte transendothelial migration were disturbed. In particular, abundances of histone H1, H2A, H3 and H4 were significantly decreased only in CVD. On the other hand level of vinculin, talin-1, zyxin and alpha actinin-1, protein related to cell adhesion, was evidently reduced in advanced stage of CKD compared to classical CVD. These results strongly indicate on the differences between molecular mechanism of atherosclerosis related and non-related to CKD. Further research should focus on detailed targeted analysis of proteins selected in this screening study.

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P22.7

Copper binding by human cystatin C fragment. Role of histidine residues

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Non-physiological interactions of proteins or peptides with metal ions are considered to beone mechanism that triggers conformational changes in biomolecules to result in their aggregation inside or outside living cells. This might lead to various disease states, among them neurodegeneration. Human cystatin C (hCC) is one protein that can be studied in this context. Native hCC is present at particularly high concentrations in cerebrospinal fluid where is displays both neuroprotective and neurodegenerative propensities. The impact of metal-induced stress on this protein was not studied before. To check the complexometric propensities of hCC, we synthesized peptides encompassing its C-terminal fragment 86-94 (FHDQPHLKRK). This peptide contains two His residues that might serve as ligands for Cu(II) ion(s). Interestingly, the His residues are separated by Pro that opens the possibility of formation of two metal binding sites. The complexometric properties of the hCC fragment were studied by potentiometric, calorimetric and spectroscopic methods, confirming the presence of two possible metal binding sites with different affinities. The obtained data allowed us to characterize metal binding domains of the studied cystatin C fragment and suggest a model of metal binding.

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P22.8

Human serum albumin and human cystatin C complex studies

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Human serum albumin (HSA) is a protein commonly found in human body fluids. It accounts for over 50% of proteins found in plasma what makes it one of the protein with the highest concentration in the blood. The main function of HSA is the control of oncotic pressure in the body. It also plays an important role in the transport of substances - it binds and transports proteins, steroid hormones, fatty acids, drugs, porphyrins, steroids, amino acids and metal ions. All peptides and proteins that bind to HSA are called albuminom. This naturally occurring subproteome of blood can provide vital information on health status, being a source of biomarker diseases. The removal of albumin and other most abundant proteins is, however, a routine step in the proteomic studies. This allows the detection of proteins occurring at a concentration much lower than the concentration of albumin. The removed albumin containing fraction may, however, also contain diagnostically relevant peptides or proteins. One of the protein which can be found in the albuminome is cysteine protease inhibitor, human cystatin C (hCC). In our work we present the characteristics of the HSA-hCC complex and identification of the binding sites on cystatin C sequence. First results revealed that one albumin molecule is able to binds more than one cystatin C molecule.

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P22.9

Proteomic analyses reveal homocysteine modification of protein lysine residues, deregulated protein folding, oxidative stress, and apoptotic pathways in the yeast *Saccharomyces cerevisiae* model of hyperhomocysteinemia

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Introduction: Hyperhomocysteinemia (HHcy) is a risk factor for heart and brain diseases. Examination of cellular proteostasis and Hcy modifications of Lys residues (KHcy), could provide mechanistic insights into the involvement of HHcy in these diseases.
Aim: To identify HHcy-responsive proteins by analyzing yeast cellular proteome.
Methods: Cultures of the yeast *S. cerevisiae* BY 4742 (a lysine auxotroph derived from S288C) were treated with Hcy. Proteomes were analyzed using SILAC, iTRAQ, and Q Exactive MS measurements. PANTHER and STRING resources were used for bioinformatic analyses.
Results: SILAC and iTRAQ analyses reveled 38 up- and 32 down-regulated proteins in HHcy yeast. Upregulated proteins are involved in cellular amino acid biosynthetic/metabolic process, vitamin B6 and redox metabolism. Downregulated proteins are involved in protein folding (MSC82, FPR1, HSP60, KAR2), apoptotic signaling (KAR2), oxidative stress response (TRX1,2), S-adenosylmethionine biosynthesis (SAM2), and sulfate assimilation (MET10). In HHcy yeast, we identified 14 KHcy residues in 10 yeast proteins (ENO2, YRO2, GPP1, GPP2, PDC1, DIT1, PDH1, IPP1, PGI1, INO1) involved in glycolysis and biosynthesis of secondary metabolites.
Conclusion: HHcy induces KHcy protein modifications, deregulates protein folding, oxidative stress, and apoptotic pathways, processes that are implicated in the pathology of HHcy in humans and mice.

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P22.10

Glycogen phosphorylase inhibition – a new fountain of youth?

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Inhibition of glycogen degradation in brain of young animals has been shown to block memory formation and disrupt the Long Term Potentiation formation, the LTP. Unexpectedly, inhibition of glycogen phosphorylase (Pyg) activity, an enzyme indispensable for glycogen breakdown, significantly improved the LTP induction in hippocampal brain slices of old animals (in CA1 region, in HFS electrophysiological stimulation paradigm) [1]. Based on this we hypothesised that inhibition of Pyg activity using BAY U6751 (BAY; Pyg low molecular inhibitor) may be used for improvement of age-associated deficits of memory formation. To verify this hypothesis, young (one month, n=9) and old (18-21 month, n=9) mice were treated for two weeks with BAY by daily intraperitoneal injections and various behavioural, immunological and metabolic parameters were monitored and compared to these obtained using a control, BAY-untreated groups of animals. The results revealed that BAY treatment affected several biological parameters both the young and old animals.

First of all, Pyg inhibition in young animals affected neuronal plasticity and behaviour, e.g. the results of taste preference test demonstrated that BAY treatment significant decreased the saccharin consumption index in young animals ($p=0.02$) having no effect on the index in old animals ($p=0.68$). The inhibition of glycogen phosphorolysis also effected the results of the 2-novel object recognition test performed at day 0 , day 7 and day 14, which provides an index of recognition memory dependent of hippocampal formation.

Glycogen phosphorylase is an enzyme of basal energy metabolism which is ubiquitously expressed in all cell of organism thus it might have been expected that its inhibition would disturb several metabolic/biological parameters. Our study demonstrated however, that two-week treatment of animals with BAY had no effect on body mass index, blood glucose level and it even improved the fur condition in old animals. Moreover the rotarod test didn't marked any difference in YOUNG and OLD animals treated with BAY *vs* control, proving the unchanged motor skills and cerebellum depending coordination.

Unexpectedly, we found that Pyg inhibition reduced significantly the total amount of lymphocytes in spleen. The role of Pyg and/or glycogen in the regulation of lymphocytes number is a new discovery and is under study.

References:

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