Session 16: Clinical Metabolomics

Lectures

L16.1

Metabolomics meets clinics – application to maternal-fetal and neonatal medicine

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Clinical metabolomics, which aims to identify small molecule metabolites present in patient-derived samples, has attracted much attention providing new insights in the elucidation of the metabolic dysregulation underlying disease development. A rich amount of information locked in metabolites can lead to scientific breakthroughs facilitating early disease prediction, screening, and discovery of novel clinical biomarkers. Particular attention is given to the application of metabolomics technologies in the field of maternal-fetal and neonatal medicine. High-throughput analysis, unparalleled sensitivity together with advanced computational biology provide the broadest metabolite coverage and the highest quality data for deciphering metabolic programming during pregnancy and its effects on the origins of adult health and disease. To address that issue, we have applied untargeted LC-MS-based metabolomics approach to study several maternal-fetal and neonatal conditions including gestational diabetes mellitus, the pregnancy complications due to the bacterial infection of the fetal amnion and chorionic membranes, the causes of the twin-twin transfusion syndrome or the estimation of the nutritional status of the premature neonates. This global in scope metabolomics analysis has allowed us to obtain specific metabolic patterns under the disease that add a great value to the research field. The results of our studies permit to enhance the understanding of the interplay between pregnancy complications, fetus development, and the long-term health consequences as well as generate many new hypotheses for future investigations.

L16.2

Metabolomics in the clinical settings. Applications in diabetology and endocrinology

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Metabolomics is downstream of genome, transcriptome, and proteome, in the closest proximity to the phenotype. Metabolome can be affected by developing disease, genetic and epigenetic factors, lifestyle changes or medications used. In the clinical settings, among others, metabolomics can be used to search for potential disease biomarkers, to uncover biochemical pathways modified by pathological situation or to study treatment efficacy. However, in order to obtain reliable clinical metabolomics results, special attention should be paid to the process of patients' qualification, clinical data collection as well as proper sample collection and storage. These aspects will be presented together with our results of metabolomics studies in the field of diabetology and endocrinology.

We have evaluated metabolic changes in serum of patients after laparoscopic Roux-en-Y gastric bypass or laparoscopic sleeve gastrectomy. Both procedures were found similar in terms of general clinical outcome, but they strongly differ in molecular mechanisms leading to the final effect. We have also evaluated plasma metabolic changes in the progress of type 2 diabetes development. By use of untargeted metabolomics, we have found potential prognostic biomarkers of high birth weight in healthy pregnancy and biomarkers allowing for diagnosis of gestational diabetes mellitus manifested by glucose intolerance without performing an oral glucose tolerance test.

Posters

P16.1

A simple UPLC-UV method for normalization of cellular concentration of metabolites involved in an active DNA demethylation process

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It is suggested that metabolic alterations in cells caused by changes in some metabolite levels may contribute to molecular changes in DNA methylation pattern. Some of these metabolites may play a vital role as co-substrates or inhibitors of TET enzymes involved in formation of epigenetic modifications.
2-hydroxyglutarate or molecules with chemical similarity can compete with 2-ketoglutarate for TET's active site, what in turn, affect or inhibit its activity and may lead to carcinogenesis.
Commonly used total protein concentration rarely corresponds with the amount of cells in the tissue that was used for analysis, thus to compare the level of aforementioned metabolites between various types of cells and tissues, a normalization parameter reflecting cells number in the sample had to be found.
We have developed an unique UPLC-UV method for quantitative determination of thymine which concentration serves as a normalization parameter for quantity of other metabolites measured in the same sample. Our method might be useful for predicting how many cells were used to analysis based on known genome size and their ploidy. We have proved the linear dependence of thymine concentration derived from acid hydrolysis of human lymphocytes total DNA content to their cell number (in range 2.42E+05 – 7.60E+06 cells/mL) obtained with the hemocytometer, as well the automatic cell counter. This method was applied to several human cultured cell lines (HCT 116, K562, PC-3) and rat tissues.

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P16.2

Consequences of changes in serum FA composition in chronic kidney disease on liver lipid metabolism

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Introduction: Lipids play important roles in our body, from an energy source to signaling molecules. Many pathological conditions can lead to alterations of serum lipid composition, one of them is chronic kidney disease (CKD). Lipid disorders, including hypertriglyceridemia, start early in the course of CKD and worsen as the disease progresses. However, still little is known about the composition of fatty acids (FA) in CKD patients and consequences of changes in FA composition on lipid metabolism.
Materials & Methods: We obtain a wide and precise profile of serum FA in CKD patients by gas chromatography-mass spectrometry. To assess the influence of changes in FA profile on lipid metabolism, human hepatoma cells (HepG2) were incubated with a mix of FA extracted from control subjects and CKD stage V patients. After 48h mRNA levels for several genes involved in lipid metabolism were determined.
Results: The progress of CKD was associated with significant alterations in the composition of many specific FA. The treatment of HepG2 hepatocytes with the mix of FA from CKD patients resulted in significant increase in mRNA levels of enzymes of fatty acid synthesis and desaturation, TAG synthesis, as well as, VLDL formation.
Conclusion: The results presented above indicate the CKD progress is associated with significant alterations of FA composition that may induce liver lipid synthesis and release, contributing to CKD related hypertriglyceridemia.

P16.3

Metabolic profiling of free amino acids in blood plasma of experimental animals in the model of acute and chronic phase of allergic contact dermatitis

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Allergic contact dermatitis (ACD) is one of the most common among inflammatory skin diseases. Determination of biochemical markers of ACD allows diagnosing the risk of developing the disease, the degree of its progression, as well as the body's response to clinical intervention.
The purpose of this study was to determine the differential metabolic profiles of blood plasma amino acids in animals with experimental allergic contact dermatitis.
Allergic contact dermatitis was induced in rat by repeated application of 2,4-dinitrochlorobenzene. The animals were divided into 3 experimental groups: control group, group with acute phase ACD, group with chronic phase ACD. Determination of the concentration of free amino acids in blood plasma was carried out by HPLC with pre-column derivatization with o-phthalaldehyde. To identify the most informative indicators, discriminant analysis method was used in Statistica 10.0.
The levels of tryptophan, tyrosine, leucine, methionine and phenylalanine were significantly changed in ACD groups in comparison with control group and differences in the metabolite levels were seen in serum samples collected during the acute and chronic phase of ACD.
Our study identified significant differences between control and experimental groups associated systemic biochemical shifts in tryptophan metabolic pathways. The findings are that the metabolic profile depending on the phase of allergic contact dermatitis and can be used for its differential diagnosis.

P16.4

Preferential polyunsaturated fatty acids uptake by colorectal cancer cells

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Colorectal cancer (CRC) is one of the world most prevalent malignancies. Alteration in content of some lipid groups in biological samples as well as disturbances in pathways associated with lipid metabolism are established component of cancers. In this study we measured total fatty acid (FA) content in normal colon mucosa and CRC tissue samples from patients, as well as in serum from CRC patients and healthy controls using GC-MS. CRC tissue samples exhibited significantly higher n-3 and n-6 PUFA content in comparison with paired control tissue. By contrast, in CRC patients serum significantly less n-3 and n-6 PUFA than in control serum was found. These results suggested that tumor cells may preferentially uptake PUFA from blood, simultaneously decreasing their blood levels. To verify this hypothesis FA content was measured in human colorectal adenocarcinoma HT-29 cells growth medium after 48h culture. Total n-3 and n-6 PUFA content was significantly lowered in medium after culture when compared to control medium (maintained under the same conditions without cancer cells). By contrast, other groups of FA were not decreased. This experiment confirmed preferential uptake of PUFA from medium by CRC cells. Concerning obtained results, the question arises if PUFA supplementation in CRC patients is advantageous by means of compensating for shortages of blood PUFA, or rather detrimental, because preferential uptake of PUFA may lead to acceleration of tumor growth.

P16.5

Methotrexate-induced inhibition of amino acid transport via the gamma-glutamine cycle (GGC)

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Objective of the study: to assess the effects of methotrexate (MTR) on the level indicators characterizing the transport of amino acids into the cell by the GGC and/or the synthesis of GSH.
MTR at a dose of 0.1 mg/kg for 7 days leads to a decrease in Cys levels (from 138.46±0.152 to 55.45±0.089 μM), CysGly (from 3.64±0.197 to 1.68±0.209 μM) and GSH (from 88.62±8.47 to 52.48±11.14 μM), which may indicate the inhibition of the GGC of amino acid transport into the cell. The level of Hcy remains practically at the initial level, but the level of Ctn decreases from 0.79±0.138 to 0.41±0.031 μM, and Ser levels increase from 157.1±13.56 to 238.2±21.00 μM, Gly (from 156.4±11.64 to 485.7±33.85 μM), Met (from 29.36±0.89 to 44.09±2.66 μM), Tau (with 108.5±6.18 to 275.7±12.04 μM). MTR probably does not affect the remethylation of Hcy in Met, but inhibits transsulfuration of amino acids.
With the introduction of MTR for 21 days, decrease in the levels of all the studied parameters of the GGC was observed. The observed decrease in the concentrations of sulfur-containing compounds may possibly indicate the inhibition of the enzymes of the GGC, as well as the resynthesis of Met. A decrease in the concentration of Hcy (from 7.24±0.45 to 4.91±0.43 μM) in this case may indicate a low activity of methionine synthase and the consequent violation of the Hcy remelting process in Met, which is confirmed by a decrease in Ctn levels (from 0.79±0.138 to 0.38±0.033 μM), Tau (from 108.5±6.18 to 270.2±25.01 μM), Met (from 29.36±0.89 to 41.99±2.23 μM).

P16.6

Application of untargeted metabolomic approach to study biochemical changes in women with polycystic ovary syndrome

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The Polycystic ovary syndrome (PCOS) is a complex, endocrine disorder with unexplained pathogenesis, which affects about 10 percent women of reproductive age. Therefore PCOS is the main reason for infertility caused by anovulation appearance. In addition to endocrine dysfunction, it causes the development of additional metabolic disorders.
In this study, the serum samples of 30 women with PCOS and 30 healthy volunteers were assessed through liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry to investigate the serum metabolites characteristic of PCOS. The main aim of the study was to determine and compare the metabolomic profiles of both tested groups, as well as to identify metabolites specific to affected biochemical pathways in PCOS.
Univariate and multivariate statistical analysis showed that the metabolomic profiles of the serum samples obtained from the women with PCOS were distinctly different in comparison with the controls. The serum of the women with PCOS had statistically significant elevated levels of phenyloalanine, valine, tryptophan, tyrosine, lactic acid, dehydroepiandrosterone sulphate/DHEAS, phosphatidylcholine, lysophosphatidylcholine, phosphatidylinositol, sphinganine and uric acid.
We conclude that identified metabolites are associated to changes in the metabolic pathways of amino acids, carbohydrates, steroid hormones, lipids and purines.

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P16.7

Metabolomic insight into the pathomechanizm of prostate cancer through the "fingerprinting" analysis of seminal fluid

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According to worldwide statistics, prostate cancer (CaP) is one of the most common and lethal type of cancer among men. Its early detection is problematic due to non-specific symptoms. Although some factors for CaP developing can be distinguished, the pathogenesis is still unclear. Metabolomics focuses on determination of metabolites presented in biological samples. By measuring their changes, the relationship between body condition and metabolic profile can be characterized. It is assumed that the metabolic changes observed in seminal fluids may reflect the CaP pathomechanizm. Thus, the aim of the study was the development of sample preparation procedure, which enables the metabolite extraction from seminal fluid. Method development included the selection of such conditions as extraction solvents and duration. Samples were collected during the prostatectomy surgery from CaP patients. Samples were analyzed with the use of HPLC-ESI-TOF/MS technique and preparation method included homogenization, precipitation and extraction steps. Obtained data underwent uni- and multivaratie statistical analysis. Sample preparation procedure was selected based on the quality of obtained metabolic profiles as well as method repeatability. Selected method was utilized for the untargeted analysis and comparison of metabolic profiles of seminal fluid, blood and urine from CaP patients in order to select the metabolites, which may have potential impact in the mechanism of CaP development.

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P16.8

Analysis of serum lipid fraction of patients suffering from cardiovascular disease related to chronic kidney disease

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Chronic kidney disease (CKD) is defined as progressive loss in kidney function over a period of time, greater than three months. Because of non-specific symptoms that can specify mentioned disorder, the diagnosis is complicated and based on the decreasing ability to maintain the standard kidney functions. Patients suffering from chronic kidney disease since early stages are at strong risk of cardiovascular disease (CVD) progression. It is known that major cause of death for all pateints with CKD are cardiac events nowadays. It was proved at proteomic level that molecular mechanism of development of CVD related to CKD shows some kind of differences in reference to classical atherosclerosis [1]. Nevertheless, disrupted metabolic pathways differentiating classical atherosclerosis between CVD related to CKD aren't well recognized or even selected. In order to specify and to acquire knowledge about the dissimilarities in atherosclerosis development - lipid profiling of blood plasma samples was carried out.
The blood samples were taken from 24 healthy volunteers and 64 patients assigned in three groups: (1) CKD1-2 – patients at early stages of CKD and first symptoms of CVD; (2) CKD5 – patients at end-stage of CKD treated with renal replacement therapy with severe CVD symptoms; (3) CVD – patients suffering from advanced classical atherosclerosis and with normal renal function.
Extraction of lipids was performed according to protocol of Matyash and co-workers [2], using MTBE extraction . Q-Exactive Orbitrap (Thermo Fisher Scientific) coupled to TriVersa Nanomate (Advion) was used for non-targeted lipid profiling. Obtained profiles were compared in order to define the differences The abnormalities in metabolism of phospholipids and triacylglycerols were observed, that can be probably related to malnutrition or systemic inflammation leading to cardiovascular disease progression and in the effect, to cardiac events in CKD. The established conclusions will be correlated with results obtained during proteome analysis in order to recognize molecular mechanism of the disease development.

References:
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