SESSION XIII

MULTI-OMICS PROSPECTIVES IN PHYSIOLOGY

Friday (September 17, 2021; (9:00 – 11:30)

Chair:

Prof. Maciej Kurpisz
Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

DETAILED SESSION XIII SCHEDULE

Opening lectures (Friday, September 17, 2021; 9:00 – 10:49; *virtual stream B*)

S13.L1 TRANSCRIPTOMIC STUDIES FOR MALE INFERTILITY DIAGNOSIS AND THERAPY MONITORING. **M. Kurpisz[1], A. Malcher[1], N. Rozwadowska[1], P. Jedrzejczak[2]** ([1]Institute of Human Genetics, Polish Academy Sciences, Poznan, Poland, [2]Infertility and Reproductive Endocrinology Clinic of Gynaecological and Obstetric Hospital, University of Medical Sciences, Poznan, Poland).

S13.L2 MULTI-OMIC INSIGHT INTO THE PATHOMECHANISM OF CHRONIC KIDNEY DISEASE-RELATED ATHEROSCLEROSIS **J. Tracz[1], L. Handschuh[1], M. Lalowski[1,2], L. Marczak[1], K. Kostka-Jeziorny[3], B. Perek[3], M. Wanic-Kossowska[3], A. Podkowinska[4], A. Tykarski[3], D. Formanowicz[3], M. Luczak[1]** ([1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland, [2]Department of Biochemistry and Developmental Biology, University of Helsinki, Finland, [3]Poznan University of Medical Sciences, Poznan, Poland, [4]Dialysis Station Dravis sp. z o.o., Poland).

S13.L3 MULTIMODAL ANALYSIS IN THE SEARCH FOR NEW BIOMARKERS OF DIABETIC KIDNEY DISEASE - microRNA AND EXTRACELLULAR VESICLES. **E.L. Stepien** (Department of Medical Physics, Marian Smoluchowski Institute of Physics, Jagiellonian University, Krakow, Poland).

S13.L4 MULTI-OMIC APPROACH FOR PREDICTING RESPONSE TO NEOADJUVANT RADIOTHERAPY OF COLORECTAL CANCER. **A. Wojakowska[1], U. Strybel[1], L. Marczak[1], M. Zeman[2], K. Polanski[3], L. Mielanczyk[4], M. Pietrowska[2]** ([1]Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland, [2]Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland, [3]Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, United Kingdom, [4]Department of Histology and Cell Pathology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland).

Oral presentations (Friday, September 17, 2021; 10:50 – 11:30; *virtual stream B*)

S13.L5 DIETARY SUPPLEMENTATION WITH DIFFERENT SOURCES OF INULIN-TYPE FRUCTANS EVOKES CHANGES IN PROTEOMIC PROFILE OF PORCINE AORTA. **M. Marynowska[1], M. Ozgo[1], A. Herosimczyk, A. Lepczynski, J. Skomial, M. Barszcz, M. Taciak** ([1]Department of Physiology, Cytobiology and Proteomics, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology in Szczecin, Poland, [2]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna, Poland).

S13.L6 PROTEINS OF MARE'S COLOSTRUM ASSOCIATED WITH FOAL AND MAMMARY GLAND GROWTH AND DEVELOPMENT. **W. Medenska, A. Dratwa-Chalupnik, M. Ozgo, A. Cichy** (Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology in Szczecin, Szczecin, Poland).

Session summary

S13.L1

TRANSCRIPTOMIC STUDIES FOR MALE INFERTILITY DIAGNOSIS AND THERAPY MONITORING

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Transcriptomic libraries from specific organs may in future serve for molecular diagnosis, treatment and monitoring of disease more precisely than traditional histopathology. Male infertility seems to be dependent on genomic causes at least in 50% and identification of reason may provide a way for genetic correction of spermatogenesis. In order to obtain testicular library we have analyzed 20 testicular samples with Affymetrix Human Gene 1.0 ST microarrays, sixteen of them were obtained from patients with various types of non-obstructive azoospermia (NOA) syndrome and four were healthy donors with normal spermatogenesis. Six out of NOA syndrome patients were subjected to gonadotropin therapy (hCG+rFSH) and one positive therapy responder was re-analyzed in microarrays before and after successful treatment. Genes analyzed by microarrays were stratified in dendograms and were subsequently validated by qPCR or raw data validated from existing data bases. Then, Class II HLA DQB alleles were determined and sequenced. Thus all patients subjected to experimental therapy undergone histocompatibility genes expression evaluation. Among the 5000 significantly different (than background) gene expression analyzed in infertile vs. healthy individuals (gonads), 14 have been delineated with the highest range of expression from background average obtained for this organ. There were identified 7 genes most significantly downregulated (*AKAP-4*, *UBQLN3*, *CAPN11*, *GGN*, *SPACA4*, *SPATA3*, *FAM71F1*) and 7 significantly upregulated (*WBSCR28*, *ADCY10*, *TMEM225*, *SPATS1*, *FSCN3*, *GTSF1L*, *GSG1*) while differentiating between different severity of spermatogenic impairment. In respect to positive vs. negative responders of NOA patients to gonadotropins therapy, 5 transcripts were found to be significantly different. Among them was found Class II *HLA DQB1* gene which acquired statistical significance differentiating between successful and negative therapy. Thus, the microarray analysis performed demonstrated high utility prognostic value concerning negative correlation between Class II *HLA DQB1* expression in testis and successful therapy. This phenomenon could have an impact on male infertility diagnosis and treatment.

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S13.L2

MULTI-OMIC INSIGHT INTO THE PATHOMECHANISM OF CHRONIC KIDNEY DISEASE-RELATED ATHEROSCLEROSIS

J. TRACZ[1], L. HANDSCHUH[1], M. LALOWSKI[1,2], L. MARCZAK[1], K. KOSTKA-JEZIORNY[3], B. PEREK[3], M. WANIC-KOSSOWSKA[3], A. PODKOWINSKA[4], A. TYKARSKI[3], D. FORMANOWICZ[3], M. LUCZAK[1]

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A progressive loss of functional nephrons defines chronic kidney disease (CKD). Although cardiovascular disease (CVD) complications and atherosclerosis are the leading causes of morbidity and mortality in CKD, the mechanism by which the progression of CVD accelerates remains unclear. Our study used a complementary multi-omic approach to assess mild and advanced CKD patients with different atherosclerosis stages and two groups of patients with different classical CVD progression but without renal dysfunction. We utilized LC-MS/MS-based proteomics and MS-based shotgun lipidomics approach fortified with standard laboratory analytical methods and functional bioinformatic analyses to profile CKD and CVD leukocyte and plasma proteins. We revealed dysregulation of proteins involved in different phases of leukocytes' diapedesis process that is very pronounced in CKD's advanced stage. We also showed an upregulation of apoptosis-related proteins in CKD as compared to CVD. The lipidome profiling revealed the upregulation of triacylglycerols in CKD and downregulation of cholesterol/cholesteryl esters, sphingomyelins, phosphatidylcholines, phosphatidylethanolamines and ceramides as compared to CVD group and controls. The differential abundance of selected proteins was validated by multiple reaction monitoring, ELISA, Western blotting, and at the mRNA level by ddPCR. An increased rate of apoptosis was then functionally confirmed on the cellular level. Hence, we suggest that the disturbances in leukocyte extravasation proteins may alter cell integrity and trigger cell death. Our results also revealed the putative existence of a functional causative link - the low cholesterol level correlated with lower estimated glomerular filtration rate and kidney dysfunction that supports the postulated "reverse epidemiology" theory. Therefore, we suggest that the proteomic and lipidomic background of atherosclerosis-related to CKD is unique and might be associated with other factors, i.e., inflammation.

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S13.L3

MULTIMODAL ANALYSIS IN THE SEARCH FOR NEW BIOMARKERS OF DIABETIC KIDNEY DISEASE - microRNA AND EXTRACELLULAR VESICLES

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Among number of complications caused by T2DM, chronic kidney disease (CKD) is the most common complication treating more than the one third of diabetic patients and increases the mortality risk in patients with T2DM. Early biomarkers of kidney damage, including diabetic kidney disease (DKD), are still being required, mainly based on the results of proteomic and transcriptomic analyzes. The large amount of data, both qualitative and quantitative parameters, makes it difficult to select potential biomarkers. Statistical methods (canonical analysis, principal components analysis - PCA, correlation analysis) are a helpful tool, especially in cases of multivariate data matrices provided by omics techniques. Exemplary analyzes of miroRNA typing in urine extracellular vesicles (EVs) from CKD patients and metabolomic analyzes based on infrared and Raman spectroscopy data in urine EVs from patients with various degrees of severity of diabetic kidney disease, are presented. Such multimodal strategies are examples of typing the new CKD biomarkers and showing that not only upregulated, but also downregulated parameters are significant for disease recognition. As the result, the augmented or decreased microRNAs related with CKD and unique spectral signatures are presented.

Acknowledgements: All publications, collaborations and grants supporting these projects are mentioned in the webpage: http://mvs.if.uj.edu.pl/.

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S13.L4

MULTI-OMIC APPROACH FOR PREDICTING RESPONSE TO NEOADJUVANT RADIOTHERAPY OF COLORECTAL CANCER

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Radiotherapy (RT) is a common modality in colorectal cancer (CRC) treatment. Serum/plasma proteomics and metabolomics of CRC patients could provide valuable insight into the response to RT. Though small extracellular vesicles (sEVs) are an emerging type of liquid biopsy, metabolomic and proteomic changes in sEVs of cancer patients after RT have not been given as much attention. This study aimed to describe the correlation between specific molecular components of serum/plasma as well as sEVs and CRC patient's response to RT. Plasma and serum samples were collected from 40 CRC patients treated with RT before surgery. Samples were classified into two groups, depending on the response to the treatment: group A - patients whose responded (sensitive) to RT and group B - patients not responded (resistant) to RT. sEVs were isolated from serum/plasma using size-exclusion chromatography (SEC). LC-MS/MS-based approach was used for proteomic profiling of serum and serum-derived sEVs. Metabolites extracted from plasma and plasma-derived sEVs were analyzed by the GC-MS. Proteomic and metabolomic data were subjected to chemometric and functional analysis. LC-MS/MS approach allowed the identification of 276 proteins, of which 18 and 91 differentiating sensitive (group A) and resistant (group B) serum and serum-derived sEVs samples, respectively. An untargeted GC-MS-based approach allowed the identification of 110 and 50 metabolites in plasma and plasma-derived sEVs, respectively, of which 31 metabolites overlapped. Proteins that differentiated serum samples from patients with different response to RT were mainly associated with lipoprotein and cholesterol metabolism. Differentiating proteins isolated from serum-derived sEVs were connected with vesicle-mediated transport, immunological processes, complement activation, neutrophil degranulation and cholesterol metabolism. This study revealed a specific pattern of proteins and metabolites in serum/plasma and sEVs, which could distinguish CRC patients with different response to preoperative radiotherapy. Response to radiotherapy-related changes observed in the molecular pattern of sEVs was more significant than response-related changes detected in serum/plasma molecules.

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S13.L5

DIETARY SUPPLEMENTATION WITH DIFFERENT SOURCES OF INULIN-TYPE FRUCTANS EVOKES CHANGES IN PROTEOMIC PROFILE OF PORCINE AORTA

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Diet has long been known to play a fundamental role in regulating host health status by modulating the composition of the gut microbiota. The use of feed supplements may also be considered as a means to improve animal production performances while maintaining health and welfare. Prebiotics, especially inulin have been established as one of the effective methods to increase the intestinal health of various animal species, including pigs. However, in the literature there are no reports on indirect effects of inulin-type fructans on the physiology of aorta. We hypothesised that inulin supplementation will modulate the protein profile of the aorta in growing pigs. The study was conducted on twenty four 50-day-old PIC x Penarlan P76 crossbred male piglets. Animals were divided into 3 groups (n=8), and fed: control diet (group I), experimental diet with 2% water extract of inulin (group II) and the diet with 4% dried chicory root (group III). The aortic proteins were separated using two-dimensional electrophoresis. The protein spots showing statistically significant changes were excised from the gels and subjected to protein identification by MALDI-TOF MS. Diet supplemented with 4% of chicory root triggered changes in the expression of 32 protein spots in the porcine aorta. 23 protein spots were differentially expressed in the group of pigs fed the diet supplemented with 2% of inulin compared with the control one. Both sources of inulin-type fructans had the potential to induce a substantial changes in the expression of proteins involved in the cellular stress response (TXNDC5, CALR, TCP1, PDIA3, HSPA8), suggesting that they may play an important role in antioxidant protection process in the aorta of growing piglets. Moreover, both experimental diets induced a positive changes in the expression of aortic proteins involved in mechanisms of blood pressure regulation (RCN2, ORM1, TXNDC5), vascular tone regulation (EFEMP1) and in the process of vascular development (ANXA2), as well as those playing a key role in actin cytoskeleton organisation (VIM, VCL, ACTR3).

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S13.L6

PROTEINS OF MARE'S COLOSTRUM ASSOCIATED WITH FOAL AND MAMMARY GLAND GROWTH AND DEVELOPMENT

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Colostrum is the first secretion of the mammary gland, which is the exclusive nourishment for the infant. Colostrum is a source of micro- and macronutrients and regulatory compounds, including bioactive proteins. All these compounds are essential for the foal's growth and development. Furthermore, in colostrum are present proteins associated with mammary gland organization and secretion of milk components. In the presented research using 2-DE coupled *via* MALDI-TOF MS, we identified 49 proteins representing 27 different gene products. Due to the epitheliochorial placenta prevents the transfer of immunoglobulins the foal is born defenseless against pathogens. It needs a supply of antibodies with the colostrum, as well as the bioactive proteins necessary to regulate the maturation of its immune system. In mare's colostrum, we identified proteins associated with supporting the immune system, such as interleukin-24 isoform × 1, immunoglobulins, complement C3 alpha chain-like. To provide immunomodulating proteins in unchanged form along with colostrum are deliver protease inhibitors. In the mare's colostrum samples were presented: alpha-1-antitrypsin and fetuin-B. The protein associated with nervous system development (ankyrin repeat domain-containing protein 6) was also present in the mare's colostrum. Furthermore, we identified proteins involved in various components transport, including iron, calcium, and vitamin D (albumin, serotransferrin, neuron-specific calcium-binding protein hippocalcin, vitamin D-binding protein). The development of the mare's mammary gland is a complex process that occurs during embryogenesis, puberty, pregnancy, and lactation. In mare's colostrum were identified proteins that are associated with the maturation of the mammary gland and the secretion of milk components: partitioning defective 3 protein homolog isoform X2 (PARD3/Par3) and FERM domain-containing protein 4B isoform X1 (FRM4B), rho GTPase-activating protein 39 (ARHGAP39), beta-lactoglobulin-1. Further analysis of mare's colostrum and milk will allow identified more proteins that are involved in the postnatal development of foals and proteins associated with synthesis and secretion of milk components. Determine the changes in the protein profile of mare's milk following days of lactation will allow a better understanding of the processes occurring in the mare's mammary gland and will clarify how changes in the composition of the bioactive proteins of the milk affect the growth and development of the foal.

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