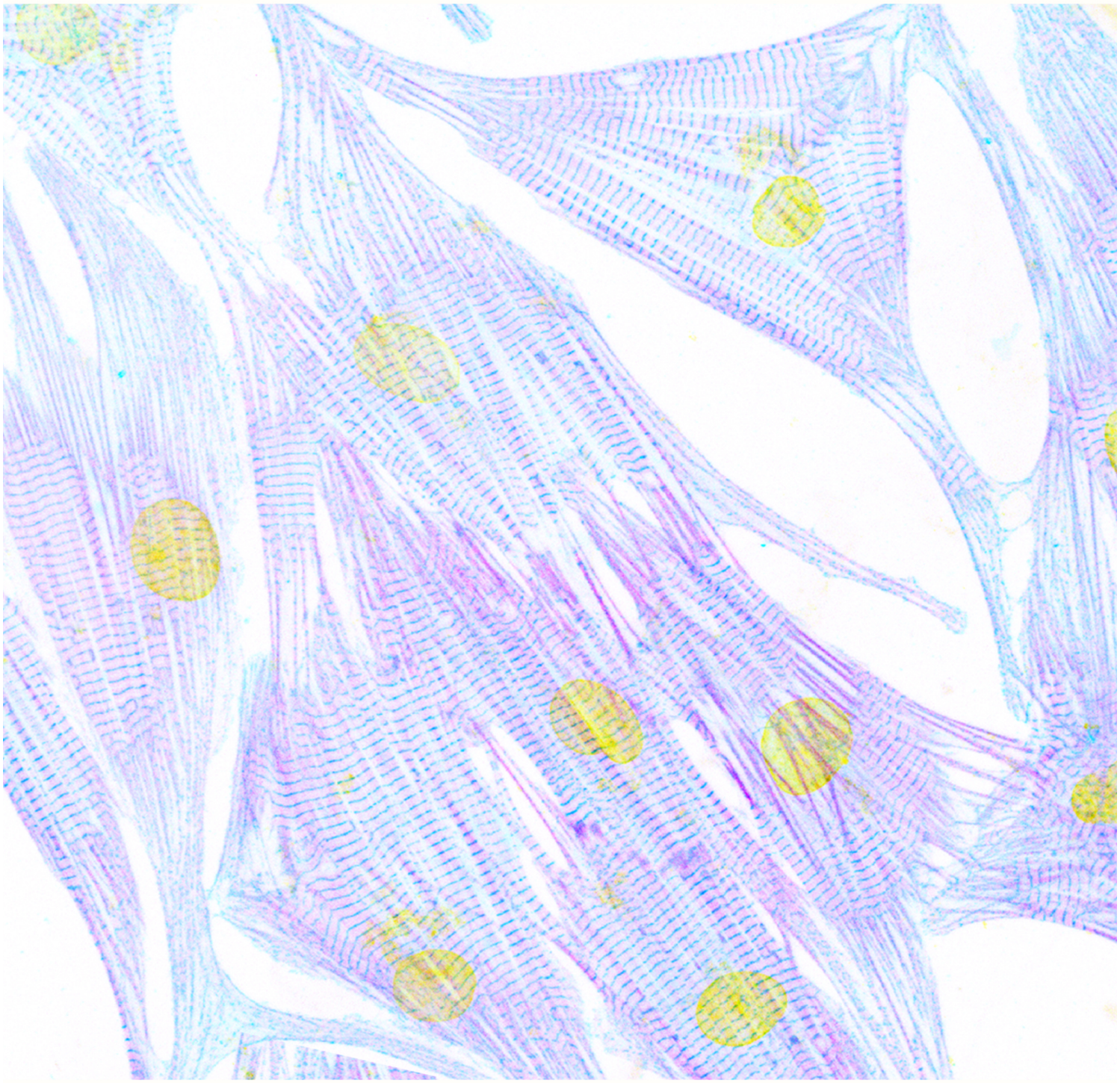
II Workshop on Recent Advances and Applications in Confocal and Widefield Microscopy

31st Aug - 4th Sep 2015



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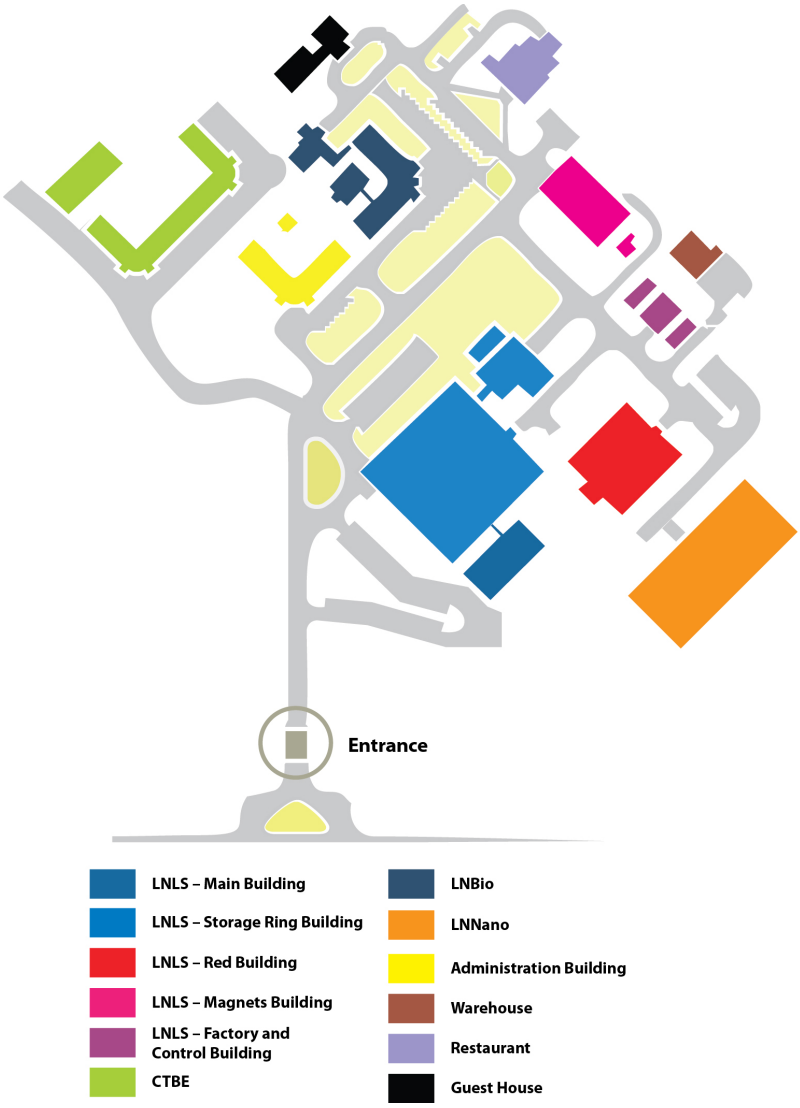
Campus Map



Summary

Presentation 4
Organizers and partnership 5
Program 6
Speakers 7
Abstracts 9

Event website: ***http://pages.cnpem.br/confocalmicroscopy2015/***

We invite you to subscribe to the LNBio newsletters and get the Laboratory news, future events and more information. To sign up, send an email to ***lnbiocomunica@cnpem.br*** .

Presentation

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

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

LIB
The Biological Imaging Facility (LIB) is focused on research and training for imaging biological samples for aspects of modern biological light microscopy, including confocal microscopy, imaging processing and analysis, and most microscopical techniques for developmental and cell biology at LNBio. One of the characteristics of the Laboratory is that users are provided with technical support in the operation of the confocal microscopy, sample preparation and data analysis, as well as training in the use of microscope

II Workshop on Recent Advances and
Applications in Confocal and Widefield Microscopy
The Workshop is organized by LNBio, in partnership with Leica Microsystems. It provides lectures from experts in the field of Confocal Microscopy, highlighting the basics of Confocal Microscopy, as well as super resolution, correlative light electron microscopy and light sheet microscopy. The presentations will also cover the use of widefield and confocal microscopy as a tool to study diverse areas in biology. The workshop is oriented towards current and future users who would like to gain practical and theoretical knowledge in confocal and advanced Widefield Microscopy.

Thank you for attending.
We hope you enjoy the Workshop,

Hozana Castillo, Silvio Consonni and Marcio Mesquita
Workshop Coordinators

Organizers and partnership

Event Coordination
Hozana Castillo – LNBio/CNPEM
Silvio Roberto Consonni – LNBio/CNPEM
Marcio M. Mesquita – Leica Microsystems

Local Committee
Maria Lívia Ramos Gonçalves - CNPEM
Tatiane Cortes - CNPEM
Pâmela Machado – CNPEM
Piero Bagnaresi – Leica
Ricardo Vilela – Leica
Joyce Felicci – Leica

Partnership

Leica
MICROSYSTEMS

Program

Minicourse on Basic Principles of Light Microscopy

31st August 2015	
14:00 - 15:30	**Principles in fluorescence, widefield and confocal microscopy** Márcio Mesquita
15:30 - 15:50	**Coffee break**
15:50 - 17:10	**Confocal and Super-resolution microscopy** Márcio Mesquita
17:10 - 18:00	**Sample preparation and troubleshooting in microscopy** Sílvio Roberto Consonni
18:00 -	**Wrap-up**

Workshop Theoretical Activities

1st September 2015	
09:00 - 09:30	**Welcome** Kleber Gomes Franchini Director of LNBio
09:30 - 10:15	**Basics of Confocal Microscopy** Piero Bagnaresi
10:15 - 11:00	**Leica TCS SP8: The Real Multiuser Confocal Platform** Ricardo Vilela
11:00 - 11:30	**Coffee break**
11:30 - 12:30	**Imaging Brain Cells to Understand Neuropsychiatric Disorders** Rodrigo Madeiro
12:30 - 13:30	**Leica TCS SP8 STED 3X: New Dimensions in Superresolution Microscopy** Ulf Schwarz
13:30 - 14:30	**Lunch**
14:30 - 15:30	**Guided tour through CNPEM**
15:30 - 16:30	**Examination of the NK cell immunological synapse by high- and super-resolution microscopy** Emily Mace
16:30 - 17:30	**Monitoring progression through mitosis and its role in preventing aneuploidy** Claudio Sunkel
17:30 - 18:00	**#1 Selected poster – Oral section**
18:00 - 20:00	**Poster Session and Cocktail**

2nd September 2015	
09:00 - 10:00	**Correlative Light Electron Microscopy** Ricardo Vilela
10:00 - 11:00	**The vertical Turn: Digital Light Sheet** Ulf Schwarz
11:00 - 11:30	**Coffee break**
11:30 - 12:30	**The unconventional secretion of stress-inducible protein 1 by a heterogeneous population of extracellular vesicles** Glaucia Hajj
12:30 - 13:30	**Retinoic acid signaling regulation: insights from an evolutionary conserved enhancer** Hozana Andrade Castillo
13:30 - 14:30	**Lunch**
14:30 - 15:30	**Guided tour through CNPEM**
15:30 - 16:30	**Quantitative analysis of F-actin clearance dynamics by non-diffraction limited STED nanoscopy at the NK cell lytic synapse** Emily Mace
16:30 - 17:30	**Polo kinase and its role in regulating microtubule-kinetochore interaction** Claudio Sunkel
17:30 - 18:00	**Poster Awards and Theoretical Activities Closing Cerimony**

Workshop Practical Activities

3rd September 2015	
09:30 - 11:00	**Practical Session I (4 Groups)** • Leica TCS SP8 • Off Line WorkStation – LAS X • Leica DM5500B • Leica M205A
11:00 - 11:30	**Coffee break**
11:30 - 13:00	**Practical Session II**
13:00 - 14:00	**Lunch**
14:00 - 15:30	**Practical Session III**
15:30 - 16:00	**Coffee Break**
16:00 - 17:30	**Practical Session IV**

4th September 2015	
09:30 - 10:30	**Hands on Session I (4 Groups)** • Leica TCS SP8 • Off Line WorkStation – LAS X • Leica DM5500B • Leica M205A
10:30 - 11:30	**Hands on Session II**
11:30 - 12:00	**Coffee break**
12:00 - 13:00	**Hand on Session III**
13:00 - 14:30	**Lunch**
14:30 - 15:30	**Hands on Session IV**
15:30 - 16:00	**Farewell**

Speakers

Claudio Sunkel
Institute for Molecular and Cell Biology – Porto, Portugal

Emily Mace
Baylor College of Medicine and Texas Children´s Hospital - Houston, USA

Glaucia Hajj
A.C.Camargo Cancer Center - Sao Paulo, Brazil

Hozana Andrade Castillo
Brazilian Biosciences National Laboratory – LNBio - Campinas, Brazil

Marcio Mesquista
Leica Microsystems **-** Sao Paulo, Brazil

Piero Bagnaresi
Leica Microsystems - Sao Paulo, Brazil

Ricardo Vilela
Leica Microsystems - Sao Paulo, Brazil

Rodrigo Madeiro
Instituto D´Or de Pesquisa e Ensino - Rio de Janeiro, Brazil

Silvio Roberto Consonni
Brazilian Biosciences National Laboratory – LNBio - Campinas, Brazil

Ulf Schwarz
Leica Microsystems - Mannheim, Germany

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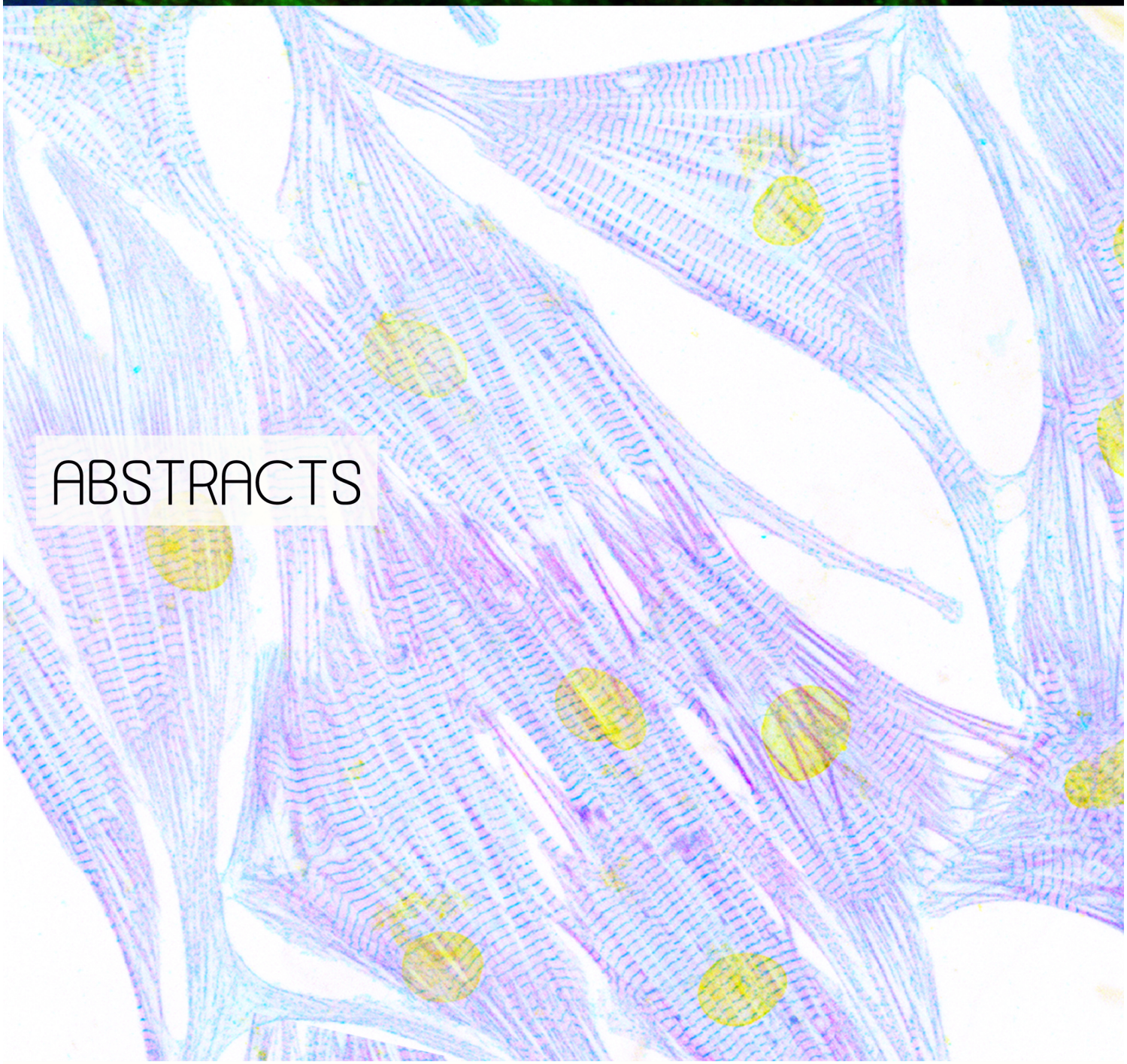

ABSTRACTS

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1. IMAGING TRIDIMENSIONAL CELL CULTURES FOR CANCER DRUG DISCOVERY: MULTIPLE WAYS TO ACCESS THE INFORMATION

1 –Adamoski, D.; 1 - Paradela, L.S.; 1 - Oliveira, K.R.S.; 1- Dias, S.M.G.
1 – Brazilian Center for Research in Energy and Materials

Cancer models using cell cultures are being used to represent the complexity of the disease in patients. Standard bi-dimensional cell culture techniques do not take into account the complexity of tumor structure and cell-cell interactions making tridimensional cell culture an indispensable tool for cancer basic research and drug discovery. However, migrating from pixel-to-voxel implies in increasing image capture, analysis and data report complexity, which may lead to data misinterpretation if not all the methodological precautions were taken.
To address those questions, we optimized 3D cell culture assays mainly focusing on High Content Screening using the capacities of three LNBio facilities –Chemical Biology and Screening Platform, Viral Vector Lab and Bio-imaging. Cancer cell lines were modified by a viral vector system to express the anthozoa-derived monomeric Kusabira-Orange fluorescent protein. Distinct methods for microsphere formation were tested based on hanging or sitting-drop on ultra-low-attachment cell culture plates. The variety of optical properties from those materials imparted the necessity of using distinct capture methods on both SP8 Laser Scanning Confocal (Leica) and Operetta (Perkin Elmer) microscopies. In all cases, dry objectives were used due the size of the imaged objects and refractive index mismatch issues. The plate reader EnSpire (Perkin-Elmer) was also evaluated as an output reader. For 3D image reconstruction of images collected on widefield, we performed a previous de-blurring or deconvolution processes using ImageJ. Sequentially, images were assembled and sphere's volume measured with Volocity (Perkin-Elmer). The fluorescence was read throughout the entire well since previous information regarding to the microsphere location was unknown.
We were successful in growing and analyzing tumor tridimensional cell cultures in order to obtain biologically-relevant information. Although the data collection could be performed on the laser-scanning microscopy, which offer low throughput capability, the Operetta Confocal mode or other conventional plate readers is by far the most adequate system for data collection on a medium-to-high-throughput mode.

2. EVALUATION OF THE EFFECTS OF MEF2C ISOFORMS γ+ AND γ- IN CULTURED CARDIOMYOCYTES

1 Pereira, A.H.; 1 Cardoso, A.C.; 1 Oliveira, R.R.; 1 Consonni, S.R.; 1 2 Franchini, K.G.
1 - Brazilian National Laboratory for Biosciences, Center for Research in Energy and Materials, Campinas, SP, 13084-971, Brazil; 2 -Department of Internal Medicine, School of Medicine, University of Campinas, Campinas, SP, 13081-970, Brazil.

Myocyte Enhancer Factor 2C (MEF2C) transcription factor plays a critical role to regulate gene expression in the pathogenesis of pathological cardiac hypertrophy and heart failure. Previous studies had been demonstrated that alternative splicing of MEF2C is critical to the regulation of MEF2C activity. However the contribution of MEF2C splicing variants to the pathophysiology of heart failure remains unclear. In the present study, we set out to gain a better understanding of how the γ domain of MEF2C can contribute to cardiomyocytes degeneration. We used adenoviral particles of MEF2C constructions that include (MEF2Cγ^{+}) or exclude (MEF2Cγ^{-}) the γ domain. Also we used as control the mutant 23/24 (MEF2C_23/24) that is defective for DNA binding and transcriptional activation. The results demonstrate that the overexpression of MEF2Cγ^{+} induces marked structural alterations in neonatal and adult rat ventricular myocytes, characterized by loss of sarcomeric organization and multinucleation, as detected by confocal and electron microscopy. Overall gene expression analyses indicated that MEF2C overexpression induced an upregulation of cell cycle genes and a downregulation of genes related to sarcomeric and cytoskeletal proteins, which are hallmarks of cardiomyocyte dedifferentiation. These alterations were accompanied by an enhancement of DNA synthesis without cytokinesis in cardiomyocytes, which culminated with enhanced apoptosis. Enhanced expressions of MEF2Cγ^{+} and markers of cell cycle re-entry were demonstrated in the cardiomyocytes of failing rat hearts. We propose that the γ domain represses the transcription activity of MEF2C and induces cell cycle re-entry and dedifferentiation of cardiomyocytes, which predispose to apoptosis and development of heart failure.

3. BOVINE PAPILLOMAVIRUS: TO MUTATION FOR METASTASIS

1,2 – Araldi, R.P., 1 – Magnelli, R.F., 1 – Modolo, D.G., 1,2 - Mazzuchelli-de-Souza, J., 3 - Spadacci-Morena, D.D., 1 - de-Sá-Júnior, P.L., 1 - Costa, A.S., 1 - Souza, D.M., 1 - Carvalho R, F., 1 - Beçak, W.; 1 – Stocco, R.C.
1 – Genetics Laboratory, Butantan Institute; 2 – Biotechnology Interunits Post-graduation Program IPT/Butantã/USP; 3 – Physiopathology Laboratory, Butantan Institute

Bovine papillomavirus (BPV) is the etiological causative agent of bovine papillomatosis, infectious disease characterized by the presence of multiple papillomas, which can regress spontaneously or progress to malignancy. Moreover, BPV is considered the best model for oncogenic process studies associated to viruses, including the HPV. Evidences have been suggested that BPV not only promote cancer initiation, inducing DNA damages, but also induces epithelial-mesenchymal transition (EMT), contributing to cell migration and metastatic process. Reactive oxygen species (ROS) are considered the major promoter of EMT. This study evaluated the role of BPV in ROS production and its association to EMT in different cell lineages: normal skin uninfected by BPV (control), three lineages of cutaneous papilloma (P01, P02 and P03) obtained from three bovines and one lineage of bovine esophagus carcinoma (EC). All samples were submitted to molecular BPV diagnosis, confirming the absence of viral sequence in control and the infection in papillomas and EC lineages. DNA damages were analyzed by comet assay, once they are always required in cancer initiation. Cell morphological changes were verified in inverted microscopy using phase contrast. Mitochondrial membrane potential (ΔΨm) was analyzed using the MitoTracker probe. Disruption of ΔΨm indicates cell metabolic changes which are associated to ATP supply. ROS production was verified using the DCFH-DA assay by. Both ΔΨm and ROS production were analyzed qualitatively by immunofluorescence and quantitatively by flow cytometer. Comet assay indicated clastogenesis in both papilloma and EC. These lineages also revealed morphological changes, including the filopodia and lamelipodia formation, which suggest cell migration and EMT. Mitochondrial membrane potential analysis pointed out reduction in ΔΨm keratinocytes of papillomas in relation to control. This data suggest a reduction of ATP production by oxidative phosphorilation, resulting in anaerobic glycolysis (Warburg effect). However, EC's cells showed an increase of ΔΨm. This data suggest a ROS production as a consequence of high metabolic demand of cancer cells, which require ATP to maintain the cell growth. DCFH-DA assay pointed out and increase in ROS production in EC's cell, reinforcing the results. This is the first study that evaluated the role of BPV in metabolic change inducement. These data suggest that BPV participate of all stages of carcinogenesis, to mutation for metastasis.

4. ANALYSIS OF THE EXPRESSION OF CUTANEOUS LYMPHOCYTE-ASSOCIATED ANTIGEN AND E-SELECTIN IN ORAL LICHEN PLANUS LESIONS THROUGH IMMUNOFLUORESCENCE

1-Silva Junior, A.; 1-Werneck, J. T.; 2-Gonçalves, L.S.; 1-Dias, E.P.
1-Pathology Pos-graduated Program – Antonio Pedro University Hospital – Federal Fluminense University – Niteroi/RJ – Brazil; 2- Dental School – Estacio de Sá University

Lichen planus is a chronic inflammatory disease with undetermined etiology. Migration of CLA T cells through E-selectin in lichen planus is few reported and there is only one study in the literature in association with oral lichen planus (OLP). The aim of this study was to analyze CLA expression and to verify if there is a relationship with E-selectin expression in oral lichen planus lesions. Material and Methods: From 37 patients with OLP, 11 were selected based on OMS criteria and modified by van der Meiji and van der Waal. All biopsy samples were obtained from buccal mucosa and an area without clinical lesion was included. The sample was divided in a half and in each of them an area with OLP (Oral Lichen Planus Group – OLPG) and an area without lesion (Perilesional Group – PG) were included. Half of the fragment was submitted to histopathologic diagnosis through H&E stain and the other was included in OCT, frozen, and them 6µm cut for immunofluorescence stain for CD3, CD4, CD8, CLA and CD62E. The frozen tissue was also stain by H&E and analyzed and the same areas of OLPG and PG were selected to correspond to the same areas for immunostain. At least 150 lymphocytes were counted from each field with five fields counted. Immunofluorescence was analyzed in Image J program and H&E stain was analyzed in APERIO system. Statistical analysis was performed by SPSS 17.0 ($p<0.05$), Wilcoxon test for OLPG and between groups analysis and Spearman test for coefficient correlation. Ethic committee approval 130/11. Results: It was observed more CD3+, CD4+, CD8+, CD3+CD4+ and CD3+CD8+ cells ($p=0.003$), CLA+ cells ($p=0.004$) and CD62E ($p=0.004$) in the OLPG in comparison to PG. CD4+CLA+ and CD8+CLA+ cells were more numerous in OLPG ($p=0.016$ and $p=0.003$ respectively). Prevalence analysis demonstrated significance only for CD8+CLA+ cells ($p=0.026$) in OLPG. None of correlation analysis among CD3+ or CLA and CD62E demonstrated statistical significance for both OLPG and PG. Conclusion: The high prevalence of CD8+CLA cells in OLPG when compared to PG suggest a possible involvement of these cells in immune mechanism of OLP. Although the correlation among CD4+CLA+ cells and CD8+CLA+ cells and CD62E were weak, it suggests an important role of CLA in immune response of OLP. It is possible that CLA is important in the pathophysiologic mechanism of OLP and a communication between skin and oral immunological compartments may exist. The higher expression of CD62E in OLPG in comparison with PG suggests its involvement in lymphocyte migration as well as in the disease etiopathogenic. Thanks the support by CAPES.

5. DIFFERENT MEDIAL GANGLIONIC EMINENCE CELLS GRAFTING PROTOCOLS RESULTED IN DISTINT PATTERNS OF ANTICONVULSIVE EFFECT AND INTERNEURONAL DIFFERENTIATION

1- Longo, B.M.; 1- Romariz S.A.; 1- Paiva D.S.; 2- Barnabé G.F.; 3- Calcagnotto M.E.
1 - Department of Physiology, Universidade Federal de São Paulo, Brazil; 2 - Ludwig Institute for Cancer Research, São Paulo, Brazil; 3 - Department of Biochemistry, Universidade Federal do Rio Grande do Sul, Brazil.

Neural stem cells from medial ganglionic eminence (MGE) have been tested to control seizure activity in animal models of epilepsy due to their capacity of differentiating in inhibitory neurons. Different methods have been proposed to prepare these cells for transplantation in order to better control their proliferation and differentiation. These cells can be dissected from MGE and immediately transplanted as “fresh cells” or cultivated and transplanted as dissociated neurospheres. Here we compared these two methods to investigate their anticonvulsive potential *in vivo*. MGE fresh cells or 7 days cultured neurospheres were obtained from GFP rat embryos (E14) and transplanted into hippocampal dentate gyrus (DG) of epileptic rats 8 days after pilocarpine induced-*status epilepticus*. A total of $5x10^4$ cells (fresh or neurospheres) were injected into the DG. Epileptic rats were observed for 540h to assess the frequency of spontaneous seizures (SRS) and their brains processed for immunofluorescence for GFP, GFAP and interneuronal markers. Our data showed a significant reduction in the frequency of seizures type V ($p<0.05$) when compared fresh cells grafted-group with non-graffed group (control). No difference in SRS type IV was found when fresh cells grafted-group and neurosphere grafted-group were compared with controls. We detected co-localization of GFP^+ cells with interneuronal markers in the hippocampus of both MGE-grafted epileptic animals. We also observed a large number of astrocytes in the hippocampus of neurosphere-grafted animals when compared with fresh cells-grafted group. Our data suggest that MGE progenitor cells, when transplanted as fresh cells, were able to attenuate the seizure frequency and preserved their original identity of inhibitory interneurons, when compared to cells cultured as neurospheres. These results might suggest that modifications in cell culture should be further investigated in order to obtain a more protective effect in epilepsy.

Financial support: FAPESP and CNPq

6. MACROPHAGE POLARIZATION PLAY A KEY ROLE AT THE RELAXATION AND RECOVERY PROCESSES OF MOUSE PUBIC SYMPHYSIS DURING LATE PREGNANCY AND POSTPARTUM

1- Castelucci.B.G.; 2- Consonni.S.R.; Joazeiro.P.P.
1- Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas; 2- Biology of the Cardiovascular System, Brazilian Biosciences National Laboratory

During mice pregnancy, pubic symphysis (PS), uterus (U) and other pelvic structures undergo an extensive remodeling for safe delivery. At the postpartum, those structures pass through a rapid recovery for birth canal homeostasis. Although recruitment and effector actions of distinct leukocytes play a key role in ECM remodeling of pelvic structures during pregnancy, there are few evidences about the involvement of phagocytic cells in PS changes during this time. Keeping in mind that the elucidation of processes about tissue remodeling in animal models can give support to choices of new targets for biomedical research, we investigate the participation of monocytes/ macrophages and the effect of their depletion over mouse interpubic ligament (IpL) relaxation and postpartum recovery. Light and electron microscopy addiction to immunohistochemistry markers of myeloid cell differentiation were used to determine the presence macrophages(F4/80+) and monocytes (Neu7/4+) in PS and IpL from 12, 18 and 19 days of pregnancy (dp), and 1 and 3 days postpartum (pp) C57bl6 mice in control and treated groups. In control group, the real-time PCR analysis showed increased expression of the characteristic inflammatory monocytes receptor (*Ccr2)* at the interpubic tissues on late pregnancy and postpartum. During pubic bones separation, evidences indicated that macrophages preferentially tend to polarize in the anti-inflammatory phenotype M2 (*Arg1*). At the relaxation of IpL pro-inflammatory M1 (*Il1a, Tnfa*) and anti-inflammatory M2 (*I110*) phenotypes was increased; in the meantime, parallel to the remodeling of the extracellular matrix necessary to the interpubic joint reorganization at postpartum (1dpp), there was an increase in the expression of genes characteristic of M1 phenotype and decreasing in the *Arg1* relative expression. Subsequently, at the 3dpp, despite the maintenance in expression level of *Il1a* and *Tnfa* there was an increased in M2 phenotype-related genes expression (*Il10, Arg1* and *Tgfb*) potentially involved in the activation of tissue repair mechanisms necessary to pubic bones tie. Depletion of monocytes/macrophages at separation and interpubic tissue relaxation, as well as 3dpp favored the M1 macrophage phenotype polarization (*Il1a*, *Tnfa*), leading to changes in the extracellular matrix disruption. This study shows that mononuclear phagocytes are important cells in the physiological process of mice PS remodeling during pregnancy, labor and postpartum. These cells act through finely regulated mechanisms to ensure the successful tissue repair of the pelvic support structures in mice

7. SUBCELLULAR LOCALIZATION OF THE TOMATO BLISTERING MOSAIC VIRUS 42K AND 66K REPLICATION PROTEINS IN *NICOTIANA BENTHAMIANA*

1 -Blawid, R.; 2 - Nagata, T.
1 – University of Brasília, Institute of Cell Biology, Laboratory of Electronic Microscopy and Virology; 2 – University of Brasília, Institute of Cell Biology, Laboratory of Electronic Microscopy and Virology.

Tymoviruses are plant RNA viruses consisting of a positive single-stranded RNA viral genome belonging to the *Tymoviridae* family, which is divided into three genera (*Maculavirus*, *Marafivirus*, *Tymovirus*). Currently, 35 species have been assigned and two species unassigned (ICTV, June, 2015). The viral genome codes for a polyprotein, involved in the viral replication (Methyltransferase/Proteinase; Met/Pro, Helicase, Hel; and RNA-dependent-RNA polymerase; RdRp), the movement and the coat protein. The coat protein is expressed from a subgenomic RNA and it is arranged into a T3 icosahedron. Tymovirus replication is known to occur in association to peripheral double-membranes of chloroplasts. The main objective of this work is to study the subcellular localization of the Tomato blistering mosaic virus (ToBMV) replication proteins, Hel and RdRp, within the cells of *Nicotiana benthamiana* plants. The ToBMV Hel protein consists of 377 aa (1131 bp, 41.85 kDa) and the RdRp of 591 aa (1776 bp; 66.5 kDa). The Hel and RdRp replication proteins were fused to the N and C-terminal of the fluorescent protein, GFP and/or YFP, from binary vectors engineered for the Gateway System. To this extend, genes were cloned into the pENTR2B vector and recombined to different pK7 binary vectors containing the fluorescent protein using the LR in vitro recombination procedure. Finally, agrobacterium cells (GV3103:pMP90 strain) were transformed with the selected binary vector and used for agroinfiltration into *N. benthamiana* plants. After 3 days post-inoculation leaf tissues were visualized *in vivo* using a confocal laser scanning microscope (CLSM SP5, Leica, Germany). Helicase proteins were found exclusively localizing to chloroplasts, expressed from both fused C- and N-terminal constructs. Sublocalization analysis of helicase using the YLoC and Plant-mPLoc servers *(http://abi.inf.uni-tuebingen.de/Services/YLoc/webloc.cgi and http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/)* predicted a chloroplast-targeting signal. On the other hand, RdRp proteins were found at the peripheral of chloroplast, cytoplasm and nucleus, (also when using both constructs), confirming the obtained results from the NetNES and PredictNLS servers *(http://www.cbs.dtu.dk/services/NetNES/and https://www.predictprotein.org/),* which predicted a nuclear export signal and a nuclear localization signal in the RdRp protein. Interestingly, we observed chloroplasts gathering around the nucleus in all agroinfiltrated leaves with RdRp and Hel constructs. It might be possible that the ToBMV Hel and RdRp replication proteins promote an exchange of signaling between the nucleus and chloroplast, which might influence tymovirus replication. This is the first study showing chloroplast aggregation around the nucleus caused by tymoviruses replication proteins. Finally, we showed that the ToBMV Hel proteins did not require the RdRp for proper recruitment to the chloroplast envelope. These results give further insights on the Tymovirus replication mechanism.

Funding support
We are thankful to the National Council of Technological and Scientific Development (CNPq 479161/2013-5) for the financial support.

8. FUBP1 ROLE IN HYPERTROPHIC HEARTS

1. Clemente, C.F.M.Z.; 2. Cordeiro, I.B.; 2. Costa, A.P.D.; 1,2. Franchini, K.G.
1. Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials, Campinas, São Paulo, Brazil.
2. Department of Internal Medicine, School of Medicine, University of Campinas, Campinas, São Paulo, Brazil

Hypertrophy is a critical event in the onset of failure in chronically overloaded hearts. Mechanical stress and neurohumoral signaling factors have been considered the main triggering stimuli for the installation of hypertrophy in cardiac myocytes in a variety of pathological process. These stimuli converge on a limited number of intra cellular signal transduction circuits to mediate the cardiac growth response. Such signaling circuits directly coordinate hypertrophic growth by altering gene expression in the nucleus and by increasing the rates of protein translation and decreasing the rates of protein degradation in the cytoplasm. Previous proteomic analysis from our lab showed increased expression of FUBP1 (3 fold) in neonatal rat ventricular myocytes (NRVMs) subjected to cyclic equibiaxial stretch (12 h, 1Hz, 8%, 12% and 15% stretch from baseline). FUBP1 was also upregulated in the left ventricle of mice subjected to 15 days of thoracic aortic constriction. FUBP1 was first discovered as a transcriptional regulator of *c-Myc*. Recent reports have indicated that FUBP1 is also an RNA-binding protein, implying FUBP1 in the translation or stabilization of mRNAs. FUBP1 has a role in apoptosis, cell proliferation and migration. FUBP1 is associated with carcinogenesis, organ development and Parkin disease. However, the function of FUBP1 in the heart is not known yet. The aim of this study is to elucidate the FUBP1 role in the hipertrophyc reponse of cardiac myocytes to mechanical stress. Indeed, we also want to evaluate the possible FUBP1 regulated genes by chromatin immunoprecipation. Primary cultures of NRVMs (1- to 2-day-old Wistar rats) were prepared by enzymatic digestion (colagenase type II and pancreatin). Myocytes were purified on a Percoll gradient and plated in type I collagen Bioflex plates (Flexcell International Corp). NRVMs were stretched in a Flexercell FX-3000 strain unit (12h, 1Hz, 8%, 12% and 15% stretch from baseline). Nonstretched NRVMs were used as control. At the end of the experimental protocol, cells were submitted to immunoblot or immunofluorescence analysis. For immunofluorescence, NRVMs were fixed with 4% paraformaldehyde/sucrose and incubated with anti-FUBP1 primary antibody (Abcam AB181111). This was followed by incubation with Cy2-conjugated secondary antibody and then with rhodamine-conjugated phalloidin. Images were obtained with microscope DMI 4000 (Leica). RESULTS: *Western blotting* analysis showed increased FUBP1 expression in stretched NRVMs, in relation to CT nonstretched. FUBP1 was located in the nucleus of CT and stretched NRVMs, as indicated by immunofluorescence data. These are the very first results of this project. We can conclude that FUBP1 is upregulated in stretched NRVMs and it is located at the nucleus. This suggests a FUBP1 function as transcriptional regulator instead of RNA-binding protein (translation or stabilization of mRNAs) in the hipertrophyc reponse of cardiac myocytes to mechanical stress .
FUNDIND SUPPORT: CNPq

9. INVESTIGATING MITOSIS IN MEDULLOBLASTOMA CANCER STEM CELLS

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Medulloblastoma (MDLB) is the most common malignant brain tumor in children up to age four. It is a very aggressive brain tumor and available therapies are not effective to more than one third of the patients. It is thought to arise from neuronal progenitors in the cerebellum, where it develops and further can initiate metastasis. Cancer stem cells (CSC) in these tumors were reported in 2003, and they correlate with tumor aggressiveness, might being important to tumor initiation and progression. Efforts have been done to understand CSC behavior and its contribution to cancer development, and recent data shows that these cells can undergo asymmetric mitosis - an important feature of stem cells. However, CSC display mechanisms to increase the rates of symmetric divisions, generating cells with high proliferative capability that can contribute to tumor growth. The present work aims to investigate mitosis of CSC in medulloblastoma, and define how asymmetric divisions occur and are regulated. DAOY and D283 cells (cell lines from medulloblastoma) were cultured as tumorspheres in low attachment dishes and defined culture medium. After 7 days the population was enriched of CSC (identified by CD133 expression). Spheres and dissociated cells were then cultured in different culture medium for 48h, fixed and submitted to immunofluorescence. Using confocal laser scanning microscopy and systems for high-content analysis and screening, the segregation pattern of proteins related to proliferation and differentiation was investigated. The segregation pattern of some of the proteins revealed the presence of asymmetric cell divisions in both cell lines. Now the influence of the different medium and culture conditions on the rates of asymmetric division are under investigation.
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10. DIFFERENT PATTERNS OF NETS (NEUTROPHIL EXTRACELLULAR TRAPS) REALEASE INDUCED BY DIFFERENT STRAINS OF PARACOCCIDIOIDES BRASILIENSIS

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NETs (neutrophil extracellular traps) action has been widely studied over the recent years. Several microorganisms are able to induce formation and release of these structures and in some cases, these NETs have antimicrobial activities. However, some pathogens have evasion mechanisms that make the entrapment only temporary. Paracoccidioides brasiliensis (P. brasiliensis) is able to induce NETs and the interaction between NETs and the fungus is still been investigated. Aims: In this context, the aims of this study were to analyse the NETs realese against different strains of P. brasiliensis. Methods: Human neutrophils (PMNs) challenged with virulent (Pb 18) and avirulent (Pb 265) strains were analyzed with the automated microscope ImageXpress® Micro XL Widefield High-Content Screening System (Molecular Devices, Sunnyvale, CA). For this, cells were labeled with DAPI (DNA) and anti-elastase (FITC). PMNs cultures were analyzed in attempt to quantify the average of extracellular DNA structures, which represents NETs released after the culture's challenge with Pb18 or Pb265 or PMA activation. The analysis consisted in thresholding the structures stained with DAPI (DNA) colocalized with FITC (elastase). The total DNA structures were quantified using a customized method with MetaXpress® Custom Module Editor and measured by Integrated Morphometry Analysis. Results: We observed an interesting different NETs release pattern from cultures challenged with Pb18 or Pb265. Analyzing NETs area (µm2), NETs from Pb18 coculture appeared to be bigger and more scattered than those released by PMNs challenged with Pb265, which seemed to be smaller and more compacted, even though both are capable of entrapping the yeast cells. Conclusion: Different strains of P. brasiliensis (Pb18 and Pb265) are able to induce different NETs formation patterns and release by human PMNs in vitro. These results can contribute to a better understanding of the role of NETs in resistance or susceptibility in paracoccidioidomycosis.

11. IMAGE-BASED CELL ANALYSIS REVEALS NEK7 AND NEK10 INVOLVEMENT IN CELL DIVISION

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The accurate mitosis execution is mainly orchestrated by the mitotic spindle apparatus – central microtubules networks organized from two centrosomes- that mediate the correct chromosomes congression. Loss of mitotic spindle organization results in cell division failure which generates aneuploidy and genomic instability, common features of most human neoplasms. The human Neks (NIMA-related kinases) -1 to11 have been emerged as important players of cell cycle. However, no detailed Nek7 and Nek10 function during cell division has been reported. Here, using systematic approaches of confocal microscopy combined with image-based cell analysis, we present the first closer inspection of the Nek7 and Nek10 functions during cell division. We showed, by using High Content Imaging (HCI), that Nek7 and Nek10 localize to mitotic spindle, spindle centrosome and midbody, indicating their potential functions during cell division. In order to address this hypothesis, we took advantage of the three-dimensional (3D) image processing and examined, in detail, HeLa cell mitosis after RNAi-Nek7 or Nek10 depletion. We noticed an increase in the spindle length average often associated with a thick metaphase plate in Nek10-depleted metaphase cells. Consistent with this, Nek7 or Nek10-depleted cells had unfocused spindle poles in addition to kinked and curved spindle morphology. Importantly, the mitotic spindle was misorientated - increased spindle angle, measured by amplitude of angle between centrosomes and the plane of cell substrate - following Nek10-depletion. Together, these results indicate that Nek7 and Nek10 participate in mitotic spindle organization. In addition, RNAi-Nek7- or Nek10 suppression induced nuclear γ-H2A.X foci formation besides severe nucleus multilobulation (seen after Nek10 suppression) and multinucleation (seen after Nek7 or Nek10 suppression) pointing to the Nek7 and Nek10 requirement in *maintaining genome integrity*. In conclusion, our findings suggest that Nek7 and Nek10 are important components for cell division accomplishment and connect them to tumorigenesis. Live-cell imaging experiments are in process in order to explore space-temporally the Nek7 and Nek10 functions in cell division and genomic instability.

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12. BACK TO HOME: A COMPLEX JOURNEY OF *DIAPHORINA CITRI* ENDOSYMBIONTS DURING HOST EMBRYOGENESIS

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Diaphorina citri is supplied with essential nutrients lacking into phloem-sap by γ-proteobacterium mutualistic symbiont *Candidatus* Carsonella ruddii and the β-proteobacterium *Candidatus* Profftella armatura, both harbored in the host bacteriome. These bacteria are transovarially transmitted during bacteriome morphogenesis during host embryogenesis. Here, the events associated with bacteriome development during *D. citri* embryogenesis are described by fluorescent in situ hybridization (FISH) and bright-field microscopy. During the initial stages of embryogenesis, bacteria were located in a symbiont-mass at the posterior pole of the egg. Energids adhered to this mass at the onset of blastulation, surrounding the symbiont-mass prior to symbiont cellularization (bacteriocyte formation). The bacteriocytes of the transitory bacteriome are organized in an inverted position if compared to the mature bacteriome, i.e. with *Profftella* externally surrounding *Carsonella*. The subsequent stages of embryonic development were marked by bacteriocyte rearrangement in the bacteriome. The cells harboring *Profftella* move inwards and become surrounded by *Carsonella*-harboring bacteriocytes. The former become devoided of the cell membrane and give rise to the syncytium while the latter is arranged around the syncytial cytoplasm. By the end of embryogenesis, the bacteriome is bilobed as observed in the nymphal and adult stages.
Keywords: bacteriome; embryonic development; morphogenesis; Asian citrus psyllid; symbionts

13. CALSARCIN-1 MAY ACT AS A NEGATIVE REGULATOR OF CALCINEURIN-A BY STERIC HINDRANCE TO THE NFAT TRANSCRIPTION FACTOR

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Signaling by the calcium-dependent phosphatase calcineurin (Cn) plays key roles in regulating cardiac hypertrophy and pathological remodeling. Cn binds to and is negatively regulated by calsarcin (CS), a family of muscle-specific proteins. However, the molecular mechanisms involved in the inhibition of Cn by CS, as well as the subcellular distribution of CS remain unclear. Additionally, there is a growing breakthrough in the development of technologies that allow the localization of proteins in cells by light and electron microscopy with the use of molecular probes. Here we combined recombinant protein expression, chemical cross-linking coupled to mass spectrometry experiments (MS/MS), a modeling strategy for structural characterization of CnA-CS1 assembly and genetic molecular probes applied to correlative microscopy and biochemical assays, which allowed monitoring through images of the spatial-temporal distribution of CS1 in the Z disc biology in cultured neonatal rat ventricular myocytes (MVRNs) subjected to cyclic mechanical stretch. The MS/MS data obtained from the cross-linked peptides of both proteins were used to guide an *in silico* docking and molecular dynamics simulation of their polypeptide models. The interacting surface of CnA was mapped in a pocket between the 1^{st} and 3^{rd} α-helixes and surrounding loops, while the corresponding surface of CS1 was mapped to the carboxyterminal loops within the Leu_{179}-Phe_{185}, Phe_{195}-Ser_{199} and Thr_{250}-Leu_{264} regions. The performed pull-down and subcellular location assays in MVRNs under mechanical stimuli showed reduced interaction between CS1 and CnA, as well as a CS1 location change. Interestingly, the cyclic stretching did not induce modification in protein or gene expression on CnA and CS1 as well as no change in the phosphatase activity of CnA. However reduced interaction between CS1 and CnA, besides CS1 location change in MVRNs under mechanical stimuli have been observed. Moreover, CS1 overexpression and silencing has supported the negative regulation of the CS1 over CnA in stretched MVRNs. Based on structural data, the region of CnA that interacts with CS1 was found to be located in close proximity, but not coincident, to the β-sheet 14, the main binding site for the PxIxIT sequence of NFAT. Therefore, it has been speculated that as the NFAT and CS1 binding sites are very close in CnA and at the same time distant from the active site of the phosphatase, it is possible that the role of CS1 in the negative regulation of CnA occurs by steric hindrance to the NFAT transcription factor. Therefore, these results could contribute to a possible pharmacological inference, whereas Calcineurin-NFAT pathway is a major mediator of hypertrophy in cardiomyocytes by pathologic stimuli.

14. CONFOCAL MICROSCOPY OF HALLER'S ORGAN AND PALP SENSILLAR PROJECTIONS TO THE SYNGANGLION OF THE LONE STAR TICK, *AMBLYOMMA AMERICANUM* (ACARI: IXODIDAE)

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Chemical communication, which requires a sensory apparatus for detection of signals, has a dominant role in the regulation of behavioral adaptations by ticks to explore its environment, search for hosts, and find partners to mate. Anatomical and histological arrangements of the nervous system in ticks have been studied by several authors; however, to our knowledge, information on the pathways between taste and olfaction peripheral receptors and the synganglion in ticks is lacking. Our project was planned to fill this knowledge gap. The front legs at the organ of Haller level (olfactory nerves), and the fourth palp segment (gustatory nerves) of unfed males and females were excised. Dextran tetramethylrhodamine (3000 MW) was used for anterograde filling of the sensory organs. The cuts were then covered with Vaseline and the preparation was kept at 4°C for 48 h. Lastly, the entire nervous system was dissected in saline, fixed overnight in paraformaldehyde, washed in saline, dehydrated in ethanol series and mounted in Vectashield medium. Whole mount synganglions were viewed using a Zeiss 710 Confocal System (Carl Zeiss, Thornwood, NY) equipped with a 488 Argon laser with a pin hole size of 26 µm. Plan Apochromat 40x/1.3NA water and 63x/1.4NA oil objectives were used for scanning. The whole synganglion was scanned every 0.26 µm from ventral to dorsal positions. Stacks resolution were of 20486 x 20486 pixels. Of the 29 synganglions and 22 palps preparations, 19 and 10 were backfilled, respectively. Stained axonal arborisations from tarsus I were confined predominantly to the first pedal ganglion and the olfactory lobes and never extended into the supra-oesophageal nervous mass. Upon entrance in the olfactory lobe, the pedal nerve arborized in single glomerular neurons and these processes penetrated a single glomerulus. Nerves localized in the palps included a sensillum process at the distal end of the palp projected in the palpal ganglion through the palpal nerve. Palpal nerve arborization was observed in the palpal ganglion, but it did not extend to the olfactory lobe neuropil. Two projections were observed in all the preparations analyzed. The anterior projection extended to a commissure where several blebs can be seen, which are likely terminal neuronal structures. Thus, it can be seen that olfactory sensilla project into the olfactory lobe, which is organized in glomeruli. Gustatory sensilla of the palpal organ projected into the palpal ganglion and did not extend to the olfactory lobe neuropil. The 3-D architecture of the olfactory lobes of male and female *A. americanum* ticks is under preparation through volume rendering using the Reconstruct sotware.

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15. ROLES OF RETINOIC ACID SIGNALING DURING SPINAL CORD DEVELOPMENT

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Retinoic acid (RA) is a metabolite of retinol that is involved in the growth and development from chordates. RA signaling participates in four major developmental programs in the central nervous system (CNS): induction of neural differentiation, anteroposterior (AP) patterning, specification of motorneurons and dorsoventral (DV) organization. Its availability is dictated by the distribution of retinaldehyde dehydrogenases (RALDHs), of which *Raldh2* plays the most important developmental role. During the spinal cord development, *Raldh2* is expressed in the motorneurons, as well as in the roof plate and dorsal interneurons 1, but little is known about the roles of RA acid signaling in the DV patterning. The aim of this study is to knockdown Raldh2 in the developing chicken spinal cord, using morpholino (Mo) electroporation, to further analyze the expression pattern of dorsal interneurons markers, by *in situ* hybridization and immunostaining, in a scenario of reduced RA signaling. This will allow us to analyze possible roles of RA signalling in the specification and differentiation of dorsal interneurons. As a preliminary result, we could observe a decrease of Raldh2 expression in the dorsal spinal cord after electroporation, detected by immunostaining and *in situ* hybridization. This result demonstrates that the designed Mos are an efficient tool for the knockdown of Raldh2 expression.

16. NETs IDENTIFICATION IN CHORIOAMNIOTIC MEMBRANES FROM PREGNANCIES COMPLICATED BY PREMATURITY AND HISTOLOGICAL CHORIOAMNIONITIS

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Neutrophils are essential to the body defense against several infections and other biological process such as inflammation. These cells could combat the infections by different mechanisms: phagocytosis, discharge of their granular components and by a recent described mechanism called NETosis. This process is characterized by the release of extracellular traps known as NETs (Neutrophil Extracellular Traps), which are composed by nuclear components (histones and decondensed chromatin) and granular components such as elastase, myeloperoxidase and other enzymes with antimicrobial activity. NETs induction is evidenced by many authors, whom correlate this process with several microbes and other particles and cells. Studies have shown that inflammatory processes with neutrophil infiltration have intense formation of NETs, often correlating the NETs with the severity of the disease. Then, this mechanism could be proposed for pregnancies complicated by histological chorioamnionitis, an infection in the chorioamniotic membranes generally associated with prematurity. Aims: In this context, the aims of this study were to identify the presence of NETs in chorioamniotic membranes from preterm pregnancies in the presence of histological chorioamnionitis and to identify the main components of these extracellular traps, correlating them with prematurity. Methods: Tissue sections from formalin-fixed biopsies were labeled with DAPI (DNA) and anti-elastase (FITC) for confocal immunofluorescence microscopy analysis. Results: The images revealed the presence of NETs constituents, nuclear and extracellular localization of DNA and co-localization of elastase and DNA, confirmed by the overlay of the two stains. Conclusion: The principal components of NETs (DNA and elastase) were identified in chorioamniotic membranes from pregnancies complicated by histological chorioamnionitis. Additionally, the analyses of other NETs components, such as histone and myeloperoxidase, are being conducted in our lab. Thus, these results are important and could contributed to the better understanding of the NETs role during prematurity.

17. GENETIC HETEROGENEITY IN WILMS TUMOURS: CLONAL EVOLUTION AND IMPLICATIONS FOR BIOMARKER TESTING

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Current Wilms tumour (WT) patient stratification for treatment and prognosis depends on clinical and histological criteria. Ongoing work aims to incorporate copy number changes with prognostic significance as biomarkers to improve the recognition of cases at higher risk of relapse, but the intra-tumoural heterogeneity of these and other genetic changes is not known. Multifocal/bilateral cases represent an additional challenge, and recognition of the clonal relationships between separate tumours/foci has significant therapeutic implications. Multiple snap-frozen samples were obtained from consecutive post-chemotherapy WT nephrectomy specimens at Great Ormond Street Hospital, May 2011-June 2013. Tumour/germline DNA was hybridised to Illumina CytoSNP-12 v2.1 arrays. Known WT loci with recurrent copynumber changes were also analysed with Multiplex Ligation-Dependent Probe Amplification (MLPA) assay of known WT genetic loci. A custom pipeline was developed for phylogenetic analysis of clonal evolution. A total of 102 samples were analysed from 21 WT. 71% (15 of 21) of cases showed intra-tumoural genetic heterogeneity, with common copynumber changes in 13/15 (87%) cases suggesting shared origins of heterogenous areas. The proposed biomarkers 1q gain, 16q LOH and 1p LOH were present in 7, 4 and 1 case(s) respectively, and when present were heterogenous in 5/7 (71%), 2/4 (50%) and 0/1 (0%) cases, suggesting that prognostically significant changes are relatively late events in WT development. Using FISH, 1q gain correlated significantly with blastemal content, but other changes were independent of clinical, radiological and histological parameters. Patterns of clonal evolution varied, without obvious correlation to current therapeutic/prognostic stratification. Our findings suggest that the current practice of testing prognostically significant genetic biomarkers in WT using one small piece of tumour and not considering the different histological subtypes is not representative and may result in inappropriate conclusions and therapeutic/prognostic stratification.

18. THE INTERACTION BETWEEN APOPTOSIS, MITOPHAGY, AND MITOCHONDRIAL BIOGENESIS INDUCED BY RESVERATROL IS CRUCIAL TO THE HEPATIC STELLATE CELLS SURVIVAL

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Resveratrol has been the focus of numerous studies reporting opposite effects that depend on its concentration. The cell line GRX is an activated Hepatic Stellate Cells model used to study the mechanisms involved in liver fibrosis development. We previously found that resveratrol triggered a dose-dependent pro-oxidant effect resulting in cell damage at highest concentrations. Here, it was studied the influence of resveratrol treatment (1, 10 and 50 μM for 24h) on altering the mitochondrial metabolism of GRX, intending to understand whether the autophagy/mitophagy induction can trigger cell death. Results from flow cytometry of GRX labelled with Annexin V and Propidium Iodide indicated that all resveratrol concentrations triggered cell death by apoptosis. The highest concentration also promoted cell death by necrosis. Results from confocal microscopy imaging analysis, fluorescence measurement, and flow cytometry of GRX co-labeled with MitoTracker Green and Orange, or labelled with JC-1, revealed that resveratrol changed the mitochondrial dynamics, reducing cell's mitochondria activity. Results from RT-PCR revealed that mitochondrial dynamics proteins had their mRNA expression altered by resveratrol. In addition, measuring the mRNA expression of mitochondrial protein ND1 revealed that 1 and 10 uM of RSV triggered mitochondrial biogenesis. Immunoblotting of LC3 I/II along with results from flow cytometry and imaging analysis of GRX labelled with Acridine Orange revealed an increase of autophagy in all treated-cells. Images of transmission electron microscopy revealed that resveratrol increased the number of autophagossomes also triggering mitochondrial swelling. Confocal imaging analysis of co-labelled GRX with MitoTracker Green and LysoTracker Red revealed that resveratrol triggered mitophagy. Altogether, we demonstrated that resveratrol changed the mitochondrial dynamics and function. Even that all treated-cells have shown an increase of mitophagy, only cells treated with 1 and 10 μM showed mitochondrial biogenesis and seemed to better respond to the resveratrol's cytotoxic effects. Since cell damage was higher and there was no mitochondrial biogenesis in GRX at 50 μM of resveratrol treatment, we suggest that these cells failed to remove and replace all damaged mitochondria. In conclusion, the interaction among the concomitant induction of apoptosis, autophagy/mitophagy, and mitochondrial biogenesis by resveratrol could be crucial to the GRX survival.
Keywords: Apoptosis; Autophagy, Cytotoxicity; GRX; Hepatic stellate cells; Liver fibrosis; Mitophagy; Resveratrol.

19. MORPHOFUNCTIONAL ANALISYS OF EMBRYONIC TICK CELLS RHIPCEPHALUS MICROPLUS CHALLENGED WITH HYDROGEN PEROXIDE

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The embryonic tick cells, BME26, were isolated for about 30 years, but described only in 2008 by Esteves et al. The BME26 lineage firmly adheres to the substrate and is morphologically heterogeneous, having a large number of vesicles with different acidification degree. It also shows glycogen inclusions and numerous dispersed mitochondria in the cytoplasm. Unpublished data of the group showed that these cells are resistant to stress induced by hydrogen peroxide compared to mammalian cells. Hydrogen peroxide (H2O2) is an unstable chemical described as a powerful oxidant that can be degraded to hydroxyl radical. These compounds are known as reactive forms of oxygen and can cause damage, leading to cell death, when there is an imbalance between oxidants homeostatic reagents and cellular detoxification. This way, it was suggested that there are protective mechanisms to prevent cell destruction, maintaining the concentration of superoxide and hydrogen peroxide under control. Previously, the use of 1 mM H2O2 caused few effect in BME26 cells morphology, proposing that there is a mechanism of resistance to H2O2 (the same concentration lead VERO mammalian cells to 90% death). Consistence with this potential mechanism of resistance, BME26 cell viability was determined by MTT after 24 hours incubation with different H2O2 concentrations (0.125 mM - 13 mM). Lethal dose was determined in 5,5 mM. We also observed intracellular ROS generation in BME26 cells after hydrogen peroxide treatment for 2 and 24 hours. The use of DHE, a fluorescent ROS probe, showed that intracellular ROS increases during a time course.

20. NEW GLUTAMINASE INHIBITOR TOXICITY EVALUATED ON 2D AND 3D CELL CULTURES

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The drug discovery process is a long and complex process composed of an initial target identification and validation, followed by a biochemical or cellular assay development, high throughput screening (HTS) of a compound library, hit identification, lead optimization and finally the selection of a candidate molecule for clinical development. Glutaminase is an enzyme that plays a key role on Myc-driven tumor cells. Glutaminase inhibition or attenuated expression have been shown to be cytotoxic to several types of tumor, in special de triple-negative breast cancer (ER-, PR- and Her2-). Even as an important therapeutic target, until the present date, there are no drugs for glutaminase. We developed and performed a biochemical HTS assay using a 30,000 compounds library of ADMET characteristics-filtered compounds. Eleven confirmed hit compounds were selected and evaluated on cellular secondary assays for differential dose-response on proliferation of a normal epithelial breast cells (HMEC), a non-transformed (MCF-10A), a non-triple negative (SKBR3) and a triple negative cell line (MDA-MB-231). A compound C1 displayed potency and cancer-selectivity over the 2D-cell cultures growth inhibition, as well as decreased the triple-negative cells glutamine consumption and glutamate secretion. Cells grown on 3D present differences in morphology and metabolism. Therefore, 3D cell culture have long been advocated as a better model for the malignant phenotype since it better preserves the in vivo cells tumorigenicity. We tested the cytotoxic effect of C1 over MDA-MB-231 3D cell culture. The cell line was modified using a viral vector system to express the anthozoa-derived monomeric Kusabira-Orange fluorescent protein. The multicellular spheroids were tested using the sitting-drop method on ultra-low-attachment cell culture plate. To test different methods of data analysis, the data was collected on the SP8 Laser Scanning Confocal (Leica), the Operetta plate reader microscopy (Perkin-Elmer) with or without the confocal module and the EnSpire Plate Reader (Perkin-Elmer). Z-stack collected images were used to reconstruct the tridimensional structures spheres and their volume measured by the Volocity Software (Perkin-Elmer) as well as the open source ImageJ Software.

21. NEK3 INTERACTOME AND ITS REGULATORY ROLE IN THE PROLACTIN RECEPTOR SIGNALING PATHWAY.

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NEK3 is a 506 amino acid serine/threonine kinase, localized in both the nucleus and cytoplasm and has a N-terminal catalytic domain and a C-terminal regulatory domain. In its C-terminal domain NEK3 contains Thr479 in its PEST domain, which becomes phosphorylated upon activation. Expression of mutants without Thr479 or PEST domain causes changes in cellular morphology and polarity of neuronal cells. NEK3 is also involved in cell motility and invasiveness of breast cancer tumor cells through interaction with regulators of the Rho GTPases Rac1 and RhoA, mediated by prolactin induced association of NEK3 to the human Prolactin Receptor (PRLR). In order to identify new interacting partners of Nek3, a Yeast Two-Hybrid using the Matchmaker Gold Yeast Two-Hybrid system was perfomed and 27 different proteins were identified. The DNA sequences were submitted to *in silico* analysis in the "Integrated Interactome System (IIS) platform" (CARAZZOLLE et al., 2014), developed at National Laboratory of Biosciences, Brazil (http://bioinfo03.ibi.unicamp.br/lnbio/IIS2/) and visualized using the Cytoscape software (SHANNON et al., 2003). The identified interacting proteins candidates are functionally involved in sumoylation, ubiquitinylation, transcriptional regulation, DNA repair, RNA processing, regulation of cell proliferation, invasiveness and metastasis. Co-localization, co-immunoprecipitation assays and mass spectrometry were performed to elucidate the consequences of interactions and mechanistic details of the interactions. Also, through migration and invasion assays in single layer and spheroid of mammary cells combined with confocal microscopy will be possible to analyze the observed interactions and understand their functional consequences *in vivo.* In addition, the effects of NEK3 over-expression, of both wild-type and mutant forms, and its knock down by shRNA on the invasive phenotype and prolactin receptor signaling will be further investigated.

22. PYRUVATE KINASE M2 (PKM2) REGULATES TH17 AND TREGS RESPONSES MEDIATED SKIN INFLAMMATION

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Psoriasis (Ps) is a chronic inflammatory disease that affects the skin, causing lost productivity, high morbidity and high costs for health systems. Evidences suggests the important role of metabolic reprogramming on the immune response, but the underlying mechanism remains unclear. Recently, the role of pyruvate kinase M2 (PKM2) was demonstrated by modulating the innate immune system through metabolic reprogramming (Warburg effect). However, the role of this pathway in the development and maintenance of the inflammatory response in Ps is unclear. According to above, we study aims to evaluate the role of PKM2 in the development on experimental psoriasis.The study consisted 3 groups: a control group, an imiquimod group and imiquimod+SKN group. C57BL/6 mice were submitted to imiquimod-induced psoriasis using imiquimod (3.25mg) on the back skin for 6 days. The pharmacological treatment with SKN (PKM2 inhibitor) was performed using a dose of 4 $mg.kg^{-1}$, i.p, started 1 day before psoriasis induction. The severity of Ps was assessed using PASI (Psoriasis Area Severity Index): erythema, scaling, thickness and histology (H&E) in skin. Furthermore, we assessed by immunofluorescence keratin 17 (K17), S100A9, HIF1-alpha and PKM2 expression. We evaluated cytokines production by ELISA levels of IL-17, IL-22, IL-10, TNF-α and IL-23. SKN-treatment ameliorates clinical aspects significantly ($p<0.05$). Histological analysis showed that SKN-treatment decreases acanthosis, leukocytes infiltration and epidermal thickness, therefore, SKN reduced expression of K17, S100A9, HIF1-alpha and PKM2. In addition, the inhibition of PKM2 decreases levels of inflammatory cytokines. To elucidate the role SKN on experimental psoriasis we evaluated by flow cytometry: Th17 cells, IL-17-producing gamma delta T cells (IL-17+γδ+), Treg and IL-10 Treg cells. Furthermore, we conducted experiments evaluating SKN on the Th17 and Treg *in vitro* differentiation. We found that SKN decreases Th17 cells *ex vivo* and *in vitro* differentiation, however, the IL-17+γδ+ does not reduces with SKN-treatment. Moreover, this compound increases Treg and IL-10 Tregs cells *ex vivo* and *in vitro* differentiation of these cells. The results set demonstrated that SKN modulates sub-sets of CD4 T cells, promoting the reduction of Th17 and increased Tregs with mechanism dependent PKM2 inhibition. Accordingly, PKM2 playing a pivotal role to development or maintenance of experimental psoriasis, with future clinical implications.

Keywords: Psoriasis, pyruvate kinase M2, shikonin, Th17, Tregs

23. BIOFILM ARCHITECTURE INFLUENCE ON MICROORGANISM VIRULENCE POTENTIAL

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Biofilm is a population of microorganisms growing on a surface, firmly attached to and enmeshed in an extracellular polysaccharide/organic polymer matrix, commonly of microbial origin. We work with a biofilm that causes dental caries, which is the most common and costly biofilm-dependent disease that affects humans (children and adult alike) worldwide. This disease results from the interaction of specific bacteria (such as *Streptococcus mutans*) with constituents of the diet (e.g., sucrose) in a biofilm known as dental plaque, which leads to demineralization of tooth surface. We use an i*n vitro* model to study cariogenic biofilms per se and how these biofilms respond to topically applied therapeutic approaches. Biofilms are formed onto hydroxyapatite (HA) disc, used as tooth enamel surrogate. The discs are placed on orthodontic wire apparatus to maintain them vertically (two discs per apparatus), and are coated with saliva (sHA) to form the pellicle. Next, the sHA discs are transferred to 24-well plates containing liquid culture media inoculated with a defined population of microorganisms (bacteria and fungi), and incubated (37^{o}C / 5%CO_2). *S. mutans* is used as a main cariogenic bacterium and *Candida albicans* as the main fungi related to caries. The biofilms are grown for distinct developmental phases and are harvested to study: (1) the architecture via confocal microscopy, (2) the microbial population, (3) the biochemical (e.g., biomass, extracellular matrix) and biophysical (e.g., viscoelasticity) characteristics, and (4) the expression of virulence factors using transcriptomic and proteomic approaches. Regarding fluorescence imaging we developed an strategy to label the exopolysaccharides synthetized by streptococcal glucosyltransferases or Gtfs using dextran labelled with Alexa fluor 647 (or other) that are added to the culture media during the biofilm development. Once the biofilms reach the age for analyses, the microbial cells are stained with Syto 9 (or other), and the biofilms are imaged via confocal microscopy. Images are acquired from five equidistant points on the disc surface (512x512), providing power for statistical analyses to quantify the biofilm components (microorganisms and matrix) using COMSTAT. In addition, at least two images are taken at a higher resolution (1024x1024) for 3D reconstruction using AMIRA software. At least three experiments are performed using duplicates. The methods are published [Klein MI, et al. *Appl Environ Microbiol.* 2009; Klein MI, et al. *J Vis Exp*. 2011; Xiao J, et al.. *PLoS Pathogens*. 2012; Falsetta ML, et al. Infect Immun. 2014], and have helped to link the biofilm architecture to its virulence potential.