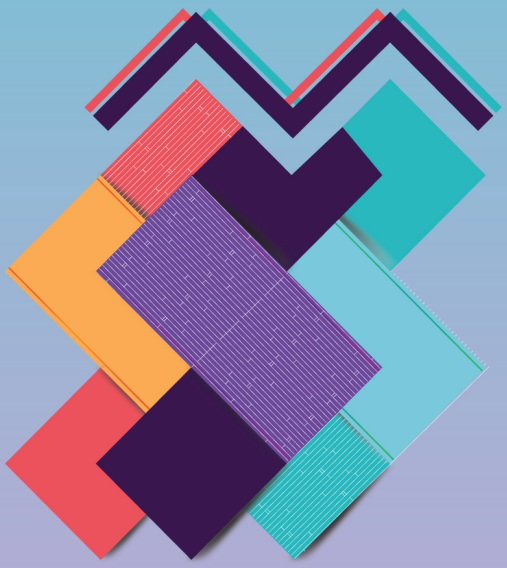


Sao Paulo School of Advanced Science on Mass Spectrometry-based Proteomics SPSAS-MS

August 28th - September 6th | 2017

Book of Abstracts

http://pages.cnpem.br/ms

FAPESP LNBio CNPEM MINISTRY OF SCIENCE, TECHNOLOGY INNOVATION AND COMMUNICATION BRASIL BRAZILIAN GOVERNMENT

Content

Presentation

CNPEM

The Brazilian Center for Research in Energy and Materials (CNPEM) is a private nonprofit organization located in Campinas, Brazil, which is funded by the Ministry of Science, Technology & Innovation (MCTI). It is dedicated to cutting-edge research in materials, nanosciences, life sciences, physics, and chemistry through four National Laboratories: Synchrotron (LNLS), Biosciences (LNBio), Bioethanol (CTBE) and Nanotechnology (LNNano). The four laboratories are open facilities for external users and companies, in Brazil and abroad. They also have teams of researchers to provide support for projects, as well as to conduct joint research programs in biomass, green chemistry, drugs and cosmetics development, characterization of advanced materials, catalysts, etc.

LNBio

The Brazilian Biosciences National Laboratory (LNBio) is dedicated to cutting-edge research and innovation focused on biotechnology and drug discovery and development. LNBio activities are organized into four areas: Open Facilities; Innovation Core; Research in-house; Training and Education. This organizational strategy was designed to encourage the sharing of infrastructure and skills with the academic and industrial sectors. Thus, LNBio optimizes and directs its resources to Science, Technology and Innovation activities.

SPSAS

The Sao Paulo Research Foundation (FAPESP) funds the Sao Paulo School of Advanced Science (SPSAS) to promote short duration courses in advanced research in different areas of knowledge in the State of Sao Paulo.

SPSAS-MS [grant #2016/18802-4, FAPESP] will offer 10-day courses in advanced themes in science and technology, contributing to the formation of the young scientists. This FAPESP initiative expects to establish in the State of Sao Paulo a globally competitive hub for talented researchers.

Sao Paulo School of Advanced Science on Mass Spectrometry-based Proteomics (SPSAS-MS)

The event is promoted by the Brazilian Biosciences National Laboratory (LNBio). The school will be hosted by the Brazilian Center for Research in Energy and Materials (CNPEM) in Campinas-SP, Brazil, from August 28th to September 6th, 2017. The SPSAS-MS program comprises classes ministered by experts in each topic as well as oral and poster sessions.

The SPSAS-MS lecturers will be ministered by 6 Brazilians and 6 foreign invited scientists. The SPSAS-MS will cover Quantitative and Spatial Proteomics, Clinical Proteomics, Targeted Proteomics, Post-translational Modifications, Structural Proteomics, Computational and Statistical methods for Proteomics. The topics will be addressed in theoretical lectures and practical activities.

Organizers and Partnerships

SPSAS-MS Organizer

Adriana Franco Paes Leme
LNBio, CNPEM

Scientific Committee

Adriana Franco Paes Leme
LNBio, CNPEM

Daniel Martins-de-Souza
Institute of Biology, Unicamp

Fabio Gozzo
Institute of Chemistry, Unicamp

Giuseppe Palmisano
Institute of Biomedical Sciences, USP-SP

Solange Maria de Toledo Serrano
Butantan Institute, SP

Vitor Faça
Ribeirão Preto Medical School, USP-RP

Local Committee

Ildéria Santos
CNPEM

Maria Lívia Ramos Gonçalves
CNPEM

Gustavo Moreno
CNPEM

Pâmela Machado
CNPEM

Bianca Alves Pauletti
CNPEM

Sami Yokoo
CNPEM

Daniela Granato
CNPEM

Romênia Domingues
CNPEM

Supported by

Organized by

Partnership

Program

Day 1: 28/08/2017		
09:00-10:00	Opening meeting	Kleber Franchini, Director, Brazilian Biosciences National Laboratory
10:00-11:00	FAPESP talk	Luiz Nunes, Associate Coordinator of FAPESP Special Programs
11:00-11:40	Coffee Break	
11:40-12:40	Welcoming of Committee Members	Scientific Committee Members
1:00-2:00	Lunch	
2:00-3:30	Quantitative and spatial proteomics	Kathryn Lilley, University of Cambridge, Cambridge, United Kingdom
3:30-4:00	Coffee Break	
4:00-4:10	SONAR: A Novel DIA Acquisition Mode for Proteomics	Alexandre F. Gomes, Waters
4:10-6:00	Quantitative and spatial proteomics	Kathryn Lilley, University of Cambridge, Cambridge, United Kingdom
6:00-8:00	Welcome cocktail	

Day 2: 29/08/2017		
9:00-10:30	Post-translational modifications	Martin Larsen, University of Southern, Odense, DK, Denmark
10:30-11:00	Coffee Break	
11:00-1:00	Post-translational modifications	
1:00-2:00	Lunch	
2:00-3:30	Post-translational modification	Giuseppe Palmisano, Institute of Biomedical Sciences, USP, Sao Paulo, SP, Brazil
3:30-4:00	Coffee Break	
4:00-6:00	Post-translational modification	

Day 3: 30/08/2017		
9:00-10:30	Protein Conformation and Dynamics by MS	David Schriemer, University of Calgary, Calgary, AB, Canada
10:30-11:00	Coffee Break	
11:00-1:00	Protein Conformation and Dynamics by MS	
1:00-2:00	Lunch	
2:00-3:30	Structural Proteomics	Fabio Gozzo, Institute of Chemistry, Unicamp, Campinas, SP, Brazil
3:30-4:00	Coffee Break	
4:00-6:00	Structural Proteomics	

Day 4: 31/08/2017

9:00-10:30	Discovery to Targeted Proteomics	Adriana Paes Leme, Brazilian Biosciences National Laboratory, CNPEM, Campinas, SP, Brazil
10:30-11:00	Coffee Break	
11:00-11:10	The iKey Separation Device as a More Efficient and Simple-To-Use Alternative for Biomarker Targeted Analysis	Alexandre F. Gomes, Waters
11:10-1:00	Targeted Proteomics in Human Samples	Vitor Faça, Ribeirão Preto Medical School, USP-RP, Ribeirão Preto, SP, Brazil
1:00-2:00	Lunch	
2:00-3:30	Computational and Statistical methods for Proteomics	Eralp Dogu, Mugla University, Turkey
3:30-4:00	Coffee Break	
4:00-6:00	Computational and Statistical methods for Proteomics	
6:00-8:00	Social Gathering	

Day 5: 01/09/2017

9:00-11:00	Discovery to Targeted Proteomics	Adriana Paes Leme, Brazilian Biosciences National Laboratory, CNPEM, Campinas, SP, Brazil
11:00-11:30	Coffee Break	
11:30-1:00	Targeted Proteomics in Human Samples	Vitor Faça, Ribeirão Preto Medical School, USP-RP, Ribeirão Preto, SP, Brazil
1:00-2:00	Lunch	
2:00-3:30	Clinical Proteomics	Thomas Kislinger, University of Toronto, Toronto, Canada
3:30-4:00	Coffee Break	
4:00-6:00	Clinical Proteomics	

Day 6: 02/09/2017

9:00-10:30	Selected 4 oral presentations
10:30-11:00	Coffee Break
11:00-1:00	Poster Session (23 STUDENTS)
1:00-2:00	Lunch
2:00-3:30	Selected 6 oral presentations
3:30-4:00	Coffee Break
4:00-6:00	Poster Session (22 STUDENTS)

Day 7: 03/09/2017

9:00-10:30	Selected 4 oral presentations	
10:30-11:00	Coffee Break	
11:00-1:00	Poster Session (23 STUDENTS)	
1:00-2:00	Lunch	
2:00-3:30	Selected 6 oral presentations	
3:30-4:00	Coffee Break	
4:00-6:00	Poster Session (22 STUDENTS)	

Day 8: 04/09/2017

9:00-10:30	Degradomics	Oliver Schilling, University of Freiburg, Germany
10:30-11:00	Coffee Break	
11:00-1:00	Degradomics	
1:00-2:00	Lunch	
2:00-3:30	Snake Venom Proteinases Degradomics	Solange Serrano, Butantan Institute, São Paulo
3:30-4:00	Coffee Break	
4:00-6:00	Snake Venom Proteomics	

Day 9: 05/09/2017

9:00-10:30	Visit at CNPEM	
10:30-11:00	Coffee Break	
11:00-13:00	Visit at CNPEM	
13:00-14:00	Lunch	
14:00-15:00	Institutional Videos	Institute of Biomedical Sciences, USP Sao Paulo, SP, BrazilButantan Institute, São PauloRibeirão Preto Medical School, USP-RP, Ribeirão Preto, SP, Brazil
15:00-15:30	Coffee Break	
16:00-18:00	Visit at Unicamp	Dalton Laboratory, Fabio Gozzo, Institute of Chemistry, Unicamp, Campinas, SP, Brazil
		Neuroproteomics Laboratory, Daniel Martins-de-Souza, Institute of Biology, Unicamp, Campinas, SP, Brazil
6:00-8:00	Social Gathering	

Day 10: 06/09/2017

9:00-12:00	Visit at CNPEM
12:00-1:00	Meeting Evaluation
1:00-2:00	Lunch
2:00-3:00	Awards for the best presentations and Closing Remarks

Speakers

Adriana Franco Paes Leme
Brazilian Biosciences National Laboratory (LNBio) – CNPEM
Campinas, SP, Brazil

David Schriemer
University of Calgary
Calgary, AB, Canada

Eralp Dogu
Mugla University
Mugla, Turkey

Fabio Gozzo
Institute of Chemistry, Unicamp
Campinas, SP, Brazil

Giuseppe Palmisano
Institute of Biomedical Sciences, USP
Sao Paulo, SP, Brazil

Kathryn Lilley
University of Cambridge
Cambridge, United Kingdom

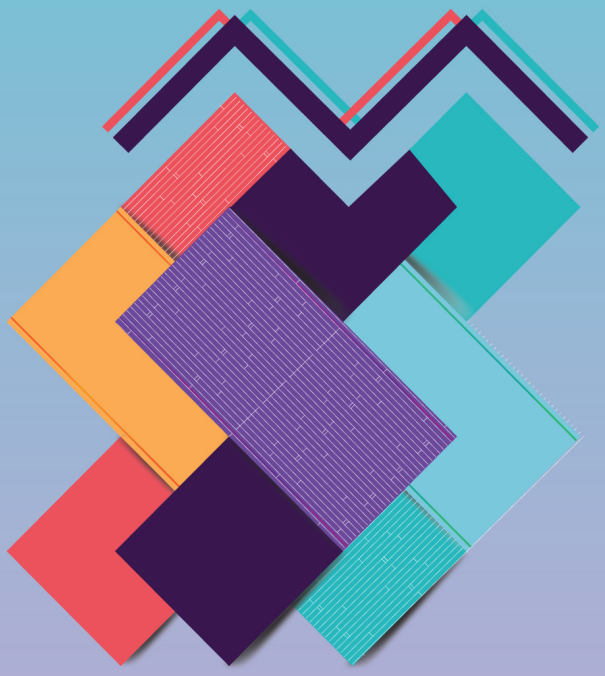
Martin Larsen
University of Southern
Odense, DK, Denmark

Oliver Schilling
University of Freiburg
Freiburg, Germany

Solange Serrano
Butantan Institute
Sao Paulo, SP, Brazil

Thomas Kislinger
University of Toronto
Toronto, Canadá

Vitor Faça
Ribeirão Preto Medical School, USP
Ribeirão Preto, SP, Brazil



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FAPESP LNBio CNPEM MINISTRY OF SCIENCE, TECHNOLOGY INNOVATION AND COMMUNICATION BRASIL BRAZILIAN GOVERNMENT

1. A STRUCTURE BASED PLASMA PROTEIN PRE-FRACTIONATION USING CONJOINT IMMOBILIZED METAL/CHELATE AFFINITY (IMA) SYSTEM

Karan Karkra, Kishore K.R. Tetala, M.A. Vijayalakshmi

1.PhD Scholar , Advanced Centre for Bioseparation Technology (CBST), VIT University, Vellore, Tamilnadu 632014, India

2. Assistant Professor, Advanced Centre for Bioseparation Technology (CBST), VIT University, Vellore, Tamilnadu 632014, India

3. Director, Advanced Centre for Bioseparation Technology (CBST), VIT University, Vellore, Tamilnadu 632014, India

The potential of immobilized metal/chelate affinity (IMA) in a continuous fashion, referred as conjoint approach, to pre-fractionate plasma proteins (in their native state) prior to LC–MS analysis was investigated in this study. Four transition metal-ions (Co (II), Zn (II), Ni (II) and Cu (II)) were individually chelated with IDA (iminodiacetic acid) coated CIM (Convective Interaction Media) disks and placed in a single housing in the following sequential order: IDA-Co (II) → IDA-Zn (II) → IDA-Ni (II) → IDA-Cu (II). The rationale behind this order is to retain proteins based on their specific requirement for surface exposed histidine topography. This structural pre-fractionation hypothesis was successfully proven using four human plasma proteins (fibrinogen, IgG, transferrin, and albumin) with varying histidine topographies. This conjoint IMA pre-fractionation strategy not only fractionated proteins (from plasma) based on their native surface histidine topography, but also identified 157 proteins from human plasma. The advantage of our conjoint IMA is its ability to fractionate proteins in their native state and reduce plasma complexity in a single step by employing single buffer system. We also investigated role of matrix in selection of proteins using 3 different matrixes.

2. AGGREGATION STRATEGIES OF SCORING FUNCTIONS FOR THE IMPROVEMENT OF THE STRUCTURE-BASED VIRTUAL SCREENING METHODS PERFORMANCE

Sotomayor-Burneo Stellamaris

Universidad Técnica Particular de Loja

The discovery of new crystal structures undoubtedly can help us understand the molecular basis of cellular processes and disease. These structures also have been used for the drug discovery process. My research is focused on molecular docking, which is a computational method that uses the tridimensional forms of proteins to measure protein-ligand interactions. Because of inaccuracies in the current molecular docking methods (scoring functions), pharmaceutical industries spend much time and millions of dollars on research, looking for active compounds (ligands with good biological activities) to test. Therefore, my research aims at developing novel structure-based (target protein-based) tools that can predict the ligands that will bind with the highest affinities to proteins with more accuracy and in less time so that industry and academia can invest their money more efficiently and effectively. In my research, I select the crystal structures of proteins and ligands. Then, I prepare them computationally according to the requirements of the computational tools (scoring functions) that I am using. Next I use the scoring functions to find the conformation of protein and ligand in which they fit best (as a complex) and then I use them to "score" the interaction within the complex. The score is a measure of how strong the intermolecular interaction is (binding affinity). Whether there will be a biological activity or not will depend on how much they fit each other and the binding affinity they have. Some of the structures I am working with are Cytochrome P450, Thyroid hormone receptor β, Glutamate receptor ionotropic AMPA-2 and Adenosine A2a receptor. I use the protein-ligand interaction results (measurements) to develop new computational methods. Mass spectrometry is and has been an indispensable tool for drug discovery and for structure-based virtual screening methods like molecular docking. It has permitted the discovery of more and more new potential drug-targets so that their function and molecular mechanisms can be studied. The number of drugs that has been discovered thanks to mass spectrometry increases exponentially with time. I am working on structure-based drug discovery and mass spectrometry is essential for that process. Without it, molecular docking would be impossible! There is no doubt that experimental (mass spectroscopy) and computational tools can achieve great things together!

3. ANALYTICAL METHODOLOGIES FOR DIFFERENTIAL DIAGNOSIS OF ZIKA, CHIKUNGUNYA AND DENGUE BY MASS SPECTROMETRY

1 Santos, F.N.; 2 Mesquita, P.R.R.; 3 Khouri, R.; 4 Martins, A.M.A.; 4 Magalhães, K.G.; 3 Barral, A.; 1 Eberlin, M.N.

1 University of Campinas;

2 Federal University of Bahia;

3 Oswaldo Cruz Foundation (FIOCRUZ);

4 University of Brasilia

Virus Zika, Chikungunya and Dengue have been a major public health challenge in Brazil due to climatic conditions favorable to the dissemination of the mosquito vector Aedes aegypti and its difficult control. The reappearance of Dengue, the sudden epidemic of Zika and Chikungunya, coupled with the large number of underreporting, inaccurate and false diagnoses have placed diagnostic methodologies as one of the central aspects of arboviruses research. Serological tests of virus Zika based on immunological reactions have low specificity and sensitivity. Molecular tests are effective in detecting RNA of the virus, but only in the short time after infection. Thus, the metabolomics and proteomics-based methods for detecting biomarkers of the virus or their interaction with the host appear as complementary tools for diagnosis, since they are discriminatory and can detect co-infections. The metabolomics and proteomics-based methods allow the detection of physiological variations at all stages of the infection, the immunological responses and the resulting clinical modifications. High resolution mass spectrometry (HRMS) is the most used technique in metabolomic/proteomic studies for the identification and characterization of biomarkers from various diseases as well as viral infections due to: (i) high sensitivity and specificity that allow obtaining reliable results in a short time; (ii) simplicity of execution of the analysis that is easily performed after standardization of the methodology, and can be automated; (iii) the high initial cost of the equipment can be quickly recovered due to the low cost of the analyzes and very high frequency of analysis. Therefore, in this work we apply metabolomics and proteomics-based in high resolution mass spectrometry to identify specific biomarkers of the Zika, Chikungunya and Dengue virus infections, perform the differential diagnosis of these arboviruses and closer to the actual clinical state of the patient, through quantification of biomarkers.

4. AUTOMATIC SELECTION OF DISCRIMINATIVE TOP-DOWN MASS SPECTRA WITH DIAGNO-TOP: APPLICATION TO THE DIFFERENTIATION OF BACTERIAL PATHOGENS

Lima, DB; Dupré M; Silva, ARF; Carvalho, PC; Chamot-Rooke, Julia

1. Structural Mass Spectrometry and Proteomics Unit CNRS USR2000 Mass Spectrometry for Biology, Paris, Île-de-France, France

2. Computational Mass Spectrometry & Proteomics Group, Carlos Chagas Institute, Curitiba, Paraná, Brazil, Curitiba, PR, Brazil

Classification of bacteria by MALDI profiling has become a widespread application. However, the approach has shown several limitations, such as in identifying microorganisms that are very much alike (e.g., enterobacteria) or classifying bacteria that derive from resistant strains or not. Here, we overcome these shortcomings with an approach called DiagnoProt for top-down proteomics (Diagno-Top), which branches from an existing software, but tailored towards top-down proteomics data. Diagno-Top can correctly classify proteomic profiles originating from collections of tandem mass spectra of intact proteins (LC-MS/MS experiments), thanks to an innovative algorithm, which applies quality control filters and clusters top-down fragmentation spectra by comparing different datasets. Diagno-Top can also interface with existing spectrum identification search engines to attribute IDs for the identified clusters.

5. BIOLOGICAL EMBEDDING OF ENVIRONMENTAL RISK. CHARACTERIZATION OF STRESS RELATED PROTEIN REGULATION FOLLOWING EARLY LIFE ADVERSITY

1 Zang, J.C.S.; 2 May, C.; 3 Kumsta, R.; 4 Marcus, K.

1 Ruhr University Bochum, Department of genetic psychology;

2 Ruhr University Bochum, Medizinisches Proteome Center (MPC);

3 Ruhr University Bochum, Department of genetic psychology;

4 Ruhr University Bochum, Medizinisches Proteome Center (MPC)

Early trauma experience has been linked to a wide range of psychological and physical health problems in adulthood. While research constantly describes dysregulations of the physiological response to stress as a strong contributor, the molecular mechanisms mediating observed correlations remain poorly understood. As sensitivity of epigenetic modifications towards environmental influences has been demonstrated in rodents, changes within the epigenetic landscape represent a promising model of how early adversity becomes embedded on the molecular level. A growing body of research integrates the study of epigenetic effects into epidemiological studies. However, most investigations rely exclusively on quantitative levels of DNA methylation, without consideration of downstream processes. The presented project aims to integrate proteomics into the investigation of the long-term effects of early adversity. A sample of adults (n=30) with a history of childhood trauma and a matched control group (n=30) were subjected to a psychosocial laboratory stress protocol. Prior and post stress induction three blood samples were taken for the isolation of CD 14+ monocytes via immunomagnetic cell separation. Following cell homogenization and isolation of protein, the concentration of extracted proteins was determined in duplicates by amino acid analysis. Proteome analysis was performed by LC-MS/MS using a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer coupled to a nano-electrospray ion source. Of 3846 protein groups, 3519 were identified by a minimum of 2 unique peptides. 1162 protein groups that were present in at least 80% of the trauma and/or the control group were accepted for further analysis. Overall, 262 protein groups showed a significantly different expression between trauma and control group and might play a role in the mediation of trauma-induced health risk. So far, these preliminary results demonstrate a functional impact of early trauma experience on the protein level of monocytes and highlight the feasibility of studying protein expression following stress exposure in the context of bio-psychological trauma research.

6. CARCINOEMBRYONIC ANTIGEN CARRIES A2-3 LINKED SIALIC ACID TYPE II CHAINS AND IS IMPLICATED IN THE METASTATIC POTENTIAL OF GASTRIC CANCER CELLS

1 Catarina Gomes; 2,3 Andreia Almeida; 1 Ana Cintia Barreira; 1,3 Hugo Osório; 2 Hugo Sousa; 1,3 João Pinto-de-Sousa; 2 Daniel Kolarich; 1,3,4 Celso A. Reis

1 Institute of Molecular Pathology and Immunology of the University of Porto. IPATIMUP, Porto, Portugal

2 Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

3 Free University of Berlin, Berlin, Germany

4 Faculty of Medicine, University of Porto, Porto, Portugal

5 Institute of Biomedical Sciences of Abel Salazar, ICBAS, Porto, Portugal

Alterations in glycosylation are an important hallmark of cancer cells. Due to their implications in disease and cancer, they currently constitute a main source of tumour markers. There are distinctive alterations that are consequence of the deregulated expression of glycosyltransferases such as α2-3 sialyltransferases that lead to expression of the SLeX antigen (Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc-R). Previous work has demonstrated that overexpression of ST3Gal IV enzyme in MKN45 gastric carcinoma cells culminated in alterations in the cell glycome by instance, switching of α2-6 linked sialic acid towards an increased expression of α2-3 linked sialic acid. The expression of these altered glycan structures promoted the activation of signalling pathways that were involved in cancer cell malignancy both in vitro and in vivo. Herein, we aimed to identify protein carriers of altered glycan structures associated to the overexpression of ST3Gal IV. Carcinoembryonic antigen (CEA) was identified by MALDI-TOF-MS as a major protein target of glycan alterations in MKN45 cells. The presence of α2-3 terminal sialylated structures like SLeX was confirmed by immunoprecipitation and proximity ligation assays (PLA) in CEA. In addition, selectin ligand binding assay further demonstrated that ST3Gal IV expressing cells have the capacity to bind to E-selectin and CEA was detected as a major ligand. Furthermore, the glycan composition of immunoprecipitated CEA was assessed by nanoPGC-LC-ESI-MS/MS analysis. The detailed structural analysis of carcinoembryonic antigen N-glycans showed that CEA carries type II chains (Galβ1-4GlcNAc) capped with α2-3 linked Neu5Ac. Ultimately, it was evaluated the expression of CEA and SLeX in a series of gastric carcinoma cases demonstrating that 83.9% of the total cases co-expressed both CEA and SLeX. This association was further confirmed by CEA/SLeX molecular in situ expression by PLA in 80.6% of the cases. The in situ expression of CEA/SLeX molecular complexes in the tumour tissues was correlated with the pattern of tumour growth and venous invasion. In conclusion, in this work we identify for the first time CEA as one of the major glycoprotein target of glycan alterations in gastric cancer. This major finding may contribute to improve gastric cancer specific diagnosis and monitoring disease and open a window of opportunities to clarify the role of CEA altered glycosylation in gastric cancer cell behaviour.

7. CHANGES IN THE PROTEIN COMPONENTS OF RADICULAR SYSTEM OF ELAIS GUINEENSIS PLANTS SUBJECTED BY FATAL YIELLOW

NASCIMENTO, S.V; CUNHA, R. L; VALADARES, R. B. S; MAGALHÃES, M. M.; OLIVEIRA, G. C.

1 Federal Rural University of Amazonia

2 Embrapa eastern amazonia

3 Vale Institute Of Technology (ITV)

4 Embrapa eastern amazonia

5 Vale Institute Of Technology (ITV)

Several studies have been carried out aiming to identify the initial cause of fatal yellowing (FY) in oil palm. However there is still no consensus on the true origin of this anomaly. Thus, analyzes are needed to obtain more convincing results. Proteomic analyzes have allowed to obtain the qualitative and quantitative protein profile with speed and precision. This work aimed to perform analyzes by bi-dimensional liquid cromatography coupled to mass spectrometry (2D-UPLC/MSE) to identify changes in protein profiles in oil palm afected by FY. For the analyzes, Elaeis guineensis Jacq. roots of plants with and without aerial symptoms were sampled from two growing areas. The differential proteome was obtained by comparing plants with and without symptoms and between plants at different stages of FY symptoms. There was a differential accumulation of stress and energy metabolism related proteins. Proteins of energetic metabolism, including the enzyme alcohol dehydrogenase, showed a high accumulation at all stages of FY development. Alcohol dehydrogenase and aldehyde dehydrogenase were identified in both conditions, the latter being more abundant in asymptomatic plants. Proteins as ferritin, tranketolase, annexins, lipoxygenase, S-adenosylmethionine synthase, alcohol dehydrogenase, aldehyde dehydrogenase and cinnamyl alcohol dehydrogenase, among others, were only identified in asymptomatic and therefore have pontencial to be used in the future as markers for selection of oil palm (Elaeis guineensis Jacq.) FY tolerant genotypes. Stress related proteins showed enhanced accumulation in asymptomatic plants when compared to plants with FY symptoms. Between different stages the stress related proteins were more abundant at the more advanced stage. These results suggest that changes in abiotic factors could trigger FY development and biotic agents may not be the only ones responsible for the FY development.

8. CHARACTERIZATION OF CHANGES IN PROTEOME PROFILE OF BOVINE SKELETAL MUSCLE WITH EXTREME VALUES FOR CONJUGATED LINOLEIC ACID AND OLEIC ACID CONTENTS

1 Poleti, M.D.; 1 2 Simas, R.C.; 1 Cesar, A.S.M.; 1 Oliveira, G.B.; 3 Andrade, S.C.S.; 4 Souza, G.H.M.F.; 5 Cameron, L.C.; 6 Regitano, L.C.A. Regitano; 1 Coutinho, L.L.

1 ESALQ USP College of Agriculture Luiz de Queiroz (Av. Pádua Dias,11 Cx Postal 9 Piracicaba/SP CEP 13418 900),

2 UNICAMP University of Campinas (R. Sérgio Buarque de Holanda, S/N, Campinas/SP CEP 13083 859),

3 IB USP Genetics and Evolutionary Biology Department (R. do Matão, 277, São Paulo/SP CEP 05508 090),

4 WATERS MS Applications & Development Laboratory, Waters Corporation (Alameda Tocantins, 125, Barueri/SP CEP 06455 020),

5 LBP UNIRIO Federal University of State of Rio de Janeiro (Av. Pasteur, 296,Urca Rio de Janeiro/RJ CEP 22290 240),

6 EMBRAPA Embrapa Southeast Livestock (Rodovia Washington Luiz, Km 234 São Carlos/SP CEP 113560 970)

Fatty acids (FA) composition in muscle considerably contributes to various aspects of beef quality and nutritional value. Studies have demonstrated that oleic acid (OA) is related with meat's overall palatability and reduced risk for metabolic disease in humans, and conjugated linoleic acid (CLA) has anti carcinogenic and anti obesity properties. The objective of this study was to identify differentially abundant proteins and biochemical pathways associated with CLA and OA synthesis in bovine skeletal muscle. Proteins were extracted from Longissimus dorsi muscle of 40 animals (20 for each FA) selected according to the extreme genomic estimated breeding values (GEBV) for conjugated linoleic acid (CLA cis9 trans11) and oleic acid (OA, C18:1 cis9) contents. For analysis of differentially abundant proteins, the animals were divided into two groups, High (H, 10 animals) and Low (L, 10 animals) GEBV. An integrated transcriptome assisted label free quantitative proteomic approach by High Definition Mass Spectrometry (HDMSE) was used to characterize the proteome profile (protein abundance) of the bovine skeletal muscle. Mass spectrometry data were acquired with the use of a nanoAQUITY 2D chromatography coupled to a nanoESI (+) source on a Synapt G2 S HDMS mass spectrometer controlled by Waters MassLynx v4.1 and processed using Progenesis QI for Proteomics v2.0 (Nonlinear Dynamics, UK). A total of 2,862 and 2,645 proteins were identified, from these 1,657 and 1,532 proteins were quantified in CLA and OA samples, respectively. Comparative analysis for CLA and OA groups revealed 113 and 102 differentially abundant proteins (DAP, P

9. CHARACTERIZATION OF THE FACTORS INVOLVED IN THE REGULATION OF FAS I SYSTEM IN MYCOBACTERIA

1 Cabruja, M.; 1 Gramajo, H.; 1 Gago, G.

1 Institute of Molecular and Cell Biology of Rosario (UNR - CONICET)

Our group is studying the regulation of lipid biosynthesis in mycobacteria, which includes the causative agent of tuberculosis. The genera mycobacterium unlike other microorganisms has two fatty acid synthase (FAS) systems, FAS I and FAS II, working in concert with the polyketide synthases (PKS) to produce the vast diversity and complexity of their lipids. Briefly, FAS I performs de novo biosynthesis of acyl-CoAs and FAS-II elongates them to synthetize very long-chain meromycolyl-ACPs (up to C56), which become the precursors of mycolic acids. These mycolic acids are the main component of the cell envelope which is a key factor related with the persistence of M. tuberculosis within the cell and the intrinsic resistance of this microorganism to a broad array of antibiotics. Interestingly, despite extensive literature on the biosynthesis, and biological function(s) of the major cell envelope components of M. tuberculosis, little is known regarding the mechanisms allowing the bacterium to modulate the expression of these components in response to environmental changes. Therefore, the overall aim of this project is to unveil the global regulatory network controlling de novo synthesis of membrane lipids in M. tuberculosis and to demonstrate that the regulatory components involved in such regulation could become drug-targets for the development of conceptually new anti-mycobacterial agents. Our research group has recently identified and characterized two essential transcriptional regulators involved in lipid biosynthesis: FasR controls the expression of fas, the gene encoding for the FAS I enzyme and MabR is a transcriptional regulator of the fasII operon (FASII system) in mycobacteria. Phosphorylation of proteins by Ser/Thr protein kinases (STPKs) has recently emerged as a major physiological mechanism of regulation in mycobacteria and is particularly associated with the modulation of several proteins involved in lipid biosynthesis. In order to evaluate if FasR is post-translationally regulated, we performed in vitro phosphorylation experiments using purified M. tuberculosis FasR and several STPKs. We found that FasR is efficiently and specifically phosphorylated in vitro by the STPK PknB. For the determination of the exact phosphorylation residue(s) we plan to perform an MS-based methodology. The identification of these residues will allow us to analyze the consequence of phosphorylation on FasR activity in vitro and in vivo through site-directed mutagenesis.

10. COMPARATIVE PROTEOMICS STUDIES BETWEEN MYCOPLASMA HYOPNEUMONIAE AND MYCOPLASMA FLOCCULARE

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Mycoplasma hyopneumoniae and Mycoplasma flocculare cohabit the porcine respiratory tract. However, M. hyopneumoniae is a pathogen, causing the porcine enzootic pneumonia, while M. flocculare is a commensal bacterium. Comparative genomic-scale studies demonstrated high similarity between these species, which includes the sharing of all predicted virulence factors. In this context, it can be assumed that the virulence-related differences between M. hyopneumoniae and M. flocculare may be associated to post-transcriptional steps. The aim of this study was to perform a comparative analysis between the soluble secretomes and the whole proteomes of the pathogenic Mycoplasma hyopneumoniae and its closely related commensal Mycoplasma flocculare. In the secretome analyses, 62 and 26 proteins were identified as secreted by M. hyopneumoniae and M. flocculare, respectively, being just seven proteins shared between these bacteria. In M. hyopneumoniae secretome, 15 proteins described as virulence factors were found; while four putative virulence factors were identified in M. flocculare secretome. The comparative analysis of the secretomes of M. hyopneumoniae and M. flocculare uncovered, for the first time, clear differences between these two species that can be associated with pathogenicity and commensalism, respectively. Now, a comparative study between pathogenic and non-pathogenic strains of M. hyopneumoniae and the commensal M. flocculare whole proteomes is being carried out, to found new differences that support the secretome results and help to elucidate the pathogenic nature of M. hyopneumoniae to swine hosts.

11. COMPARATIVE VENOM PEPTIDOME AND CONSTRUCTION OF PEPTIDE LIBRARY FOR SCORPIONS OF THE GENUS TITYUS (T. SERRULATUS, T. BAHIENSIS AND T. OBSCURUS).

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The arthropods constitute one of the most abundant and widely distributed groups of animals on earth. In Brazil, Tityus is the main genus of scorpions responsible for envenomation incidents. This genus belongs to the family Buthidae and is represented by 35 species. Lethal scorpion venoms primarily consist of peptide toxins which target a variety of voltage-gated sodium, potassium, calcium or chloride ion channels in neuronal cells, and in cardiac, smooth and skeletal muscle. T.serrulatus, T.bahiensis and T.obscurus are the most important species causing envenoming of medical importance. Several technological innovations such as the combination of liquid chromatography and mass spectrometry, high-resolution systems with quadrupole and ion-trap analyzers are being used for detection and sequencing of peptides. This study aims to identify and sequence a series of neglected peptides from T. serrulatus, T.bahiensis and T.obscurus venoms, allowing the construction of a library of novel peptides. The peptides were extracted in presence of 50% (v/v) MeCN, analyzed with a LCMS-IT-TOF system, which allows the use on-line used of a semi-micro chromatography, coupled to high speed separation and analysis under high accuracy and high mass resolution. The analysis of the venom was carried out under gradient of MeCN from 5 to 95% (v/v) [containing 0.05% TFA (v/v)] for 90 min, using a column XBrigdeTM BEH300 C18 [3,5µm 2,1x100mm (WATERS), with pores of 120 Å]. The elution of components was monitored at 214 nm with a flow of 0.2mL/min during 90 minutes. The eluents were analyzed in positive mode (ESI+) continuously throughout the experiment. More than 60 peptides were detected and sequenced with the help of different algorithms (MASCOT DISTILLER; PEAKS) and manual deconvolution and interpretation. The peptides were classified in important groups of toxins, like hypotensins, allergens and antimicrobial peptides. Financial Support: BIOPPROSPECTA/FAPESP, CNPq, CAPES Toxinology Program.

12. DEVELOPMENT AND IMPLEMENTATION OF NEW PROTEOMIC TECHNIQUES AT CEQUIBIEM (MASS SPECTROMETRY CORE FACILITY)

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The main objective of this project is to organize and promote the development and expansion of the services provided by the Mass Spectrometry Facility CEQUIBIEM. At CEQUIBIEM we provide support to the entire scientific community in Argentina and neighboring countries in the field of mass spectrometry applied to proteomics.

This includes set up and development of new techniques, and consulting services for an appropriate experimental design, data interpretation and analysis of results. Among our equipment we have a Bruker MALDI-TOF/TOF ultraflex II and a Thermo Scientific ESI-Q-Orbitrap Q Exactive coupled to a nanoLC. We provide services of protein identification by MS and MS/MS, molecular weight determination of proteins and peptides, post-translational modifications studies, relative quantification analysis (LFQ) and biosimilar studies.

My main activity is the operation of Q Exactive, including sample processing and analysis, data processing and reporting for clients. Since the acquisition of the Q Exactive in our laboratory, I spend a lot of my time setting up and developing new techniques that can be performed with this instrument, in order to meet the needs of researchers and to expand the scope of experiments and results obtained.

The first technique implemented was Label Free Quantification, as well as molecular weight measurement of proteins and peptides up to 30 kDa. We are currently developing the Paralell Reaction Monitoring technique and in the future we intend to start developing a Data Independent Acquisition technique.

13. DEVELOPMENT OF A SPECTRAL DATABASE BY MALDI-TOF RESULTING FROM A RAPID CHARACTERIZATION OF THE PROTEIN PROFILE OF CONTAMINATING BACTERIA PRESENT IN THE FIRST-GENERATION ETHANOL FERMENTATION PROCESS

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The sugar and alcohol industry moves a significant part of the Brazilian economy. The valorization of ethanol as an alternative to fossil fuels motivated the expansion of sugarcane planting and, coupled with high-yield fermentation strains, provided an increase in productivity in mills, making the Brazilian sugar and alcohol complex one of the most competitive in the world. The process of producing ethanol from the by-products of sugarcane is called fermentation, which occurs through specific microorganisms, which are capable of producing ethanol (C2H5OH) from fermentable carbohydrates, such as glucose (C6H12O6), releasing CO2. The microorganism most used in fermentation processes, due to its high performance and adaptability, is Saccharomyces cerevisiae yeast. As large-scale fermentation does not take place in an aseptic environment, the presence of contaminants, largely bacterial, is a recurring process and may adversely affect the fermentation yield, causing great losses. Thus, identification of contaminating bacteria by mass spectrometry techniques allows a rapid and efficient identification of contaminating microorganisms, which can prevent drastic falls or interruption of the process. Thus, this work aims to construct a spectral database by MALDI-TOF referring to the characteristic protein profile of each contaminating microorganism from the previous characterization of lactic acid bacteria at the species level based on the sequencing of the 16S rDNA region together With the sequencing of three housekeepings genes pheS, rpoA and groEL by the MLSA technique.

This identification will serve as a basis for the identification of the characteristic protein profile made by MALDI-TOF, whose results will be accessible in-house database, Which will subsequently be made public.

14. DEVELOPMENT OF EXTRACTION METHODS FOR THE PURIFICATION AND IDENTIFICATION OF PROTEINS FROM GOAT MEAT

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Development of extraction methods for the purification and identification of proteins from goat meat Human beings need nutrients for their daily survival, with food being the main route for the acquisition of nutrients. The metabolism of nutrients provides the energy necessary for the body to perform its vital functions, for example: production, locomotion, growth, among others. The nutritional quality of food is related not only to the concentration of minerals, but also to the concentration and type of proteins present. In this context, the demand for goat meat has been increasing in relation to the other red meat consumed in the market, since it has played a significant role in human nutrition because it presents essential amino acids such as lysine, threonine, tryptophan, low fat and cholesterol, low calorie and high digestibility, in addition to high levels of protein and iron.According to USDA estimates, in 2004 the world herd of goats was around 849,065,423 animals. In 2014, there was a significant increase, with the amount of animals around 1,006,785,725, 2.2% in Europe, 0.4% in Oceania, 4.3% in the Americas, 32.9% in Africa and 60.2% in Asia. Regarding to the proteins, these are present in the bodies of living beings in many possible ways, it has been characterized as very complex macromolecules with polar regions, hydrophobic and charged, which have functions: catalytic, transport, structural, among others. When working with proteins, they are not static and modify according to the conditions and stimuli of the medium which makes the study a complex task. In light of the above, the extraction of these biomolecules in goat meat was obtained through the optimization of four variables (Extraction time, extraction temperature, concentration extractor and extractor volume), this step was done using factorial planning and surface response methodology. The optimum conditions for the proposed method were: 0.05 mol L-1 for the concentration extractor (Tris-HCl pH 7.3),extraction time of 10 minutes, temperature of 44 °C and extractor volume of 3.5 mL.The protein content extracted under optimum conditions was significantly higher than some results reported in the literature. The next stage of this study requires the knowledge to be acquired with the participation in SPSAS-MS, once it will be the separation of the proteins by SEC-DAD, collection of the fractions, realization of the triptic digestion for later identification of the proteins using nESI-Q-Tof.

15. DIET IMPACTS PREIMPLANTATION HISTOTROPH PROTEOME IN BEEF CATTLE

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In ruminants, the period from oocyte fertilization to implantation is relatively prolonged, and survival of embryos depends on uterine secretions, or histotroph. Diet impacts conception rates following timed artificial insemination (AI) in beef cattle; we thus hypothesized diet affects histotroph. Our objective was to determine if diet fed prior to timed AI treatment impacted histotroph proteome in Angus-Simmental cattle. Cows were assigned to 1 of 4 isocaloric diets: silage-based total mixed ration (CON), high protein (PROT), high fat (OIL), or high protein and fat (PROT+OIL). After ~190d on diets, an intravaginal progesterone implant (CIDR) was inserted into cow for 7 days for timed AI treatment. At 9 days post CIDR removal, animals were selected (n = 16; 4/treatment) for presence of a corpus luteum using ultrasonography. Histotroph was collected by flushing 30cc of a sterile saline solution into the uterine horns using a Foley catheter. The uterus was massaged via palpation, and the saline flush was collected. Samples were frozen, freeze dried, and reconstituted for digestion with Trypsin/Lys-C protease. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was run using a Q Exactive HF. Proteins were identified by comparing against a bovine specific database (UniProt) using MaxQuant software, and differential expression of proteins was determined using analysis of variance (ANOVA) and Tukey adjustments (P-adj) run in 'R'. DAVID Bioinformatics Resources 6.8 was used for functional analysis. Over 2000 proteins were considered expressed (n≥3 cows in a treatment), with 1239 proteins common among every group. There were 20, 37, 85, and 123 proteins unique to CON, PROT+OIL, PROT, and OIL, respectively. Relative to the CON group, 23, 14, and 51 proteins were found to be differentially expressed (P-adj

16. DIFFERENTIAL PROTEIN EXPRESSION IN THE PREFRONTAL CORTEX OF MICE CHRONICALLY EXPOSED TO THE ANTIPSYCHOTIC DRUGS HALOPERIDOL AND CLOZAPINE

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Psychotic disorders are very serious and costly mental diseases. Throughout the years the understanding of the genetic basis of these illnesses has improved considerably but there is only rudimentary knowledge of its pathophysiology, mostly due to inconsistent results and confounding factors. In the case of schizophrenia, several explanations for the origin of the disorder have emerged, such as the dopaminergic, glutamatergic or serotoninergic hypotheses. Having this in mind, 2 antipsychotics were selected: haloperidol and clozapine, first and second-generation antipsychotics, respectively. Haloperidol acts essentially in D2 receptors, and clozapine, along with softer action in D2, also has affinity for 5HT and other receptors. The main goal of this project is to investigate the differential protein expression in the rodent prefrontal cortex following chronic exposure to commonly prescribed psychotropic medication, with the objective of distinguishing between pharmacological and disease-related changes and correlating expression profiles with drug effects. Two different quantitative mass spectrometry proteomics approaches (label-free SWATH and stable isotope labelling (iTRAQ)) were used to investigate the differential protein expression in the mice cortex, following chronic exposure to haloperidol and clozapine. Moreover, a subcellular fractionation was performed giving rise to two distinct fractions, membrane-enriched and soluble fractions. In total, over 2000 proteins were quantified, where about 60% of the quantified proteins in the membrane-enriched fraction are annotated as "membrane". As expected, the majority of the quantified proteins in each drug injected mice maintained a stable expression when compared with the control mice, nonetheless some statistically meaningful differences were observed. Using a principal component analysis with these significantly altered proteins it was possible to distinguish between the control and medicated groups. Moreover, when gene ontology analysis was performed for these proteins, the majority are related with metabolic processes, suggesting a metabolic shift caused by the two drugs. Also, some proteins involved in synapse and synaptic vesicle cycle and calcium signaling show altered expression.

In summary, this study can give clues about the mechanism of action of long-term antipsychotic exposure and depict target molecular pathways for the investigation of psychotic disorders' physiology and treatment.

17. DIFFERENTIAL PROTEOME ANALYSIS OF PRICKLY PEAR (OPUNTIA STRICTA) IN RESPONSE TO INFESTATION BY CACTUS SCALE (DIASPIS ECHINOCACTI)

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Prickly pear is a relevant crop as forage for the semi-arid region in the Brazilian Northeast during periods of drought. It has suffered reduction in productivity due to the attack of cactus scale (Diaspis ehinocacti). This work aimed to verify the differential accumulation of Opuntia stricta proteins in response to cactus scale infestation. One year aged clones of O. stricta (variety IPA 200016) will submitted or not (treatment control) to cactus scale infestation, which occurred by contact with previously infested plants fragments. Secondary cladodium will be harvested and proteins extracted by phenol-based method. By two-dimensional gel electrophoresis, allowing the identification of exclusive or common proteins significantly differential between treatments (DEPs) through software Image Master 2D Platinum programs v.7.05 (GE Life Sciences). DEPs will be analyzed by mass spectrometry (AutoFlex III ToF/ToF, Bruker Daltonics, Bremen, Germany) for putative identification. Subsequent analysis will allow the elucidation of their function, location and metabolic mechanism. The identification of proteomic markers in prickly pear provides information at the molecular level and may be an useful tool in this crop breeding.

18. DIFFERENTIAL PROTEOMICS USING STABLE ISOTOPE DIMETHYL LABELING AND PATTERNLAB FOR PROTEOMICS

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Accurate quantification of protein expression in biological systems lies within the heart of proteomics. In an effort to improve on quantitation, several widely adopted proteomic approaches incorporate differential stable isotopes in samples for relative protein quantification. In particular, dimethyl labeling has shown to be reliable; besides, it uses inexpensive reagents and is applicable to any sample type.

This poses dimethyl labeling as an attractive solution for any core facility or proteomics lab. Here, we present a new module, soon to be incorporated into the widely adopted PatternLab for proteomics, that can statistically pinpoint differentially abundant dimethyl labeled peptides, and ultimately, infer changes at the protein level according to a rigorous statistical approach.

19. DISCOVERING THE ROLE OF BACTERIAL MICROVESICLES IN INFLAMMATORY BOWEL DISEASE (IBD)

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Inflammatory bowel disease (IBD) manifests in chronic inflammation of the intestinal epithelium. The main symptoms include severe abdominal pain, vomiting, diarrhea, rectal bleeding and weight loss. IBD is commonly diagnosed as two forms, namely ulcerative colitis (UC) or Crohn's disease (CD).

To date IBD is already considered as a global disease, for its prevalence has been increasing world-wide since the 1930s, and at the moment it is set to appr. 1 % in western countries. IBD is a multifactorial disease that is affected by moderate genetic predisposition and several environmental factors including diet, socioeconomic status, antibiotic use and exposure to microbes. Still, the specific disease pathogenesis remains unknown. The recent rapid progress in the study of human microbiota has revealed its significance in both health and disease. In fact, there is already evidence of intestinal microbiota affecting the development of inflammation and ulcers in IBD. However the detailed mechanisms are yet to be resolved. One possible candidate operating on the pathogenesis is the bacterial microvesicles (MVs).

These membrane vesicles assist bacteria in adapting to and communicating with their environment. The interactions are mediated via a targeted cargo of e.g. DNA, RNA and proteins carried in the MVs. Bacterial MVs have been shown to regulate innate and adaptive immune responses. They have also been affiliated with malignant processes such as gastric ulcer and sepsis by releasing virulent factors or toxins into their target cells. Hence they could be implicated in also other inflammatory diseases. Here we aim to elucidate the role and function of bacterial MVs in IBD. We plan to harvest the bacterial MVs from fecal samples of IBD patients and healthy controls. We will further apply multiomics approach to identify the contents of the MVs, including their proteome, with the goal of identifying components behind the malignant process. The project is currently under planning, but most likely the proteome will be discovered using LC/MS-MS and the raw data processed using MaxQuant and MS/MS spectra searched using the Andromeda search engine against the corresponding Protein Database. Finding IBD specific proteomic profiles of bacterial MV origin could serve as a non-invasive diagnostic tool in the future.

20. DISCOVERING THE ROLE OF EXTRACELLULAR VESICLES AND THEIR MOLECULAR CARGO IN ORAL CANCER PROGRESSION

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Introduction Oral cancer mortality rates have increased by 10% in the last decade. Efforts to reverse this are hampered by a limited understanding of the underlying molecular complexity of the disease. Recently, interest has grown in the contribution of extracellular vesicles (EVs) to cancer pathogenesis. Since the discovery of mRNA and miRNA in EVs, they have been considered a signalling system capable of delivering a range of potentially behaviour changing biological molecules to local or distant cells. Developing tumours exist as a complex milieu comprising multiple cell types which necessitates a large amount of cross talk and communication between the different cell types. This is a role that EVs have been shown to play in other cancers. The aim of this work is to explore the role of EVs, and their cargo (protein and RNA), in oral cancer progression. Methods EVs were extracted from the culture media of oral cancer cell lines by ultracentrifugation or size-exclusion chromatography and characterized by transmission electron microscopy (TEM), tuneable resistive pulse sensing (TRPS) and western blotting. The ability of these EVs to transfer from cancer cells to other cell types was visualised using a fluorescence microscopy. Any impact of this uptake on the phenotype of the recipient cells was tested by a range of functional assays. In order to identify potential mechanisms responsible for any observed effects on the cells, RNA and protein cargo of the vesicles were identified by ion torrent sequencing and mass spectrometry, respectively.

Results Cell lines representative of different stages of tumorigenesis produced between 1500-4000 EVs per cell per 24 hours ranging in size from 50-200 nm and bearing EV markers. The uptake of EVs by normal stromal cells was shown to exert a range of biological effects including fibroblast activation and stimulation of endothelial cell tubule formation. Analysis of sequencing and mass spectrometry data has revealed protein and RNA candidates which could lead to identification of mechanisms for the observed effects. Conclusion Our data indicates that oral cancer cell-derived EVs are able to transfer miRNA and proteins to other cells of the oral cancer microenvironment leading to changes in stromal cell phenotype that are potentially beneficial to a developing tumour.

21. EARLY-STAGE BLOOD-BASED PROTEOMIC DIAGNOSIS OF COLORECTAL CANCER

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Background: Colorectal cancer (CRC) is the 2nd most common cause of cancer-related mortality worldwide. 5-year survival rates of CRC patients found with early tumors is >90% following colonoscopy and surgical resection. Development of high compliance blood-based diagnostic tests for the early and sensitive detection of tumors remain key factors for improving CRC patient survival. Low abundance proteins released directly from the tumour or from the tumour microenvironment into plasma may provide a source of minimally-invasive diagnostic/prognostic biomarkers and aid in our understanding CRC pathobiology.

Methods: A comprehensive exploratory study was performed using SWATH MS on 100 patient plasma samples (i.e., pools n=20 for each of four CRC stages: Dukes' stage A-D) against healthy, age- and sex-matched individuals (also pooled from n=20 healthy individuals). For deeper insights into the plasma proteome,pooled plasma samples were doubly ultradepleted using an in-house IgY chicken antibody depletion method (depletes ~ 200 high to medium abundance plasma proteins) and commercially-available Agilent MARS-14 system that depletes the 14 most abundant plasma proteins.

Results: Statistical analysis was performed on technical triplicates run on SWATH. Out of 250 quantifiable proteins, 6 protein candidates F2, HGFAC, PON1, CST3, ADAMDEC1 and CFD exhibited differential expression across all CRC stages compared to healthy controls. We are currently progressing towards target validation for all 6 candidates. We have successfully established a CST3 western blot assay that validates decreased expression in the earlier stages of CRC in pooled plasma as was observed by SWATH-MS.

Conclusions: The Human Protein Atlas illustrates that ADAMDEC1 mRNA is highly expressed in small intestine, rectum and colon. CST3 and PON1 has been reported as downregulated proteins in later stages of rectal cancer, thereby making the 6 plasma protein candidates we discovered as MRM assay targets for CRC early diagnosis prior to accurate confirmatory colonoscopy followed by surgery. Future work will involve quantification and validation of identified targets on individual plasma samples and development of immuno-MRM based assays. Keywords: Colorectal cancer, plasma, SWATH-MS, DIA, immuno-MRM

22. EFFECT OF GASTRIC PLICATION SURGERY ON PROTEIN PROFILE OF OBESE PEOPLE USING MASS SPECTROMETRY TECHNIQUES

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The prevalence of obesity and being overweight and also their associated metabolic disorders such as type 2 diabetes is increasing globally. Among the various existing options for the obesity treatment, bariatric surgery is the most effective procedure with long-term effects on weight loss and also can cause resolving or improving type 2 diabetes.

Laparoscopic gastric plication is a new bariatric surgery procedure in which no portion of stomach is removed. The precise weight loss mechanism by which patients experience less hunger is not well understood. It has been suggested that food restriction may not be the only reason for weight loss, and neuro-hormone changes may also be playing a role. My focus is on investigating serum proteomic profiles of obese patients to reveal the molecular mechanisms involved in the regulation of appetite, and glucose metabolism, following the mentioned surgery. Based on surgery outcomes and understanding the pathways involved in appetite modulation, it may be possible to develop alternative therapies for obesity. Untargeted quantitative proteomic analysis of obese sera will be performed on serum of 15 patients before surgery, and 40 days and 4 months postoperatively, using MStern blotting and timsTOF as samples preparation method and mass spectrometry technique respectively.

23.EFFECT OF SELENIUM ON PROTEOME OF ALZHEIMER'S DISEASED MICE CORTEX AND HIPPOCAMPUS

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Alzheimer,s diasease (AD) is responsible for neurodegeneration resulting in loss of memory and cognition. Over 35 million people throughout the world are affected from this disease. In addition to the burden on the population, it is hypothesized that the considerable health and care burden of AD will impose a large economic burden throughout affected economies especially China with huge population above age 60. Previous economic studies estimate the 2009 worldwide cost of dementia to be US$421 billion and attribute 10% of the global burden (US$41 billion) to China.

The hallmark pathological features of AD are loss of synaptic connections, neuron death, intracellular precipitation of hyperphosphorylated tau protein causing neurofibrillary tangles, and extracellular deposition of beta-amyloid (Ab) protein aggregates (plaques). Among the known mechanisms of AD, oxidative stress is believed to be the most important cause of AD. Several human studies supported the hypothesis of correlation of low Se levels and AD. A study on 65 years old Chinese population revealed the fact that low selenium levels in the body have deep association with lower cognitive functions and memory loss highlighting the importance of Se in AD. To further confirm this hypothesis, some of Se containing compounds were supplemented to the AD subjects and it was found quite effective causing improvements in cognition functions. Similarly, some of animal studies showed reduced Tau phosphorylation when supplemented with sodium selenate in cell cultures and transgenic animal models. None of the study revealed the proteomics associations with selenium within the brain tissues. Being the actual and the most important players responsible for biological functions within the cells and tissues, proteins cannot be neglected to understand the phenomenon of selenium efficacy during AD. To fulfill this gap, this study is designed to observe the proteomic alternations in cortex and hippocampus of 3xTg-AD transgenic mice after supplementing with sodium selenate by using iTRAQ comparative proteomics strategy. This study will elaborate the actual proteomics changes when treated with Se in the treatment of AD and will be helpful to better understand the mechanism underlying these changes.

24.EVALUATION OF ANTI-INFLAMMATORY, IMMUNE-MODULATORY AND ANTIMICROBIAL ACTIVITIES OF CINEMALDEHYDE IN A RAT MODEL OF LIGATURE-INDUCED PERIODONTITIS

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The clinical/surgical treatment for periodontitis currently used has some limitations mainly associated with the host immune response. However, to date, only doxycycline has been introduced as an immune-modulatory and co-adjuvant in periodontal therapy. Cinemaldehyde is an essential oil obtained from plants of the genus Cinnamomum, which can be found in the Brazilian Amazon region. This bioactive compound has shown promising pharmacological effects, including anti-inflammatory, immune-modulatory and antimicrobial activities. Hence, we aim to evaluate the influence of this natural product on the immune-inflammatory response related to periodontitis in rats. The ligature-induced periodontitis model will be utilized by placing ligatures around the lower first molars of male Wistar rats, aged 8 weeks and weighing 300-400g. Cinemaldehyde will be obtained commercially from Sigma® (St. Louis, MO, USA). Animals will be divided into 4 experimental groups: Group 1 (n = 10 animals): rats without ligature that will receive daily gavage with placebo (NaCl 0.9%, control group); Group 2 (n = 10 animals): rats with ligature that will receive daily gavage with placebo (0.9% NaCl, ligature group); Group 3 (n = 10 animals): rats with ligature that will receive daily gavage with DMSO (vehicle group); Group 4 (n = 10 animals): rats with ligature that will receive daily gavage with Cinemaldehyde (50 mg/kg, Cinemaldehyde group). After 15 days, animals will be killed and their hemimandibulaes will be destined to the following evaluations: levels of proinflammatory cytokines (IL-1β and TNF-α) in gingival tissue, blood and saliva, using enzyme-linked immunosorbent assay (ELISA); production of nitric oxide (NO) in gingival tissue, blood and saliva, using Griess reaction assay; expression of genes related to the inflammatory process (IL-1β, TNF-α, COX-2, NOSi, MMP-9) in gingival tissue by RT-qPCR; expression of RANKL and OPG in periodontal tissues by RT-qPCR and by immunohistochemistry; histological analysis of alveolar bone loss and integrity of gingival collagen; evaluation of the presence of Porphyromonas gingivalis, Tannerella forsythia and Aggregatibacter actinomycetemcomitans in the ligatures by PCR. The results of the present study are expected to reveal novel biological properties of Cinemaldehyde, evaluate its potential as a modulator of the host immune-inflammatory response, and develop a pre-clinical investigation of a possible adjuvant drug therapy for periodontitis.

25. EVALUATION OF BIOMARKERS IN DENTAL CARIES PRESENT IN SALIVA BY THE TECHNIQUE MALDI TOF

Regiane Cristina do Amaral, Lidiany Karla Azevedo Rodrigues Gerage Universidade de Fortaleza Despite the decline in dental caries be observed in developed and developing countries, this disease shows up still prevalent, taking as risk factors socioeconomic and education levels.

The recent development of proteomic techniques has allowed the analysis of protein profile of biological systems, allowing understands the physiology of organisms and microorganisms, which causes us to have biomarker discovery for early detection of diseases.

This study will use proteomic techniques to analysis of protein expression in saliva. Saliva is an important factor in the process of remineralization of dental enamel and others. So saliva of children (3 - 5 years) will be collected, divided into caries-free (dmft = 0) and caries (dmft> 0), whose families have similarity in economic and socio educational levels and these will be analyzed by mass spectrometry (MALDI-TOF) for identification of the proteins. Thus, this study aims to analyze the most prevalent proteins in these children and associate with the caries index found.

26. EXAMINING THE ROLE OF ADAM PROTEINASES IN THE TUMOUR MICROENVIRONMENT

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It is becoming increasingly clear that stromal cells play an important role in tumour initiation, progression and metastasis. Fibroblasts, the most abundant stromal cell, play an important role in the tumour microenvironment. Cancer-associated fibroblasts (CAFs) are phenotypically different to normal fibroblasts and express and exhibit divergent gene expression and protein secretion profiles. The molecular mechanisms of CAF trans-differentiation remain unclear. ADAMs (A Disintegrin And Metalloproteinase) are involved in myriad biological pathways through ectodomain shedding of transmembrane proteins, an important event in many different signalling cascades. We hypothesise that changes in ADAM expression and/or function in fibroblasts contributes to the formation of CAF in the tumour microenvironment.

We aim to test this by first analysing the expression of ADAMs in normal and CAFs at RNA, protein and functional levels using a variety of molecular and cell biology techniques. We will then alter the expression of ADAMs and observe the effects on CAF formation/ Finally, we aim to test the effect of altered ADAM expression and/or function on tumour formation in vivo. This project will generate novel data about the role of ADAMs in the tumour microenvironment and may identify opportunities to therapeutically intervene in signalling pathways that promote tumour growth and metastasis.

27. FINE REGULATION OF MAIZE PHOTOSYNTHETIC PHOSPHOENOLPYRUVATE CARBOXYLASE ACTIVITY - TOWARDS THE ESTABLISHMENT OF NEW BIOTECHNOLOGICAL TOOLS

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Directly, or indirectly, food production largely depends on plant productivity, and photosynthesis efficiency directly relates to plant growth. Some plants possess a more efficient photosynthetic system, the C4-photosynthesis. In C4-plants, a CO2-shuttle is established to concentrate CO2 around the enzyme responsible for carbon fixation, increasing this enzyme efficiency. A key player in this shuttle establishment is the enzyme phosphoenolpyruvate carboxylase(PEPC). PEPC uses the solubilized atmospheric CO2 to carboxylate phosphoenolpyruvate, producing oxaloacetate that will be the first carrier in the CO2-shutle. PEPC is a very abundant protein in C4-leaves and its total amount varies little along the photoperiod.Thus, PEPC has to be tightly regulated to address the different carbon fixation needs along the day.

Posttranslational modifications(PTMs) are able to rapidly modulate cellular responses, by tuning protein activity and/or stability. Regarding PEPC, it is known that the phosphorylation of a serine residue in the N-terminal is important to diminish its allosteric inhibition by malate. However, C4-plants that are unable to phosphorylate this serine residue display normal photosynthetic capacity. This suggests that novel PTMs regulating PEPC are not yet described. To discover novel PEPC PTMs we analyzed leaf protein extracts from maize, a C4-plant. Using an LC-MS/MS-based approach we identified putative phosphorylated and ubiquitinated residues. SWATH-MS analyses were performed to assess the variation of putative PTMs along the day. Harnessed in this preliminary results suggesting that PEPC is a target for massive phosphorylation and ubiquitination, our hypothesis is that PEPC turnover, together with phosphorylation, regulate carbon fixation in maize. To prove this hypothesis we will follow a multidisciplinary approach, strongly relying on MS-based experiments, to: understand the effect of novel PTMs in PEPC enzymatic activity; identify PTMs involved in protein stabilization; unveil possible crosstalk between PTMs; identify the kinases and E3-ligases responsible for modifying PEPC.

Results obtained in this project will provide more knowledge on the regulatory mechanisms modulating PEPC activity and stability and will reveal the interactors responsible for PEPC modification. This will establish the basis to uncover regulators behind the C4-metabolic pathways, which are still mostly unknown players within the complex re-arrangement of the C4-leaves.

28. FUNCTIONAL PROTEOMIC ANALYSES OF BOTHROPS ATROX VENOMS REVEALS PHENOTYPES ASSOCIATED WITH HABITAT VARIATION IN THE AMAZON

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Venom variability is commonly reported for venomous snakes including Bothrops atrox, the most common species in the Amazon region. In this study compared the composition of venoms from B. atrox snakes collected at Amazonian conserved habitats (terra firme upland forest and varzea) and human modified areas (pasture and degraded areas). Venom samples were submitted to shotgun proteomic analysis as a whole or compared after fractionation by reversed-phase chromatography.

By means of a species venom gland transcriptome and the in silico functional prediction of each isoform, we were able to predict the principal venom activities. Our predictions were validated by experimental approaches including both enzymatic and in vivo assays, and indicated restrictions in respect to Bothrops antivenom efficacy to variable components. Whole venom proteomes revealed a similar composition among the venoms with predominance of SVMPs, CTLs, and SVSPs and intermediate amounts of PLA2s and LAAOs. However, when distribution of particular isoforms was analyzed by either method, the venom from varzea snakes showed a decrease in hemorrhagic SVMPs content and an increase in SVSPs, procoagulant SVMPs, and PLA2s. There were remarkable differences in the venom pool from snakes collected at the floodplain (varzea habitat). Not only was this venom less hemorrhagic and more procoagulant, when compared to the venom pools from the other three habitats studied, but also this enhanced procoagulant activity was not efficiently neutralized by the antivenom. These results suggest that proteomic analysis at the isoform level, with functional characterization in silico, may predict snake venom biological activities. The prevalence of functionally distinct isoforms contributes to the variability of the venoms and could reflect the adaptation of B. atrox to distinct prey communities in different Amazon habitats. Thus, using a functional proteomic approach, were highlighted intraspecific differences in B. atrox venom that could impact both in the ecology of snakes but also in the treatment of snake bite patients in the region.

29. GLYCOPROTEOME PROFILING OF BOTHROPS VENOMS BY SELECTIVE ENRICHMENT USING LECTIN-AFFINITY CHROMATOGRAPHY AND MASS SPECTROMETRIC IDENTIFICATION.

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Protein glycosylation is one of the major post-translational modifications in viperid venoms and affect significantly protein folding, conformation, stability, pharmacodynamics, as well as increase the size of venom proteomes and diversify functions of toxins.

We have recently shown that Bothrops venom proteomes are markedly defined by their content of glycoproteins. To further investigate proteome venom variation and the mechanisms involved in the generation of different venoms by related snakes, in this investigation we are analyzing the subproteomes of glycoproteins selected by lectins with different saccharide specificities and mass spectrometry. Venom from B. cotiara, B. insularis, B. jararaca, B. moojeni, B. neuwiedi, B. jararacussu, B. erythromelas, B. atrox and B. fonsecai was subjected to affinity chromatography using the lectin SNA (Sambucus nigra; SNA-agarose), which binds to sialic acid attached to terminal galactose in N-glycans.

Protein profiles were compared by SDS-PAGE and identification was carried out by in-solution trypsin digestion (LC-MS/MS). Metalloproteinases (SVMP) and serine proteinases (SVSP), which are the most abundant glycosylated Bothrops venom components, were identified among the SNA binding toxins, and only a few L-amino acid oxidases (LAAO) were identified in four venoms, indicating that this toxin class is not rich in N-glycans containing sialic acid attached to terminal galactose. Notably, the electrophoretic profiles of components with affinity for SNA were different between the venoms, and, interestingly, in most venom profiles a protein band of ~14 kDa was observed, although with variable intensity, which typically correspond to components that are not glycosylated, such as phospholipases A2 (PLA2) and C-type lectins (CTL). Accordingly, CTL and PLA2 were identified in the SNA-bound fraction of seven venoms.

This finding is in agreement with our previous studies using other lectins (ConA, WGA and PNA). Thus, in order to better understand the interaction between non-glycosylated toxins and these lectins, a further aim of this project is to analyze the sites of interaction between phospholipases A2 (isolated from Bothrops venoms) and the lectins ConA, WGA and PNA, using cross-linking-MS approaches.

30. GLYCOPROTEOMIC APPROACH OF SURFACE LAYER POST-TRANSLATIONAL MODIFICATION IN LACTOBACILLUS KEFIRI'S NON-AGREGGATING STRAINS

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Surface-layer (S-layer) proteins are ubiquitous in both Bacteria and Archaea. S-layers are two-dimensional arrays of a single protein that constitutes the outermost cell envelope and have been considered to function as protective coats, maintenance of cell shape. The presence of S-layer proteins in L. kefiri strains isolated from kefir, a probiotic fermented milk, was described some years ago. It has been demonstrated that these S-layer proteins are involved in the interaction of bacterial cells with yeasts present in kefir grains. They are also able to inhibit the invasion of Salmonella enterica to Caco-2 cells, to antagonize the effect of Clostridium difficile toxins and to protect bacterial cells against the deleterious effect of lead ions. These glycoproteins present apparent molecular masses ranging from 66 to 71 kDa and a high heterogeneity among aggregating and non-aggregating strains of L. kefiri have been shown. Since the amino acid sequence of the S-layer glycoprotein from L. kefiri CIDCA 83111 was recently reported, the aim of this work was to characterize in detail the glycosylation of this protein. To achieve this purpose,the reduced fraction obtained by alkaline treatment of the glycoprotein was subjected to HPAEC-PAD after acid hydrolysis, showing Glucose as the main component. When the oligosaccharides were subjected to MALDI-TOF m.s. analysis we found that the glycoprotein was substituted with oligosaccharides bearing up to six hexose residues.In order to get deeper into the O-linked sugar structure,the excised gel band from SDS-PAGE corresponding to the S-Layer glycoprotein was subjected to a sequential enzymatic digestion with trypsin and Glu-C. After cotton-Hilic chromatography, an enriched glycopeptide fraction was obtained. The analysis on a Q-Exactive nanoHPLC-ESI-Orbitrap equipment showed that the PTM was linked to peptide 147-160.

31. GLYCOPROTEOMICS EVALUATION OF CELL SURFACE TARGETS IN CANCER: THERAPEUTIC APPLICATIONS

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Immunotherapy is one of the most promising fields in cancer treatment and developments in immunotherapy field are evolving very rapidly in Gastrointestinal (GI) cancers.

Currently, this type of treatment focuses mainly on targeting glycoproteins expressed on the surface of cancer cells such as EGFR (Epidermal growth factor receptor), HER2 (Human Epidermal growth factor Receptor 2) and others (Gill, S., Dowden, S., et al. 2014; Jonker, D.J., O'Callaghan, C.J., et al. 2007). The response to therapies depend on the absence of oncogenic activation of signaling pathways downstream the receptor, however these only partially explain the clinical response to these directed treatments (Cunningham, D., Humblet, Y., et al. 2004).

One of the main problems that needs to be solved is the resistance and inefficiency of the current treatments in subgroups of cancer patients. Our hypothesis is that variation in the glycosylation of the protein targets at the tumor cells surface can interfere with the recognition of therapeutic MAbs, therefore determining the therapeutic response of cancer patients. Also, the use of this therapy still lacks pending new evidence on specific biomarkers to enable the selection of patients and the monitoring of response. This project will both evaluate if variation on the glycosylation of targeted proteins can interfere with recognition of therapeutic MAbs and if specific glycoforms can serve as potential biomarkers.

We propose to apply genomic editing tools to develop glycoengineered GI cancer cells and advanced mass spectrometry in cells and tissues to identify and characterize the glycoprotein biomarkers of response to cancer therapy. These results will be crucial for the understanding glycosylation changes mediating resistance to treatment in cancer that will ultimately lead to the development of novel therapeutic strategies with major relevance and potential applications with technology valorization foreseen.

32. HYPERLOPIT: HIGH RESOLUTION MAPPING OF THE SPATIAL PROTEOME

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The organisation of eukaryotic cells into distinct sub-compartments is vital for all functional processes and aberrant protein localisation is a hallmark of many diseases. Microscopy methods, although powerful, are usually low throughput and dependent on the availability of fluorescent fusion proteins or highly specific, sensitive antibodies. One method which provides a global picture of the cell is LOPIT (localization of organelle proteins using isotope tagging), which combines biochemical cell fractionation and multiplexed-quantitative proteomics to allow simultaneous determination of the steady-state distribution of hundreds of proteins within organelles.

We have recently significantly re-developed this protocol to enable the subcellular localization of thousands of proteins in a single experiment (hyperLOPIT) including spatial resolution at the sub-organelle and large protein complex level. We have also created an open-source infrastructure to support analysis of quantitative mass-spectrometry-based spatial proteomics data. Here we provide the integrated protocol for all elements of the hyperLOPIT pipeline.

33. IMPLEMENTATION OF TOP-DOWN PROTEOMICS AT PROTEOMICS UNIT – UFRJ AND DEVELOPMENT OF NEW METHODOLOGIES FOR TOP-DOWN VENOMICS

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Protein identification in venomics studies are generally performed by bottom-up approaches. However, these approaches have inherent disadvantages: impossibility of proteoform identification and poor post-translational modification characterizations. Top-down approaches applied to venom studies seeks to eliminate these problems analyzing and fragmenting intact proteins in a high throughput fashion and using reliable scores and statistics methods for proteform identification. Rapid advances in instrumentation by most manufacturers over the past decade have rendered targeted and high-throughput denaturing top-down feasible for proteins until 30 kDa. Nowadays, it is possible to run denaturing top-down experiments in almost any proteomics laboratory that uses modern high-resolution mass spectrometers. Thus, the venom of the Brazilian yellow scorpion, a secretion rich in biological active proteins >30 kDa, was chosen with the purposes of implement top-down proteomics at the Proteomics Unit – UFRJ and develop a new method for intact protein analysis of scorpion venom. This method will be applied to identify the venom proteoform composition of two different populations of Tityus serrularus, one from Brasilia-DF and other from São Paulo-SP.

The venom of eight individuals from Brasília – DF were reduced before be submitted to LC-MS/MS. These runs were used to optimize different MS parameters such as resolution, AGC, injection time, number of microscans, minimal ion intensity of precursor, HCD energy, m/z range, maximum number of ions in MS1 and MS2 events. Raw data were searched against a database containing Uniprot protein entries for the order Scorpione and a deep sequence transcriptome from the venom gland of T. serrulatus. Search was performed using ProSight PC 3.0 in absolute mass mode and in these preliminary data were possible to identified 173 proteins and more than 200 proteoforms with high confidence.

The principals group of toxins identified were sodium and potassium channel toxins followed by anionic peptides, antimicrobial peptides, cytolytic peptides, and protease inhibitor peptides. According to the amount of proteins identified in each run was possible to obtain the best parameters for the final method that will be used to compare the proteoform composition of T. serrulatus venoms from Brasília and São Paulo.

34. INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF OVOTRANSFERRIN (OFT)

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Studies have shown on top of antimicrobial activity due to iron sequestering effects, ovotransferrin (Oft) may play an additional role in the prevention of infection and invasion at epithelial surfaces in an iron independent

manor. My primary work involves investigating the mechanism of the inhibitory effect Oft has on the infection and invasion of two key pathogens, Campylobacter jujuni and Pseudomonas aeruginosa. C. jujuni is the leading cause of acute gastroenteritis in the world. P. aeruginosa related pneumonia is a life limiting infection in cystic fibrosis patients and the elderly. Ultimately, the mechanism of action of Oft's inhibitory effects could lead to the development of novel therapeutic agents which could be expanded to other organisms.

My initial results have shown that C. jujuni binds directly to Oft, and understanding its exact binding partners is key to understanding its exact mechanism of action. I have also recently observed Oft's inhibitory effect on biofilm formation, which is a key aspect of colony formation and infection by P. aeruginosa. Working with the Mass Spectrometry Resource (MSR), Conway institute, UCD, we will investigate Oft's binding partners to provide a platform for bioactive protein discovery.

Secondly, another member of the transferrin family - Lactoferrin (Lf) - a homolog of Oft, is found in milk secretions of many mammals. It is known to act as part of the innate immune system having intrinsic antimicrobial activity. Interestingly, Lf has been shown to have proteolytic activity with a non-conventional Ser-Lys dyad. It has been reported in the literature that Oft also acts as a protease, however, our data obtained to date do not support this hypothesis. We would like to utilise mass spectrometric methods to help to confirm our data and understanding of the Lf cleavage motif.

35. ISOLATION OF MICROORGANISMS RESISTANT TO HEAVY METALS FROM SEDIMENTS OF AN OLD MINING ZONE. RESISTANCE MECHANISMS STUDY

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"Mining environmental liabilities are installations, effluents, emissions or waste deposits produced by mining operations now abandoned that constitutes a permanent and potential risk for population health and for the ecosystem. The most documented water pollution associated with abandoned mining areas is the acidic water produced by the oxidation of sulfides ores exposed to surface. This acidic water is known as Acid Mine Drainage (AMD) and enhances mobility of heavy metals from soil, sediments or rocks.

AMD affected environments are low-complexity natural systems regarding to biodiversity. Pollutants produce the elimination of the most sensitive microbial populations in varying degrees depending of the nature of the AMD. However, some microorganisms have developed sophisticated cellular machinery which consists of proteins and metabolites, responding to deficiency or excess of heavy metals. A possible approach to understanding metal-microorganism interaction is proteomics. Comparison of protein profiles in presence and absence of heavy metals allows the identification of proteins induced or repressed by heavy metals presence and concludes about resistance mechanisms. In San Luis-Argentina, a gold mine was exploited until 1894. After its exploitation, galleries and facilities were abandoned without having an appropriate closure process. Currently, the drainage provenient from this mine is released to “La Carolina” stream. Because of these reasons, this project proposes as main objective the isolation and characterization of microorganisms resistant to heavy metals, isolated from sediments of this ancient gold mine, and the analysis of homeostatic molecular mechanisms that microorganisms possess against heavy metals. Specific objectives:

1- Evaluation of the acid mine drainage influence on physicochemical parameters (pH, sulfate content and heavy metals concentrations) of La Carolina stream sediments, and on the microbial biodiversity through metagenomic analyses.

2- Isolation and molecular characterization of microorganisms that possess resistance to heavy metals (mainly Cu and Fe). 3- Characterization of extra and intracellular proteins associated with the presence of heavy metals in selected microorganisms through shotgun proteomic analysis and gel-dependent techniques such as two-dimensional electrophoresis.

4- Identification and characterization of non-protein metabolites involved in microbial homeostasis mechanisms against heavy metals."

36. LIVER PROTEOME OF NELLORE BULLS WITH HIGH AND LOW RESIDUAL FEED INTAKE

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Because the liver is a central organ of energy metabolism, our hypothesis was that hepatic protein profile differed among Nellore bulls with high and low residual feed intake (RFI), one of the most used indexes to measure feed efficiency (FE). The RFI values were obtained by regression of dry mater intake (DMI) in relation to average daily gain (ADG) and mid-test metabolic body weight (BW). Thus, 18 animals (9 in each group) were individually fed in a feedlot for 98 days. Immediately after slaughter, liver samples were collected and the proteome of hepatic tissue was investigated by two-dimensional electrophoresis (2D-PAGE) in combination with mass spectrometry (electrospray ionization, ESI/MS/MS). For each RFI group (high and low), a pooled sample was created from equal amount from all samples of the liver. The gels of the experimental groups (all in triplicate) were then scanned and the images analyzed using the ImageMaster Platinum (v. 7.0). The protein spots to be characterized by ESI-MS/MS from the polyacrylamide gel were extracted using a scalpel blade, cut into approximately 1-mm3 segments, and transferred to two microcentrifuge tubes. In brief, the tryptic digestion was performed using a specific kit (In-Gel DigestZP Kit) that digests proteins and purifies the obtained peptides. The detection of peptides was performed online, and proteins were identified by their homology to proteins in the UniProt database using the ''Otophysi'' series (UniProt 2015). A total of 279 and 215 liver protein spots were found in high and low RFI bulls, respectively. From this total, 71 spots were found to be differentially expressed (P < 0.05) between RFI groups and 35 could be identified by mass spectrometry. The proteins were related to lipid metabolisms such as acyl-CoA dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase type-2. Additionally, other proteins such as myosin light chain 2 and tropomyosin 3 fast-twitch myosin light chain were also found in protein spots. In the current study, the expression changes may be associated with the differences in fatty acid oxidation between RFI phenotypes.

37. LOSS OF FUNCTION OF ATXN3 ALTERS THE UBIQUITOME OF NEURONAL CELLS, NEGATIVELY IMPACTING ON THE SPLICING PROCESS

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2 ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal;

3 Vrije University Medisch Centrum (VUMC), Amsterdam, The Netherlands Ubiquitylation is a tightly controlled process that has been shown not only to mediate protein degradation, but also to modulate protein function and subcellular localization, playing a crucial role in the function and development of the nervous system.

Deubiquitylating (DUB) enzymes have been recognized as central players in the maintenance of the correct ubiquitylation/deubiquitylation balance in cells. Ataxin-3 (ATXN3) is a protein with DUB activity mutated in Machado-Joseph disease (MJD). To date, besides the involvement of ATXN3 in the Ubiquitin-proteasome pathway (UPP) and its potential involvement in transcription regulation and DNA repair, its normal cellular functions are known and no substrates for its DUB activity have been identified. In this work, in the attempt to identify potential candidates of the DUB activity of this protein, we characterized the ubiquitome of neuronal cells lacking ATXN3 (ATXN3shRNA cells) by mass-spectrometry. We found that a large proportion of the proteins with altered polyubiquitylation in ATXN3shRNA cells were proteins involved in RNA post-transcriptional modification. By transcriptomic analysis and using reporter minigenes we confirmed that splicing was globally altered in cells lacking ATXN3.

Among the targets with altered splicing were genes encoding components of the spliceosome, involved in the ubiquitin mediated proteolysis, in axon guidance and MAPK signaling pathway, findings that we need to further explore. These findings lead us to propose that ATXN3 plays a role in splicing regulation in neurons, a novel function for this protein.

38. MALDI-TOF MASS SPECTROMETRY PROTEIN PROFILE FROM HEMOCYTES OF PACIFIC WHITE SHRIMP UNDER THERMAL SHOCK

1 Blank, M.; 1 Schleder, D. D.; 1 Vieira, F. N.; 1 Hayashi, L. 1 Federal University of Santa Catarina Rapid change in water temperature contributes to immune suppression of aquatic animals affecting their productivity and susceptibility to disease causing great economic losses especially for shrimp farm industry. Here, we employ Matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS) to investigate protein profiles of shrimp hemocytes submitted to cold stress. Shrimps were cultivated for 15 days in home tanks with constant aeration and seawater temperature of 28.0 ± 0.3°C. At the 16th day, a group of 10 shrimps were submitted for 1h to thermal shock (TS) in a tank with seawater at 11.5 ± 0.1 °C. Hemolymph was collected in ammonium buffer (0.45 M sodium chloride, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA, pH 4.6) containing 10 mM b-cyclodextrin (1/2, v/v) to prevent from clotting. Subsequently, samples were centrifuged at 1,000xg for 15 min at 4 °C. For determining the best sample preparation for MALDI-TOF MS study, four different shrimp hemolymph materials from control group were initially investigated: plasma, intact hemocytes and lysed hemocytes cells supernatant and pellet. Different matrix substances for MALDI-TOF MS and optimal matrix/sample proportion (v/v; 1/1 and 3/1) were also evaluated: 2,5-Dihydroxybenzoic acid (20 mg/ml) in 70/30/0.2% MeOH/H2O/TFA, Sinapinic Acid (38 mg/ml) and α-Cyano-4-hydroxycinnamic acid (10 mg/ml) in 70/30/0.2% ACN/H2O/TFA. All mass spectra were acquired in positive linear mode within the mass range of m/z 4,000–20,000 on an Autoflex III (Bruker Daltonics, Germany). FlexAnalysis and ClinProTools (Bruker Daltonics) were used to process spectral data. Our MALDI-TOF MS analysis revealed that supernatant of lysed cells and Sinapinic Acid in a proportion of 1/1 (matrix/sample), gives the best mass signals and coverage. Comparison of supernatant protein profiles from control shrimp hemocytes and those submitted to TS detected a total of 56 distinct proteins. Statistical analysis indicated that 11 peaks were significantly different (p

39. MALDI-TOF MS PROTEOMIC PHENOTYPING OF WOOD-DECAY BASIDIOMYCETES (HYMENOCHAETACEAE, BASIDIOMYCOTA): A NEW APPROACH/TOOL TO IDENTIFY ENVIRONMENTAL FUNGI

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Hymenochaetaceae (Imazeki & Toki 1954) is an important family in the Phylum Basidiomycota (Fungi). Many species of this family are medicinal fungi and also produce many enzymes of great industrial interest.

They plays a fundamental role in wood decomposition, which are an essential part of the carbon cycling in forest ecosystems. Furthermore, some of them are important pathogens of trees exploited by paper and cellulose industry. The presence of setae, septa in the generative hyphae, permanent positive xanthochroic reaction, absence of clamp and yellow to deep brown basidiomata are the main caracteres that define this group. Most of the species have a typical tropical distribution, are saprophytes or parasites (facultative and/or obligatory) on a wide diversity of trees (angiosperms and gymnosperms). Currently, the species identification of this family and others of the Phylum Basidiomycota is carried out based on morphological caracteres (macroscopic and microscopic) and DNA molecular identification (the gold standard): the first one is expertise dependent and the last one has high cost and are time consuming and error prone (multistep workflow). During the last decade, a new and powerful analytical tool called matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been employed to identify fungi, mainly with clinical interest, with high efficacy and reliability, short and quick workflow, and cost-effective. Currently, the major restriction to use this tool is the database representativity, because many fungi have no reference spectra in the database or are insufficiently represented. Therefore, our objective in this work is to use the MALDI-TOF MS to identify environmental fungi, building a reference database to the Family Hymechaetaceae with reference strains (identified both morphologically and molecular by DNA sequences), and evaluating the constructed database with isolates not previously identified, as well as to standardize the methodology (growth media, growth time period and the protocol to protein extraction) to obtain good spectra. At the end of this work, we hope to have an extended reference spectra library for Hymenochaetaceae species developed using MALDI-TOF MS. The application of this methodology will allow for a much quicker identification of environmental fungi with economic and biotechnological potential.

40. MITOCHONDRIAL ADAPTATION THROUGHOUT THE TRANSITION AND ESTABLISHMENT OF THE WARBURG EFFECT IN INDUCED HEPATOCELLULAR CARCINOMA IN A RAT MODEL

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Cell Biology Department, Center for Research and Advanced Studies of the National Polytechnic Institute. Mexico City, Mexico." The mitochondria participates in multiple cellular processes, mainly in energy supply to the cell by the synthesis of ATP. Additionally, mitochondria participates in several metabolic pathways, apoptosis and calcium homeostasis. Disruption of mitochondrial functions has been associated to different pathologies, including cancer. In cancerous cells, a significant metabolic alteration is observed, cytosolic pyruvate is fermented to lactate (known as the Warburg effect), decreasing its entry into the mitochondria to continue its oxidation in the Krebs cycle. Then, mitochondria have to adapt its metabolism in response to this alteration. A few metabolic pathways have been identified as relevant in the mitochondrial response to the altered metabolism, mainly glutaminolysis. However, the role of mitochondria during cancer development and the metabolic change observed in cancerous cells remains unclear, perhaps due to the lack of a proper model in which the mitochondrial alterations can be followed during cancer progression. A useful model is the hepatocyte resistant model in rat, in which hepatocellular carcinoma (HCC) is induced by diethylnitrosamine administration. In order to achieve this goal, we chronologically analyze mitochondria during the development of chemically induced hepatocellular carcinoma in rats. We conducted a massive comparative proteomic analysis by tandem MS/MS coupled to liquid chromatography to study the mitochondrial adaptation during the transition and establishment of the Warburg Effect. To complement the proteomic analysis we are using genetic expression data obtained from the same stages of HCC development.

41. MOLECULAR INTERACTIONS AND FUNCTIONALITY OF PROTEINS INVOLVED IN Plasmodium falciparum ERITROCYTIC INVASION THROUGH APEX-2-BASED-PROXIMITY TAGGING, CRISPR-CAS9 AND PROTEOMICS APPROACHES

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2 Ph.D. student at Medicine School, University of Brasília, Molecular Pathology Graduate Programme, Laboratory of Biochemistry and Protein Chemistry

3 Malaria Programme, Wellcome Trust Sanger Institute, Cambridge, England My Ph.D. project is entitled: "Molecular interactions and functionality of proteins involved in Plasmodium falciparum eritrocytic invasion through APEX-2-based-proximity tagging, CRISPR-Cas9 and proteomics approaches", and it is an extension of a post-doc internship project of my Ph.D. supervisor, Dr. Sébastien Olivier Charneau, at Wellcome Trust Singer Institute, in the Malaria Programme in Cambridge, England. Initially, the major goal of Dr. Charneau in his post-doc internship was to standardization of CRISPR-Cas9 technology in malaria parasites. The initial trials to properly construct both a donor and expression plasmids for two well-known genes, easily manipulated previously, Pfkahrp and Pfmahrp (Ghorbal et al, 2014; Wagner et al, 2014; Lu et al, 2016) were successfully achieved, as a control of the genome-editing system.

The protein expression of both genes were observed by fluorescence microscopy in the parasite cells, through using a 3'-APEX2-flag linked to AF488-straptavidin after chemical activation, followed by specific-antibody staining. All constructions were analysed by PCR for positive and negative inserctions, including Western-blot assays to garantee protein-partners viability. After realized that CRISPR-Cas9 system is efficient, he invited me, to continue with other 4 genes, that we strong believe, have a significant importance for parasite infect new red-blood cells through gliding movement. Two of these four genes were successfully obtained at Wellcome Trust Singer Institute by Dr. Charneau, but, there are no confirmation assay, about protein expression and immunofluorescence inside parasites, which will be performed by me. The other two constructs will be constructed through protocol stablished already. Briefly, after a proper transfection, the new mutant-lineage of parasites will express a specific targeted-protein labeled with a 3'-APEX2-flag, which in the presence of adequate substrate, such as hydrogen-peroxide, forms biotin-phenol radicals, that will after being treated on a straptividin-beads column, colecting with great specificity the partner-proteins. After that, all samples will be submitted for mass-spectrometry analysis for protein identifications.

42. MONITORING OXIDATIVE STRESS AND PROGRESSION TO CELL DEATH: FROM SECRETOME TO BLOOD DIAGNOSIS

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Oxidative stress is perhaps the most common factor and the main cause of cell death involved in several disorders. It may occur through diverse mechanisms leading to different responses, which can be reflected in the secreted molecules. Under controlled conditions, these responses can be reflected by the molecules secreted by cells, therefore the secretomes can be an important source of potential biomarkers, more likely to be reflected in biofluids. The aim of this work was to obtain a panel of markers from the secretome analysis which can be used to distinguish the changes caused by oxidative stress regulation or induction of cell death, and be able to monitor the indicators of cell death progression in blood. A cell model was treated with different stimulus of hydrogen peroxide to induce oxidative stress with and without cell death. The newly generated secretome spiked with the proper internal standards was analyzed by a quantitative mass spectrometry approach (SWATH-MS) and the markers identified were validated in cerebrospinal fluid and serum from animals from a model of Parkinson's disease. A large number of molecules were quantified between control and mild oxidative stress conditions. Four groups of molecules were highlighted by allowing a clear distinction between the conditions and can be considered indicators of stress previous to cell death. In addition, 24 proteins markedly increased in the cell death condition were considered good extracellular indicators of cell death. From these, three reveal to be able to distinguish the 6-OHDA injected animals from the controls in both CSF and serum samples. In summary, an integrative approach was introduced and successfully applied to identify oxidative stress biomarkers in the secretome. To transpose the proposed method to clinical diagnosis, these potential biomarkers were further validated in CSF and plasma from an animal model subjected to an oxidative stress insult, revealing to be capable of distinguishing the 2 groups, and will be further validated in plasma of patients with neurodegenerative disorders. Additionally, cell viability assays will be conducted in cells to correlate with the MS data and, time-course analysis using the PD model will be performed to establish the profiles of these biomarkers according to the establishment of the lesions.

43. NATURAL (Δ9-THC) AND SYNTHETIC (JWH-018) CANNABINOIDS INDUCE SEIZURES THROUGH CANNABINOID CB1R.

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Natural cannabinoids and their synthetic substitutes are the most popular recreational drugs. Numerous clinical cases describe strong aggravating toxic symptoms and neurological consequences upon inhalation of the synthetic cannabinoids mixture, known as “Spice”.

We discovered that an intraperitoneal administration of the natural cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC), one of the main constituent of marijuana, and the synthetic cannabinoid JWH-018 both triggered electrographic seizures in mice recorded by electroencephalogram (EEG) and video. Pretreatment with AM-251, a cannabinoid receptor 1 (CB1R)-selective antagonist, completely prevented cannabinoid-induced seizures. In addition, CB1RKO mice show no electroencephalographic or behavior seizures after cannabinoid administration. This data, first of all, shows that abuse of cannabinoids can be extremely dangerous and presents an emerging public health threat. Additionally, our data strongly suggests that AM-251 could be used as a crucial abortive therapy for cannabinoid-induced seizures or similar life-threatening conditions. Finally, we discuss a further ways of deciphering neural circuitry underling neurotoxic seizures, which including c-fos staining and neuroproteomic-based approach.

44. NEISSERIAL OUTER MEMBRANE VESICLES (OMVS) ARE POTENT VIRULENCE FACTOR CARRIERS.

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Neisserial Spieces are Gram-negative bacteria that cause meningitis and gonorrhea in immunocompetent individuals. How they evade immune attack is poorly understood. Neisseria express a number of cell surface enzymes and toxins, which are thought to be important for virulence. In particular, the bacterial toxin PorB was shown to target mitochondria to modulate host cell death. However, PorB (a trimeric beta –barrel protein) is the major protein of the outer membrane of Neisseria and it is unclear how it targets host pathways. We now show that Neisseria spp, similar to many other Gram-negative bacteria; also secrete large numbers of outer membrane vesicles, ranging from 20-200 nm in diameter. These extracellular vesicles secreted by Neisseria contain a number of secreted proteins along with major outer membrane protein "PorB". Aims of this project are to investigate whether OMVs are the major secretion system for the pathogenic form of PorB and also to investigate the effects of OMVs on host cells.To verify that OMVs are enriched for outer membrane proteins, we probed OMVs along with cellular fractions for markers of the inner and outer membrane as well as the periplasm. As expected the outer membrane protein BamA and PorB were present in OMV enriched fractions, which lacked detectable levels of inner membrane protein F1β. Gel staining and Mass spectrometric analysis also confirmed that PorB is major protein of Neisseria secreted OMVs. We next determined whether OMVs affect macrophages, as they clear invading pathogens. Bone-marrow derived macrophages (BMDM) from mice were treated with increasing concentration of purified OMVs and host cell viability was monitored after 24 hrs. We have been able to characterize purified OMVs on protein level and could show that PorB is highly abundant on/in secreted vesicles and when bone marrow derived macrophages are treated with OMVs the metabolic activity of the cells significantly decreases.

45. NEURODEGENERATION: (PHOSPHO) PROTEOMICS APPLIED TO THE STUDY OF THIAMINE DEFICIENCY AND ITS ASSOCIATION WITH SPATIAL COGNITIVE DEFICITS

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3 Department of Psychology (LCV, JMH, KRJ, LMS), Behavioral Neuroscience Program, Binghamton University, State University of New York, Binghamton, New YorkThiamine deficiency (TD) has been used as experimental model in rodents to study the mechanisms of neurodegeneration and the involvement of specific proteins in the molecular processes, such as neuronal death that leads to cognitive changes. The molecular machinery related to learning and memory process is highly dynamic and tightly associated to the phosphorylation of pre- and postsynaptic proteins required for neurotransmission and synaptic plasticity. Even knowing the importance for neuronal functions, the extent of synaptic protein phosphorylation during neurodegeneration remain undetermined. In this study, we aim to verify the TD-dependent changes in phosphproteome dynamic at synaptic terminals (synaptosomes) obtained from the brain regions more affected by TD, Hippocampus, Thalamus and Prefrontal Cortex. The brain tissues will be dissected from Wistar male rats treated or not with pyrithiamine hydrobromide, an inhibitor of the enzyme responsible for the phosphorylation of thiamine. After rest period of 15 days, rats will be challenged in the Morris Water Maze task to assess spatial memory. The experimental procedures will includes subcellular fractionation to isolate the synaptosomal fraction and phosphopeptide enrichment of synaptic proteins using immobilized metal affinity chromatography followed by mass spectrometry.

46. NEW SUBSTRATES AND PROCESSES REGULATED BY THE MYCOBACTERIUM TUBERCULOSIS SER/THR PROTEIN KINASE PKNG REVEALED BY PROTEOMICS AND INTERACTOMIC ANALYSES.

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The success of Mycobacterium tuberculosis as human pathogen resides mostly in its ability to maintain a latent infection in the host. It has been demonstrated that the Ser/Thr-kinase PknG from M. tuberculosis regulates critical processes in bacterial physiology and pathophysiology by still poorly understood mechanisms. To further characterize the role of PknG in mycobacteria, we carried out interactomic and comparative proteomics approaches. Using a novel strategy for affinity purification and sequential elution of PknG partners coupled to shotgun proteomic analysis, we obtain a list of potential kinase substrates that participate mainly in the control of nitrogen metabolism. To validate these substrates as physiologically relevant, we performed quantitative comparative proteomic studies of M. tuberculosis pknG knockout mutant (delta-pknG) and wild type (WT) strains using 2-D Fluorescence Difference Gel Electrophoresis (2D-DIGE) and shotgun strategies. 2D-DIGE approach showed differences in the relative abundance of spots of the same protein but with different pI, pattern consistent with potential changes in the phosphorylation status. We corroborate that some of the substrates identified by the interactomic approach that participates in nitrogen assimilation process were indeed physiological substrates of PknG. In addition quantitative global changes in the proteome of delta-pknG and WT strains of M. tuberculosis were analyzed by shotgun approach and spectral counting. We identified more than one-thousand proteins for each replicate of each condition, and more than one-hundred proteins were differentially detected between strains. Our results show that delta-pknG lacks a set of proteins that are expressed in the WT and that are crucial for adaptation to host nutritional conditions. Altogether interatomic and proteomics data suggest that the role of PknG in intracellular survival could be attributed to the regulation of essential bacterial pathways required for its adaptation to host environment.

47. NOVEL PATHOGENIC PATHWAYS INVOLVED IN THE DEVELOPMENT OF CHRONIC KIDNEY DISEASE: ROLE OF SIRTUIN 3, A NAD+ DEPENDENT DEACETYLASE.

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1 Institut Pasteur de Montevideo "Chronic Kidney Disease (CKD) has become a major public health problem because of its high frequency and impact on the medical budgets. A highly prevalent factor in the pathogenesis of CKD is arterial hypertension (HTA), in which the Renin-angiotensin-aldosterone system plays an important role. Angiotensin II (Ang II), which has a vasoconstrictive effect on smooth muscle vascular cells; it´s a stimulant of myocardial contractility and SNS. However, its role exceeds these effects and actually, Ang II is a multifunctional peptide with a crucial role in the generation of low grade chronic inflammation processes. Such effects are associated mainly with the activation of NF-kB among other pro-inflammatory transcription factors. Recent evidence demonstrates that the expression of NAD+ glycoprotein CD38 is under control of NF-kB. CD38 controls the activity of Sirt 3, a NAD+-dependent deacetylase involved in the control of mitochondrial & metabolic activity in response to cellular stress. The fact that histones are modified by acetylation has been known for almost 30 years.

It is important to highlight that acetylases are now known to modify a variety of proteins, including transcription factors, nuclear import factors and alpha-tubulin. Acetylation regulates many diverse functions, including DNA recognition, protein-protein interaction and protein stability. The study of the acetylome is becoming an important issue in proteomics. Our hypothesis is that at the renal level Ang II promotes an increase in the expression/activity of CD38 mediated by NF-kB, which would determine an inhibition of the renoprotective effect of Sirt 3, thus affecting the acetylome of the tissue. Our objective is to evaluate whether treatment with inhibitors of the angiotensin converting enzyme or the Ang II AT1 receptor (ARAs) has an effect of decreasing vascular and renal mitochondrial dysfunction based on decreased expression of CD38 and consequently in an increase in the activity of Sirt 3, resulting in a global change in the renal cells acetylome."

48. NS4B-DENV2 INTERACTIONS WITH HOST CELL PROTEINS FOR PROTEOMICS

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Dengue, the most important arbovirus in Brazil, is serious public health problem. The non-structural protein 4B (NS4B) is related to viral replication and may be involved in the modulation of NS3 helicase activity and inhibition of interferon in Dengue virus (DENV) infections. Proteomics has been applied in several instances to the study of the interaction between virus and the host cell proteome.

The aim of this study was to identify interactions between NS4B-DENV2 protein and host cellular proteins by Proteomics. The NS4B-DENV2 protein was expressed using the pGEX-5X-1 vector, containing Glutathione S-Transferase tag (GST), in E. coli BL21. Synthesis of the fusion protein was confirmed by Western blot and NS4B-DENV2 was purified by affinity column. We performed a pull-down assay and SDS-PAGE to compare the HeLa cell extract proteins that interacted with NS4B-GST and GST alone. The bands were cut out of the gel and submitted in situ digestion using trypsin for subsequent analysis by mass spectrometry (MS) to identify proteins differentially precipitated. The MS data were performed a search against NCBInr database and subsequently Scaffold 3.6 software analysis.

Thus, it was possible to confirm the interaction of 74 proteins with NS4B-DENV2-GST, with protein threshold to 90% and between these proteins was identified polyprotein DENV2, which identification validates the pull-down assay. We also performed analysis using the peptide threshold to 95% and the Scaffold identified 26 proteins. Among these proteins are myozenin-2 and stanniocalcin-1, which has been poorly studied and have functions related to the immune system. These proteins can be targeted for future validations, since the NS4B is involved on the interferon signaling inhibition. Therefore, this study may be the starting point to produce new knowledge about the NS4B role in DENV2 pathogenicity and replication and help in developing new strategies for Dengue prevention.

49. OMIC ANALYSIS OF THE NEUROPROTECTIVE EFFECTS OF TIBOLONE AGAINST LIPOTOXICITY FOLLOWING EXPOSURE TO PALMITIC ACID

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Obesity is an excessive accumulation of fat in adipose tissue in the form of free fatty acids. This condition involves a series of pathological cellular responses that occur following exposure to elevated levels of fatty acids (lipotoxicity). In the last decade, the association between neurodegenerative diseases (ND) and obesity has been studied. Where obesity and related metabolic disorders exacerbate the phenomena of neurodegeneration. In that sense, a large number of studies have shown that subjects suffering from obesity show an increased risk of developing different EN. These diseases represent a heterogeneous group of disorders that are characterized by progressive dysfunction of neurons and astrocytes. In that regard, recent findings suggest that astrocytes play a critical role in central nervous system (CNS) protection. For this reason, loss of normal astrocytic function may be a primary contributor to neurodegeneration. On the other hand, several reports have shown the association between estrogen levels and the correct functioning of astrocytes. In that sense, neurosteroids such as tibolone are seen as a therapeutic goal that could prevent, delay onset, prolong survival and improve the quality of life of patients with EN. However, the mechanisms of action and metabolic effects of this substance at the level of the nervous system remain unknown, due to poor understanding of the biochemical basis of its action. In this respect, omics are approaches that allow biochemical analysis from an integral perspective of cellular physiology. These techniques allow the determination of cellular metabolic profiles in different biological contexts such as those associated with EN, specific metabolic insults and exposure to protective therapeutic substances, which can provide valuable information for the compression of the metabolic behavior of astrocytes in different contexts. However, the separate omics studies do not provide a deep understanding, because the behavior of biological systems is determined by the complex interactions constructed between its components; Therefore, an integrated approach is needed to study biological systems. In recent years, innovative developments have been generated in computational and analytical technologies that have allowed the integration of large-scale post-genomic data into computational systems models, an approach known as systems biology.

50. PEPTIDOMICS-BASED PHENOTYPE OF THE HEMOLYMPH PEPTIDES FROM SPODOPTERA FRUGIPERDA LARVAE (LEP., NOCTUIDAE)

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(2)Laboratório Especial de Toxinologia Aplicada – LETA, CeTICS, IBu, SP, Brazil INTRODUCTION: Bioactive peptides are present in various metabolic pathways.

Peptides present in biological samples are indicators of a normal biological system, pathogenic processes or the effect pharmacokinetics in the organism. OBJECTIVE: To carry out phenotypic analyses of the constitutive expression of hemolymph peptides from S. frugiperda larvae by mass spectrometry. MATERIAL AND METHODS: Larvae were reared on tubes with artificial diet at 27±1°C and a 16:8 (light:dark) hour photoperiod. Hemolymph collected from 4th or 5th instars larvae was centrifuged (10,000 x g for 30 min at 4°C). The supernatant was lyophilized, followed by precipitation methods (organic solvent and acid) and stored at -80 °C until peptidome analysis. Three biological replicates were desalted by solid-phase extraction (Sep-Pak Vac C18) and analyzed on an LTQ-Orbitrap Velos mass spectrometer interfaced with an Easy-nLC II (Thermo Fisher Scientific). Peptides (10 µl injection volume) were loaded onto a pre-column (C18 Jupiter particles, Phenomenex) and separation was carried out on an analytical column (Acqua C18, Phenomenex). The raw data files were processed by Matrix Science MASCOT software and submitted to search against NCBInr database sequences using PatternLab for proteomics 4.0. RESULTS AND DISCUSSION: LC/MS data showed the expression and processing of the α-helical peptides (cecropin), cysteine-rich peptides (defensin and diapausin), and glycine-rich peptides/proteins (gloverin). The peptides identified are involved with the insect immune system, and have direct antimicrobial activity against pathogens such as bacteria and fungi. Antimicrobial peptides/insect proteins (AMPs), like defensins, cecropins, were found in several orders of insects, but gloverin was only discovered in the order Lepidoptera. CONCLUSION: This analysis adds knowledge and new insight into the function of biological systems, as well as the understanding of endogenous peptides in the control of the protein-protein interaction and the discovery of peptide biomarkers. Keywords: Lepidoptera, Hemolymph, Phenotype, Peptidomic.

51. PHOSPHOPROTEOME OF RHODNIUS PROLIXUS ANTENNAE

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Rhodnius prolixus is a vector of Chagas disease. Insects detect environmental molecules through olfaction, a process that trigger essential behaviors to insect survival. Olfaction is mediated by different proteins. Among them, OBPs (odorant binding proteins) transport hydrophobic semiochemicals across the sensillum lymph to the olfactory receptors (ORs). Protein phosphorylation is a biologically important post-translational modification that regulates a variety of essential biochemical phenomena such as signal transduction. The purpose of this study was to identify phosphorylated proteins involved in olfactory signaling pathways and phosphorylated OBPs expressed in antennae of R. prolixus.

For phosphoprotein profile analysis, proteins were extracted from the antennae of 120 adult male and separated into two groups. In the control group, proteins were extracted using 25 mM HEPES buffer pH 7.4 supplemented with protease and phosphatase inhibitor cocktail (Sigma), in order to preserve phosphate groups. In the treated group, proteins were extracted using the same buffer without the phosphatase inhibitor. To identify the presence of phosphorylated OBPs, proteins of the treated group were dephosphorylated with rAPid alkaline phosphatase (Roche). The proteins of both groups were analyzed separately by 2-DE gel electrophoresis with a pH range of 3-10. The phosphatase-treated proteins migrated to more basic areas of the 2-DE gel, indicating that there was a change in their pI, probably due to the loss of the phosphate group. This indicates that Rhodnius OBPs may be phosphorylated. Indeed, we identified phosphorylation sites in 5 OBPs (OBP1a, OBP2b, OBP3c, OBP4d and OBP5e) using MS/MS and database search with Mascot v2.3.02 software. Furthermore, phosphorylated peptides were also identified in olfactory signal transduction proteins: two olfactory receptors – OR1a and OR2b; a gustatory receptor – GR1a; two transient receptors potential channel (TRPs) – TRP pyrexia and TRP gamma; two metabotropic signaling proteins - diacylglycerol kinase eta-like and calmodulin. These results will be refined using TiO2 phosphopeptide enrichment. Therefore, this work is the first report of the identification of post-translational modification in Rhodnius OBPs and it is believed that phosphorylation of OBPs may act as a mechanism of action for the release of odors or may be involved in increasing the specificity and selectivity of OBPs to different chemical molecules.

52. PROTEIN MODULATION IN CHRONIC MYELOGENOUS LEUKEMIA CELLS UNDER NUTRITIONAL DEFICIENCY REVEALED BY MULTIPLEXED MASS SPECTROMETRY-BASED PROTEOMICS

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The formation of a cancer cell is a multistep process in which there are several genetic and epigenetic changes. The environmental stress induced by tumor nutritional deficiency causes disruption of cell metabolism, and increases the release of cytokines, chemokines and growth factors. There are many studies describing the effects of environmental stress on tumor progression and acquisition of resistance; however, most of them focus on the effect of hypoxia and hypoglycemia.

Although serum restriction can also be a source of microenvironmental stress, little is known about the effects of these restriction on cancer cells. In the present study, we evaluated the influence of serum restriction in chronic myelogenous leukemia cells K562 in vitro. Plasmatic restriction reduced cell viability, increased cell death and the amount of cells in the G2 phase of cell cycle. Nutritional stress induced resistance to environmental stress factors such as pH and the presence of reactive oxygen species. In order to elucidate the molecular mechanisms underlining this pathway rewiring, we used a multiplexed mass spectrometry-based proteomics approach. More than 8000 proteins were identified and quantified revealing important changes in expression of proteins related to cell cycle such as the CDK1, CDK5 and proteins involved in the mTOR signaling pathway. Collectively, these results show a modulation of specific pathways upon serum starvation that lead to acquired resistance

53. PROTEINS AND PEPTIDES IN MILK OF THE ADOLESCENT MOTHERS: PROTEOMICS, ANTIMICROBIAL ACTIVITY AND INFLUENCE OF THE LACTATION PERIOD.

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Human milk contains several components such as immunoglobulins and bioactive peptides that have specific properties such as disease protection and stimulation of the immune system of the infant. The changes in milk composition occur during the lactation. The protein fraction of milk originates bioactive peptides that have numerous functions. Among the features already described, the peptides have the capacity to exert anti-inflammatory activity, regulation of gastrointestinal function and antimicrobial activity. The aim of this study was to analyze proteins and bioactive peptides of mature human milk in two distinct groups of healthy adolescent mothers in two stages of lactation: up to 3 weeks (group 1) and over 5 weeks of lactation (group 2). The samples were collected from adolescent mothers (14-19 years) with no chronic diseases, in two different moments of lactation, after consent. In milk collected protease inhibitor was added to minimize protein degradation. The milk was centrifuged at 1,500 × g at 4 ° C for 20 minutes. The cream was separated and the skim milk was stored at -80 ° C. The peptides and smaller proteins were separated by ultrafiltration (10 kDa). These proteins were analyzed by liquid chromatography and mass spectrometry. The fraction of low molecular weight (≤ 10 kDa) was tested against two microorganisms: Escherichia coli and Staphylococcus aureus, and the peptides were identified by mass spectrometry. We identified a total of 532 proteins in this study. The proteomic approach revealed a high degree of variability of mature human milk proteins, between adolescent mothers, and also between the two groups at different times of lactation. Samples of peptides were able to inhibit the growth of E. coli. This study also suggests that the bioactive peptides detected in several samples were digested before being secreted, because the precursor proteins of these peptides weren't found on protein identification analysis of the skim milk. These bioactive peptides can be derived from less abundant fraction of human milk proteins. This strategy proved to be appropriate, since it allowed to compare the protein composition between the two groups lactation. It was also able to investigate bioactive peptides present in adolescent mothers mature milk. Keywords: Human Milk, Lactation, Proteomics, Bioactive Peptides.

54. PROTEOME ANALYSIS OF PHENOL-DEGRADING ACHROMOBACTER SP. STRAIN C-1, ISOLATED FROM AN INDUSTRIAL AREA

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This paper reports the successful isolation and characterization of a new phenol-degrading bacterium, Achromobacter sp. strain C-1, from an industrial area (Cubatão, Brazil). Achromobacter sp. is a non-motile, strictly aerobic, gram-negative, short-rod or coccobacillary bacterium, which can occur singly, in pairs or in clusters. 16S rRNA gene sequence analysis revealed that Achromobacter sp. strain C-1 belongs to the gamma group of Proteobacteria, with 99% similarity to 16S rRNA gene sequences of Achromobacter xylosoxidans. Strain C-1 can grow aerobically on a number of aromatic compounds, such as phenol, catechol, m-cresol and o-cresol. In particular, it can degrade up to 600 mg/L of phenol at 37°C. In order to understand the phenol degradation pathway used by strain C-1, phenol or glucose cultured C-1 proteomes were comparatively analyzed with two-dimensional SDS-polyacrylamide gel (2D SDS PAGE). Nine protein spots were exclusively induced from phenol-cultures of strain C-1. Of these 9 spots, three phenol-degrading enzymes (phenol degradation meta-pathway protein, hydroxymuconic semialdehyde dehydrogenase and 4-hydroxy-2-oxovalerate aldolase) were identified by peptide mass fingerprinting, and the results were confirmed by tandem mass spectrometry of selected peptides, which suggests that strain C-1 degrades phenol via the -ketoadipate pathway. Analysis of the metabolite produced from phenol proved that this enzyme is a catechol 2,3 dioxygenase, which is able to utilize phenol as a substrate. These results suggest that comparative proteomic analysis of biodegrading bacteria cultures under different conditions may be a useful initial step toward the elucidation of an aromatic compound degradation pathway and the strain C-1 could be an excellent candidate for the biotreatment of phenol-containing industrial wastewaters.

55. PROTEOMIC ANALYSIS ABOUT THE INFLUENCE OF CELL CULTURE STIMULATION OF DERMAL FIBROBLASTS WITH TUMOR SECRETORS

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The development, establishment and propagation of tumor processes is conditioned by a series of ubiquitous conditions for the most diverse types of cancer, such as the independence of signs of cell growth, insensitivity to inhibitory signs of growth, resistance to cell death, unlimited replication, promotion of angiogenesis, invasion and acquisition of metastatic capacity. For these conditions to be attained, a large number of genes involved in functions essential for normal growth, survival and cellular function have their expression deregulated. The interaction of tumors with their microenvironment depends on molecular signals that mostly modulate the malignant phenotype, especially during the early invasive stages, when cells need to activate a mechanism that allows them to migrate, invade, and survive outside their original niche under Conditions and settle in a new location even under initially unfavorable conditions. Thus, stromal cells are often recruited by tumor cells to participate in the tumorigenic process and contribute to tumor development by, for example, inducing the production and release of molecular signals responsible for tumor progression. In this context, this project aims to identify the repertoire of newly synthesized proteins, associated with the malignant phenotype, through the stimulation of cell cultures with tumoral secretoma. The importance of this work lies in the possibility of better understanding the influence of proteins secreted by tumor cells on the protein expression of a specific biological model (a cell line from the normal fibroblasts of a patient with melanoma secreted by the lineoma derived from tumor lines) using approaches proteomics as cell cuture, metabolic labeling and mass spectrometry. Key words: proteomics, cancer, secretoma, melanoma.

56. PROTEOMIC ANALYSIS BY MALDI-TOF MS AS A TOOL TO IDENTIFY ENDOPHYTIC FUNGI

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Endophytes are microorganisms living in the internal tissues of the plants without causing any symptoms.1 Studies tending to characterize libraries of endophytic fungi isolated from plant species of Argentina acquire relevance, due to their potential to synthesize bioactive metabolites, and from ecological point of view. The identification of microorganisms by MALDI-TOF MS is based on the comparison of the spectral profiles of their ribosomal proteins (range of m/z 2,000 to 20,000 Da) with reference profiles. Today, MALDI-TOF MS is adapted for use in clinical microbiology laboratories, where it serves as a paradigm-shifting, rapid, and robust method for accurate microbial identification. Multiple instrument platforms are beginning to displace automated phenotypic identification instruments and in some cases genetic sequence-based identification practices. 2 In addition, classical taxonomy based on morphological characters is now widely understood to pose a series of challenges, including poor resolution and variability, also requiring relatively lengthy identification processes.3 Therefore, a rapid method requiring minimal sample preparation and analysis for the identification of these organisms is appealing. MALDI-TOF MS has been evaluated to potentially fill this niche. Nevertheless, there is no overall reference database of MALDI-TOF MS profiles comparable to GenBank or other genomic databases.4 The aim of the present study was to obtain MALDI-TOF MS spectral data from a library of 46 endophytic fungi isolated from Peperomia arifolia Miq. (Piperaceae) and Ficus carica L. (Moraceae); and to compare them with the results from sequencing of conserved ribosomal DNA regions and comparison with GenBank.5 The fungi were processed by a previously described methodology.4 The MS analysis was performed on a MicroFlex LT MALDI-TOF mass spectrometer (Bruker Daltonics). Data were processed by Principal Components Analysis (PCA, Bionumerics v7.6), and allowed to group fungal isolates in clusters that matched with the molecular identification at genera level (68.2% of the variance explained for the three first PC). The results of molecular biology were reinforced with those obtained by MALDI-TOF, high intra-genus homogeneity was observed for MS data obtained for Thielavia, Cladosporium and Chaetomium genera. Ref1-DOI:10.1146/annurev-phyto-080508-081831.

2-DOI:10.1128/CMR.00072-12. 3-DOI:10.1111/j.1365-2672.2009.04448.x. 4-DOI:10.3852/13-401. 5-DOI:10.1128/JCM.01685-08.

57. PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES FROM RESISTANT GASTRIC CANCER CELL LINE

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Background: Gastric cancer is the fourth most common cause of cancer death worldwide and one of the tumors with higher mortality rates in Brazil. Chemotherapy is one of the major treatments for Gastric Cancer, but drug resistance limits the effectiveness resulting in treatment failure. The mechanisms of Gastric Cancer pathogenesis are largely unknown what causes limitations in the personalized treatment. Neoadjuvant therapy has been largely applied in these tumors because it can improve tumor resectability and survival of patients. However, tumors can develop resistance during chemotherapy or this resistance can be present intrinsically before chemotherapy. Indeed, mechanisms associated to chemotherapy resistant in gastric cancer are complex and multifactorial, understanding the variety of this factors that are involved in chemoresistance is of great relevance. Material and methods: Gastric adenocarcinoma cell line (AGS) was treated with increased concentration of 5-fluorouracil to generate a resistant cell (rAGS_FU). Extracellular vesicles (EVs) secreted from AGS and rAGS_FU cell lines were isolated by ultracentrifugation, quantified and evaluated regarding their aggressiveness through invasion and colony assays. Proteomics analysis of EVs and secreting cells were performed to identify potential markers for chemotherapy resistance in gastric cancers cell lines. Results: We found proteins involved in resistance to chemotherapy upregulated in EVs derived from resistant cells; parental cell treated with EVs derived from resistant cells were able to promote high colony formation and increased invasion potential. Thus, cells resistant to chemotherapy have a more aggressive phenotype and are able to transfer these acquired characteristics to the non-resistant ones using EVs. We compared the proteomes of EVs derived from the two cell lines and revealed important pathways that can be associated with chemoresistance in Gastric Cancers. Conclusion: It is crucial to understand these chemoresistance mechanisms and develop efficient treatment strategies to overcome these; A deep investigation of these data is needed to understand and create new opportunities for the discovery of new biomarkers of response to chemotherapy in gastric cancers and contribute to the better understanding of the biological role of molecules shuttled by EVs.

58. PROTEOMIC ANALYSIS OF THE DIFFERENTIALLY EXPRESSED PROTEINS OF A549 CELLS INFECTED WITH HUMAN ADENOVIRUS TYPE 2

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Adenovirus delivers their DNA into the nucleus of the infected cell for replication. They accomplish this task by hijack host cell machinery whilst avoiding host defense. During adenovirus infection, the virus controls the host cell to fuel their own replication and production, while the cells have evolved mechanisms to defense and eliminate the virus. This evolutionary interaction between virus and host cell is poorly understood. In recent years, extensive studies have been done utilizing suffix “omics” including genomics, transcriptomics, and proteomics, to elucidate the interactive relationship between host and viral pathogens that revealing more details about viral strategies to modulate cellular machinery, as well as the corresponding cell defensive mechanisms. One of the most revealing approaches to study the interaction between the virus and the host cell is to understand the alternation in the proteomic profiling during virus infection and this has been the focus of our research. This study aimed to investigate the virus-cell relationship from protein expression induced or repressed by human adenovirus serotype 2 (HAdV-2) infection of the A-549 cell line. Two biological replicates of samples collected at 24 and 48 h post-infection (hpi) were investigated for both infected and uninfected cells. The proteins were extracted and then digested with trypsin, followed by separation and identification of the peptides in a liquid chromatography system (nano-ESI-UPLC-MSE). In total, 874 and 921 proteins were quantified at 24 and 48 hpi, respectively. Among them, 243 and 209 were deregulated (>1.3-fold for Up-regulated and

59. PROTEOMIC AND GLYCOSILATION MODIFICATION CHARACTERIZATION OF LEISHMANIAS

L. (V.) BRAZILIENSIS, L. (L.) AMAZONENSIS AND L. (L.) CHAGASI USING A SHOTGUN PROTEOMICS APPROACHES.

1 Saad, J. S; 1 Kawahara, 1 R; Sauter, I. P; 1 Cortez, M; 1 Palmisano, G.

1 University of São Paulo Leishmaniasis is a tropical diseases present in more than 98 countries. It is the second most common parasitosis after malaria, resulting in 20,000-30,000 deaths annually. It is caused by the flagellate protozoan of the genus Leishmania, which comprises more than 20 species. Leishmaniasis is characterized by, different clinical manifestations such as visceral leishmaniasis, cutaneous, mucocutaneous and cutaneous-diffuse. The Leishmaniasis diagnosis is currently based on immunological and molecular methods. Mass spectrometry-based proteomics has aided the immunological and molecular methods to better understand the biological role during the illness, the virulence-pathogenicity and pathogen-host interactions. This study compared the proteome and glycoproteome of three species of Leishmania: L. amazonensis, L. braziliensis and L. chagasi. A total of 2459 proteins were identified with an overlap of only 11% among the three species, demonstrating differences in protein expression between species. Clustering analyses were performed using the differentially regulated proteins among the three species, which were retrieved by ANOVA test and further visualized as heatmap and PCA. Protein expression profile revealed 69 up-regulated proteins in La and Lb compared to Lc and 78 up-regulated proteins to Lc compared to La and Lb. In the glycoproteome dataset of L. amazonensis, L. braziliensis and L. chagasi, 734 N-glycosylation sites were identified within the NxS/T/C motif (where X is not proline) covering 386 glycoproteins. Furthermore, site-specific glycan heterogeneity information was obtained by analyzing the intact glycopeptides fraction of the three leishmania species.

A total of 1560 N-glycopeptides were identified with a glycan composition consisted mainly by high mannose (80%), followed by fucose (7%). We also identified 290 O-glycopeptides with a different glycan distribution, which were composed in greater extension by fucose (39%) followed by Neu5Ac (29%).

These datasets opened exciting findings of in depth characterization of protein expression and post-translational modification in leishmania, which can be further explored in the parasite-host interaction context as well as candidate biomarkers for diagnosis and therapeutic targets.

60. PROTEOMIC CHARACTERISATION OF PRIMARY BREAST TUMOUR EXTRACELLULAR MATRIX

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Breast cancer is the most commonly diagnosed type of cancer in women, with a high mortality rate seen among breast cancer patients of African descent. High mortality rates are mostly due to late stage diagnosis and the lack of appropriate personalised therapy. Breast tumours are complex tissue masses that are made up of a number of distinct cell types that are surrounded by an intricate tumour microenvironment. Several studies have highlighted the role of the tumour microenvironment, more specifically the extracellular matrix (ECM), in tumour development and progression to invasion or metastasis. The ECM consists of numerous protein components that provide a scaffold for cellular growth and binding of growth factors that exhibit tumour promoting properties. As a result of this, ECM components can potentially be targeted by new or existing anticancer therapies and characterised as prognostic or staging markers for breast cancer. In this research project, tumour biopsies resected from patients diagnosed with stage II-IV invasive ductal carcinoma will be used to characterise the ECM that will be decellularised using optimised extraction methodologies. Proteomics involving innovative and cutting-edge techniques, such as liquid chromatography tandem mass spectrometry (LC-MS/MS) based methods that are at the fore-front of drug target validation, drug discovery and prognostic marker identification, will be used to acquire proteomic data from the ECM isolated from both tumour mass biopsies and equivalent non-tumorous breast tissue. Samples will be subjected to in-solution tryptic digestion and tryptic digests will be analysed using SWATH technology on a 6600 TripleTOF mass spectrometer from SCIEX. Advanced technology such as sequential window acquisition of all theoretical mass spectra (SWATH) will be used for the data independent acquisition and analysis of amino acid sequence data. The data will be used to identify low abundance and modified proteins as well as provide relative quantitation of ECM proteins present in different samples. Tumour associated ECM changes will be identified through comparison of the relative protein levels between healthy and tumour masses. The data from this study will be used to identify potential biological signatures of tumour development and progression as well as identify viable therapeutic drug targets for chemotherapy. Further applications for the characterised ECM would be to produce a library of defined ECM for cell culture.

61. PROTEOMIC CHARACTERISATION OF PROCESSED EXTRACELLULAR MATRIX SCAFFOLDS FOR WOUND HEALING

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1 University of Pretoria An open wound is defined as a breach in the integrity of the integument, resulting in the protective functions being compromised. Wound healing is a dynamic, complex process consisting of distinct sequential but overlapping phases that re-establish an intact integument. In an acute wound, healing proceeds in a predictable fashion but this can be hindered by external and intrinsic factors and patient pathologies resulting in the formation of a non-healing or chronic wound. Chronic wounds have a negative impact on patient quality of life, which is estimated to be comparable to that of other chronic diseases. As a result, skin injuries require immediate and effective treatment to prevent morbidity and mortality.

Current interventions for wounds are aimed at restoring the protective function while circumventing microbial infections and the effects of excessive oxidative damage at the wound site, and where appropriate bringing the edges of the wound together to eliminate tissue gaps. Hindrance of the progression of the healing process can lead to the formation of a chronic wound. Emerging therapies such as use of platelet rich plasma are centred on the stimulation of natural wound healing processes. Platelet rich plasma, isolated from the patient is rich in growth factors, cytokines, chemokines and scaffold forming fibrin which has been reported to assist the wound healing process. This research project will investigate the effect of an autologous platelet rich plasma in combination with an extracellular matrix-like scaffold, in an attempt to stimulate and guide natural healing processes. This will be assessed using a porcine wound model where different treatments will be applied to full thickness skin excision wounds.

Gross anatomical, histological and proteomic differences in the wound bed from different treatments as compared to untreated controls will be assessed at different times during the wound healing process photographic imaging, specific staining techniques and MALDI-TOF/TOF followed by comparative bioinformatics analysis. Treatments to be compared will include space filling scaffold alone and in combination with platelet rich plasma and space filling scaffold with platelet rich plasma and seeding with epithelial cells. Any significant observed differences will provide insight into the efficacy of the applied treatments and build a platform for the advancement wound healing therapies.

62. PROTEOMIC CHARACTERISATION OF SCHISTOSOMA MANSONI OESOPHAGEAL REGION TO PINPOINT PROTEINS INVOLVED IN BLOOD PROCESSING THAT COULD SERVE AS NOVEL VACCINE CANDIDATES

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Occupying the 4th position on the list of DALYs for neglected tropical diseases, Schistosomiasis represents a major health problem in developing countries worldwide. Schistosomes, the causative parasitic flatworms, possess a gland tissue located between the oral sucker and transverse gut that actively participates on the first steps of blood processing. Although it was firstly described in the 70's there is limited knowledge about the gland function and composition of its secretory products. Recent studies with schistosome-infected Rhesus macaques, known to exhibit a self-cure response, have highlighted the importance of the gland integrity for successful worm feeding to guarantee its survival in the host bloodstream. In this context, this project aims to perform an extensive proteomic profiling of the Schistosoma mansoni oesophageal region to pinpoint proteins involved in blood processing that could serve as vaccine candidates. We firstly performed a shotgun comparative analysis of the oesophageal region and the parasite´s posterior end, as a reference proteome. Surprisingly, label-free quantitative analysis revealed that a group of proteins with no homology outside Schistosomes known as Micro-exon genes (MEG) and Venom-allergen like (VAL) were overrepresented or uniquely expressed in the glandular region. This allowed the design of a QconCAT for absolute quantitation of gland constituents. At total of nine proteins were successfully quantified revealing MEG-12 as the most abundant, with a concentration of ~3ηg/μg, almost 7x more abundant than the second, MEG-4.1; the other proteins ranged from 0.03 to 0.36 ηg/μg of oesophageal proteome. Protein turnover analyses are also being carried out to reinforce the biosynthetic potential of the gland. After incubating parasites for up to 4 hours in SILAC medium containing [13C6]-Lysine/arginine the rate of stable isotope incorporation was assessed by mass spectrometry. Preliminary analysis revealed that MEGs reached >60% of isotopic incorporation whilst two gut secreted proteins presented ~50-60%. In contrast, constitutively expressed intracellular proteins barely hit 3%. Future analysis aims to evaluate the occurrence of the oesophageal gland products in worm's vomitus confirming their likely availability to the host immune system. We expect that our results contribute to a more rational selection of new molecules for vaccine design considering their abundance, subcellular location and secretory properties.

63. PROTEOMIC COMPARISON OF HYPOXIC AND NORMOXIC EXTRACELLULAR VESICLES

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The cancer cells in malignant tissue do not grow autonomously but recruit immune cells which are reprogramed not to damage but to support the the tumor growth. This conversion of immune cells requires a strong interaction between tumor and supporter cells. In addition to cell contact, the cancer cells release membrane-enclosed particles. They were characterized by electron microscopy, mass specrtometry and other biochemical methods and contain critical signalling proteins of the donor cell. Using the characteristic membrane proteins and luminal payload from the donor cell, they are able to communicate with distant cells in a cell contact-like manner. Such extracellular vesicles crucially participate in the communication between different cell types of the tumor microenvironment and support the proinflammatory state of the tumor microenvironment. However, a hallmark of such proinflammatory tissue is the low oxygen concentration, and consequently the inflammatory cells generate their energy by anaerobic glycolysis. Hypoxia strongly stimulates the release of EVs and we here pose the question whether hypoxia also influences the phenotype and molecular composition of the EVs. In this project, we cultivated Hodgkin lymphoma cell lines under normoxic (19% O2) and hypoxic conditions (1% O2), isolated the cell supernatants and isolated EVs by ultracentrifugation at 100000g. Nanoparticle tracking analysis reveiled that cells released significantly more EVs under hypoxia. Preliminary proteomics of the mass spectrometry showed that most proteins did not differ much but hypoxia generated a significant increase of proteins involved in protrusion formation, such as filopodia, ruffles and lamellopodia. Although further investigations are necessary, these data suggest a relationship between protrusions and EVs under hypoxic conditions. Together, these data suggest that hypoxia not only influences the amount but maybe also the site of release.

64. PROTEOMIC INFORMED BY TRANSCRIPTOMIC FOR THE CHARACTERIZATION OF HYALOMMA DROMEDARII SALIVA PROTEINS

1Chaima

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The camel tick, Hyalomma dromedarii, is common in regions with Mediterranean steppe vegetation and in desert climates in Africa, the Near East, Middle East, Far East, India, Mongolia, and Tibet. As a hematophagous ectoparasite, it requires blood feeding on a vertebrate host. Eventually, host defenses mechanisms are induced and ticks might be rejected. Ticks infuse saliva in to their blood host consisting of a cocktail of anti-hemostatic, anti-inflammatory, immunomodulators, which enable blood fluidity and enable ticks to succeed their blood meal. Recent transcriptomic analysis, enhanced by next generation sequencing, shed the light on insights into the molecular mechanisms involved in tick hematophagy, pathogen transmission, and tick-host-pathogen interactions. These sialotrascriptomic analysis lead to better proteomic studies, of unknown genome species, aiming to identify pharmaceutically active proteins. In a previous work, we made a de novo transcriptomic analysis of Hyalomma dromedarii tick salivary glands, using next generation sequencing. In this work, we used the generated transcriptomic data set, to identify secreted proteins of Hyalomma dromedarii tick salivary glands extracts and saliva using LC MS/MS. Our results confirmed the complexity of saliva and salivary glands complexity saliva of H. dromedarii tick, and the remarkable differences in saliva salivary glands composition between male and females ticks. The main protein families identified in H. dromedarrii were including enzymes, lipocalin, protease inhibitors, glycine rich, metastirate specific, immunity related and host protein. Our results contributes in the understanding of differences between H. dromedarrii male and female salivary glands, the tick-host relationship and opens perspectives novel drug discoveries.

65. PROTEOMIC PROFILE OF GRANULOMAS FOUND ON BIOPSIES OF LOCAL CUTANEOUS LEISHMANIASIS PACIENTS INFECTED WITH LEISHMANIA BRAZILIENSIS

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Leishmaniasis remains one of the major tropical diseases Neglected.

This pathology is caused by an intracellular parasite Transmitted to mammals through the bite of infected female sandflies By Leishmania. Leishmaniasis presents a wide spectrum of Clinical manifestations, localized cutaneous leishmaniasis (LCL) being a Of the main forms of this disease. LCL is characterized by the presence of Ulcerated lesions and erythematous borders found at the site of parasite. During the early stages of LCL, several cells of the system Immune system act in the Leishmania inoculation region and trigger Appropriate responses against the pathogen.

An intense process of activating Infected tissue-resident macrophages contributes to the development of Granulomas, structures possibly related to the elimination of Leishmania and healing of the lesions. Many factors influence the establishment Of granulomas in LCL lesions, especially the immunological responses of host. Despite the great relevance, little is known about the Importance of the formation of granulomas in the immunopathogenesis of LCL.

Thus, the Knowledge of the differentiated protein profile expressed in granulomas Containing parasites would contribute to the understanding of the mechanisms Pathological conditions caused by Leishmania braziliensis. Our proposal is the Qualitative and quantitative characterization of the changes in the In granulomas containing parasites and granulomas without parasites in the Lesions of patients with LCL by mass spectrometry technique. The elucidation of key proteins in LCL immunopathogens has the potential To generate new diagnostic methods and to identify possible targets Therapies.

66. PROTEOMICS APPLIED IN THE SUBCELLULAR CHARACTERIZATION OF THE ORBITOFRONTAL CORTEX FROM PATIENTS WITH SCHIZOPHRENIA

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Schizophrenia (SCZ) is a complex neuropsychiatric disorder characterized by three principal symptoms: positive (hallucinations, disordered thoughts and speech), negative (anhedonia, social withdrawal) and cognitive (difficulty in attention, working memory). This debilitating disorder affects about one percent of the world population and its heritability rate is estimated to be around 64-81%. The complexity in the inheritance is given by the contribution of several genetic variations which confer genetic susceptibility to this disease, but only a part of these have been identified so far. In the last decade, efforts were focused on transcriptomics and proteomics studies using post-mortem brain tissues of patients with SCZ and controls. However, despite the efforts of the scientific community, the pathophysiology of SCZ is still not fully understood. In this context, the present study proposes to map the proteomes of subcellular fractions (nucleus, cytoplasm, mitochondria and synaptosome) of postmortem brain tissues from the orbitofrontal area of patients with SCZ and control group. In our proteomic approach, 737 protein groups in the synaptosome fraction, 631 in the mitochondria, 904 in the nucleus and 1654 in the cytosol, were identified and quantified by iTRAQ labeling and LC-MS/MS analysis. The major groups of dysregulated proteins are related to oxidative stress, apoptosis, calcium metabolism, specifically those involved in the regulation of calmodulin. Also, were found proteins that are involved in regulation of cytoskeleton, processing, trafficking and degradation of proteins, synaptic plasticity and dopamine synthesis.

Currently we are working in the characterization of phosphoproteome for the elucidation of signaling pathways not yet elusive in this disease. This approach will be helpful to expanding the vision of proteomic studies available, providing a more complete view on SCZ pathogenesis.

67. PROTEOMICS IN ASSOCIATED WITH SECOND-HAND SMOKE EXPOSURE

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Non-smokers exposed to second-hand smoke (SHS) are at risk to develop tobacco smoke associated diseases and improved risk prediction and understanding of SHS-induced pathogenesis are needed. Herein, to better investigate the molecular effects of SHS we performed a proteomic study at the respiratory level of those occupationally exposed subjects. Nasal brushing samples (n=51) were selected from those worker's biobank for proteomics study. All samples were from healthy subjects with normal spirometry values (FVE1/FVC).

68. QUALITATIVE AND QUANTITATIVE FECAL PROTEOMICS OF COLORECTAL CANCER PATIENTS FED ON FLAVONOID RICH FOOD

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The range of proteomics analysis for the biological samples varies from in vitro cultured cancer cell lines to in vivo sources including tissues, organs, feces and a range of body fluids. In vitro cultured colorectal cancer cell lines have been widely used in preclinical studies; however, it cannot mimic the complex in vivo tumour microenvironment, restricting comparative studies. Suitable alternative biological samples such as body fluids e.g. blood, urine, lymphatic fluid, interstitial fluid and cerebrospinal fluid and including feces are needed for it understanding. Although many people are currently unwilling to handle their own feces, the significant advantages of using fecal samples cannot be ignored.

They offer accessible alternative biological samples for investigating a range of diseases of the gastrointestinal tract as collection is non-invasive and excessive sample and can be easily done. Feces contain not only information on the gastrointestinal tract (GI) and organs feeding into it, but also food and gut bacteria, and essentially sample the whole GI tract as they move down its length. Proteins and peptides present in stools may arise from leakage, secretion or exfoliation.

Such proteins and peptides have potential as biomarkers, especially for colorectal cancer and other bowel related diseases. Colorectal cancer have very high incidence in the human population and the majority of evidence stated a significant survival benefit with surgical resection is very low for reoccurrence. Recent studies have characterized physical activity, sedentary behavior, and cardiorespiratory fitness as distinct, interrelated constructs that influence the risk of colorectal cancer and related outcomes. Variety of chromatographic and biochemical technique will be employed for the extraction of the total proteins content followed by high-through-put MS/MS analyses.

A standardized fecal biobanks compatible with proteomics analysis will be acquired. In addition, next generation sequencing will be employed for metagenomics analyses. These investigation will help in the understanding of change in microbiota and identification of potential biomarkers for colorectal cancer.

Our preliminary data suggest that beside microbial proteins, there are many proteins of human origin that are released possibly due to the rapture of tumor cells inside. Human proteins content significantly reduced upon feeding with honey and anticancer drug compared to anticancer drug alone.

69. QUANTITATIVE PROTEOMICS UNRAVELING THE EFFECTS OF FGF2 ON CHROMATIN OF TUMOR CELLS

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Despite being a growth factor, FGF2 has anti-proliferative and tumor suppressive functions in some contexts.

In Y1 murine adrenocortical carcinoma cell line, the FGF2 promotes G0→G1 transition but delays S phase and permanently block cells in G2/M. Chromatin plays an important role in essential cellular processes such as mitosis, transcription, replication, repair, among others. To better understand the molecular mechanism unusually induced by FGF2 we performed mass spectrometry based quantitative proteomics (Label free) focusing on chromatin associated proteins. To this end, Y1 cells growing in DMEM medium were stimulated with FBS (fetal bovine serum) and FGF2 (10ng/ml) by 0, 1 and 5 h. Chromatin was extracted (from three biological replicates), digested with trypsin, desalted using Seppak and detergent removal by using HILIC columns. Peptides were further fractionated into five fractions using SCX-Stage Tips. About 180 fractions were loaded on a C18 column and analyzed by a high resolution mass spectrometry LTQ-Velos Orbitrap in an acetonitrile gradient. The data were processed against a UniProt database of Mus musculus by Andromeda/MaxQuant.

Further analyses were performed by Perseus, DAVID, STRING and Cytoscape. After stringent filtering, about 1664 proteins were identified and 169 of them were found differentially expressed (FDR 1%, p)

70. REGIOSELECTIVE SULFENYLATION OF IMIDAZOHETEROCYCLES UNDER GREENER CONDITIONS: USE OF AN IODINE/DMSO SYSTEM, AND DETERMINATION OF REACTION MECHANISM USING MS TECHNIQUES.

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Metal-free reaction for functionalization of C–H bonds to access C–C and C–heteroatom bonds is a fast emerging area.

1 On the other hand, the synthetic versatility of organosulfides is explored by great amount of research articles, reviews, and books.

2 Functionalization of the imidazoheterocycles scaffold is an important synthetic task since imidazoheterocycles are widely used for pharmaceutical, biological and material applications.

3 In this context, as part of our wider research program aimed at designing and developing eco-friendly processes,5 herein we report, for the first time we report a straightforward, mild, and environmentally benign protocol for the direct thiolation of imidazoheterocycles, using diorganyl disulfides as well as thiols. Furthermore, we are successful in capturing for intermediates using HPLC-MS and GC-MS in order to have some insight of the reaction mechanism.

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71. REVEALING MISSING PIECES OF THE PUZZLE: THE FUNCTION OF TURGOR SENSITIVE CANDIDATE GENES IN THE COMPLEX MECHANISM OF CELL WALL INTEGRITY MAINTENANCE IN ARABIDOPSIS THALIANA

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As sessile organisms, plants cannot avoid stress by moving to a more favourable environment. They have evolved the ability to adapt to stressful conditions by altering physiological and developmental processes. An important component here is the plant cell wall, which is a dynamic structure involved in environmental sensing, signalling, intracellular communication, transport and mechanical protection against environmental stress. While cellulose microfibrils form the main load-bearing elements many different polysaccharides and proteins ensure the cell wall can meet the various functional requirements during development and interaction with environment (Hamann 2015). The available data suggest that a dedicated system monitors the functional integrity of the cell wall and maintains it by inducing specific changes in structure and composition in response to cell wall damage (Hamann 2015). Knowledge about the underlying regulatory mechanism is limited but receptor-like kinases and ion channels have been implicated (Hamann 2015). This also suggests that post-translational processing and changes in protein levels could be important regulatory elements in cell wall integrity (CWI) maintenance.

The responses to cell wall damage seem to be also very sensitive to changes in turgor pressure levels (Hamann 2015). Recently my host lab, has identified turgor sensitive candidate genes implicated in CWI maintenance through extensive transcriptomics experiments. My host group has started following up by functionally characterising the candidate genes and possible interaction partners. The focus of my MSc thesis is to functionally characterise several of these candidates. The phenotypic effects of loss of candidate gene expression have been investigated for two of candidates already. KO alleles for a receptor like protein and a glucuronosyl transferase exhibit reduced CWD responses implicating the candidates in CWI maintenance. For both candidates the available evidence suggests that their activity is regulated both on the transcriptional level and by post-translational modification.

Therefore thorough training in quantitative proteomics is of particular interest to me since it will help me to understand how the candidates are regulated by the CWI maintenance mechanism.

Hamann, T. 2015, The plant cell wall integrity maintenance mechanism–A case study of a cell wall plasma membrane signalling network. Phytochemistry, 112, 100-109.

72. SCREENING OF PROTEINS ON PROSTATE CELL LINE (PC3) TREATED WITH COMPOUND FROM PTEROCARPAN (PTC) GROUP

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The malignant neoplasms are increasing with the progressive control of other diseases and the consequent population aging. The search for anticancer agents remains necessary because the drugs available so far are not sufficient to control the cancer. Also, many drugs have some fairly pronounced side effects or cells develop resistance, which limits the therapeutic.

Previous studies indicate that the pterocarpans have important antifungal, antibacterial, insecticidal and antitumor activities. Studies show other interesting biological activities such as: against snake venom; anti-HIV properties; activities against protozoa, among others. Recent advances in applied genomics helped in the target identification process, since it allowed for high throughput screening of expressed genes. However, studies have shown that there is a poor correlation between the regulation of transcripts and actual protein quantities. The reasons for this are that genome analysis does not account for post-translational processes such as protein modifications and protein degradation. Therefore, the methods employed in the drug-discovery process started to shift from genomics to proteomics. Proteomics is large-scale study of proteins, particularly their structures and functions. Here, a shotgun proteomics approach was used in the identification of differentially expressed proteins of PC3 cells (an adenocarcinoma-derived lineage) treated with a pterocarpan. The treatment here studied (PTC) modified the proteome of cultured prostate cancer cell line (PC3).

The differentially expressed proteins were analyzed in terms of their biological processes, molecular function, cellular component, pathways and protein class. Previously, studies from our lab showed antiproliferative activity of pterocarpans with the inhibition of spindle pole separation during mitosis, leading to cell cycle arrest at prometaphase in prostate cancer cell lines. In the present study, proteins associated to cytoskeletal, folding/unfolding, actin filament, and cell division processes were mostly down-regulated under treatment with PTC. We conclude that the treatment with pterocarpan causes disruption of the cell cytoskeleton of the PC3 during prometaphase, resulting in cellular responses such as the expression of molecular chaperones and various transcription factors. The analysis of this system is still being refined triggering the new conclusions and proposition of possible mechanisms of action.

73. SIMULTANEOUS ANALYSIS OF MYCOTOXINS IN CORN FLOUR USING LC/MS-MS: REDUCING THE EFFECT OF MATRIX

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The present study was developed to simultaneously quantify of different mycotoxins including Zearalenone (ZEA), T2-toxin, Aflatoxin B1 (AFB1), Deoxynivalenol (DON), and Ochratoxin-A (OTA) in thirty and ten corn flour samples collected from local Gristmill Corn in Ardabil province and local markets in Tehran, respectively. A modified method QuEChERS (quick, easy, cheap, effective, rugged, and safe) in combination with an LC-MS/MS technique was applied for sample preparation and measuring the mycotoxins levels, respectively. Spiked calibration curves based on external and internal standards were used to overcome matrix effects and were reported as linear between 2-50 ng g-1 for Aflatoxin B1, T-2 toxin, Ochratoxin A; 50-1250 ng g-1 for Zearalenone and 75-1800 ng g-1 for Deoxynivalenol.

With the aid of Spike calibration curves and acidic condition, the absorption of OTA by PSA (Primary, secondary amine) was reduced, and consequently, the percentage of recoveries was improved (in the range of 92.98-103.8). AFB1, OTA, and ZEA were detected and quantified in 23 (76.6%), 6 (20%) and 14 (46%) out of thirty samples, with average contamination of 154.1 ng g-1, 25 ng g-1, and 358.7 ng g-1, respectively. The co-occurrence of AFB1+ZEA and AFB1+OTA+ZEA was noted in 20% and 23% of corn samples, respectively. The measured level of contamination for DON and T-2 toxin of corn flour samples did not exceed the maximum tolerated level (MTL).

74. STRUCTURE ELUCIDATION OF THREE SEMI-SYNTHETIC CHITOSANS SULFATES AND UNDERSTANDMENT OF THEIR POTENTIAL ANTICOAGULANT AND ANTITHROMBOTIC ACTIVITIES THROUGH PROTEOMIC APPROACH

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The use of chitosan has attracted much attention due to its physicochemical and biological properties. The chemical modification of chitosan increased the applications of these compounds on medicine and pharmaceutical industry. In the present study, we produced a chitosan sulfate (CS) composed by glucosamine (GlcN) and N-acetyl glucosamine (GlcNAc), which were 3,6-O sulfated. Following, low molecular weight chitosan (LMWC) were produced by chitosanolysis with HCl then, centrifuged to separate the supernatant and precipitate. LMWC were submitted to sulfation producing LMWC supernatant sulfate (LMWC-SS) and LMWC precipitate sulfate (LMWC-PS), both were 2-N and 3,6-O sulfated. Despite same sulfation pattern, LMWC-SS and LMWC-PS have difference in molecular weight, 3.2 and 11.2 kDa, respectively. Both CS and LMWC were tested for anticoagulant and antithrombotic activity. At the dose of 333.33 µg/mL, whereas CS had a milder activity extending up to five times plasma clotting time, LMWC-SS and LMWC-PS extended 6 and 13 times, respectively. The results suggest that such difference on anticoagulant and antithrombotic is not only due the molecular weight, but the resulting charge density. These features can be observed on the plasma proteins, identified by proteomic analysis, that interact with these sulfated chitosans. CS and LMWCS were immobilized on affinity chromatography resin and eluted with human plasma and the proteins which interacted with each sulfated chitosan were digested and derivatized to nanoHPLC-MS analysis. Mass spectrometry spectra showed that CS interacts with IgG and IgM. LMWC-SS with IgG, and also with ApoA2, a tissue factor inhibitor. And LMWC-PS interact with ApoA1, ApoA2 and fibrinogen, inhibiting the tissue factor and preventing formation of fibrin. These results indicate that prolonging of clotting time might be related to sulfated chitosans and specific plasma protein interactions.

75. SYSTEMIC PROTEIN CHANGES DURING HUMAN AGING AND EFFECTS ON THE HSC NICHE

1 Congrains, A.; 1 Niemman, F.; 2 Ferrioli, E.; 1 Saad, T.O

1 Unicamp, University of Campinas; 2 USP , Faculty of Medicine Riberao Preto Solid scientific evidence from heterochronic parabiosis experiments (connecting the circulatory system of old and young animals) revealed the existence of factors in the blood of young animals that are able to ameliorate several aging-related phenotypes in old animals. These rejuvenating factors are able to restore the regenerative capacity of stem cells in various tissues and reverse cardiac hypertrophy, cognitive dysfunction, decreased muscle regeneration among other aging-associated conditions in aged mice. The secretion of rejuvenating compounds in the circulatory system is expected to decrease and deleterious factors to increase during aging leading to reduced regeneration capacity and general degeneration. Our study intends to deepen our understanding of the changes in the systemic composition during human aging and the identification of these aging-regulatory molecules. This study proposes the use of a proteomic approach to identify proteins that increase or decrease gradually during aging using serum samples from volunteers of different age groups. A set of candidate proteins will be validated in a sample of more than 40 individuals (10 per age group) using specific ELISA microplates. Validated proteins that showed decrease during aging will be administered to aged mice to achieve systemic concentrations comparable to those found in young mice. Changes in hematopoietic stem cells, number of myeloid and lymphoid progenitors and other phenotypes related to aging will be evaluated. Our results aim to contribute to the development of new therapies for debilitating diseases in the elderly, particularly those related to the hematopoietic compartment.

76. TARGETED ANALYSIS OF CLONOTYPIC PEPTIDES IN PLASMA CELL DISCRASIAS

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{Introduction} Plasma cell dyscrasias are produced as a result of abnormal proliferation of a monoclonal population of plasma cells that may or may not secrete detectable levels of a monoclonal immunoglobulin (paraproteins). Multiple myeloma (MM) is a disseminated malignancy of monoclonal plasma cells that accounts for 1.3 % of all malignancies and 15 % of hematologic cancers. Its treatment is costly, prone to adverse events and can trigger comorbidities. The decision to withdraw the drug regimens is based upon negativity of the routine tests (immunofixation, protein electrophoresis, flow cytometry). However, many cases relapse and are defined as positive minimal residual disease (i.e. residual amount of cancer cells that remain after treatment).

Our objective is to develop a method that can detect smaller amounts of paraprotein than the currently used ones, in a less invasive way for the patient. {Methodology} Serum samples are drawn from myeloma patients at the diagnosis as well as during and after treatment, to monitor minimal residual disease. Variable regions of immunoglobulin light chain are sequenced by RACE PCR at the MM diagnosis (i.e. when the clonotypic cells are highly expressed). Translational data identify the unique peptides characteristic for the clone: those peptides are considered as specific for the patient and are followed by mass spectrometry-MS- (Ultimate 3000 coupled to a QExactive).

In order to simplify the discovery and identification processes by MS, here different sample preparation methods are compared, either enriching for the immunoglobulins or decreasing the serum complexity by removing selectively albumin and few other blood abundant proteins. Finally, we are investigating an alternative approach for the identification of unique peptides, by using peptide De Novo sequencing. Optimization of this approach will lead to a procedure based only on MS and will allow to overcome the need of RNA sequencing.

{Preliminary results} Our preliminary results show that specific enrichment of the variable regions of immunoglobulin light chains allow more sensitive clonotypic peptide detection than current methods. Systematic analysis of both unique and non-unique peptides before and during treatment will be investigated for the detection of cases with minimal residual disease or in full remission. This will be extremely important for individualized treatment decision.

77. TARGETED PROTEOMICS APPLIED TO the RESEARCH IN PUBLIC HEALTH: IMPLEMENTATION OF the LATEST TECHNOLOGICAL ADVANCES IN FIOCRUZ

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Targeted proteomics is an approach based on mass spectrometry that accurately detects and quantifies pre-selected analytes. This method is considered the new gold standard technique to verify/validate shotgun results. Ions with specific m/z ratios are monitored in a fast, selective, sensitive and reproductive manner, even when these analytes compose highly complex samples with large dynamic range (e.g.: plasma). Therefore, it represents a robust analytical technique complementary to immunoassays and it is crucial to advances in clinical proteomic research. In the last year, I worked as a post-doctoral researcher under the supervision of Dr. Bruno Domon (Luxembourg Institute of Health) who is a specialist in the field and the creator of PRM (Parallel Reaction Monitoring) analysis workflow. The current post-doctoral project will enable the implementation of the targeted proteomics approach at Fiocruz/RJ, including the latest developments of Dr. Domon's group in PRM technology (e.g., on-the-fly mode), which will address the pressing demand of the studies in public health. The PRM approach will be applied to two independent research projects from the Laboratory of Toxinology-Fiocruz/RJ to: a) monitor the abundance of proteins with antiophidic properties from the plasma of snake bite-resistant animals to better characterize the natural immunity phenomenon; b) quantify a panel of proteins related to invasiveness and aggressiveness of breast cancer in NAF (Nipple Aspirate Fluid) samples, which is a biological fluid closely related to the tumor microenvironment. So far, the digestion protocol was set and the high quality experimental spectral library was built. The PRM method was successfully stablished for 21 peptides from three proteins with antiophidic properties: alpha-phospholipase A2 inhibitor, gamma-phospholipase A2 inhibitor, and antihemorrhagic factor BJ46a.

At this moment, peptides from these proteins with post-translation modification (PTM) are being experimentally evaluated to compose an additional PRM list. After implementation of the PRM method at Fiocruz/RJ, the approach will be employed not only to diverse institutional projects, but also to other projects from collaborative institutions.

78. THE ANTI-INFLAMMATORY AND ANTI-MICROBIAL PROPERTIES OF NOVEL METAL BASED COMPLEXES

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A central problem in the health system is the threat posed by multi-drug resistant organisms with Pseudomonas aeruginosa being one of the most pathogenic, Gram-negative bacteria found in hospital and community environments. It is known to be multiresistant due to its intrinsic/acquired antibiotic resistant mechanisms, biofilm formation properties and its vast repertoire of virulence factors signalled by Quorum sensing (QS) genes. There is a need for new therapeutic agents that can overcome antibiotic and drug resistance through different mechanistic pathways. In parallel, the construction of novel metallotherapeutics as potential anti-inflammatory drugs is one of the promising strategies employed for the treatment of carcinogenesis and other inflammatory related diseases. The coordination of Ag+1 ions with bioactive hydrophobic N-donor ligands such as 1,10-phenanthroline, can increase their bioavailability through improved cell membrane permeability yielding significant improvements in anti-bacterial, anti-fungal and anti-biofilm capabilities when compared to free Ag+1 ions. The synthesis, in vitro antimicrobial activities, a study of their interactions with DNA, and their pro-oxidant and antioxidant capabilities of the silver(I) complexes [Ag2(udda)] (where uddaH2 = undecanedioic acid) and its water-soluble and highly photo-stable 1,10-phenanthroline derivative [Ag2(phen)3(udda)] have been recently reported. My research is dedicated to furthering the insight into the mechanistic action of these complexes activity utilising two in vitro models; mammalian (peripheral blood mononuclear cells) and bacteria (clinical resistant strains of P. aeruginosa), and an in vivo model, larvae of the insect Galleria mellonella. The profiles of [Ag2(udda)] and [Ag2(phen)3(udda)] were assessed against clinical resistant strains of P. aeruginosa along with a reference strain (ATCC 27853). The Minimum Inhibitory Concentration (MIC) for each compound was determined. All strains were susceptible to the compounds with one strain showing a lower MIC to MD5 (MIC=8µg/ml) compared to the common antibiotic Ceftazidime (MIC=32µg/ml).

The in vivo Galleria mellonella model has been used to identify the key genomic and proteomic cellular signalling cascades induced by selected metallotherapeutic drugs at very low active concentrations to elucidate their mechanistic mode of action and their potential as alternative chemotherapeutic drugs for future development.

79. THE FUNCTIONAL ROLE OF CHEMOKINE RECEPTOR XCR1 AND ITS BIOENGINEERED LIGAND IN ORAL CANCER

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Introduction: Oral squamous cell carcinoma (OSCC) has a poor prognosis which further worsens after metastasis to cervical lymph nodes. Expression of the chemokine human lymphotactin (hLtn) and its receptor XCR1 has been shown in OSCC and is thought to facilitate cell migration, proliferation, invasion and metastasis. hLtn is also biologically interesting being the only metamorphic chemokine. The aim is to determine the effect of hLtn on XCR1 regulation expression, adherence to basement membrane constituents which is vital in invasion, as well as producing different functional hLtn conformations. Methods: Immunohistochemistry for XCR1 and hLtn was performed on tissue sections from normal lymph nodes, primary tumour and metastases. Oral cancer cell lines (OCCL) were incubated with hLtn and effect on XCR1 expression determined using qRT-PCR and flow cytometry. Adhesion to collagen I and IV with and without hLtn stimulation was also studied using a range of concentrations. Due to the unique nature of hLtn, bioengineered hLtn (wild type, CC3 and W55D mutant) were designed and produced. CC3 has the active conformation of chemokine while W55D exists as a dimer. Chemotaxis assays using OCCL were used to determine protein functionality. Results and Discussion: Strong ex vivo expression for XCR1 and hLtn was seen in primary and metastatic OSCC. All OCCL variably expressed XCR1 and the highest XCR1-expressing OCCL (SCC4) showed a significant reduction in receptor expression after treatment. OCCL showed significant attachment to collagen I and IV and further increased after hLtn stimulation.

80. THE POTENTIAL USE OF MASS SPECTROMETRY IN THE DIAGNOSIS OF PRIMARY AMYLOIDOSIS

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Introduction/Objectives: Amyloidosis comprise a group of disease characterized by fibrillar protein deposition in extracellular matrix of several organs and tissues. The amyloid formation causes damage to cells and tissues and dysfunction of the affected organ. Thirty six proteins have been described as amyloid precursor which generally accumulate in heart, kidney, adipose tissue, nervous system and liver. The amyloid fibers are identified by biopsy of affected organ followed by two possible techniques: optical microscopy after Congo red staining and immunohistochemistry. The former is sensitive but unable to predict the protein identity. On the other hand, tests based on immunohistochemistry allow identification of deposited proteins. However, it is necessary a large panel of antibodies for all known variants. In addition, this technique presents limitations related to cross-reactivity and loss of epitope resulting from the structural alterations suffered by deposited proteins. Therefore, it is necessary the development of new methods with high sensitivity and specificity for amyloidosis diagnosis.

Our objetive is to apply mass spectrometry technology for unequivocal identification of proteins accumulated in primary amyloidosis. Material and methods: Amyloid deposits were submitted to laser microdissection. The proteins from excised fragments were extracted using eFASP optimized method. The peptides generated were analyzed by nanocromatography coupled to the Q-Exactive mass spectrometer.

The spectral data acquisitions were performed using the DDA mode (data dependent acquisition) with the selection of the 10 most abundant ions for sequencing. The data was processed in MaxQuant software. Clinical data from each patients were used to confirm the type of protein involved in the deposit. Results/Conclusion: We analysed twenty samples from different organs which included heart, kidney, adipose tissue, bone marrow and stomach. It was possible to unequivocally identify the amyloid protein in all analyzed biopsies. Extraction using eFASP increased by approximately 200% the number of sequenced proteins. Futhermore, laser microdissection allowed the analysis of non affected areas inside the same sample as negative control. The association of laser microdissection and mass spectrometry, as well as patient clinical analysis are powerful tools for definitive amyloidosis diagnosis.

81. THE SIZE OF THE PARTICULATE MATTER ADDRESSES THE PATHWAYS TO THE BRAIN

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Air pollution has been negatively implicated in pulmonary, cardiovascular, and central nervous system. However the influence resulting from exposure to air pollutants on brain cancer remains unclear1. Among all air pollution components, which includes particulate matter (PM), gases, organic compounds and metals, PM seems to be the most widespread threat to the society. PM is mostly characterized by their size, e.g., regarding to their diameter,

82. TOWARDS AN UNDERSTANDING OF THE EVOLUTION OF THE HPV E6 PDZ BINDING MOTIF

1, 2 Sarabia, V.; 1 Banks; L.

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2 Open University The cancer causing HPV E6 oncoproteins have PDZ binding motifs (PBMs) that allow the virus to interact with PDZ domain-containing proteins of the cell.

The E6 PBMs are quite diverse in their sequences, minor variations in the PBM can have a dramatic effect on PDZ targets. The ability to interact with PDZ proteins was thought to be only found in cancer-causing HPV types, but certain benign E6s have also ancestral PBMs. The list of potential substrates for E6 is high, and this reflects the diversity in the PBM composition between HPV types, such that each E6 from HPV16 and 18 will have a number of PDZ targets that differ from those of HPV 31, 33 and 58 E6s. We have found significant variation in the numbers of PDZ substrates that can be bound by each HPV E6 PBM. HPV16 and HPV18 E6 proteins are the most promiscuous, HPV66 which is not classified as cancer-causing has an E6 PBM with a more restricted target range, and benign HPV40 E6 has an ancestral PBM, with a restricted profile of potential PDZ targets. Furthermore, the PBM has a phospho-acceptor site (PAS), which adds to the functional diversity of E6 and its phospho-regulation (PR). It is possible that the ability to target multiple PDZ substrates has co-evolved with PR of the PBM. We have done pull-down (PD) assays with the PBM of the HPV18/66/40 E6 and the corresponding mutant PBMs to evaluate the range of targets by mass spectrophotometry (MS) analysis. The PD assays were verified by western blot for the different PDZ proteins that only bind to E6s that have been phosphorylated within the PBM. Furthermore HPV 18/66/40 E6 mutated proteins have been expressed to assess their phosphorylation by AKT or PKA.

These E6s will be used in immunoprecipitation with HA-tagged; and also fused with BirA* protein for proximity-labeling assays on 293 cells, coupled with MS, to determine whether the evolution of a PAS is accompanied by an increase in the number of PDZ substrates that can be bound by each PBM. Any potentially novel PDZ proteins that are identified will also be analysed in cells derived from cervical tumours in order to evaluate their potential relevance for HPV induced malignancy. We expect to identify key residues whose mutation reduces HPV18 phosphorylation and correspondingly reduces the functional flexibility of the E6 PBM; and conversely, identify residues in HPV66/40 E6 PBMs that, when mutated, generate a PAS and increase the functional flexibility of the E6 PBM.

83. TOWARDS PERSONALIZED TREATMENT OF ORAL SQUAMOUS CARCINOMA VIA DEVELOPMENT OF A RELIABLE ORGANOTYPIC IN VITRO METHOD

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Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide.

Despite the advances in its treatment, the 5 years survival did not improve significantly with an average of 50% survival.

Treatment approaches for the OSCC include surgery, radio-, chemo-, and targeted therapy. Traditionally, in vitro studies using cancer cell lines cultured on plastic are used to test the efficacy of new anti-cancer compounds, unfortunately such studies are missing the important aspects of cancer cells-tumor microenvironment (TME) interaction.

Recently, organotypic in vitro culture techniques preserving the 3D histological structure of the tumor including TME have been developed. These methods aim to preserve the normal morphological tumor appearance, and they could also be used as preclinical platforms to individually measure the outcome of the radio- and/or chemotherapy. New anti-cancer compounds are commonly first tested using viability assays in 2D cell culture. In this PhD-project we aim to develop a reliable in vitro model which could be used to test different cancer treatment modalities on the OSCC patients´ samples before giving them to the patients. This model could be used to detect cancer cells proliferation and invasion potential, enabling the treatment plan towards "personalized medicine" approach. We´ll use OSCC tumor pieces and/or isolated cells from tissue, culture them on top of human based extracellular matrix "Myogel", extracted from human leiomyoma, and supplied them with patient´s serum. Myogel provide a TME mimicking human tumor matrix, including e.g. soluble cytokines and growth factors. The second approach is to show whether drugs testing in 3D cell culture on top of Myogel could provide more reliable results than 2D cell culture on top of plastic. Preliminary results showed that cancer cells cultured in 3D Myogel gave different response to anti-cancer drugs than those cultured in 2D on plastic. Our plan is to test the efficiency of anti-cancer drugs and radiotherapy against several OSCC cell lines cultured in Myogel coated dishes vs uncoated dishes. We aim also to develop a novel human based extracellular matrix derived from metastasized lymph node "Lymphogel" to provide more reliable environment to culture metastasized cancer cells. Development of reliable 3D in vitro models could significantly improve the selection of the best treatment option for the OSCC patients.

84. TOXICOLOGICAL ASSESSMENTS OF NANOPRODUCTS USING ANALYTICAL, BIOCHEMICAL AND MOLECULAR APPROACHES

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Nanotechnology is undergoing an explosive expansion, with more than 1000 nanoproducts already in the market.

Emerging biomedical applications of manufactured nanomaterials (NMs) include their exploitation as biosensors or drug-delivery agents, often coated with bioconjugates such as DNA and proteins to target specific cell types. However, the same properties that make NMs so attractive may also contribute to their toxicological profile in biological systems. NMs have greater surface area per mass compared to the raw material, rendering them more reactive and potentially more toxic in the cellular environment. In fact, concerns about the carcinogenic potential of NMs have been justified by observations that they display higher toxicity in rodents than equal mass doses of their non-ultrafine counterparts and are tumorigenic in rodents after high dose exposures. Since toxicity testing in animals is extremely expensive and should be reduced for undeniable ethical reasons, it becomes important to find alternative methods to test NMs' toxicity. Toxicological studies focused on the growing number of NMs become even more relevant considering that presently no clear guidelines are available for their testing/evaluation. To fill this gap, several new initiatives such as Tox21 have begun to utilize in vitro models and a variety of new technologies to develop in vitro signatures predictive of in vivo response. To answer this emergent need, and following the current trends, the major scientific objectives proposed here will be i) gathering toxicological data for several NMs based on current cytological/genotoxic assays, ii) identifying cellular stress response pathways and their key endpoints/proteins, and iii) generating a knowledgebase with relevant information regarding NMs' diversity and cellular toxicity to retrieve protein signatures associated with the toxicological effects of NMs. Based on this results, we expect to contribute for further development of new highthroughput testing strategies that could be used for risk assessment at industry and legislator level.

85. TUMOUR MICROENVIRONMENT IN BONE INVASIVE ORAL CANCER

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1 The University of Sheffield. Oral squamous cell carcinoma (OSCC) frequently invades maxillofacial bones, worsening prognosis. The mechanism of OSCC bone invasion is poorly understood; in particular, the role of the fibrous stroma and cancer-associated fibroblasts (CAFs), the existence of which accounts a poor prognosis. Demographic and clinicopathological data from OSCC cases (n=277) with bone resection were reviewed and 32 analysed in detail histopathologically. Immunohistochemistry (IHC) for receptor-activated nuclear factor kappa-β ligand (RANKL), osteoprotegrin (OPG) and α-smooth muscle actin (α SMA) was also carried out on matched cases with cortical and lamellar OSCC invasion (n=5). Osteoblastic cell lines (SAOS and TE85) were co-cultured with conditioned media from OSCC cells (H357) and cancer-associated fibroblasts (CAF) followed by RANKL and OPG mRNA detection using quantitative real-time polymerase chain reaction (qPCR). Primary oral fibroblasts were treated with transforming growth factor-β (TGF-β) to induce myofibroblastic transdifferentiation, and an enzyme-linked immunosorbent assay (ELISA) used to detect RANKL protein secretion. Osteoclastogenesis was conducted by culturing murine monocytes (RAW 264.7) in H357, NOF, and CAF conditioned media. No direct contact between tumour and bone was seen in most cases, as fibrous stroma appeared to intervene. In addition, αSMA-positive myofibroblasts were seen infiltrating bone ahead of main tumour. Strong RANKL and OPG staining was also seen in tumour and stroma adjacent to bone. A significant increase in RANKL and decrease in OPG mRNA by osteoblasts was observed after indirect co-culture of osteoblasts with both OSCC cells and CAF. RANKL secretion by fibroblasts was significantly up regulated following TGF-β treatment. Indirect co-culture of murine macrophages with conditioned media from CAF (isolated from human OSCCs) resulted in a marked increase in osteoclastogenesis, which was verified by tartrate-resistant acid phosphatase, multinucleation and osteoclastic resorption pit formation. These findings suggest an important role for CAF in OSCC bone invasion.

86. UMBILICAL CORD TISSUE AS A NEW MATRIX FOR THE TOXICOLOGICAL ASSESSMENT OF FETAL EXPOSURE TO CANNABINOIDS AND COCAINE

1 Silva, J.P; 1 Yonamine, M.

1 University of São Paulo, Faculty of Pharmaceutical Sciences Drug abuse affects approximately 324 million people around the world.

This high number of users has generated concern because of its devastating potential, offering serious risks to human health, fatal poisoning, psychosocial and economic problems and especially keeping a large number of individuals to a condition of dependency and social marginalization. In Brazil, the number of users has grown mainly for the widespread drugs marijuana and cocaine, including women in pregnancy as emergent group of users with a great impact on public health. Drug abuse by pregnant women brings irreversible damage to the neonate, promotes maternal neglect and increases fetal mortality rates. However, this issue is poorly explored in Brazil. Toxicological, molecular and biochemical data are scarce and the clinical evaluation of in utero exposure depends largely on the mother's report, which often omits or denies drug abuse. Traditionally, to detect the exposure, screening tests in conventional samples (urine and blood) are useful tools but have some limitations for collecting and processing as well as small period of detection. The development of new approaches to predict the use of drugs during the pregnancy has been a challenge for the toxicological assessment. This research project aims to study the applicability of umbilical cord tissue (UCT), in comparison with meconium, as an alternative human sample for diagnostic of in utero exposure to cocaine and cannabis. For this, the main metabolites of these drugs will be assessed by mass spectrometry in order to establish the better biomarker in this alternative matrix. Further, we intent to prospect the impact of drug abuse in the tissue at molecular level, using proteins as possible novel biomarkers to toxicological evaluation in pregnancy. Once the umbilical cord is the main interface between the mother and the fetus it can provide a comprehensive history of contact with intoxicants in gestational period. Being available in all babies, the collection can be performed immediately after birth in sufficient quantity for analysis, bringing analytical advantages from the collection and processing steps. Until now, were collected 39 paired samples of UCT and meconium. The UCT samples were excised at three portions (proximal to the fetus, placenta and intermediary). The urine drug screening was positive to THC and cocaine in three samples, corroborating with maternal report of abuse.

87. UNRAVELING PERITONEAL DIALYSIS EFFLUENT PROTEOME EVOLUTION

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7 UMIB/ICBAS/University of Porto ;

8 Department Internal Medicine IV (Nephrology and Hypertension), Innsbruck, Austria Up-to-date there is a focus of interest in peritoneal dialysis (PD), as it provides a better quality of life and autonomy of the patients than other renal replacement therapies such as haemodialysis.

However, long-term PD leads to morphological and functional alterations in the peritoneum, reducing the lifespan of this dialysis up to five years, and forcing the replacement of PD by other renal replacement therapies. Peritoneum failure does not occur in every patient in the same sequence and to the same extent. The possibility to identify and follow changes in the PM at the molecular level by proteomics has recently been proposed through using longitudinal studies, as of prime importance to unravel morphological and biochemical changes in the long-term PD [1][2]. This work aims to develop longitudinal studies to unravel the evolution of the proteome of the peritoneal dialysate with time, so biomarkers and molecular profiles for diagnosis and prognosis can be obtained. Experimental description: Peritoneal dialysis effluent (PDE) samples from anonymous patients, already at different stages of dialysis were taken (patients in the beginning of PD and long-term PD patients). The PDE from the patients was analyzed using 2D gel electrophoresis [2][3]. Gel comparison and statistical analysis using Progenesis SameSpots indicated the gel spots differentially expressed for each patient. All the detected spots were excised digested and identified by MALDI-MS. In addition, an open source software developed by our research team was used for fast and automatic processing of 2D-gel and MALDI-based mass spectrometry protein data[4]. Conclusion: All the identified proteins were studied for their biochemical function. Identification of specific molecular changes can be particularly interesting for the understanding and early detection of long-term peritoneum alterations as well as for the development of new therapies to increase the lifespan of the peritoneal dialysis. References:

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Clinical Proteomics, 2014, 11,17 4 www.sing-group.org/s2p Acknowledgements: This work was supported by PROTEOMASS Scientific Society (Portugal).LAQV/REQUIMTE (UID/QUI/50006/2013) and UCIBIO/REQUIMTE (UID/Multi/04378/2013). J.E. Araújo acknowledges Portuguese Foundation for Science and Technology under doctoral grant number SFRH/BD/109201/2015.

88. USED OF IMMUNOPROTEOMICS TO INTERPRET ANTIGEN PROCESSING DATA

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Epithelial cells are the targets of autoimmune processes in many diseases, such as diabetes or Graves' thyroiditis, or in organ transplant rejection.

Inflammatory environment, in this case autoimmunity, induce major histocompatibility complex (MHC) class I and class II expression in epithelial cells, so the peptides presented by the MHC molecules may represent the candidate targets of the autoimmune responses. The sequence of each MHC allele's peptide binding site determines the structure of the peptides bound so that peptide repertoires associated are characteristic of each allele. We are currently studying the peptides constituting the repertoire associated to HLA-DR3, a MHC-II allele associated to a large number of autoimmune diseases. The aim is to compare the peptide repertoire of HLA-DR3 and their dependence on the two main MHC-II associated chaperones, the invariant chain (Ii) and HLA-DM, in comparison to other alleles. We are using classical approaches, i.e., immunoprecipitation of MHC molecules, peptide elution and sequencing, using MS techniques. Previous analysing of the DR3 associated repertoires in human dendritic cells as well as human thymus, spleen and autoimmune organs, showed differential characteristics of the DR3 repertoires compared to other alleles. We will now compare the repertoires of a rat epithelial cell line (RINm5F) transfected with HLA-DR3, HLA-DM and Ii genes in different combinations. Peptide repertoires will be identified by high resolution mass spectrometry techniques using LC-MS/MS in an Orbitrap XL instrument together with database search using several search engines. The results will be compared with previous results from a similar set of transfectants carrying the HLA-DR0401 allele. Thus, by using immunoproteomic techniques, we are expected to identify potential alternative pathways involved in peptide binding to HLA-DR that may differ between different alleles.

89. WHICH PROTEINS ARE RESPONSIBLE FOR THE SELECTIVE NEURONAL VULNERABILITY IN PARKINSON'S DISEASE?

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Within neurodegenerative disorders specific neuron populations are degenerating, e.g. dopaminergic neurons in the substantia nigra during Parkinson's disease [1, 2].

Furthermore, not all dopaminergic neurons within the substantia nigra are equally affected. Studies revealed that ventral located neurons within the substantia nigra have a higher prevalence to degenerate during Parkinson's disease compared to neurons in the dorsal tier of the substantia nigra [3]. Reasons for this selective neuronal vulnerability are still an open issue [4]. To gain a better understanding of molecular differences between these types of neurons, they were isolated specifically with laser microdissection [5]. While methods in the past had the disadvantage that it was not possible to isolate specific cell populations of a complex tissue, laser microdissection overcomes this problem. Using this approach in combination with data independent acquisition mass spectrometry dorsal and ventral located neuron populations within the substantia nigra were analyzed. The results of this study revealed proteomic differences between these two types of neurons. With that hints a deeper understanding of the protective mechanisms against neurodegeneration in specific neuron populations can be gained.

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