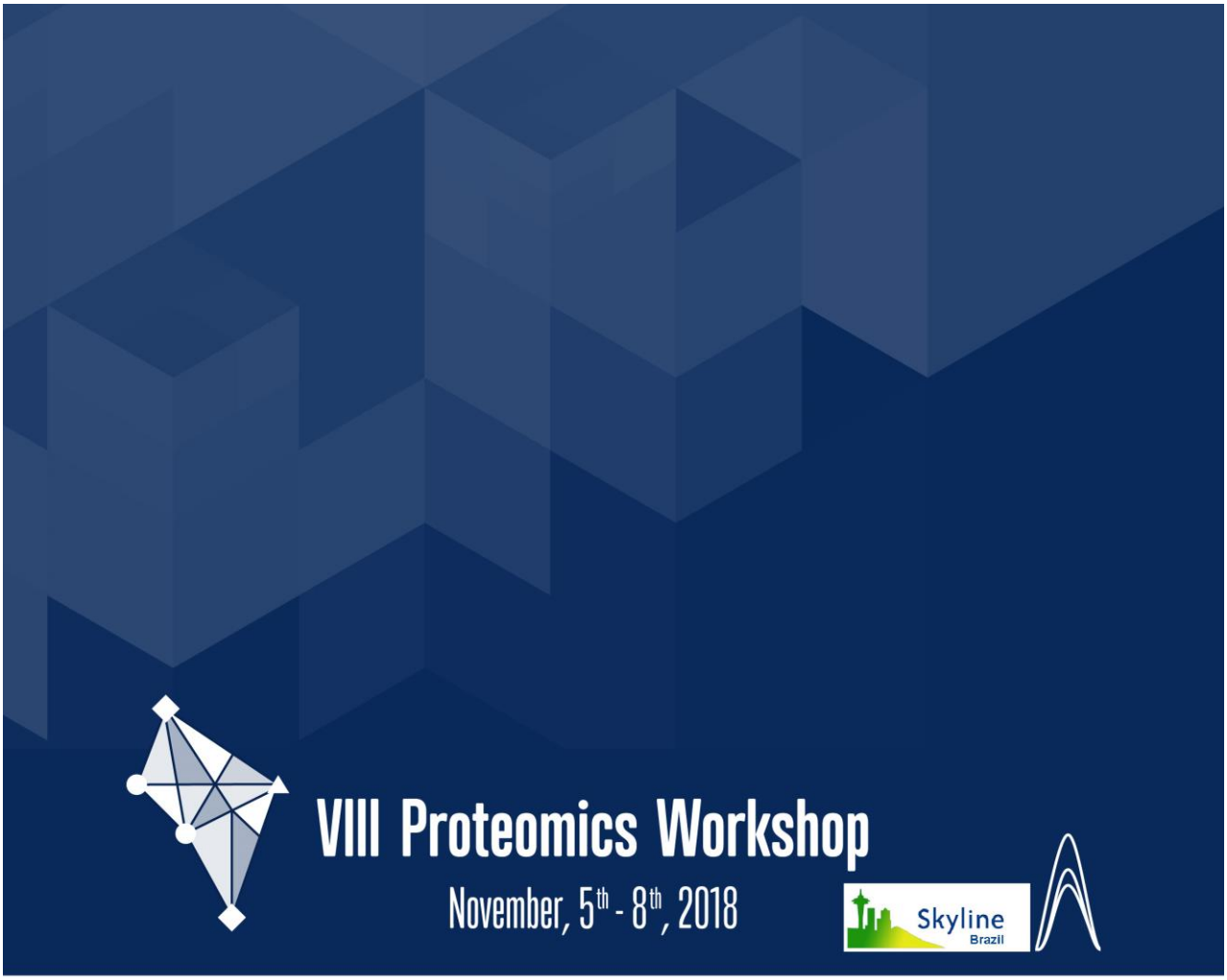

http://pages.cnpem.br/proteomicsworkshop

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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, discovery and development. LNBio´s activities are organised into four areas: Open Facilities; Innovation Core; Research in-house; Training and Education. This organisational strategy was designed to encourage the sharing of infrastructure and skills with the academic and industrial sectors. Thus, LNBio optimises and directs its resources to Science, Technology and Innovation activities.

VIII Proteomics Workshop Skyline

The VIII Proteomics Workshop Skyline will be held by the Brazilian Biosciences National Laboratory (LNBio) at the Brazilian Center for Research in Energy and Materials (CNPEM) Campus in Campinas-SP from 5th to 8th November 2018.

The event is focused on highlighting the recent developments and the state-of-the-art of Mass Spectrometry-Based Proteomics. It is divided in three-days workshop and the lectures cover several of MS applications.

This meeting represents a training opportunity for current and future users of the LNBio´s Mass Spectrometry Facility and for researchers in the area of proteomics and mass spectrometry. It also promotes the exchange of scientific knowledge, as well as the formation of new research groups in these thematic areas.

Following the example of the previous editions, held in the last six years, the workshop also promotes the exchange of new technologies, the closer relationship between Brazilian and international researchers and dissemination of knowledge in the community.

Adriana Franco Paes Leme,
VIII Proteomics Workshop Skyline Coordinator

Organizers

Coordinator

Adriana Franco Paes Leme

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Agenda

Theoretical and Hands-on Program

Day 1 – 05/11 – Introduction into targeted proteomics – SRM

8:30 AM	**Registration**
8:45 AM	**Welcome** Adriana Franco Paes Leme and Birgit Schilling
9:00 AM	**Basics of Quantitative Proteomics – from relative to absolute** Tina Ludwig
10:30 AM	**Coffee break – Poster presentation 1-12**
11:00 AM	**Introduction into targeted mass spectrometry** Tina Ludwig
12:00 PM	**The biological relevance of targeted proteomics (case study)** **Ariel Bensimon**
1:00 PM	**Lunch**
2:00 PM	**Introduction to Skyline** Ariel Bensimon
2:30 PM	**Tutorial: SRM method development** Tina Ludwig/ Ariel Bensimon
4:00 PM	**Tutorial: Data analysis** Tina Ludwig/ Ariel Bensimon
6:00 PM	**Science Slam – Poster presentation 1-12 (30 min)**
8:30 PM	End of day

Day 2 – 06/11 – Building prior knowledge for targeted proteomics

9:00 AM	**Building prior knowledge for targeted proteomics** Birgit Schilling

10:00 AM	**Coffee break – Poster presentation 13-24**
10:30 AM	**Hands-On: Method refinement** Lindsay Pino / Tina Ludwig
12:00 PM	**Panorama Introduction and Hands-On: Panorama targeted knowledge base** Birgit Schilling
1:15 PM	**Lunch**
2:15 PM	**System suitability and Panorama QC** **Lindsay Pino**
3:30 PM	**Coffee break – Poster presentation 13-24**
4:00 PM	**Hands-on retention time prediction with iRT in Skyline** Ariel Bensimon / Lindsay Pino
6:00 PM	End of day
6:30 PM	**Extra time for installation of necessary R scripts and MSstats**

Day 3 – 07/11 – Study design and statistical considerations in targeted proteomics

9:00 AM	**Hands-On: Grouped study data processing with Skyline** Ariel Bensimon
10:15 AM	**Coffee break – Poster presentation 25-36**
10:45 AM	**Study design and statistical considerations** Lindsay Pino
12:00 PM	**MSstats and its integration with Skyline** **Lindsay Pino**
1:00 PM	**Lunch**
2:00 PM	**Tutorial: MSstats for statistical analysis** Lindsay Pino
3:00 PM	**Targeting proteomics at high throughput (research examples)** Lindsay Pino

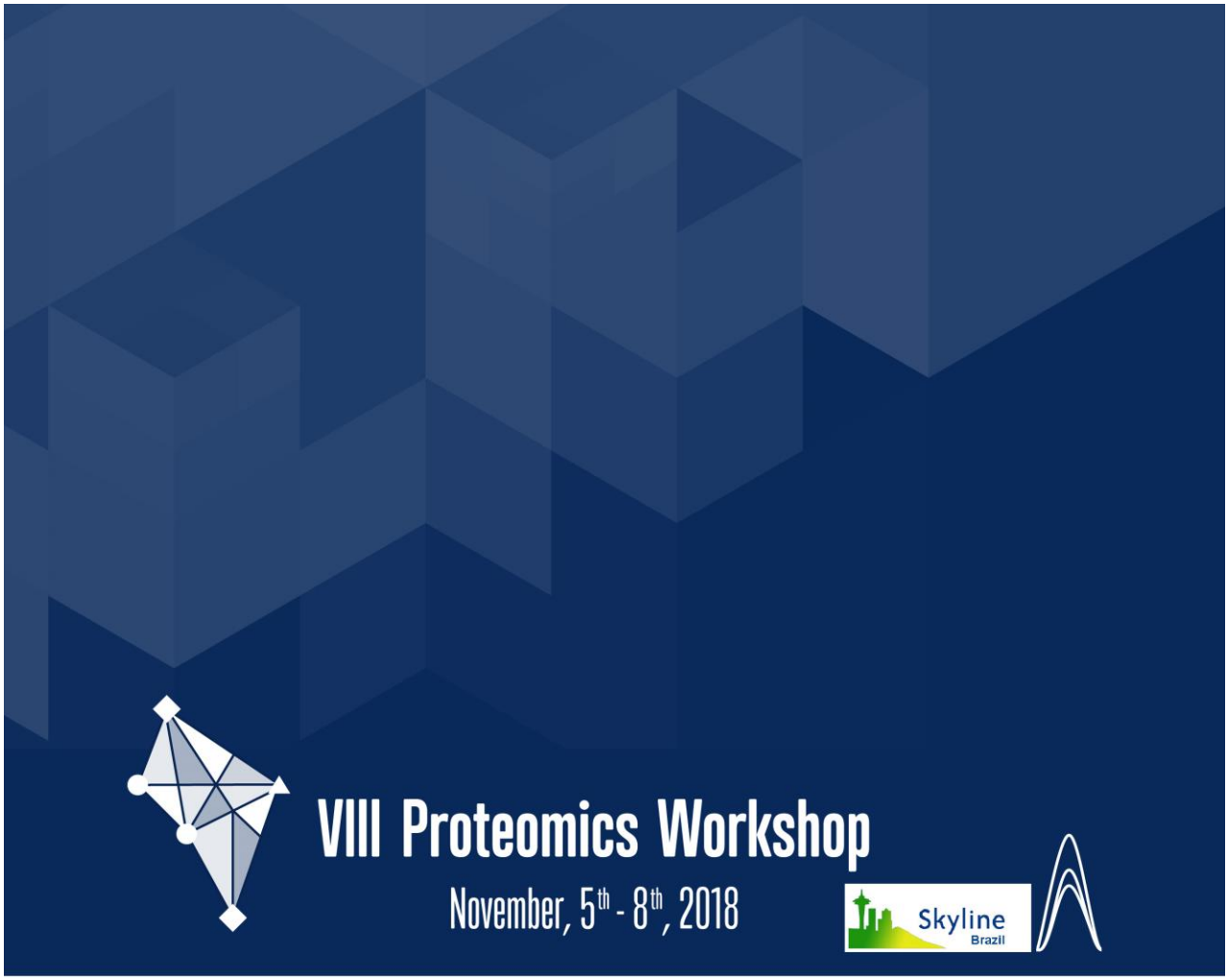
4:00 PM	**Coffee break – Poster presentation 25-36**
4:30 PM	**Waters – The Science of What's Possible** Alexandre Gomes – Mass spectrometry Specialist
4:45 PM	**Introduction to Calibration Curves / Absolute Quantification** Birgit Schilling
5:15 PM	**Hands-On: Calibration Curves / Absolute Quantification** Tina Ludwig / Birgit Schilling
6:45 PM	**End of day**

Day 4 – 08/11 – Performing PRM and DIA measurements

9:00 AM	**Intro to MS1 Filtering (from DDA data) and from DDA to PRM** Birgit Schilling
9:30 AM	**Hands-On: MS1 Filtering with Skyline** Birgit Schilling
10:30 AM	**Coffee break – Poster presentation 37-41**
11:00 AM	**Hands-On: PRM with Skyline** Birgit Schilling
12:30 AM	**Introduction to Data-Independent Acquisition (DIA)** **Lindsay Pino**
1:30 PM	**Lunch**
2:30 PM	**Hands-On: Analysis of DIA data in Skyline** Lindsay Pino
4:00 PM	**Coffee break**
4:30 PM	**Future of targeted proteomics** Birgit Schilling an all
5:00 PM	**Feedback and Course Certificates** all
5:30 PM	**Kahoot – final quiz and contest** all
6:30 PM	**End of course**

Speakers

- **Birgit Schilling,**
  Buck Institute for Research on Aging, USA
- **Lindsay K. Pino,**
  University of Washington, USA
- **Ariel Bensimon,**
  CeMM, Austria
- **Christina Ludwig,**
  Technical University Munich, Germany



Poster Session

1.Adriele Ferreira Gouvêa Vasconcellos
Universidade Estadual de Campinas (UNICAMP)/CAPES

Radiotherapy treatment for head and neck cancer presents several colateral effects and radiation-related caries is one of these side effects. Direct influence of radiation on dental structure stills a controversial subject, but some researches show morphostructural changes due to accumulated doses. The aim of the present study is to define the proteomic profile of in vivo irradiated enamel, dentin, cementum and pulp. This is a retrospective study, approved by the local UNICAMP Ethical Comitee (protocol #2.425.127), in which 22 teeth with clinical indication for extraction will be divided in two groups: control and in vivo irradiated. All selected patients presented oral and oropharingeal squamous cell carcinoma and irradiated patients received 60 to 70 Gy total doses prior to teeth extraction. The radiotherapy planning will be obtained following Morais-Faria et al. [2015] dosimetric distribution protocol. Teeth will be macroscopically evaluated and paired according to post-radiation dental index proposed by Walker et al. [2011]. Proteomic profile will be analyzed following the protocols previously described in the literature and evaluated by liquid chromatography coupled to mass spectrometry. Expected results: To correlate the possible proteomic alterations caused by radiotherapy in the dental structure with the development of radiation-related caries.

2. Alex Castro
Universidade Estadual de Campinas (UNICAMP)

Responsivity Biomarkers of Cardiorespiratory Fitness to aerobic training:
A Systems Biology Approach

Cardiorespiratory fitness (CRF) is positively associated with maintenance and health enhancement. However, despite of regular aerobic training be recommended to the CRF improvement, the interindividual responses to standardized doses of exercise are widely heterogeneous. In this sense, to investigate molecular determinants such as the plasmatic and skeletal muscle proteomics profile associated with CRF trainability have the potential to generate new biomarkers and clarify the biology of adaptations to different training regimes. Therefore, the aims of this study are: (1) to investigate baseline serum and skeletal muscle metabolomics and proteomics profile and its associations with intrinsic CRF; (2) to investigate baseline serum and skeletal muscle metabolomics and proteomics profile associated with CRF trainability (acquired CRF) in response to 8 weeks of continuous endurance training (ET) and high-intensity interval training (HIIT) programs; (3) to investigate the changes in the serum and skeletal muscle metabolomics and proteomics profile associated with CRF trainability (acquired CRF) in response to 8 weeks of ET and HIIT.

3. Aline Guimarães Santana
Universidade Estadual de Campinas (UNICAMP)/FAPESP

Finding binding parterns of Agrin fragments to study their role on the oral cancer progression
Advisor: Adriana Franco Paes Leme

Agrin is a high molecular weight heparan sulfate proteoglycan found with high protein expression in various types of cancer, such as squamous cell carcinoma (SCC), liver cancer,

lung cancer, among others. We have recently shown that agrin knockdown (shAgrin) in oral SCC cell lines (SCC9 and HSC3) decreases about 50% the cell proliferation, migration and invasion, as well as decreases the formation of cell colonies and the tumour spheres. These processes are essential for the development, progression and tumor aggressiveness. These results were further corroborated by the in vivo orthotopic model, performed by the injection of control and shAgrin HSC3 cells on the side of the tongue of mice. The results showed that the decrease of agrin expression interferes negatively on angiogenesis and vascular invasion, cell morphology, cell growth pattern and keratinization, slowing down the tumor progression, decreasing its severity. The same study shows that some interaction partners of agrin, especially the C-terminal portion may be associated with the development of cancer. However, it has been observed that agrin undergoes a post-translational proteolytic processing generating 90 kDa (1103-1863 aa) and 22 kDa (1864-2067 aa) proteins (neoproteins) of the C-terminal region. These neoproteins were observed overexpressed in the secretome of the SCC9 and HSC3 cell lines, and they have been suggested to play an independent role on tumour development, although their role is unclear. In this way, this work seeks to continue the studies of our group (Kawakara et al., 2015 and Rivera et al, under evaluation), understanding the mechanisms of neoproteins signalling in the progression in oral cancer, as well as to investigate if the N-terminal region also plays a role in this process. For this, we will use strategies by structural proteomics to: i) identify interaction partners for the different constructions of agrin; ii) interfere with agrin direct binding partner through synthetic peptides that modulate agrin activity using protein-protein interaction experiments and functional experiments on cells. Thus, we expect that this project i) explain the mechanisms by which agrin in its different processed forms acts on tumour development and progression in oral cancer and ii) advance in the search for molecules that may interfere in the effect of agrin.

4. Aline Larissa Gonçalves
Universidade de São Paulo (USP)/CAPES

Prospection and development of thermophilic fungi as cell factory and biochemical characterization of endoglucanases

The progressive growth in the world population has increasing the energy demand, mainly by fossil fuels. However, the limitation of oil reserves and the contribution to the greenhouse effect led to the use of alternative and sustainable energy source. In this context, the lignocellulosic biomass emerges as alternative as raw material to the production of biofuels and other compounds. However, the enzymatic saccharification of these materials is a challenge due to the recalcitrance making necessary the improvement in this process. In this context, the prospection of thermophilic microorganisms as a source for proteins has been performed once they can produce enzymes with thermal stability which can be explored in the process optimization. Among these microorganisms, the thermophilic fungus Aspergillus niveus has emerging as a candidate due to its ability to secrete enzymes that act on the cellulose chain, such as endoglucanases. The secretome of A. niveus grown in sugarcane bagasse as a carbon source identified an endoglucanase from glycosyl hydrolase family 5. The gene that encodes this enzyme was cloned, heterologously expressed in A. nidulans A773 and biochemically characterized. The analysis allowed to evaluate the best substrate of action (β-glucan), the optimal temperature (65°C), pH (4.5), thermostability (relative activity above 80% at 65°C for 120 minutes) and the stability in a range of pH values. In addition, aiming the development of a more efficient cell factory, the use of A. niveus as a chassi organism for the heterologous expression of oxidative enzymes was proposed, thus complementing its predominantly hydrolytic secretome. In this way, a genetic knockout was performed at PyrG gene generating an auxotrophic selection mark that will allow future transformations of A. niveus with sequences of interest.

5. Amanda Cristina Baldassi

Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP) / CNPq

Probing Eucalytpus photosynthetic response to atmospheric carbon dioxide levels by targeted mass spectrometry analysis of Calvin-Benson enzymes

The last IPCC report predicts a drastic increase in the atmospheric CO_2 concentrations. This increasing CO_2 scenario may be beneficial for most plant species, especially for those employing the C_3 photosynthetic pathway as they depend on a high CO_2:O_2 ratio to counterbalance losses due to photorespiration process. Understanding how key enzymes involved in carbon assimilation respond to an increase in the atmospheric CO_2 may assist in genetic engineering strategies that aim to increase photosynthesis and plant yield. We aim to monitor the photosynthetic behaviour of Eucalyptus grandis plants grown in different CO_2 environments. For that, instead of quantifying the net photosynthetic rates, we plan to specifically target the changes in the abundance of the enzymes (and its proteoforms) related to carbon assimilation. We intend to carry out a first discovery, unbiased, proteomics approach in order to select specific peptides from the selected proteins. Then, protein extracts will be analysed in the mass spectrometer in different concentrations in order to determine the best acquisition method, the linear response range and transitions to be monitored for each of the peptides from the selected proteins. Finally, optimized method will be used to quantify the selected enzymes in plants grown in different CO_2 concentrations. Successful design of the targeted assay has the potential to be used for any other scientific project, including those targeting metabolic adaptation to future climate changes or assessment of photosynthetic performance in different environments.

6. Amanda Ribeiro Martins da Silva

Universidade de São Paulo (USP) / FAPESP

Targeted quantitative proteomics is a powerful approach to overcome problems associated with the stochastic nature of untargeted methods, along with the high rate of missing values, which hamper detection and accurate quantification of low abundance proteins. Parallel reaction monitoring (PRM) has been an appropriate strategy in this sense, and has proven sensitivity and specificity to quantify concomitantly multiple peptides present in a sample with wide dynamic range. Data independent acquisition (DIA) is another methodology that has been used to quantify peptides in complex biological samples. However, a comparison of PRM and DIA methodologies in a clinical scenario is still missing. We therefore evaluated the potentialities of the DIA methodology to be employed in the clinical area, and compared to PRM approach, by quantifying proteins present in high-density lipoprotein (HDL). HDL is a plasma lipoprotein responsible for the removal of excess cholesterol from periphery. HDL composition is highly heterogeneous, containing many low abundance proteins linked to cardioprotective effects. These characteristics make HDL an attractive target for clinical proteomics. We employed a mixture of labeled peptides for retention time calibration (iRT) and HDL of apparently healthy humans to compare the analytical performance of DIA and PRM methodologies. DIA yielded quantitative results for 50 proteins in HDL against 43 proteins determined by PRM. We analyzed the coefficient of variation of 150 peptides, and both methods provided results below the 25 % recommended for clinical studies. PRM is more specific, but DIA requires less method development, without the need of a preselection of the precursor ions. In this way, DIA is an attractive strategy for quantifying HDL proteins in translational studies.

7. Ana Paula Masson

Universidade de São Paulo (USP)

Identification of protein involved in post-translational modifications related to the ubiquitin-proteasome system during the epithelial-mesenchymal transition (EMT).

Silvestrini, V.C.[1,2]; Palma C.S.[1,2]; Freitas, A. C [1,2]; Poersch, A; Thome[1,2], C; Delsin, L.E.A[2]; Masson, A.P[2]; Lanfredi, G.P[2]; Faça, V.M.[1,2]
[1] Dept. Biochemistry and Immunology, Ribeirão Preto Medical School - University of São Paulo, Ribeirão Preto - SP/Brazil.
[2] Cell-Based Therapy Center, Ribeirão Preto Blood Center, Ribeirão Preto Medical School – University of São Paulo, Ribeirão Preto - SP/Brazil.

INTRODUCTION: The biological process termed epithelial-mesenchymal transition (EMT) is characterized by the loss of epithelial markers and the increased expression of mesenchymal markers. Such mechanism facilitates cell migration and invasion, culminating with the acquisition of invasive capacity (metastasis) in tumors. During these processes, target proteins are post-translationally regulated by ubiquitination, sumoylation or neddylation using specific enzymes.

OBJETIVE: The aim of our work is to identify representative peptides from proteins related to key proteins involved in EMT and evaluate changes in the pattern of sumoylation/neddylation/ubiquitination. MATERIAL AND METHODS: The cell line MCF10A was used as a model to EMT induction in response to TGF-βII, simulating the critical stage of metastatic triggering/development. Multiplex Proteomic analysis based on highthroughput LC-MS/MRM turned possible monitoring 26 proteins altered during EMT induction in cells fractionated to subcellular compartments. This could be possible by monitoring most intense transitions and retention time optimization for each peptide.

RESULTS AND DISCUSSION: During the global proteomic analysis, sets of functionally correlated and regulated molecules were observed. One of the sets that stood out was related to enzymes and proteins involved in ubiquitination, sumoylation and nedylation. This result highlighted the regulatory role of NEDD8, indicating a decrease in proteins involved in this pathway. The SUMO2 protein was also regulated, especially in subcellular fractions enriched with nuclear proteins, suggesting a possible control in this compartment. Targeted proteomics, validated the SUMO2 alterations, supporting its regulatory role during EMT.

CONCLUSIONS: The EMT process triggers cellular alterations correlated to protein turnover and localization, altering SUMO2, NEDD8 and other important proteins involved in the cellular control. With these data, our research will further investigate through chemical inhibitors the effects of the modulation proteins involved in the ubiquitin-proteasome system during EMT marker using modern proteomic tools strategy.

Keywords: EMT, Ubiquitin-proteasome system, proteomics.

Supported by: FAPESP (Proc. 2017/03960-6 and 2016/03809-3) , CNPq (Proc. 308561/2014-7 and 402697/2016-2), CTC¬CEPID (Proc. 2013/08135-2), FAEPA, FMRP-USP.

9. Ariane Fidelis Busso Lopes

CNPEM

Mass spectrometry-based proteomics of the invasive tumor front and matched lymph node metastasis in head and neck cancer

Ariane Fidelis Busso-Lopes[1], César Rivera[2], Barbara Pereira de Mello[3], Luisa Lina Villa[3,4], Wilfredo González-Arriagada[5], Adriana Franco Paes Leme[1]

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[2] Department of Biomedical Sciences, Faculty of Health Sciences, University of Talca, Talca, Chile
[3] Department of Radiology and Oncology, School of Medicine, University of São Paulo, São Paulo, Brazil
[4] Cancer Institute of São Paulo – ICESP, School of Medicine, University of São Paulo, São Paulo, Brazil
[5] Department of Oral Pathology and Diagnosis, Faculty of Dentistry, Valparaiso University, Valparaiso, Chile

Lymph node metastasis is the main prognostic factor in patients with head and neck squamous cell carcinoma (HNSCC) and it is associated with worse outcome. However, the mechanisms underlying the local spread are poorly understood and no molecular markers for HNSCC are currently used in clinical practice. Herein, we explored the mechanisms associated with lymph node metastasis through the protein profile of the neoplastic cells from the invasive tumor front (ITF) of primary site and from matched lymph node metastasis. The ITF comprises the most aggressive tumor cells, giving insights about the invasion and metastasis processes. We isolated neoplastic cells from formalin-fixed paraffin-embedded (FFPE) ITF from patients positive (N+, n=14) and negative (N0, n=15) for lymph node metastasis, and from matched FFPE metastatic lymph nodes using laser microdissection combined with mass spectrometry-based proteomics. Twenty-seven percent of the cases were positive for HPV16. In total, 2,491 and 2,392 proteins were identified in tumor cells from the ITF and from the lymph nodes, respectively. Firstly, the proteome comparison for ITF between positive (N+) and negative (N0) lymph node metastasis patients indicated ninety-five differentially abundant proteins (Student´s t-test; P-value≤0.05), from which thirty-three proteins were associated with advanced T and clinical stages, recurrence, surgical margins and pattern of invasion (Mann-Whitney test; P-value≤0.05). Secondly, the protein pattern of neoplastic cells from ITF and matched lymph nodes showed an individual patient-specific metastasis profile through hierarchical clustering analysis, with thirty-one differentially abundant proteins associated with tumor invasion by the regulation of immune system processes. In summary, our results identified potential prognosis markers of tumor invasion and lymph node metastasis for HNSCC. Financial support: FAPESP (Process number 2015/19191-6)

10. Bianca Alves Pauletti

CNPEM

Effects of gradient times on protein and peptide identifications associated with an enrichment on biological pathways and networks

Quality control is a crucial point to successful analysis in mass spectrometry. In our laboratory routine we evaluated the instrument conditions by the standard sample angiotensin peptide injection. In order to improve our quality control with a more complex standard sample and to obtain reference values of protein identification and quantification we extract total protein from HEK 293 cells (Human Embryonic Kidney 293) (10 µg) reduced, alkylated and trypsin digested. The peptides were desalted using reverse phase column Sep-Pak® (Waters, Milford, MA, US). An aliquot of 4.5 µl containing 1 µg proteins was analyzed on an ETD enabled Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) connected to the EASY-nLC system (Proxeon Biosystem, West Palm Beach, FL, USA) through a Proxeon

nanoelectrospray ion source. The samples were analyzed in five different run times (20, 45, 60, 120 and 212 minutes) and submitted to two different fragmentation methods: CID and HCD. Peak lists (msf) were generated from the raw data files using Proteome Discoverer version 1.3 (Thermo Fisher Scientific) with Sequest search engine and searched against SwissProt human database with false discovery rate of less than 1% for proteins and peptides. The results will provide the number of proteins and peptides identified in each condition and furthermore the correlation between spectral counts and intensity in quantitative label free analysis.

11. Clarice Izumi
Universidade de São Paulo (USP)

PROTEOMIC ANALYSIS OF EXOSOME AND TRANSFECTED HUMAN MENSECHYMAL STEM CELL hTERT+ DURING CELL DIFFERENTIATION: THE ROLE IN GLIOBLASTOMA (GBM).

Clarice Izumi; Sabrina de Fátima Comin, Maristela Orelana, Hélen Julie Laure and José César Rosa
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Extracellular vesicles are secreted by cells under various conditions and have been the target for discovery of new disease markers. During stimuli by drugs in general occurs an increase of the secretory process and consequently the elimination of extracellular vesicles or exosomes. In order to isolate and characterize these vesicles by proteomic strategy, we stimulated cells derived from glioblastoma with Thapsigargin (THP) and Tunicamycin (TUN) eliciting Unfolded Protein Response. EV was characterized by several markers like CD9, CD63, CD81 and HSP70. EVs have cytoplasmic and membrane proteins, fragments of DNA, RNA, miRNA, mRNA and MHC receptors, and can be strong candidates for biomarkers of diseases and may also be related to cellular communication and cell differentiation. We investigated the proteome profiling of exosome from U87MG and T98G, GBM cell lines. Proteomic exosome of U87MG was rich in proteins related to cell differentiation. As proof of principle, HBMS, human mesenchymal stem cell transfected with hTERT, was treated with isolated exosome of U87MG. No evidence of morphological change was detected in HBMS treated with exosome. Quantitative label free proteomics of HBMS showing up-regulated and down-regulated proteins affected by the exosome, with emphasis on the reduction of vimentin (VIME) considered to be marker for mesenchymal cell phenotype. The relationship between the up and down regulated proteins identified in the exosomes and in the HBMS proteomes are being placed in a context of cellular intercommunication and their importance for the study of the cancer will be explored.
Supported by FAPESP, CNPq, FAEPA

12.Daniela Cajado O. S. Carvalho
Butantan Institute /FAPESP

Proteomic analysis of the effects of PA-BJ, a serine proteinase from Bothrops jararaca venom, on endothelial cells.

Daniela Cajado-Carvalho, Débora Andrade Silva, Milene Cristina Menezes, Dilza Trevisan Silva, Solange M. T. Serrano.
Laboratório Especial de Toxinologia Aplicada, Instituto Butantan, Brazil.

PA-BJ is a thrombin-like serine proteinase from Bothrops jararaca that promotes platelet-aggregation by cleaving Protease-Activated Receptors (PAR) on platelets. Using proteomic approaches, we showed that although both PA-BJ and thrombin induce platelet aggregation via PAR-1 and PAR-4, these enzymes activate different pathways to cause platelet secretion and aggregation. Thrombin and vitamin K-dependent coagulation proteinases, are able to selectively activate cell surface PARs on the vasculature. The aim of the present project is to characterize the molecular effects of PA-BJ upon endothelial cells. To characterize the molecular effects of PA-BJ upon endothelial cells (primary and immortalized cultures) using proteomic approaches based on mass spectrometry. PA-BJ was isolated from B. jararaca venom after two chromatographic steps using weak and strong cation-exchange columns. The PA-BJ proteoforms were identified by LC-MS/MS and evaluated by platelet aggregation assay. The primary culture of blood outgrowth endothelial cells (BOECs) was obtained using fresh human blood, which was submitted to Ficol-centrifugation gradient, and the buffy-coat was seeded into gelatin-coated 48-well plates and cultivated with specific Endothelial Generation Media with 20% fetal bovine serum. Immunofluorescence assay using anti-PECAM and anti-vWF Abs was used to confirm BOEC identity. Protein extraction from Human Umbilical Vein Endothelial Cells (HUVECs) was tested using two extraction buffers (RIPA and PTS) and compared by proteomic identification using LC-MS/MS. Viability of HUVECs upon treatment with PA-BJ (0.7 nM-400 nM) for 2 h was evaluated by MTT assay. Next, HUVECs were incubated with PA-BJ (100 nM) or PBS (control) for 1, 2 and 4 h for analysis by proteomic approaches. First, two PA-BJ proteoforms were obtained and identified by LC-MS/MS, and showed slightly different molecular masses and isoelectric points. Both proteoforms were able to aggregate platelets at 100 nM concentration. Second, we successfully established BOEC cultures, for the first time in our laboratory, using two different blood donors. The cells were detected only after 15 days of culture and were viable after six passages. BOEC identity was confirmed by immunofluorescence assay. Moreover, frozen BOECs were viable after thawing and re-seeding. Lastly, since protein extraction is critical to obtain optimal proteomic results, extraction methods were evaluated using HUVECs. The proteomic analysis revealed that both extraction buffers (RIPA and PTS) resulted in the identification of a similar number of proteins from cytoplasm and nucleus, however extraction using RIPA resulted in 20% more total proteins. On MTT assay, HUVECs were still viable after treatment with PA-BJ in all tested concentrations. Since PA-BJ at 100 nM is able to efficiently aggregate platelets, this concentration was chosen as a sub-cytotoxic dose for proteomic studies to evaluate the intracellular events trigged by PA-BJ.

13. Daniela Granato

CNPEM/CAPES

Squamous cell carcinoma (SCC) is the most common type of oral cancer (OSCC) and is a malignity derived from the squamous stratified epithelium of the oral mucosa. Regardless of the advances in the treatment of OSCC along the years, the rate of survival is very low, with 50% of the advanced cases dying in the period of 5 years. Besides, the prognosis and treatment strategies are defined mostly based on TNM classification. Thus, the identification of biological signatures may complement the clinical decision. Discovery based proteomics combined with Targeted proteomics, associated with computational biology are robust techniques currently used to identify, quantify and classify prognostic signatures in different types of cancer. Considering that the most common prognostic factor in cases of patients with SCC involves the lymph node metastasis, this project aims in the discovery phase to (i) characterize the proteins (panel of signatures) with prognosis meaning, extracted from (1) saliva and (2) tear fluid of oral SCC patients and from healthy control individuals; in a second phase (ii) verify signatures, also in plasma, and in a small cohort of patients, through targeted proteomics; and on a third phase (iii) validate the signatures, in a large cohort of patients, for clinical application through targeted proteomics, immunohistochemistry (IHC) and Enzyme-Linked Immunosorbent Assays (ELISA). It is expected that the signature with prognostic value provided from the analysis of liquid biopsies may improve the clinical decision for treatment modalities and, with that, improve the survival and quality of life of the patients.

14. Dilza Trevisan Silva

Butantan Institute / FAPESP

Metalloproteinases are abundant in viperid venoms and are implicated in several pathological effects. Hemorrhage at the site bite is a dramatic effect, however, in severe cases, bleeding in internal organs may occur. Hemorrhagic factor 3 (HF3) is an extremely hemorrhagic metalloproteinase from Bothrops jararaca venom and shows a minimum hemorrhagic dose of 2.2 pmoles on the mouse skin. Previous studies have shown that HF3 participates in local effects of envenomation, such as hemorrhage and inflammation. Here, we are applying transcriptomic and proteomic approaches to evaluate the effects of HF3 on kidney from mice submitted to injection of HF3 on the thigh muscle. After 2 h and 6 h of injection, the kidneys were collected and immediately frozen for further procedures. For transcriptomic analysis total RNAs were extracted from all mice kidneys, and the mRNAs were purified. The converted cDNAs were used to perform an RNAseq analysis using Nextera® XT DNA Sample Preparation Kit and paired-end sequencing on Illumina HiSeq™. The generated BCL files were used for demultiplexing and conversion to fastq files for pre-processing by quality control, followed by an alignment with the reference genome of Mus musculus, estimating the abundance for the RefSeq genes. By applying the DESeq2 methods for differential expression analysis, considering a fold change of 3 and a p-adjusted value of 0.01, after 2 h of HF3 treatment 14 genes were differentially expressed (DE) and after 6 h of HF3 treatment 73 genes were differentially expressed. Most of the DE genes were found to be related to acute renal failure. For proteomic analysis, equal amounts of kidney tissue protein from six mice used in each experimental group (control and HF3-treated, 2 h and 6 h) were pooled and submitted to in-solution trypsin digestion followed by LC–MS/MS analysis. The resulting spectra were analyzed for label-free-quantification using MaxQuant and Perseus. The analysis of the HF3-treatment for 2 h revealed the relative quantification of 822 proteins, from which 50 were detected as differentially abundant between the control and HF3-treated groups. Furthermore, the analysis of the HF3-treatment for 6 h resulted in the relative quantification of 986 proteins, from which 108 were differentially abundant. Considering a fold change of 0.25, in the 2 h treatment most differentially abundant proteins are down-regulated and most of them are mitochondrial proteins involved in oxidative phosphorylation processes, such as cytochrome c oxidase subunit VIb polypeptide 1 and distinct NADH dehydrogenases. In contrast, in the 6 h treatment most of the differently abundant proteins are up-regulated, including mitochondrial proteins, structural constituent of ribosomes and granulin, that plays a role in inflammation, wound healing. These results indicate that HF3 is able to induce systemic effects on mouse kidneys and the potentially induced signaling pathways are under analysis.

15. Douglas Ricardo de Souza Junior

Universidade de São Paulo (USP)/FAPESP

Obesity and incidence of cardiovascular diseases (CVD) are closely related, and obese people tend to have high levels of the atherogenic low-density lipoprotein (LDL) in plasma. Other than plasma cholesterol concentration, the composition of the diet seems to be an important factor in the development of CVD. Lipids are one of the main components of diets and are essential for cellular homeostasis and several metabolic processes. Besides the role as the main energetic source, lipids are also bioactive compounds. Thus, saturated fatty acids induce metabolic stress and inflammation, but polyunsaturated fatty acids seem to have the opposite effect. Unlike LDL, high-density lipoprotein (HDL) is considered cardioprotective, in part due to its ability to mobilize the excess cholesterol from macrophages of arterial walls and

transport it to the liver for biliary excretion. HDL has also anti-inflammatory properties that contribute with its protective role. In this view, our goal is to use quantitative proteomics and statistical tools to evaluate macrophages response when these cells are exposed to different lipids such as cholesterol, palmitic acid (16:0) and docosahexaenoic acid (DHA, 22:6) and then incubated with HDL. Macrophages exposed to acetylated LDL showed incorporation of cholesterol as measured by staining with oil red-o. Incubation of these macrophages with HDL significantly reduced the cellular cholesterol content. Proteomic analyses revealed increased amount of many inflammatory proteins in macrophages exposed to excess cholesterol. Identifying networks of differently expressed interacting proteins could help us better understand the effects of different lipids on cellular homeostasis, and the protective role of HDL in this process.

16. Edione Canzi

UNA

Exossomes (EXOs) are extracellular vesicles secreted by all cell types that play an important role in intercellular communication. These membrane vesicles are present in most body fluids, as well as blood, saliva, breast milk and sperm. Besides that, exosomal release from cancer cells contributes to metastasis through intercellular communication. EXOs are regarded as potent biomarker for many kind of diseases and therapeutic targets. Considering this, effective EXOs isolation protocols are required to assure its purity assessment. We focused to develop a protocol to isolate and purify cellular exosomes from colon adenocarcinoma (SW 480) cells, quantifying exosome biomarkers by advanced targeted quantitative proteomic SRM mass spectrometry analysis. Here, the successful quantitation of specific exosome markers was developed and validated through orthogonal Western Blotting approach. In addition, we aimed to obtain a protocol to determine that exosomes purification can be distinguished from the differential protein abundance from its original cell. Experimental. The colon adenocarcinoma cells (SW 480) lines were grown in L-15 media supplemented with 10% FBS, according to ATCC recommendations. 24 hours pre-harvest, dishes were washed twice with 10mL of unsupplemented media, once with 10mL of dPBS (no calcium or magnesium), and a final time with 10mL of unsupplemented phenol red free media (same formulation as the growing media). Cells are then given 12.5 mL of phenol red free media supplemented with 0.8% ITS-A (Life Tech 51300-044, 100X) and incubated for 24 hours (+/- 15 min) at the same temperature (37°C) and CO2 level used during the growth and expansion phase. The EXOs preparation was performed by the following steps: 300 mL of Culture Medium (CM) was collected in conicals tubes and added one cOmplete protease inhibitor tablet to each tube in ice. The CM was centrifuged at low speed (2000 x G, 10 min, 4° C) and the supernatant was separated from the pellets debris. The supernatant was filtrated twice (0.22 µm and 0.1 µm filter) and concentrated using 4 Amicon Ultra-15 100K tubes. After that, the enriched EVs fraction was submitted to ultracentrifugation steps to obtain the purified EXOs. It was flash freezed on dry ice bath and store at -80°C, until analysis. Proteomics preparation: BCA quantification was performed for EXOs and cell pallets (10uL). Cell pallets (300 uL) and EXOs (100uL) were submitted to alkylation and reduction steps, followed by trypsin digestion and desalting peptides using C18 SepPak cartridges (Waters). All experiments were prepared in biological triplicate to assure reproducibility. SRM quantification: the samples were suspended in 2% ACN (0.1% formic acid) to achieve 1ug/uL and injected in the LC-MS/MS system. Five proteins were evaluated: CD9, IL6, TSG101, EPCAM and CD63. For each protein three peptides were used and for each peptide was used at least three transition. The peak area integration was performed using Skyline (3.7.0.10940) (University of Washington). Western blot: this analysis was conducted according the regular protocol. Results. Comparing the relative protein levels in cell pallets and EXOs we found the major difference in CD9 protein. Its content is increasing in EXOs and decreasing in cells pallets, In addition, the same pattern was visualized for the Western blot analysis. Which proves that the purification could be achieved, once CD9 is a great marker for exossomes.

17. Elisa Castaneda Santa Cruz

Universidade Estadual de Campinas (UNICAMP)

Purpose: The diagnosis of severe psychiatric disorders such as bipolar disorder (BD) and schizophrenia (SCZ) relies on the clinical assessment of symptoms. Until now, there is not a defined biomarker panel at the pathological, cellular, or molecular level for these diseases, which might support their diagnosis. Thus, the aim of this work was to evaluate the serum proteomic profile of BD and SCZ patients in order to propose potential biomarkers related to each disease and/or to their clinical treatments. Experimental design: Major serum proteins were depleted by affinity chromatography and label-free proteomic analyses was performed comparing samples from BD and SCZ patients in relation to healthy controls (HC). Proteomics data were processed by MaxQuant software in order to define statistically significant proteins among the groups. The differential protein list was obtained by online search databases for protein classification regarding to the biological processes. Results: 15 proteins were listed; more than 50% has the transport and protease inhibitors activity. 11 proteins make a part of the High-Density Lipoproteins (HDL) associated proteins due to chronic inflammatory diseases as the studied, or are involved in inflammatory processes, which could be related to the effect of antipsychotic drugs Conclusions and clinical relevance: The protein different levels showed will help to improve our understanding of molecular mechanisms between the diseases. Moreover, additional studies are required to determine the relevance of these proteins as a marker of therapeutic response.

18. Fabio Mitsuo Lima

Grupo Fleury

Identification and quantification of amyloid proteins in cardiac tissue using mass spectrometry

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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 objective is to apply proteomics approach for unequivocal identification and quantification of amyloid proteins. 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 DIA mode. The data was processed using Skyline software where a spectral library was constructed. Results/Conclusion: We analyzed twenty samples from different heart necropsies. It was possible to unequivocally identify the amyloid protein in all analyzed biopsies, confirming the clinical suspicion. Quantification was based on the addition of a labeled internal standard and the total microdissected area. Futhermore,

laser microdissection allowed the analysis of non affected areas inside the same sample as negative control. The association of LMD and mass spectrometry, as well as patient clinical analysis are powerful tools for definitive amyloidosis diagnosis.

19. Felipe Jun Fuzita

Universidade de São Paulo (USP)

Introduction: Lepidopteran midgut presents two secretion mechanisms. In anterior midgut, small vesicles surrounded by microvilar membrane are released (microapocrine secretion) while exocytosis is observed at posterior midgut. There is little knowledge about which proteins are secreted by each mechanism. Objectives: Obtain the global protein composition of secreted and membrane-bound proteins from Spodoptera frugiperda midgut using shotgun proteomics, to further establish the secretory route used by each protein. Methodology: From the midgut we isolated microvillar membranes, soluble and membrane fractions of microapocrine vesicles, loosely or tightly bound proteins from peritrophic membrane, and the endoperitrophic content. Each sample was in-gel digested and analyzed in a LTQ-Orbitrap Velos ETD (Thermo). Alternatively, they were used for in-solution digestion in the presence of RapiGestTM surfactant (Waters) with subsequent analysis in a Q Exactive (Thermo). Results and discussion: The global analysis of all fractions identified 836 proteins present as secreted or membrane-bound proteins. From these, 134 are secreted by exocytosis, while 272 are present in microapocrine vesicles (100 membrane bound and 172 soluble). Exocytosis is the main route used by serine endopeptidases (30 isoforms), triacylglycerol lipases (18 isoforms) and peritrophins (13 isoforms). Most of the aminopeptidases (11 isoforms) are bound to microvilli, but are secreted with microapocrine vesicles and found in the peritrophic membrane. Carboxypeptidases are secreted in microapocrine vesicles, bound to the membrane (six isoforms) or in a soluble form (4 isoforms). Maltases are released by both mechanisms, in soluble and membrane-attached forms, whereas two amylases are secreted solubilized inside microapocrine vesicles. Conclusions: It appears that enzymes involved in initial protein and lipid digestion use the exocytic route, whereas the enzymes for final protein digestion are secreted in microvesicles. On the other hand, enzymes from initial carbohydrate digestion follows the microapocrine route whereas enzymes from carbohydrate final digestion are secreted by the both studied mechanisms.

20. Fernanda Janku Cabral

Universidade Estadual de Campinas (UNICAMP)

Schistosomiasis is caused by the parasite Schistosoma mansoni and is prevalent in tropical and subtropical areas of the world. It is estimated that it affects 200 million people and more than 1,53 million people are disabled by the disease. S. mansoni presents a complex life cycle with an intriguing development involving changes in the metabolism, protein synthesis and acquisition of a highly specialized tegument. Although several efforts have been made to study genes, transcripts and epigenome of the parasite, little is known about how gene expression is regulated. In the attempt to understand the control of the gene expression in the life cycle of the parasite, the aim of this project is to investigate the importance of posttranslational histone tail modifications and whether these epigenetic marks are dynamically acquired and lost during stages as well as the putative transcriptional co-regulators that contribute to the gene expression control of the parasite. Specifically, it is proposed to investigate: 1) the dynamics of chromatin modification using mass spectrometry; 2) elaboration of a comparative map between histone PTMs and gene expression of annotated modifying enzymes in the stages; 3) putative transcriptional co-regulators such as HP1 that may play a role in the regulation of silencing in cercariae.

22. Francisca Nathália de Luna Vitorino
Universidade de São Paulo (USP) / FAPESP

Chromatin response under proliferative and anti-proliferative stimuli in tumor cell

Chromatin plays an important role in essential cellular processes such as mitosis, transcription, replication, among others. The chromatin response to stimuli is not well characterized. Despite being a growth factor, FGF2 has anti-proliferative and tumor suppressive functions in some cellular 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. To better understand the chromatin response to proliferative and anti-proliferative stimuli induced, respectively, by Fetal Bovine Serum (FBS) and 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 in the presence or absence of FGF2 by 0 and 1 h. Chromatin extracts (from three biological replicates) were digested with trypsin, detergent-removal by using HILIC columns and desalted using Sep-Pak. Peptides were further fractionated into five fractions using SCX Stage Tips. All 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 using MaxQuant and further analyzed using Perseus and DAVID platforms. We performed the experiment three independently times and the results were analyzed separately. More than 1500 proteins were identified and more than 150 were found to be differentially expressed (FDR 1%, $p<0.05$) in FGF2 treated samples. DAVID clustering analyzes identified that terms associated with transcriptional regulation and mitochondria were down and up-regulated, respectively, in FGF2-treated samples. To analyze the transcription rate after the FGF2 stimulus, we performed run-on assays using uridine analogs (EU), and we found that FGF2-stimulated cells show decreased transcription rates. Using EU or anti-fibrillarin, at immunofluorescence assays, we found that transcripts from nucleolus were preferentially affected by FGF2 stimulation. Proteomics analysis indicated that the chromatin abundance of cdc42 (cell division control protein 42 homolog) protein increase upon FGF2 stimulation, however, we could not validate its location and abundance due to problems at commercial antibodies specificity. Nevertheless, Y1 cells expressing the dominant negative for cdc42 (pCM – N17) are resistant to FGF2 anti-proliferative effects. Currently, we are optimizing proteomics data analysis in order to normalize and analyze the three experiments combined (which were performed in independent times) to obtain more robust results and to increase reproducibility. Preliminary results, with the analyzes of the three experiments combined, using the maxquant platform and applying more restrictive filters on the Perseus platform, we obtained a list of proteins identified more reliable. These analysis are still in progress and we are looking for better ways for this type of data processing.

23. Gisele Adriano Wiezel

Universidade de São Paulo (USP)/FAPESP

Identification of phospholipase A2 inhibitors in the venom gland of the snake Crotalus durissus terrificus and heterologous expression of the inhibitor rCdtPLI2 in Pichia pastoris

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The rattlesnake C. d. terrificus (Cdt) presents high medical relevance in Brazil due to the severity of the envenomings caused by this snake. Its venom is mainly comprised by crotoxin, a heterodimeric phospholipase A2 (PLA2) with neurotoxic actions and may lead the victim to respiratory paralysis and death. The aims of this study include to identify and produce the recombinant form of a PLA2 inhibitor (PLI) to be further studied as adjuvant in the antivenom therapy. For this purpose, Cdt venom (dissolved at basic and acid pHs) was separated by SDS-PAGE and the protein bands were reduced with dithiothreitol, alkylated with iodoacetamide and digested with trypsin. Then, digested samples were analyzed by high resolution LC-MS/MS using HCD fragmentation mode and data were searched against a database with protein sequences from Crotalus genus. PLIs were identified in bands between 30-66 kDa. Furthermore, a transcriptomic analysis of Cdt venom gland revealed 4 PLI sequences (CdtPLI1-CdtPLI4) belonging to the 3 PLI classes (alpha, beta and gamma) with abundance of 0.17% (Wiezel et al., The in-depth venome of the Brazilian rattlesnake Crotalus durissus terrificus: an integrative approach combining its venom gland transcriptome and venom proteome; in submission). A synthetic gene with the CdtPLI2 sequence was designed and cloned into the expression vector pPICZαA. P. pastoris cells (KM71H strain) were transformed by electroporation with the pPICZαA-CdtPLI2 vector and expression of rCdtPLI2 was induced by methanol in BMMY medium. After 120 h, rCdtPLI2 was purified from the supernatant by affinity and reversed-chromatography. In addition, rCdtPLI2 was incubated with a PLA2 from Cdt venom and the inhibitor was able to complex with PLA2, as verified by a native PAGE gel. In conclusion, this study paves the way to the discovery of novel biomolecules in snake venom glands that may be used as pharmacological tools.

25. Humberto Gonczarowska-Jorge

Leibniz Institute for Analytical Sciences

Results As Soon As Possible (rASAP): 2 Hours from Lysis of Cells and Tissues to Data Analysis Using Subtilisin

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Current bottom-up techniques for mass spectrometry-based proteomics rely on digestion of proteins into peptides that have, compared to proteins, more similar phyco-chemical properties, ionize better and possess a higher sensitivity for the mass spectrometer. This technique, however, comes with the expense of incomplete sequence coverage and, by its turn,

loss of information on post-translational modifications. It has been shown that combination of two or more enzymes can give a boost in proteome coverage. The use of the enzyme-of-choice trypsin with the recently characterized broad specificity enzyme subtilisin showed an increase of the proteome coverage, but more surprisingly an augmentation of 33% in the phosphosproteome coverage in a TiO2/HILIC-based approach. It was demonstrated as well that subtilisin can incredibly almost fully digest cell lysate samples down to 1 single minute incubation. Subtilisin-based sample preparation protocol was evaluated if compatible with a super-reduced preparation time for LC-MS. Cell pellets or tissue samples were [1] lysed by ultrasonication in the presence of 6 M guanidine hydrochloride (GuHCl) (2 min), [2] meanwhile sample was carbamidomethylated, protein concentration was determined by BCA (40 min), [3] sample diluted to 0.5 M (GuHCl) with 50 mM ammonium bicarbonate and preheated to 56 ˚C for 5 min (6 min), [4] digested with subtilisin (2 min) and [5] digestion stopped by acidification and further centrifugation at 18,000 x g for 1 min (2 min), totalizing 52 min of sample preparation. An additional 50 min LC-MS measurement plus 10 min database search totalize almost 2 h from cell lysis to data analysis. Measurement of HeLa cells, mouse heart tissue and yeast mitochondria rASP prepared generated an identification of 2179, 1011 and 863 proteins respectively with at least 1 unique peptide with 1% FDR. The rASP protocol can also be used for label free quantification, demonstrating consistent results and quantification when analyzing human control and IBMPFD1 fibroblasts. Also, demonstrated a high correlation to trypsin-based quantitative data. Thus, subtilisin is presented now as powerful tool for fast sample preparation down to 2 h, showing reproducible digestions, likewise being compatible with label free quantification.

26. Jéssica Luana Chechi

Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP) / FAPESP

Proteomic approach for studying Pythium insidiosum

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Fapesp (Grants 2016/10804-8 and 2018/08009-0) and CAPES (finance code 001)

Pythiosis, whose etiological agent is the oomycete Pythium insidiosum, is an emerging and life-threatening disease that occurs most frequently in tropical and subtropical countries, affecting several animal species, including humans. The disease is difficult to diagnose, since the pathogen's hyphae are often confused with zygomycetes fungi in histological sections. The treatment of pythiosis is also difficult because the pathogen does not respond satisfactorily to the available antifungals due to the absence of ergosterol in the cytoplasmic membrane, and it is necessary to perform surgical procedures, often extensive, when possible. There are few studies on the protein characterization of this pathogen. Studies conducted in Thailand, where the disease is endemic in humans, reported a 74 kDa protein as an immunodominant antigen because it was recognized by sera from patients with pythiosis. This protein was later identified as exo-1, 3-β-glucanase, an important enzyme that helps in cell wall modeling and considered as virulence factor. Characterization of P. insidiosum by proteomic approach has been also performed in our group, focusing on virulence factors. We analysed 186 spots on 2D–PAGE by Image Master 2D Platinun v 7.05 (Ge Healthcare) software and found proteins ranging from 12 to 89 KDa and isoeletric points from 4 to 7 and identified β 1,3 glucan synthetase, Hsp 70 and enolase, proteins already described in the literature related to fungal virulence. At the moment we are studying immunodominant proteins in equine pythiosis. Using analytical techniques such as 2D-PAGE, Western blot, mass spectrometry and bioinformatics, we expect to understand the pathogenesis of this disease in this animal species, the most one affected by pythiosis, and also to contribute with some accurated rapid serological tests to help in the early diagnosis, as well as to investigate such proteins for future treatment approaches.

27. João Paulo da Luz Silva
Universidade de São Paulo (USP) /CNPq

Diurnal rhythms in sugarcane histones post translational modification

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Circadian rhythms regulate a wide variety of physiological and metabolic processes. The circadian clock consists of complex signaling networks at transcriptional and translational level mainly thought inhibition feedbacks by transcription factors. This system modulates the expression of more than one third of transcripts in sugarcane. The overall regulation of genetic expression might involve regulation under the chromatin level. Indeed, in mammals, circadian oscillatory gene promoters have shown rhythms in histone modifications. These findings point to a connection between energetic balance, chromatin remodeling and circadian physiology. In plants it was observed that rhythmic modifications in histones occur in circadian clock genes. Therefore, we hypothesize that post-translational modifications (PTMs) are rhythmic in histones in a diurnal fashion in sugarcane hybrid cultivar SP80-3280. To test that hypotheses, we carried a proteomic study of the sugarcane histones extracted from leaf +1 in 5 time points (4,7,12,17 and 22 hours after sunrise). Histones were purified from isolated nuclei and propionylated before investigating PTMs present in our sample using nano liquid chromatography coupled to tandem mass spectrometry (LC-MS). We performed an untagged approach to quantify more than 70 peptides from histones H3 and H2A. We found that H3K27me and H3K36and37me are enriched at night in leaf+1 of sugarcane. We also found that H2AK10me are enriched at night. This result is the first evidence that there might be diurnal accumulation of histone PTMs in sugarcane. These results also point to a possible chromatin regulation of the circadian clock

28. Leandro Xavier Neves
CNPEM

Proteomic Analysis of Extracellular Vesicles from Saliva and Plasma of Patients with Squamous Cells Carcinoma for the Study of Post-Translational Modifications and Identification of Disease Markers

The squamous cell carcinoma (SCC) is a malignancy derived from stratified squamous epithelium and represents the most common type of oral cancer. In Brazil, 600,000 new cases of cancer are expected to occur in 2018, of which 14,700 would be affections of oral cavity. The prognosis is mostly based on the tumour, node and metastasis (TNM) staging system. However, the TNM classification assumes that tumours in the same stage and similar morphology are biologically alike although evidences reinforce that subtle molecular aspects may affect the disease progression. In this context, extracellular vesicles (VEs) are strongly related with intercellular communication processes by carrying signalling molecules. In fact, VEs secreted by cancer cells are able to reprogram both adjacent and distant cells, creating a pro-oncogenic environment and preparing premetastatic niches. Unfortunately, the knowledge regarding the composition and roles of VEs in tumour development is scarce and the characterization of VEs has not proved a trivial task. Extracellular vesicles posses a complex proteome prone to post-translational modifications (PTMs), such as phosphorylation, ubiquitination and proteolysis. Therefore, an extensive proteomic characterisation of VEs in SCC patients is urgently required for a better understanding of their physiological role. The present project proposes the application of MS-based quantitative analyses of proteins and endogenous peptides from VEs isolated from plasma and saliva of patients divided into the following groups: (a) No oral SCC, (b) premalignancy lesion, (c) SCC

pre-surgery and (d) SCC post-surgery. The employment of enrichment techniques to capture phosphorylated and ubiquitinylated peptides will increase analytical sensitivity allowing for the profiling of these PTMs in the VEs proteome and peptidome. In parallel to conventional protein identification by searching for tryptic peptides, sequence deduction using unspecific cleavage sites is expected to reveal others protease substrates processed in the VEs. In addition, de novo sequencing may constitute a strategy to study putative sequence variation of such molecules. Bioinformatics analyses will be used to predict regulated cellular processes, active proteases involved in endogenous peptide production, and other functional and structural features. At last, biomarkers of biomedical interest will be proposed and validated using targeted proteomics after development of method with adequate sensitivity, selectivity and throughput. We expect our data will (i) reveal the profile of proteins and endogenous peptides present in VEs isolated in saliva and plasma in different SCC stages, (ii) expand the understanding about the role of PTMs and alternative sequences in VEs isolated from patients with oral SCC, (iii) correlate these information to clinical-pathology aspects, (iv) provide a quantitative method for the analysis of SCC markers using targeted proteomics.

29. Leticia Signori de Castro
Universidade de São Paulo (USP) / FAPESP

Bovine sperm nucleus isolation and proteomic characterization for fertility studies: preliminary results

Mature spermatozoa present a unique chromatin compaction characterized mainly by protamine-DNA binding and other nuclear proteins that contributes to paternal DNA activation. Studies already described the whole sperm bovine proteome but, in these cases, the nuclear proteins could be masked by most abundant proteins or lost during protein extraction due to their high affinity for DNA. The aim of this study was to standardize for bovine sperm the isolation and enrichment of nuclear protein extraction for mass spectrometry analysis. For that, 4 straws from different bulls were thawed, pooled and washed twice to remove the extender (200g/5 min). Twenty million sperm were incubated 45 min on ice with 10 mM DTT and 0.1% CTAB to remove the tail and membranes. After incubation, the sample was washed twice in 50 mM Tris-HCl, one part was fixed in glutaraldehyde for transmission electron microscopy (TEM) and the other part submitted to high salt (0.65M NaCl) and acid (0.5M HCl) concentration for nuclear protein extraction and precipitation with TCA. Precipitated proteins were digested with trypsin and Lys-C mix, desalinated and submitted to mass spectrometry analysis (nano-LC/MS). Optical microscope images revealed after CTAB treatment, showed 99.9% of sperm heads were with a normal shape and without tail. TEM images showed that most part of plasma and acrosome membrane were removed. Proteome data were compared to UniProt database being identified 327 different proteins. Based on subcellular localization described at UniProt, 25% of proteins have nuclear localization, being 9% exclusively nuclear and 16% with both cytoplasm and nuclear localization. Nucleus was the second most represented cell compartment, behind only of cytoplasm group, which contemplate proteins from other organelles like flagellum and cytoskeleton. We also could identify mitochondria (14%), membrane (11%), cytoplasm (24%) and other organelles (26%) proteins. Between nuclear proteins, we identified 8 different histones variants and protamine 1 and 2. Although the presence of extra-nuclear proteins, also presented in this same protocol for humans, we could get a representative number of nuclear proteins and this methodology of sperm nuclear isolation and protein extraction could be used to future studies of bovine sperm nucleus to evaluate the whole of this proteins on bull fertility characterization.

30. Lorenna Rocha Reis
Universidade de São Paulo (USP) / FAPESP

Neutrophil extracellular traps (NETs) are fine threads of DNA coated with proteases, granular and cytosolic proteins, released by activated neutrophils in order to "trap" invading pathogens. The neutrophil-triggered process that gives rise to NETs is called NETosis, and it is a type of cell death that differs from apoptosis and necrosis. Recent studies indicate that there are multiple NETosis activation mechanisms, and depending on the NETosis pathway, the cell may or may not continue to perform its functions despite NETs release. Different stimuli-triggered NETosis are not fully characterized, making discovery and quantitative proteomics excellent tools to differentiate distinct NETosis pathways. The goal of this work is to provide a complete characterization of the proteins present in neutrophil NETosis induced by different stimuli. Using multiplexed techniques of qualitative and quantitative proteomics, we aim to identify host proteins released during the inflammatory process prompting NETosis. With biochemical approaches, we have characterized the time course of NETs release under a variety of neutrophil stimuli. Preliminary proteomic results showed many inflammatory proteins released during NETosis. Further work will focus on developing quantitative proteomic strategies to differentiate NETs mechanisms driven by distinct stimuli.

31. Luciana Daniele Trino
CNPEM

Immobilization of multiple potential biomarkers, pure and obtained from liquid biopsies, in metal-organic frameworks (MOFs) as biosensors applied in head and neck cancer diagnosis and prognosis

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Among the different types of tumors, head and neck cancer is the sixth most common form of malignant tumor, and early diagnosis of the disease is a decisive factor in controlling its progression. Due to the need of a multidisciplinary medical team and different types of interventions, this disease is associated with high costs and invasive treatments, generating a high economic and social burden. Thus, the identification of additional parameters or biological markers that can assist in the prognosis of patients with head and neck cancer is essential for the reduction of mortality rates and to improve the strategies of treatment of these patients. An ideal cancer biomarker must have high sensitivity and clinical specificity for early diagnosis and efficient prognosis. Saliva has been well studied in the search for biomarkers since it is a simple and fast fluid to be collected, as well as non-invasive. One of the most promising techniques in the identification and verification of biomarkers of head and neck cancer is the proteomics-based in mass spectrometry (MS). More specifically, selective reaction monitoring (SRM) in MS has emerged as a promising strategy capable of quantifying potential biomarkers with high reproducibility, sensitivity, and accuracy simultaneously. Therefore, from the knowledge of specific biomarkers for head and neck cancer identified and validated by MS, it is possible to develop other techniques that can be implemented in the clinic. Among them are the biosensors combined with the techniques of immobilization of these biomolecules in nanomaterials, which are able to identify these biomarkers with high sensitivity, specificity, and greater speed. In addition, biosensors have a high potential for the simultaneous detection of multiple biomarkers. Among the types of nanomaterials are the metal-organic frameworks (MOFs) which have several active sites

available. Thus, the response of the interaction between biomarkers validated by MS in MOFs can be detected by means of an optical or electrochemical response. Therefore, the project aims to immobilize simultaneously multiple biomarkers candidates of head and neck cancer and proteins from liquid biopsies (saliva) in metal-organic frameworks, ZIF-8 and NMOF-1, to develop biosensors for diagnosis and prognosis of the disease. Considering the retrospective presented, this proposal can provide a significant advance in the diagnosis and prognosis of cancer, since in addition to the direct benefit to anticipate treatment planning, it is noted that no similar strategy, specific for head and neck cancer using multiple biomarkers or biopsies, was found in the literature. Therefore, combining the development of mass spectrometry strategies to select robust and promising candidates with point-of-care devices will result in both improving the quality of life of the patient and reducing the treatment cost.

32. Luciana Godoy Viana

Fleury Group

Targeted proteomics in clinical laboratory routine – The case of quantification of serum chromogranin A

Luciana Godoy Viana, Débora Lima, Jessica Silva Salgueiro,Rodrigo Andrade Schuch, Karina Helena Cardozo, Valdemir Melechco Carvalho

ChromograninA (CGA) is a glycoprotein secreted by different neuroendocrine tissues and therefore is an important a biomarker for neuroendocrine tumors. Quantification of CGA has been performed by immunoassays in clinical laboratories. However, these assays are susceptible to interference from autoantibodies, heterophilic antibodies and hook effect. To circumvent such bioanalytical limitations, we developed a novel method for CGA dosing by targeted proteomics. Methods: Quantification is based on Protein Standard Absolute Quantification where a fully isotopically labeled recombinant protein is used internal standard. Biomarker enrichment is achieved by a solid phase procedure based on a polymeric sorbent and then subjected to the protein digestion. Digested extract is analyzed by a nanocromatography system (NanoAcquity, Waters) followed by detection and quantification by parallel reaction monitoring in a hybrid quadrupole-orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific). Three peptides are monitored at 70000 resolution, maximum injection time of 250 ms and 50000 for automated gain control. The method was fully validated according to Clinical Laboratory Improvement Amendments and National Committee for Clinical Laboratory Standards. Results: The assay was designed to detect unmodified and non-cleavable segments of the protein sequence. The assay requires only 4 µL of serum and the limit of quantification is 50 ng/mL. The assay is linear up to 2058 ng/mL and the imprecision obtained were 8,6% and 9,8% for 82,05 ng/mL and 291,0 4ng/mL, respectively. The targeted proteomics assay was compared with a immunofluorescent test and presented good accuracy: bias = 4,3%, slope = 1,106 (1,033-1,179) and intercept -12,7 (-33,80–8,4). Conclusion: The application of targeted proteomics in clinical laboratory is innovative and has the potential to achieve new limits of analytical performance for protein determination and to benefit the patients with a more precise diagnostic.

33. Marcos Alejandro Sulca López
Universidade de São Paulo (USP)

In my doctorate we had a rising interest to study new biological compunds as antimicrobials. I studied snake venoms from Peruvian snakes never studied for search of antimicrobials, in this case, specially from Bothriopsis oligolepis venom. For first time, some antimicrobials proteins were isolated from this venom, and identified by trypsinization and sequencing of the peptides by MALDI-TOF/TOF MS. Proteins such as a metalloprotease, a serine-protease and a C-type Lectin were identified. These inhibited the growth of Gram-positive and Gram-negative bacteria, but not of Candida species. Later, some antimicrobial peptides (AMPs) were designed having as a reference base the amino acid sequences of the sequenced peptides of these proteins, the peptides were synthesized by solid phase peptide synthesis. All the peptides synthesized were purified and analysed by LC/ESI-MS. Two peptides showed to have activity against Gram-negative and yeasts of the genus Candida. Our results show that proteins from new biological sources can act or be the origen of new antimicrobials compunds.

34. Maria Juliana Calderan Rodrigues
Universidade de São Paulo (USP)

Investigation of the plant cell wall, membrane and apoplast molecular roles in the interaction Sporisorium scitamineum x sugarcane: mining altered pathways through -omics and data integration

The smut disease is one of the most damaging to sugarcane crop with widespread occurrence, caused by the fungus Sporisorium scitamineum, generating losses of up to 62%. Against this fungus, there are resistant, tolerant and susceptible sugarcane varieties. After infection, both plants and pathogens secrete proteins that will determine the disease progression. Pathogens secrete molecules involved with processes of infection and pathogenicity, while the plant host secretes molecules that play fundamental roles in resistance. Although the secreted molecular components play a predominant role, and recent advances have been made in the elucidation of the interaction sugarcane x S. scitamineum, molecular differences related to the cell membrane, wall and apoplast are not yet known. These cell structures are the first barriersto the fungus, and thus by depicting the molecules that take part in this interaction, we can elucidate both the molecular pathways used by the early plant response and the fungus strategy. This project aims to generate information for the understanding of the interaction between sugarcane x S.scitamineum, detailing the modifications of the cell wall and relating them to the defense mechanisms, thus contributing to the generation of resistant varieties and new disease markers. The results will be obtained through omics: extraction and analysis of secreted proteins, analysis of specific transcripts, mining and integration of already existing and new data. The identification and functional analysis of proteins directed to the secretory pathway, as well as an integrative analysis, open up new possibilities that will contribute both to the understanding of plant metabolism and to the intrinsic responses of each variety and its resistance degree.

35.Milena Monteiro de Souza
A.C. Camargo Cancer Center / CAPES

The major cause of long-term morbidity and mortality after HSCT is the development of graft versus host disease (GVHD). In order to identify saliva proteins associated with GVHD, we performed a discovery and validation proteomic study. The total study cohort included 23 consecutive patients: 3 patients already diagnosed with cGVHD and another group with 20 patients (submitted to four saliva collections: prior to the transplant, 30, 100 and 200 days after HSCT). We first used two-dimensional gel electrophoresis to screen salivary proteins

from the patients with cGVHD and healthy, which showed different region expression between them. The Orbitrap mass spectrometry identified salivary proteins from all groups studied. The clustered analysis of significant proteins based on their patterns of expression (heatmap) was used on cGVHD and healthy, that revealed 7 proteins associated with Th1/Th17 cells; these proteins were then compared to the protein profile acquired from HSCT patients at different stages, which showed three proteins that could be associated with cGVHD development. Additionally, the cytokines concentration analysis in the saliva from cGVHD patients was performed to evaluate their inflammatory profile. The multiplex assay verification detected positive concentration for most cytokines associated with the Th17 immunological response. These results suggest that the proteins: Isoform H14 of Myeloperoxidase, Alpha-2-Macroglobulin and Complement C3, might be associated to the increased concentrations of IL-6, TNF-a, IFN-y and IL-17, and may be a potential biomarker for the early diagnosis and development prediction of cGVHD in HSCT patients.

36. Mirele Daiana Poleti

A.C. Camargo Cancer Center / CAPES

Understanding the biological mechanisms associated with the deposition and composition of intramuscular fat (IMF) in models and domestic animals, as well as in humans has been the focus of several scientific studies in the world. It is well known that for human health the presence of IMF is related to metabolic diseases and endocrine processes. In the meat production, the primary concern of the deposition of IMF is with the meat quality traits. Meat is the source of protein most consumed by humans and has high nutritional value, is rich in essential amino acids, iron, zinc, B vitamins and essential fatty acids such as palmitoleic, oleic, linoleic and linolenic. In recent decades, a great deal of effort has been devoted to better understanding the genetic role in the fat deposition process in animals and humans. However, the active role of proteins at the phenotype level is still underutilized due to the technological limitations of the past. Currently, use of label-free quantitative proteomics data obtained by high definition mass spectrometry (HDMSE) has allowed greater robustness and reliability of the results. The objectives of this study are (1) to identify the association between SNPs (single nucleotide polymorphisms) and protein level, and (2) to associate them with the deposition and composition of IMF. For this, the genotyping data of 400,000 SNPs and protein abundance of 105 animals will be used. The association analyzes between SNPs and protein abundance will be performed using the Matrix eQTL statistical package available in program R. Obtaining these results will allow a better understanding of the molecular mechanisms involved in the biological processes of IMF deposition and composition and its relation with metabolic diseases such as type 2 diabetes and obesity.

37. Natália Pinto de Almeida

Universidade federal do Rio de Janeiro (UFRJ)/FAPERJ

Targeted Proteomics Approach for the study of diterpene metabolism in Jatropha curcas

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Jatropha curcas L. is a Euphorbiaceous plant commonly found in tropical and subtropical regions and has a great biotechnological value since the seed contains high levels of oil that

exhibits essentials features to be an alternative source of biodiesel. However, among others toxins, the seed also accumulates phorbol ester (PE), a diterpenoid compound that confers toxicity to the oil and seedcake. Although it is known that PE accumulates in seeds, there is scarce information on its synthesis. It has already been shown that casbene synthase (CS) is considered a key enzyme for the synthesis of this toxin. The aim of this project consists in the application of targeted proteomics techniques for the analysis of CS and enzymes related to the PE synthesis pathway and diterpene metabolism in different tissues of J. curcas. Root, endosperm, leaf and integument samples were analyzed. Two genotypes of J. curcas, one containing a high levels of phorbol ester and one containing reduced levels were analyzed. For the development of SRM method, we stablished the target proteins based on previous J. curcas proteomic studies and literature information available. Isotopically labeled synthetic peptides from CS were optimized for the development of the SRM method and used for identification and quantification of the target peptides. Samples were reduced with DTT, alkylated with iodoacetamide and digested with trypsin. The optimization and SRM analysis was performed on the EASYII-nanoLC coupled to nESI-TSQ Quantiva. The results were analyzed with the software Xcalibur v. 2.2 and Skyline v. 3.7. . We optimized the detection of 35 CS peptides and it was possible to identified 6 peptides that comprehend 6 gene models of CS isoforms in root. Among the identified peptides, one is more abundant in the genotype with higher leves of PE and one in the genotype with lower levels of PE. Probably, there is a relation between the correspondent CS isoforms and the regulation of PE synthesis. We also observed no evidence of this enzyme in endosperm, leaf and integument. From our results, we can confirm the presence of CS in root, and suggest that PE synthesis can occur in this tissue.

38. Natália Rangel Palmier
Universidade Estadual de Campinas (UNICAMP)

Aims: Lately there's been a great search for customized treatments regarding the molecular profile of diseases in order to increase treatment success rates and decrease long term morbidity rates. Thus, the present study aims to characterize the salivary proteomic profile of patients treated for head and neck cancer (HNC) aiming to correlate the proteomic profile results with the risk of developing oral toxicities of radiotherapy (RT) as well as the correlation with the severity. Patients and Methods: 50 patients diagnosed with OSCC that will initiate curative protocols of adjuvant radiotherapy (RT) or chemoradiotherapy (CRT), will be prospectively assessed. Acute toxicities will be daily evaluated during radiotherapy and in the dental follow-up visits 1 and 3 months after the end of RT (D+30 and D+90, respectively). For the chronic toxicities additional assessments will be performed on a quarterly basis according to the post-RT follow-up protocol of ICESP / FMUSP. Dysphagia, dysgeusia, mucositis, and osteoradionecrosis will be classified according to the Common Toxicity Criteria for Adverse Events (NCI, Version 4.0, 2010). Xerostomia will be assessed according to the scale adapted from Eisbruch et al., 2003. Radiation-related caries (RRC) will be classified according to the Post Radiation Dental Index (Walker et al., 2008). For the molecular analysis, whole saliva will be collected in 5 different times for acute toxicities (D-1, D17, D35, D+30RT and D+90) and 8 times for chronic toxicity (additional collections D+180; D+270; D+360). The saliva samples will be centrifuged and digested in trypsin. Bradford test kits will be used for quantification of proteins prior to analysis by means of mass spectrometry. After processing, the protein analyzes will be performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS / MS) on the Velos Orbitrap (Thermo Fisher Scientific) LTQ mass spectrometer connected to the EASY-nLC liquid chromatography system (Proxeon Biosystem) through the nanoeletrospray (Proxeon) source. The raw data from the LC-MS / MS analysis of the saliva samples from all patients will be processed by the MaxQuant v1.3.0.3 program and the MS / MS spectrum will be searched

against the Human Uniprot database through Andromeda tool. Bioinformatics analyzes will be performed with the help of Perseus software v1.2.7.4, available in the MaxQuant package. The abundance of proteins will be calculated based on the normalized intensity of the spectrum (LFQ intensity) and its values converted to log2. Finally, a comparison of the proteomic profile of saliva before, during and after RT will be performed, as well as a comparison of the proteomic profile and the presence and severity of post-RT oral toxicities. Expected results: Characterize biomarkers that allow the identification of patients that are more likely to develop severe oral toxicities of the HNRT.

39. Romenia Ramos Domingues

CNPEM

Biomarkers are essential in diagnosis, prognosis and drug development. It demands high sensibility and specificity to obtain results that confirm or exclude diseases, as well as high quality in analytical, computational and statistical methods. However, the success of biomarkers in clinical trials has been limited, and some reasons are that few candidate biomarkers are rigorously validated by statistical models, and likely, the use of small numbers of biological samples together with high biological variability results in many false positives. Therefore, the goal this study is to apply statistical models to help select candidate biomarkers in discovery phase and classify them for further application in a new verification step such as targeted proteomics. For that, human saliva samples originated from patients with and without Oral Squamous cell Carcinoma (OSCC) were analyzed by LC-MSMS with four recombinant proteins spiked in the samples. After protein identification and quantification performed with Andromeda search algorithm within MaxQuant, the data were analyzed by Kruskal-Wallis, Wilcoxon, Nearest Shrunken Centroid (NSC), Random Forest and Support Vector Machine – Recursive Feature Elimination (SVM-RFE), followed by validation analysis such as crossvalidation, permutation and ROC curves. The results showed that Kruskal-Wallis and Wilcoxon were able to detect the spiked recombinant proteins in saliva samples in increasing concentration as true positives. There was an improvement in sample classification in Random Forest and NSC analyses when the input data were proteins that were previously filtered by p-value, except for SVM-RFE. Similarly, in relation to the ranking analysis, the recombinant proteins improved their position when previously filtered by the p-value for the NSC analysis, but not for the SVM-RFE. ROC curves showed the signal noise limit for the correct discrimination of each patient class. The results suggest that data filtered by p-value improve the correct classification of patients for Random Forest and NSC analyses, but not for SVM-RFE analysis.

40. Sheyla Batista Bologna Lopes

Universidade de São Paulo (USP) / FAPESP

Could serous acinar metaplasia in minor salivary glands in Sjögren's syndrome and lupus erythematosus be responsible for the MUC5B and MUC7 decrease?

Sheyla Batista Bologna Lopes, Milena Monteiro Antunes, Giovanna Florezi, Wanessa Cavalcante, Sandra Pasoto, Silvia Vanessa Lourenço

Xerostomia is the subjective feeling of dry mouth mainly caused by salivary gland damage or dysfunction and it can be present in inflammatory diseases such as primary Sjögren's syndrome (pSS) and lupus erythematosus (LE). Minor salivary glands (MSG) are

responsible for 10% of the saliva daily flow. Due to their mucous acinar composition, the saliva secreted from these cells present the most efficient lubricating component, the mucins. Thus, the present study aimed to investigate whether it is possible to relate specific morphological findings in MSG of xerostomic LE and pSS patients to the salivary proteomic quantitative analysis. Thirty cases pSS according to the 2016 the American-European Consensus Criteria for Sjögren´s syndrome and thirty cases of LE diagnosed according to the American College of Rheumatology criteria were included in the study; MSG biopsy was performed and unstimulated saliva was collected. The LE samples were grouped according to their subtype (systemic lupus erythematosus - SLE -, discoid lupus erythematosus - DLE - and subacute lupus erythematosus - SCLE) and the pSS cases were organized according to their focus score in two groups (SS1 and SS2). The histopathological aspects of all cases were examined using a conventional optical microscope and the mass spectrometry proteomic analysis was performed on LTQ-Orbitrap Velos ETDTM coupled with Easy nanoLC II System. The peptides were separated on a C18RP column on a 115 min gradient and the quantitation was performed using the softwares MaxQuant (v:1.4.1.2) and Perseus (v:1.6.1.1). Regarding to the mucins detected it was possible to highlight the Mucin-5B (MUC5B) and the Mucin-7 (MUC7). Both were remarkably present in the controls and diminished in the pSS and LE groups. Mucins are important proteins responsible for the lubrication and they are secreted only in the saliva produced by the mucous acini. The salivary gland damage may incite the acinar serous metaplasia and this finding was detected in a highly percentage of the cases: in the SLE group, 75% presented this aspect; in the SS1 cases, 87.25%, the DLE group, 90%; the SS2 samples, 92.85% and in the SCLE cases, 100%. Although the MUC5B and MUC7 were diminished in all disease samples compared to the control group, the most affected groups were the SCLE and SS2, presenting a fold change of 0.41 and 0.66 consecutively for the MUC5B, and 0.20 and 0.06 for the MUC7. Acinar serous metaplasia is not the unique cause of the xerostomia sensation, but based on our results, this finding may influence in the mucins secretion and it should be an aspect to be considered due to a less efficient lubricant effect produced by the saliva secreted by the serous acini.

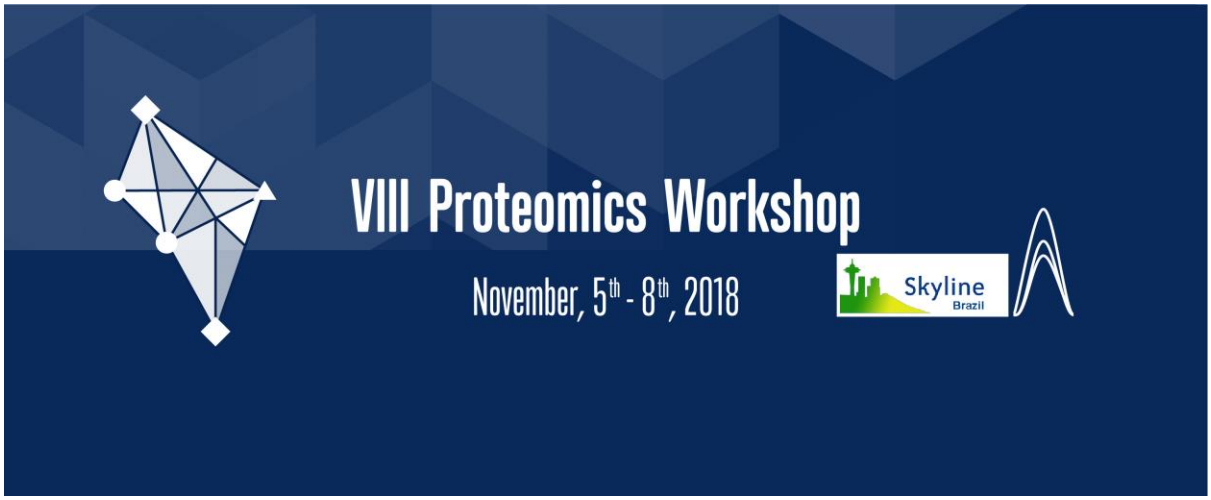
41. Tatiane De Rossi Mazo

Universidade Estadual de Campinas (UNICAMP) / FAPESP

The most common oral cancer in the world is squamous cell carcinoma (OSCC), accounting for more than 90% of all cases of cancer in the oral cavity. Thus, the research on molecular markers associated with the development and progress of human diseases has been the subject of intense research. The successful biomarkers are applied to the routine practice, both in diagnosis and prognosis, as well as guiding therapeutic strategies for intervention. The findings that saliva has molecular profiles indicating systemic diseases urge the study of non-invasive diagnosis using saliva as source of potential diagnosis, prognosis and predictive based on proteomics. In the discovery-based proteomics studies that have been developing by our research group showed (1) the differential abundance of 137 secreted proteins, in OSCC cell lines compared with a non-cancer cell line, and (2) differential abundance of 44 proteins in saliva of patients with OSCC compared with patients that who had the tumor removed. Based on these large-scale data, in a subsequent study of targeted based proteomics, we verified the abundance of 10 proteins in the saliva of patients with OSCC. However, although these studies indicate potential candidates for markers, their correlation with the prognosis cannot be performed due to the heterogeneous distribution of the patients between the different stages. In the SRM method, we monitored 68 proteins and 24 proteins were found decreased in patients with lymph node metastasis in comparison with patients without lymph node metastasis. In conclusion, this study indicates a panel of potential OSCC marker proteins, which can contribute to the prognostic evaluation, the patient's risk profile and the possibility of recurrence, and guide therapeutic intervention strategies.

42. Thomas M. Halder
Blau Farmacêutica

Development of an LC-MS based assay to determine the kinetics of Vibrio proteolyticus amino peptidase (VpAP), removing the N-terminal methionine of the human growth hormone (hGH) precursor VpAP is a commercially available, soluble enzyme which cuts specifically the N-terminal methionine from the precursor protein of hGH (somatropine). It is used in the final step of the production of hGH as API (Active Pharmaceutical Ingredient) resulting in the final hGH sequence. An LC-MS based assay was developed to define the optimal parameters for the kinetics of this enzyme reaction. Most critical parameters turned out to be pH, and the enzyme/protein ratio. Less critical parameters were temperature, enzyme freezing and thawing cycles, and the use of different protein batches.



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