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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Two thousand 19: Outcomes of a decade of antimicrobial resistance proteomics

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ABSTRACT

Antimicrobial resistance (AMR) is today acknowledged to be one of the most concerning threats to global human health and the international community has now started to act concertedly to tackle this problem. AMR is not a recent problem dating back to the discovery and first use of antibiotics. For the last 10 years, the Functional Genomics and Proteomics Unit based at the University of Trás-os-Montes and Alto Douro, Vila Real, Portugal, has aimed to understand more about the mechanisms that equip bacteria to survive antibiotic action by investigating the entire complement of proteins expressed by resistant strains. The proteome can reveal the complex profiles of expressed proteins underlying phenotypic characteristics, and provide an excellent approximation of the information contained within a microorganism's genome. Coupled with bioinformatics, proteomics has led to significant progress in the characterization of bacterial pathogens. From different starting points, the proteomics studies performed have contributed to knowledge on over 2000 proteins that individually or as a complex whole participate in AMR. Since 2009, the proteomes of different *Salmonella, Enterococcus, Escherichia coli* and *Staphylococcus aureus* strains have been thoroughly studied with the purpose of identifying the main proteins present or those that are differentially expressed between strains. By looking at the whole proteome or subfractions thereof, resistant strains with different levels of resistance have been compared to related non-resistant strains both in the presence and absence of antibiotic stress. High-resolution protein separation has been achieved with two-dimensional gel electrophoresis (2-DE) and shotgun analysis has overcome some of the limitations related to the low solubility of membrane proteins. Comprehensive coverage of the proteins present has thus been attained by using 2-DE followed by either matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) together with shotgun LC-MS/MS approaches. The study of strains recovered from clinical human samples provided a better understanding of extended-spectrum beta-lactamase (ESBL) producing *E. coli*, new insights into pleural empyema methicillin-resistant *S. aureus*, and an in-depth examination of the mechanisms of quinolone resistance in *Salmonella Typhimurium* causing acute gastroenteritis. Other proteomes studied were those of multidrug resistant (MDR) *E. coli* and ciprofloxacin resistant and vancomycin resistant enterococci recovered from pigs slaughtered for human consumption. Also, the role of resistant bacteria as environmental reservoirs of AMR was better elucidated through studying the proteomes of ESBL-positive *E. coli*, vanA-positive enterococci and MDR *Salmonella* recovered from a variety of free-ranging wild animals including boars, rabbits, seagulls, red foxes, Iberian wolves and lynxes. Proteomics techniques have considerably improved during the past decade in parallel with advances in bioinformatics, and together have proved to be the appropriate research tool with which to tackle the major challenge that AMR poses in modern medicine.

Acknowledgments:

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Comparative membrane-associated proteome of *Leishmania* (*Leishmania*) *infantum* and *L.* (*L.*) *amazonensis*

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ABSTRACT

The species *L. (L.) infantum* and *L. (L.) amazonensis* are causative agents of visceral and cutaneous leishmaniasis, respectively. Most proteome analyses of Leishmania have been carried out on whole-cell extracts, but this approach tends to underrepresent membrane proteins because of their high hydrophobicity and low solubility. Due to the great importance of membrane proteins in biological processes, including host–parasite interactions, virulence and invasiveness, this study applied label-free shotgun proteomics to characterize and evaluate abundance levels of plasma membrane proteins of promastigotes life-stage. The total number of proteins identified in *L. (L.) infantum* and *L. (L.) amazonensis* was 2033 and 2243, respectively, and both species shared 1908 of these proteins quantified. After cell localization prediction of all identified proteins, 394 were described as plasma membrane proteins and their majority (320 proteins) was shared between both species, 18 were exclusively detected in *L. (L.) infantum* and 56 in *L. (L.) amazonensis*. Proteins with qualitative (present or absent) or quantitative (p-value < 0.05) differences were classified as "regulated" proteins. Thus 106 regulated plasma membrane proteins were selected, being 32 from *L. infantum* and 74 from *L. amazonensis*. These proteins were representing considerably distinct processes in the species, such as regulation of cell communication and cell adhesion in *L. (L.) infantum*, and localization and transport metabolism in *L. (L.) amazonensis*. The proteins involved in these processes included GP63, a well-known virulence factor, as well as members of the ABC transporter superfamily. The identification of numerous proteins with uncharacterized roles highlights the importance of investigating Leishmania proteins. They could contribute to better understand the parasite biology and may act as new potential therapeutic targets.

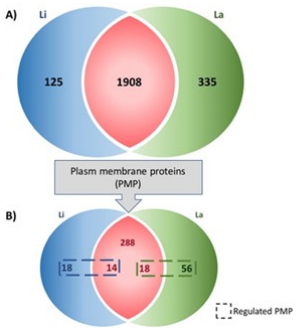

Figure 1 - Distribution of identified proteins. *L. (L.) infantum (L.i)* and *L. (L.) amazonensis (L.a)* exclusive and shared proteins before and after plasm membrane protein (PMP) selection and PMP up regulated in each species.

Acknowledgments:

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (PPM00639-15), Conselho Nacional de Desenvolvimento Científico e Tecnológico (446275/2014-0), the National Institute of Science and Technology for Vaccines (CNPq-573547/2008-4/FAPEMIG/MS-CBB-APQ 00077-09) and Rede Mineira de Biomoléculas (CCB-RED00012-14). H. M. A is CNPq fellows (PQ).

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In pathogen bacteria *Staphylococcus aureus* MazEF Toxin-Antitoxin System (TAS) regulates cell dormancy in response to environmental stress

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ABSTRACT

Bacterial cells can resist an antibiotic treatment resulting in chronic bacterial infections. To survive a lethal dose of antibiotics, bacteria change their physiological state to a non-growing state, called dormancy. The mechanisms of entry into bacterial dormancy are not understood. Toxin-antitoxin system (TAS) is a stress-inducible functional unit that comprises a toxin component and a corresponding antitoxin that blocks toxin activity. In *E. coli*, overexpression of the toxin component leads to cell growth defects, suggesting that bacterial TASs can be involved in development of bacteria dormancy to resist antibiotic treatment. We are working with pathogen bacteria Staphylococcus aureus and investigating the role of an endoribonuclease MazF toxin on bacterial dormancy. We identified genome-wide RNA targets cleaved by MazF endoribonuclease and demonstrated that MazF overexpression causes S. aureus dormancy. To further understand MazF effect on *S. aureus* cell metabolism, we applied RNA-seq, RIBO-seq (ribosome profiling) and quantitative mass spectrometry. Our results show that activation of MazF toxin reduces ribosome biogenesis, translation, and induces formation of ribosome dimers, that decreases cell metabolism and provokes bacterial dormancy. Thus, our data suggests that MazF toxin may be a key regulator of dormancy and antibiotic resistance in S. aureus. To understand how MazF toxin can be activated under natural conditions, we investigated an upstream pathway regulating MazE antitoxin degradation. Our results suggest that environmental stress, such as heat shock, oxidative stress or high concentration of antibiotics induce aggregation of adaptor protein YjbH, that may result in activation of MazF toxin. We proposed the model (Figure 1) where S. aureus MazEF Toxin-Antitoxin System is modulated by adaptor proteins in response to environmental stress and regulates bacteria dormant state. Our model suggests a potential pathway to regulate antibiotic resistance in pathogens

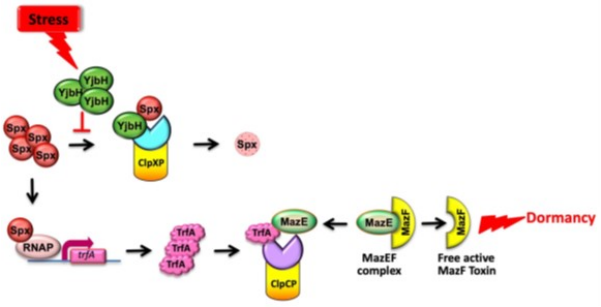

Figure 1 - In bacteria *S.aureus* MazEF Toxin-Antitoxin System regulates cell dormancy in response to environmental stress, such as heat shock, oxidative stress, and presence of antibiotics.

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Comprehensive metabolomics studies to identify biomarkers of type 1 diabetes onset and pancreatic islet stress

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ABSTRACT

Technological advances now allow quantitative assessment of biochemicals in small enough samples to make possible longitudinal studies involving multiple sample collections in rodents or small human tissue cultures. To investigate biochemical changes caused by onset of type 1 (juvenile-onset) diabetes and identify biomarkers of islet stress, we carried out a longitudinal metabolomics study in NOD mice, the most commonly used animal model of this diseases. Using complex LC-MS analyses for metabolomics profiling, we were able to quantify the concentration time-profile of more than 650 biochemicals in blood and feces [1]. In animals that became diabetic, several of them showed considerable (>4x) change, and some, such as 3-hydroxybutyrate (BHBA), maltose, and 1,5-anhydroglucitol, changed by more than 10-fold (Figure 1). While there were no strong differences between the metabolic signatures of progressors and non-progressors before the onset of diabetes, we found compounds that had significantly different levels and show promise as possible early biomarkers, maybe as part of a combined metabolic signature. In a separate study, we investigated the effects of stress factors such as inflammation and hypoxia on the biochemical profile of isolated human islets under both basal and hyperglycemic conditions [2]. Using metabolomics profiling of media and cell samples via the same method, we were able to identify and quantify a total of 241 and 361 biochemicals, respectively. One of the most notable observations was the consistently large changes induced by an inflammatory milieu in kynurenine and kynurenate, suggesting that they might serve as biomarkers of islet inflammation and that indoleamine-2,3-dioxygenase (IDO) on the corresponding metabolic pathway could be a therapeutic target to modulate the effects of inflammation in islets.

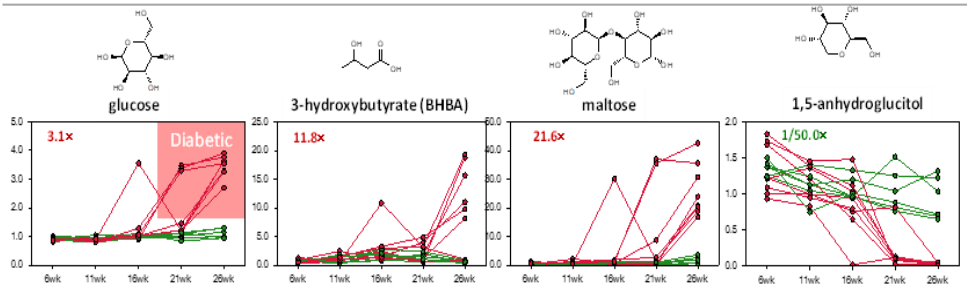

Figure 1 - Spaghetti plots illustrating the longitudinal time-profile of some of the small-molecule biochemicals showing the largest fold-change in diabetic progressor NOD mice (red) versus non-progressor controls (green) in blood samples. Individual data (scaled intensity) are shown with numbers indicating the average fold-change value at week 26, when all progressors were already diabetic.

Acknowledgments:

Financial support by the National Institutes of Health (NIH/NIDDK, 1UC4DK104208) and the Diabetes Research Institute Foundation is gratefully acknowledged.

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Engineering bacterial monooxygenases to produce human drug metabolites

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ABSTRACT

Flavin monooxygenases are a large family of enzymes carrying out oxidative reactions. One of these group of enzymes, Baeyer-Villger monooxygenases (BVMO), are wide spread in nature especially in bacteria and fungi but absent in humans. These enzymes are of great biotechnological interest due to their stereo- and regio-specific conversion of ketones into the corresponding esters and lactones, for green chemistry applications. Previously we have identified and cloned a BVMO (Ar-BVMO) from the genome of Acinetobacter radioresistens and found that it is closely related not only to a medically relevant monooxygenase (ethionamide-prodrug activator) [1] but also by itself capable of inactivating the imipenem antibiotic [2]. By multi-alignment of several known primary sequences of BVMOs, a conserved arginine thought to be located in the active site of these enzymes was also found to be present in Ar-BVMO. Since this conserved amino acid is crucial for the enzymatic activity of other BVMOs, we proceeded by replacing the latter arginine, R292 in Ar-BVMO (Fig. 1), with glycine or alanine. After expression and purification of the mutant proteins, their activities were compared to that of the wild type Ar-BVMO. As expected, the results obtained demonstrated the drastically reduced Baeyer-Villiger activities of both mutants. But somewhat unexpectedly and to our surprise, one of the two mutants, R292A, was still capable of heteroatom oxidation, reactions similar to those carried out by human hepatic drug metabolizing enzymes. For example, it could convert the anti-tubercular drug ethionamide to its corresponding S-oxide form (Fig. 1). In this way, the advantages related to stability and solubility of a bacterial enzyme are combined with the catalytic specificity of a human membrane-bound enzyme

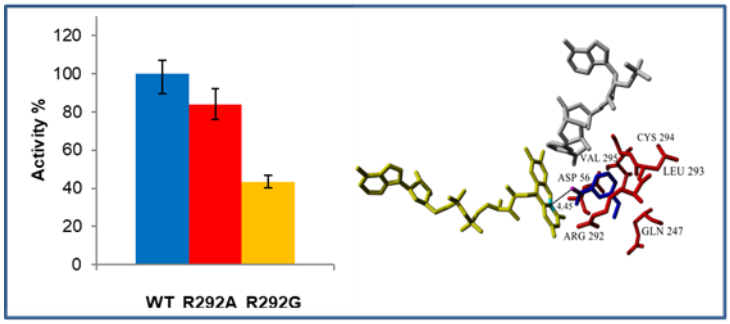

Figure 1 - Left: Graph showing the difference activities of the wild type and the two engineered mutants towards ethionamide (anti-tubercular drug); Right: 3D model of Ar-BVMO active site with flavin cofactor in yellow and ethionamide in dark blue.

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Proteogenomic analysis of Mycobacterium tuberculosis Beijing B0/W148 cluster

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ABSTRACT

Today, the whole genome structure and it's most complete description, annotation, are the start points in the study of life organisms. At the same time experimental datasets obtained on proteomic and transcriptomic level can improve the annotation, providing evidence of new genes and correcting known ones. The aim of this study was to improve the annotation of RUS_B0 strain of M. tuberculosis Beijing B0/W148 cluster using the proteogenomic analysis. The resulting (circular) genome of RUS_B0 strain was obtained using Illumina HiSeq2500 platform and Sanger sequencing (GenBank accession number is CP030093.1). LC -MS/MS proteome analysis of 58 Beijing B0/W148 cluster strains was performed on a Q Exacitve HF mass spectrometer (Thermo Fisher Scientific, USA). According to proteomic data we identified 31,527 peptides corresponding to 2,782 proteins. For Genome Search Specific Peptides (GSSPs) identification six-frame translation of RUS_B0 genome carried out. According to proteogenomic analysis 67 GSSPs were identified. Among them 31 GSSPs were used for translation star site (TSS) correction of 32 annotated genes; 32 GSSPs - crossed along the coordinates with annotated pseudogenes and 4 GSSPs - corresponded to new, not annotated genes. Based on obtained data we confirmed the presence of peptides (n=32) for 8 RUS_B0 pseudogenes. Additionally cluster-specific singe amino acid polymorphism (Ala253Ser) in the oxalyl-CoA decarboxylase protein (TBPG_RS00635) was shown (Fig. 1). For GSSPs verification independent proteome analysis of two strains in two biological replicate was performed. According to identification of spectra obtained in targeted HR-MRM analysis performed on TripleTOF 5600+ mass-spectrometer 23 GSSPs were verified. The results allowed us to get the most complete annotation of RUS_B0 strain of Beijing B0/W148 cluster which is widespread in the Russia.

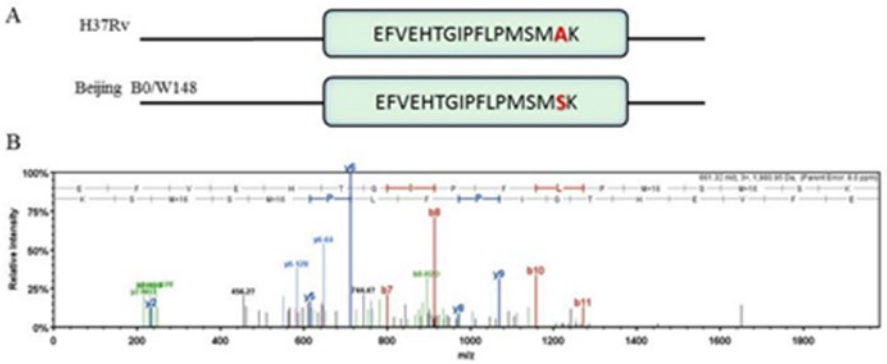

Figure 1 - Identification of Beijing B0/W148 cluster-specific SAP with representative MS/MS spectrum. A. The sequence of peptide which contains Beijing B0/W148 cluster-specific SAP and sequence of such peptide annotate in H37Rv. B. A representative MS/MS spectrum of peptide EFVEHTGIPFLPMSMSK.

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Grapevine leaf proteome reveals specific adjustments leading Plasmopara viticola resistance

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ABSTRACT

Grapevine downy mildew is an important disease affecting crop production leading to severe yield losses. We have conducted a leaf proteome study to identify specific adjustments on tolerant and susceptible grapevine genotypes to P. viticola (0h, 6, 12 and 24 hpi). Leaf proteome analysis was performed using 2D difference gel electrophoresis followed by protein identification via mass spectrometry. We have also accessed reactive oxygen species, antioxidant capacity, lipid peroxidation and gene expression. By analyzing the constitutive differences, proteins related to photosynthesis and metabolism allowed the discrimination of resistant and susceptible grapevine cultivars [1]. Following inoculation, increase of hydrogen peroxide levels, cellular redox regulation, establishment of ROS signalling and plant cell death seem to be key points differentiating the resistant genotype [1]. Lipid associated signalling events, particularly related to jasmonates appear also to play a major role in the establishment of resistance [1]. Aiming at a better understanding of the genotype-specific differences that account for a successful establishment of a defense response to the downy mildew pathogen we have developed an apoplastic fluid extraction method through vacuum infiltration compatible with both proteome and metabolome analysis. To perform this analysis, APF was extracted from susceptible and tolerant cultivars/genotypes, Trincadeira and Regent, respectively. Our preliminary results show that both genotypes are inherently different at the intracellular space composition. Label-free proteome sequencing by LC-MS approach will be conducted at several inoculation time-points in order to define the key events associated to pathogen recognition and signalling activation that lead to the establishment of the incompatible interaction.

Acknowledgments:

Work supported by UID/MULTI/04046/2019 Research Unit grant from FCT, Portugal (to BioISI),by PEst-OE/BIA/UI4046/2014, PEst-OE/QUI/UI0612/2013 PTDC/BIA-BQM/28539/2017, investigator FCT program IF/00819/2015 to AF, PD/BD/131030/2017 to ARC and SFRH/BD/137066/2018 to JF

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Exploration of the platelet proteomic signature associated with adverse transfusion reactions

Sandrine Laradi[1,2*], Danielle Awounou[1#], Chaker Aloui[1,2#], Celine Barlier[1#], Stephane Claverol[3], Jocelyne Fagan[1,2], Emmanuelle Tavernier[4], Christiane Mounier[4] Hind Hamzeh-Cognasse[2], Fabrice Cognasse[1,2], Olivier Garraud[1,5]

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ABSTRACT

Grapevine downy mildew is an important disease affecting crop production leading to severe yield losses. We have conducted a leaf proteome study to identify specific adjustments on tolerant and susceptible grapevine genotypes to P. viticola (0h, 6, 12 and 24 hpi). Leaf proteome analysis was performed using 2D difference gel electrophoresis followed by protein identification via mass spectrometry. We have also accessed reactive oxygen species, antioxidant capacity, lipid peroxidation and gene expression. By analyzing the constitutive differences, proteins related to photosynthesis and metabolism allowed the discrimination of resistant and susceptible grapevine cultivars [1]. Following inoculation, increase of hydrogen peroxide levels, cellular redox regulation, establishment of ROS signalling and plant cell death seem to be key points differentiating the resistant genotype [1]. Lipid associated signalling events, particularly related to jasmonates appear also to play a major role in the establishment of resistance [1]. Aiming at a better understanding of the genotype-specific differences that account for a successful establishment of a defense response to the downy mildew pathogen we have developed an apoplastic fluid extraction method through vacuum infiltration compatible with both proteome and metabolome analysis. To perform this analysis, APF was extracted from susceptible and tolerant cultivars/genotypes, Trincadeira and Regent, respectively. Our preliminary results show that both genotypes are inherently different at the intracellular space composition. Label-free proteome sequencing by LC-MS approach will be conducted at several inoculation time-points in order to define the key events associated to pathogen recognition and signalling activation that lead to the establishment of the incompatible interaction.

Acknowledgments:

This work was supported by grants from the French National Blood Service–EFS (Grant APR 14 2016-32), France; the Association Recherche Transfusion (ART) (2018-19) and the Association "Les Amis de Remi", Savigneux, France. We thank the EFS delivery department of Saint-Priest-en-Jarez for their precious collaboration in the collection of PC bag sampling. We also thank the blood donors for agreeing to take part in this study.

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Chromosome-centric proteomic QR-code: promising tool for personal health molecular monitoring and health analytics.

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ABSTRACT

Purpose: for the practical use of the International Chromosome-Centric Human Proteome Project (C-HPP) results in medicine, it is necessary to uncover which part of the human plasma proteome could be identified and measured.

Experimental description: within the framework of the C-HPP, the Russian Consortium is developing plasma analysis technology, which combines the chromosome-centric approach with bottom-up Selected Reaction Monitoring with Stable Isotope-labeled peptide Standards (SRM SIS). This study was aimed to quantitatively analyze the proteins encoded by 643 genes of the four selected chromosomes in the blood plasma of healthy, clinically well-examined people using SRM SIS technologies. Fifty-four male subjects (age 20-47) were examined at the Institute of Medico-Biological Problems (Moscow, Russia) and approved for space-related simulations and experiments.

Results: the concentration of 205 proteins was accurately measured with SRM SIS assay (1), while quantitative proteomic profile of each sample was presented as personal QR-code. The concentration range covered by the SRM SIS technology was six orders of magnitude (from 10^{-6} to 10^{-11} M) in case of the analysis of whole plasma, and five orders of magnitude (from 10^{-7} to 10^{-11} M) in case of the analysis of depleted samples. Unexpectedly, from the obtained data we derived no correlation between protein abundances and corresponding number of samples in which this protein was detected.

Conclusions: there are only about 30 proteins encoded by examined 643 protein coding genes, which abundance levels are more or less stable (inter-individual CV ≤20%). It seems that those proteins could be used as a pillar for creation SRM-assays for personal health analytics.

Acknowledgments:

This study was supported by the Program of the Presidium of the Russian Academy of Sciences ("Proteomic and Metabolomic Profile of Healthy Human"). Data storage and analysis was performed using equipment of "Human Proteome" Core Facility (Institute of Biomedical Chemistry). Ek.I. and Ek.P. acknowledge the Leading Scientific School of Prof. Andrey Lisitsa (grant the Russian Federation of President NSh -6313.2018.4)

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Computational approach to detect interference in SRM data

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ABSTRACT

Purpose: Proteomic technologies, particularly SRM target mass-spectrometry, enable to detect thousands of proteins in biological samples. These data require appropriate means for storage, analytical processing and interpretation to decipher the new knowledge. This work reviews modern state of target mass-spectrometry in proteomics. However, the most of SRM data is distorted by interference. The main reason of the interference is that some components in a complex biological sample may have the same precursor and fragment masses as the target peptide 1. Currently, manual inspection is used to find the interference. To formalize expert's inspection results we have developed an algorithm to detect the interference and to estimate its influence on the quantitative protein analysis.

Experimental description: In the present work we collected and normalized chromosome 18 protein detection SRM results. 2247 peptides of 275 chromosome 18 proteins were detected in human plasma and HepG2 cell line. Three SRM transitions for each peptide were monitored. Each experiment was repeated in 3 technical runs. For the selected peptides stable isotope labeled internal standard peptides was used. Data processing and interference detection were programmed in Perl. The measurements were segmented according to the within-run CV of transitions' intensity and peptides; measured concentrations. The most confident data were revealed by removing the transitions with CV>30%.

Results: We performed analysis of the huge SRM data set, including 275 proteins of chromosome 18. Data array was processed using cascade filters based on the analysis of mass-spectrometric parameters reproducibility and consistency. In total, 23 from 275 (chr.18) proteins were detected with high confidence. For the most confident and most variable changeable proteins we performed meta-analysis to find properties, that unite them.

Conclusions: In conclusion, we presented a method for detection of interference based on reproducibility in technical runs and technical replicates. SRM detection results are discrepant because of technical and biological variability. In addition, we showed, that the difference between measured concentrations of two peptides of one protein may be explained by the fact, that at least one of them conforms not only to the canonical isoform of the target protein, but to splice isoforms of other proteins too.

Key Words: proteomics, SRM, mass-spectrometry, bioinformatics, database

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HAX1 interactome mapping based on two complementary approaches reveals new functions in oxidative phosphorylation and protein aggregation

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ABSTRACT

HAX1 protein is involved in the regulation of apoptosis, cell migration and calcium homeostasis, but the exact mechanisms of its action remain elusive. HAX1 expression level has medical implications; its deficiency causes severe neutropenia and neurological symptoms, while its overexpression is observed in several types of cancer [1]. In this study we aimed to elucidate HAX1 role in the cell by characterizing its protein interactome using two different approaches: yeast two-hybrid system (Y2H) and affinity purification coupled with mass spectrometry (AP-MS). The results revealed a substantial variability of the protein subsets, depending on the method and the specific cancer cell line used. Detailed analysis of the results using STRING protein association networks revealed a large subset of mitochondrial proteins involved in metabolic regulation, namely proteins of malate-aspartate shuttling system and respiratory chain proteins, possibly functioning in metabolic shift, observed in cancer cells. However, the strongest and the most reliable interaction partner, confirmed in all approaches and by additional co-immunoprecipitation and microscopic studies was the mitochondrial chaperone CLPB (caseinolytic peptidase B). CLPB belongs to the AAA superfamily of ATP-ases and is involved in the disaggregation of protein aggregates. Its deficiency is lethal, due to significant neonatal neurologic disorders and severe neutropenia, which partially conforms to the observed HAX1 deficiency symptoms. Thus, further analysis of these results should contribute to a better understanding of HAX1 functions and their medical implications.

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A new insight into cryptobiosis: Cell repair in ametabolism

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ABSTRACT

Cryptobiosis is a physiological state of reversible ametabolism. During cryptobiosis, organisms show no visible signs of life; their metabolic activity becomes almost impossible to measure. Many organisms, for example African chironomids, tardigrades, brine shrimp eggs, and single-celled eukaryote protozoa such as Colpoda, undergo cryptobiosis. During cryptobiosis, they acquire extreme tolerance to stresses by halting their metabolism. Colpoda is a single-celled eukaryote protozoa adapted to terrestrial environments. Its strategy involves formation of a resting cyst (encystment) in response to desiccation stress. This encystment is a form of cryptobiosis. Therefore, they also possess the ability to revert to a vegetative state when the stressor has passed. In addition, they exhibit extreme tolerance to many environmental stresses, for example high and low temperatures, acids, organic solvents, and so on. In the processes of encystment and excystment, the expression of some proteins has been reported to be altered [1]. In general, cell injuries caused by gamma rays are either direct or indirect injuries; the latter are caused by ROS stress. In our study, we demonstrated that cell injuries caused by gamma ray irradiation were repaired during cystic state as cryptobiosis in C. cucullus R2TTYS strain [2]. In this presentation we will discuss cell repair during cryptobiosis.

Keywords: Cryptobiosis, Cyst, Colpoda

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Neutrophils response to surgery and ischemia from a proteomic standpoint

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ABSTRACT

Neutrophils play an important role in the mechanism of traumatic injuries, ranging from simple surgical traumas to polytrauma patients. The mesenteric ischemia/reperfusion (IR) model in laboratory animals is well suited to represent the systemic inflammatory events following severe trauma. The ischemic preconditioning (IPC), characterized by short IR events preceding a longer ischemia, shows a protective effect against the inflammatory damage. Although neutrophils are known to be key players in such systemic inflammatory response, the molecular mechanisms underlying their function is yet controversial and there are no viable treatments to modulate their response in patients. In the studies presented here we compared the proteomic profile of neutrophils from control rata and rats subjected to abdominal surgery (laparotomy), IR alone and IR preceded by IPC. After database searches, normalization and statistical analysis our proteomic analysis resulted in the identification of 2437 protein groups that were assigned to five different clusters based on the relative abundance profiles among the experimental groups. Cluster based KEGG pathway analysis revealed significant regulation of directional migration and chemotaxis after mild surgical trauma, added to phagocytosis and ROS production when the animals were exposed to IR. The inflammatory damage prevention by IPC was revealed at the molecular level to be related to related to chemokine signaling, transendothelial migration and, especially in the oxidative stress pathways (Figure 1). We identified interesting antioxidants including peroxiredoxin-6, glutathione peroxidase, and methionine sulfoxide reductase which were found downregulated after IR. Such a decrease in antioxidant activity in IR neutrophil leads to higher ROS production further contributing to tissue damage whereas IPC prevented such changes. Similarly another interesting enzyme, argininosuccinate synthase, involved in NO generation (nitrosative stress) was found significantly increased in IR and decreased in IPC.

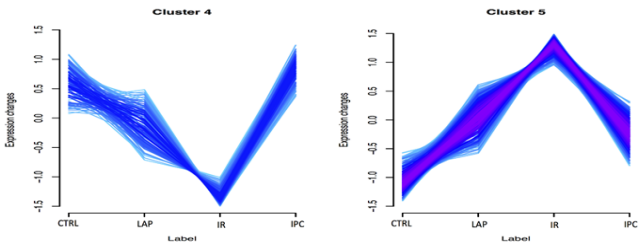

Figure 1 - Two clusters of regulated proteins showing the effect of surgery and IR, as well as the protective effect of IPC

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