VI FARM
DNA

VI FUNDAMENTAL ASPECTS OF DNA REPAIR AND MUTAGENESIS

University of Sao Paulo, SP, Brazil
September 7th to 9th, 2018

VI FUNDAMENTAL ASPECTS OF

DNA REPAIR AND MUTAGENESIS

VI FARM-DNA

Sao Paulo, September 7th, 2018

Dear Colleagues

We are delighted to welcome you to the University of Sao Paulo, Sao Paulo, Brazil, for the VI FARM-DNA meeting.

The main purpose of the meeting is to promote the areas of DNA repair and mutagenesis in Brazil, by encouraging the interaction between scientists and students working on various aspects of DNA repair, that is, **sowing the seeds of knowledge** in the mechanisms cells use to maintain genomic stability. Topics at the forefront of research in the area will be discussed and we hope fruitful ideas and collaborations will launch here. Hopefully, the highly focused program on DNA repair and Mutagenesis will facilitate deep discussions on these subjects, particularly motivating young researchers.

This is the sixth meeting of this kind in Brazil (**the first occurred in 1999**), and we are pleased to have more than 130 participants from different nationalities, and approximately 30 oral presentations and almost 80 poster presentations. **We specially thank those participants coming from far away countries**, which certainly will enrich our meeting.

As your program chair for the meeting, we hope you enjoy the science and that the meeting will trigger active debate on the frontier of discoveries on how cells deal with damaged DNA and their functional consequences. Also important, we hope that all participants enjoy the pleasant atmosphere of the university and the busy, but also exciting, life in Sao Paulo.

With our warm welcome,

Nadja Souza-Pinto
Dept. of Biochemistry
Institute of Chemistry, USP

Carlos Frederico Martins Menck
Dept. of Microbiology
Institute of Biomedical Sciences, USP

GENERAL INFORMATION

The official language of this meeting is English. So poster presentations should necessarily be in English. Please, we ask for the Native Speakers to present their work slowly, so that everyone will undertand better your message.

The posters must be displayed on the boards from Friday and removed only Sunday afternoon. The authors are asked to be around their posters at least one day (odd numbers on Saturday, and even numbers on Sunday), but they may also be required for poster presentation out of this period, by any participant.

The secretariat will be open daily from 10:00 am to 5:00 pm.

PLEASE WEAR YOUR NAME BADGE AT ALL TIMES.

SCIENTIFIC AND ORGANIZING COMMITTEE

Dr. Carlos Frederico Martins Menck, ICB, USP

Dr. Carlos Renato Machado, ICB, UFMG

Dra. Catarina Takahashi, FFCL-RP, USP

Dra. Elza T. Sakamoto Hojo, FFCL-RP, USP

Dra. Juliana da Silva, ULBRA, RS

Dra. Nadja C. de Souza-Pinto, IQ, USP

Dr. Rodrigo da Silva Galhardo, ICB, USP

PREVIOUS FARM-DNA MEETINGS

1999
Human DNA Repair Diseases: From genome instability to cancer
University of Sao Paulo, SP

2003
Fundamental Aspects of DNA Repair and Mutagenesis
University of Sao Paulo, SP, May 1st to 3rd.

2007
III Fundamental Aspects of DNA Repair and Mutagenesis
University of Sao Paulo, SP, October 25th to 27th.

2009
IV Fundamental Aspects of DNA Repair and Mutagenesis
Federal University of Minas Gerais, Belo Horizonte, MG
November 8th to 10th (Dr. Carlos Renato Machado)

2013
V Fundamental Aspects of DNA Repair and Mutagenesis
University of Sao Paulo, SP, October 31st to November 2nd
(Dr. Nadja Souza Pinto, Rodrigo Galhardo & Carlos Menck).

SCIENTIFIC PROGRAM

September 7th

9:00 a.m. **In Loco Registration**

9:00 a.m. - 1:00 p.m. **Short courses**

These courses will be in Portuguese.
Local: Ed. Biomédicas 2, Av. Prof. Lineu Prestes, 1374, Cid. Universitária, USP, São Paulo, SP

Short course 1: Aging and Neurodegeneration: the role of redox processes and DNA repair mechanisms.

Giovana Leandro and Natalia Cestari Moreno, University of São Paulo, SP

Short course 2: DNA lesions and repair in host-pathogen interaction.

Pilar Veras Florentino, University of São Paulo, SP, and Bruno Repolês, Federal University of Minas Gerais, BH, MG.

Short course 3: Mitochondrial DNA repair.
Nadja Souza-Pinto, University of São Paulo, SP, and Mateus Prates Mori, University of São Paulo, SP.

3:00 p.m. **Opening FARM-DNA presentation**

3:30 - 4:30 p.m. **Opening lecture**

Coordinator: Carlos F M Menck, USP, SP

Alain Sarasin, Institut Gustave Roussy, Villejuif, France

How History and Geography can explain the high incidence of XP-C patients in the Comorian Archipelago and their link with some Brazilian XPs.

4:30 - 6:30 p.m. **Symposium 1: DNA repair and Cancer**

Coordinator: Clarissa RR Rocha, USP, SP

Jean-Yves Masson, CHU de Québec-Université Laval Research Center, Québec City, QC, Canada

The DNA-double strand break repair protein PALB2: function, regulation, and charting synthetic lethal strategies for cancer treatment.

Luís Mariano Polo, University of Sussex, Brighton, UK.

XRCC1 mediated interactions shed light on its links with cancer risk.

Jenifer Saffi, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, Brazil.

DNA repair imbalance is associated with tumor aggressiveness and modulates response to chemotherapy in sporadic colorectal cancer.

Leonardo Karam Teixeira, Brazilian National Cancer Institute (INCA), Rio de Janeiro, RJ, Brazil

Cyclin E: Replication Stress and Genomic Instability in Human Cancer.

SHORT TALK
Yuli T. Magalhães, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil.
Responsiveness of glioblastoma cells to γ-radiation and cisplatin treatments: a RHO -p53- mediated pathway?

6:30 p.m. **Welcome Meeting!**

September 8th

8:30 - 10:30 a.m. **Symposium 2: Mechanisms of DNA damage bypass and replicative stress**

Coordinator: Alessandra Pelegrini, USP, SP

Alessandro Vindigni, Saint Louis University School of Medicine, St. Louis MO, USA

Redefining replication stress response pathways in BRCA1-deficient cells.

Vanesa Gottifredi, Fundación Instituto Leloir, IIBBA/ CONICET, Buenos Aires, Argentina

Oncogenic and antioncogenic signals arising from the depletion of pol eta in the xeroderma pigmentosum variant disease.

Rodrigo Martins, Universidade Federal do Rio de Janeiro, RJ, Brazil
Maintenance of genomic stability in central nervous system development: Regulation of replicative stress by the ATR-ATRIP signaling pathway.

Patricia Kannouche, Institut Gustave Roussy, Villejuif, France
Unexpected role of the specialized polymerase zeta in DNA replication and genome stability.

SHORT TALK

Annabel Quinet, Saint Louis University School of Medicine, St. Louis MO, USA

Redifining replication stress response pathways in BRCA1-deficient cells.

10:30 -11:00a.m. **Coffee breaks**

11:00 a.m. - 1:00 p.m. **Symposium 3: RNA and transcription on damaged template**

Coordinator: Giovana Leandro, USP, SP

Manuel J. Munoz, Universidad de Buenos Aires, Buenos Aires, Argentina

Gene expression misregulation in DNA repair deficient cells.

Lucymara F. Agnez Lima, Universidade Federal do Rio Grande do Norte, Natal, Brazil

Chemical Inhibition of APE1 and its transcriptional consequences.

Luis F.Z. Batista, Washington University, St. Louis, MO, USA
Modulation of RNA decay pathways improves functionality of cells with impaired telomere maintenance.

SHORT TALK

Jessica E.B.F. Lima, University of São Paulo – RP, Ribeirão Preto, SP, Brazil
DNA damage levels, oxidative stress and mitochondrial alterations in patients with type 2 diabetes mellitus.

1:00 - 2:00 p.m. **LUNCH**

2:00 - 2:45 p.m. **Lecture 2**

Coordinator: Nadja Souza Pinto, USP, SP

Andrés Aguillera, Universidad de Sevilla, Seville, Spain
Role of chromatin and DNA damage response functions in R loop-mediated genome instability.

2:45- 3:00 p.m. **Coffee break**

3:00 - 5:00 p.m. **Symposium 4: Nuclear stress and DNA repair**

Coordinator: Pilar Veras Florentino, USP, SP

Bruce Demple, Stony Brook University, School of Medicine, Stony Brook, NY, USA
The Long and Short of It: Mammalian Base Repair Pathways for Oxidative DNA Lesions.

Filippo Rosselli, Institut Gustave Roussy, Villejuif, France
Loss of function of the Fanconi anemia A protein leads to nucleolar stress and altered ribosomal biogenesis.

Carlos Renato Machado, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
The recombinase Rad51 plays a key role in events of genetic exchange in *Trypanosoma cruzi*.

Valeria Valente, Faculty of Pharmaceutical Sciences of Araraquara, University of São Paulo State, Araraquara, Brazil
The histone chaperone HJURP facilitates DSB repair and promotes radioresistance to astrocytoma cells.

SHORT TALK

Raphael S. Pavani, Butantan Institute, São Paulo, SP, Brazil
Replication Protein A from trypanosomatids: A new perspective for a well-known complex.

5:00 - 7:00 p.m. **COFFEE BREAK WITH THE POSTERS**

September 9th

8:30 - 10:30 a.m. **Symposium 5: Making and Mending Breaks on DNA**

Coordinator: André Uchimura Bastos, USP, SP

Bernard Lopez, Institut Gustave Roussy, Villejuif, France
RAD51 generates genetic instability through non-homologous recombination.

Francisco Meirelles Bastos de Oliveira, Universidade Federal do Rio de Janeiro, Brazil

Quantitative Analysis of DNA Damage Signaling Responses.

Keith W Caldecott, Genome Damage and Stability Centre, University of Sussex, UK

DNA single-strand breaks, genome stability, and human disease.

Nicolas Hoch, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil.
XRCC1 Mutation is Associated with PARP1 Hyperactivation and Cerebellar Ataxia

SHORT TALK

Marina Dall'Osto, Research Center of Toulouse, Toulouse, France

Role of the DNA polymerase kappa in the regulation of the kinase CHK1.

10:30 - 11:00 .am. **Coffee break**

11:00 a.m.- 1:00 p.m. **Symposium 6: Mechanisms for maintaining genome integrity**

Coordinator: Natália C Moreno, USP, SP

Ben Van Houten, University of Pittsburgh, Pittsburgh, PA, USA
Damage sensor role of UV-DDB during base excision repair: stimulation of APE1 and OGG1.

Marcos R. Chiaratti, Federal University of São Carlos, São Carlos, SP, Brazil

Mfn1 is essential to oocyte fertility in mice whereas Mfn2 is required in oocytes to filter out mutant mitochondrial DNA.

Marcos T. Oliveira, Universidade Estadual Paulista, Jaboticabal, SP, Brazil

Twinkle and the maintenance of mitochondrial DNA.

Jose Renato Cussiol, Universidade Federal de São Paulo, SP, Brazil
Cross-talk between phospholipid metabolism and DNA Damage Response in *Saccharomyces cerevisiae.*

SHORT TALK
Mateus Prates Mori, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil.

Decreased PGC-1 expression correlates with ΔTG mutation in *XPC* gene but not with decreased expression of PGC-1A target genes.

1:00 - 3:00 p.m. **LUNCH ON THE POSTER SESSION**

3:00 - 3:45 p.m. **Lecture 3**

Coordinator: Carlos Renato Machado, UFMG, BH, MG

Jan Hoeijmakers, Erasmus Medical Center, Rotterdam, The Netherlands
Genome maintenance protects from aging and cancer: the impact of nutrition.

Coffee break 15 minutes

4:00 - 6:00 p.m. **Symposium 7: Carcinogenesis and stem cells**

Coordinator: Luciana R Gomes, USP, SP

Ludmil Alexandrov, University of California, San Diego, La Jolla, CA, USA

The Repertoire of Mutational Signatures in Human Cancer.

Alysson Muotri, University of California, San Diego, La Jolla, CA, USA

Blocking LINE-1 reverse transcriptase activity in TREX1-deficient cells rescues neurotoxicity in Aicardi-Goutières syndrome.

Peter de Keizer, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, The Netherlands

Targeted Apoptosis of Senescent Cells against Aging and Cancer.

Rodrigo S. Fortunato, Universidade Federal do Rio de Janeiro, RJ, Brazil

DUOX1 silencing in mammary cells alters the responses to genotoxic stress.

SHORT TALK

Alexandre T. Vessoni, Washington University in St. Louis, St. Louis, MO, USA

DNA damage response in Human Embryonic stem cells with short telomeres.

6:00 p.m. **Closing Ceremony with Poster Awards Distribution**

LECTURES AND SYMPOSIA ABSTRACTS

OPENING LECTURE

How History and Geography can explain the high incidence of XP-C patients in the Comorian Archipelago and their link with some Brazilian XPs

A. Sarasin*, K.M. Santiago**, M.I.W. Achatz** and F. CARTAULT***
*Laboratory of Oncogenesis and Genetic Instability, UMR8200 CNRS, Gustave Roussy Institute and University Paris-Saclay, Villejuif, FRANCE.
**Centro Internacional de Pesquisa A.C. Camargo, Sao Paulo, BRAZIL
***Service de Génétique, CHU de La Réunion, Saint-Denis de La Réunion, FRANCE

Xeroderma pigmentosum (XP) is a rare, autosomal, recessive disorder characterized by sunlight sensitivity and early appearance of cutaneous and ocular malignancies. We followed black-skinned XP patients from the Comoros Archipelago located in the Indian Ocean between the African mainland and Madagascar close to the equator. Thirty-two XP patients were counted in the last 10 years and all are coming from the same island (Anjouan / Ndzuani) with a population of around 200,000 individuals. This area is therefore the highest prevalence of XP patients in the world (almost one XP in 5,000). It was possible for us to follow, at the clinical and genetic level, a cohort of 18 patients in this population. Skin and ocular features were remarkably precocious and severe. The first skin cancer (on a black skin) appeared at around 4.5 years and ocular abnormalities (cancers and blindness) were the rule before the age of 10. Following skin biopsies, we analyzed patient fibroblasts. Nucleotide Excision Repair (NER) was 90% deficient as compared to wild-type cells and cell survival was very low following UVC irradiation. Following retroviral complementation, we demonstrated that these patients belong to the XP-C complementation group. A new homozygous mutation was found at the 3'end of the intron12 (IVS 12-1 G>C) of the *XPC* gene leading to the abolition of an acceptor splicing site and therefore to the absence of the XPC protein. This unique mutation, characteristic of the Comorian Islands, where consanguinity is the rule among this cohort, is associated with a founder effect with an estimated age of about 800 years. The study of mitochondrial DNA and markers of the Y chromosome led us to identify the haplotypes of our patients, which are derived from the Bantu people. This mutation was also found in a Kenyan patient. Although the four Comorian islands were populated by the same individuals coming essentially from Mozambique / Tanzania during the 8 -10th centuries, XP-C patients were only found in the small island of Anjouan. To avoid the slavery process caused by the arrival of the Arabs around the 11-13th centuries, some people of Anjouan escape them and hid themselves inside the island protected by volcanic mountains. This population lived in a poor environment, with an endogamic life style and without connection with the other islands. XP-C patients still live now in the same villages as their ancestries several centuries ago. This may explain the high incidence of XP who are located exclusively in one island.
Surprisingly, the same unique mutation has been recently described in Brazil. We identified in these Brazilian patients similar African haplotypes confirming historical slave trade between East Africa and South America. During the 18-19th centuries, about 20% of all enslaved Africans (called the "Moçambiques") were coming from the Portuguese Mozambique toward Rio de Janeiro in Brazil. Heterozygous XP-C individuals should have been present in these seaborne trips and then migrated inside Brazil.
Since this mutation appears to be borne in Africa, it would be interesting to compare the haplotypes between African, Comorian and Brazilian XP-C patients with this mutation. These data could lead to improved knowledge of migratory roads from Africa.

SYMPOSIUM 1: DNA repair and Cancer

The DNA-double strand break repair protein PALB2: function, regulation, and charting synthetic lethal strategies for cancer treatment.

Amélie Rodrigue(1), Nadine Brahiti(1), Laura Sesma(1), Mandy Ducy(2), Timothy Wiltshire(3), Yan Coulombe(1), Guillaume Marguillan(2), Penny Soucy(2), Graham Dellaire(4), Marcelo Alex Carvalho(5), Alvaro Monteiro(6), Fergus J. Couch(3), Jacques Simard(2), and **Jean-Yves Masson(1)**

(1) Genome Stability Laboratory, CHU de Québec-Université Laval Research Center, Québec City, QC, G1R 2J6, Canada
(2) Genomics Centre, CHU de Québec-Université Laval Research Center, Québec City, QC, G1V 4G2, Canada
(3) Department of Health Sciences Research, Mayo Clinic, Rochester MN, USA(4) Department of Biochemistry & Molecular Biology, Dalhousie University, Canada
(5) Instituto Nacional de Câncer, Programa de Farmacologia, Rio de Janeiro, Brazil
(6) Cancer Epidemiology Program, H. Lee Moffitt Cancer Center and Research Institute, Florida, USA

Inherited mutations in PALB2 are associated with a predisposition to ovarian, breast and pancreatic cancers. In particular, it has been reported that mutations in PALB2 increase breast cancer risk up to 8-9 fold. PALB2 was identified as a BRCA2 interacting protein, essential for BRCA2 anchorage to nuclear structures and for its function in double-strand break repair. The basis of the tumorigenic potential of PALB2 is thought to be related to functions in homologous recombination. Our group focuses on both the regulation and activities of PALB2 during DNA damage response and the effect of cancer-causing mutations. First, we will present our work in deciphering the functions of PALB2 in DNA double-strand break repair and homologous recombination. Predicting the functional consequences of PALB2 mutations or variants has been challenging as they can lead to different biological effects. Using a novel CRISPR/Cas based homologous recombination assay, biochemical and cellular assays, we performed a structure-function analysis of PALB2 and have undertaken a systematic functional analysis of PALB2 variants of unknown significance (VUS). These studies allowed us to uncover a PALB2 regulation mechanism by which cancer cells could drive genomic instability. I will conclude the presentation with our work on novel synthetic lethal pathways affecting the viability of PALB2-deficient cancer cells.

XRCC1 mediated interactions shed light on its links with cancer risk.

Luís Mariano Polo, University of Sussex, Brighton, UK.

A critical step of single-strand DNA nicks and breaks repair is the rapid recruitment of the scaffold protein XRCC1 that interacts with, stabilises and stimulates multiple enzymatic components of the repair process, including base excision repair (BER) and single-strand breaks repair (SSBR). It contains three domains, one N-terminal domain –that interacts with Polβ– and two BRCT domains, all connected by flexible loops of ~130 residues. XRCC1 is recruited by PARP1 or PARP2, two enzymes that are activated following DNA damage and synthesise ADP-ribose polymers that XRCC1 binds directly through the interaction of its central BRCT domain. Persistence of XRCC1 at sites of interest is crucial for the repair. However, the molecular basis that stabilises XRCC1 close to the damage site remains unclear. 2D NMR experiments revealed interactions mediated by the central BRCT domain of XRCC1 simultaneously with poly-(ADP-ribose), through non-overlapping binding sites. Mutation of residues within these sites separately has distinct effects on recruitment of XRCC1 to DNA damage in vivo, potentially impacting on repair fidelity and cancer susceptibility after carcinogen exposure.

DNA repair imbalance is associated with tumor aggressiveness and modulates response to chemotherapy in sporadic colorectal cancer.

Natalia Leguisamo[1], Helena de Castro e Gloria[1], Talita Vila Martins[2], Daniel de Barcellos Azambuja[2], Antonio Nocchi Kalil[2], Lisiane B. Meira[3], and **Jenifer Saffi[1]**

1- Laboratory of Genetic Toxicology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, Brazil. 2- Oncology Surgery Service, Santa Casa de Misericórdia de Porto Alegre (ISCMPA), and Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, Brazil. 3- Department of Clinical and Experimental Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom.

Inappropriate DNA repair caused by inefficiency of one of its pathways - such as base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER) and translesion synthesis (TLS) - is known to have influence in several cancer clinical and pathological outcomes and to contribute to chemotherapy resistance. Imbalances in different steps of these pathways, as consequence of high and/or low expression of its components simultaneously, may influence cancer cells' fate in different ways, such as through metabolic intermediates disruption, toxic intermediates accumulation and cell death evasion, which, in the end, may have a determinant role in patients' survival. Our study characterized the main DNA repair pathways expression profiles in colorectal tumors and its association with clinical and pathological features. In addition, we also exploited, in vitro, the possible mechanisms behind tumor aggressiveness and response to chemotherapy (5-fluouracil (5-FU) and temozolomide (TMZ)). Study design included two arms: (1) clinical: Seventy pairs of sporadic colorectal tumors and matched adjacent mucosal specimens were assessed for BER (MPG, OGG1, APE1, Polβ, XRCC1), MMR (MLH1 and MSH2), NER (CSB, XPA, XPD, XPG, ERCC1) and TLS (DNA polymerases eta, theta and kappa) gene (qPCR) and protein expression (immunohistochemistry) and its association with pathological and clinical features commonly used for patient staging and treatment approaches. (2) In vitro: MMR-deficient colon cancer cells overexpressing MPG and XRCC1 and treated with 5-FU and TMZ were evaluated for viability and energy metabolism. Our clinical data showed that overexpression of BER components were associated with more aggressive tumor features and poor pathological outcomes in colorectal cancer patients. Regarding the NER components expression, while CSB, XPG and XPA genes have lower expression in tumor tissue, XPD, XPF and ERCC1 are overexpressed. In general, genes with lower expression in tumor tissue is correlated to features that indicate better prognosis, On the other hand, overexpressed genes are associated, in general, with clinicopathological features that indicate worse prognosis. Concerning the TLS components, also found and increase in DNA polymerases kappa and eta, but not theta. DNA polymerases kappa and theta overexpression is also associated with poor pathological outcomes features, such as poor cellular differentiation and presence of metastatic lymph nodes. Finally, we choose to reproduce the BER imbalance by overexpressing MPG (upstream pathway component) or XRCC1 (downstream pathway component) in MMR-deficient colon cancer cells to evaluate the response to 5-FU and TMZ. The overexpression of MPG, but not XRCC1, in MMR-deficient colon cancer cells increased sensitivity to 5-FU and TMZ through ATP depletion and lactate accumulation. In summary, DNA repair pathways have a heterogeneous expression pattern and plenty of associations with poor clinical and pathological outcomes. However, BER gene and protein changes in expression levels seems to lead to a pathway imbalance, which may be exploited as a tool for a more accurate therapy design.

Cyclin E: Replication Stress and Genomic Instability in Human Cancer.

Leonardo Karam Teixeira, *Brazilian National Cancer Institute (INCA), Rio de Janeiro, RJ, Brazil*

Cell cycle progression is regulated by the Cyclin-Dependent Kinase (CDK) family of proteins, so named because their activation depends on association with regulatory subunits known as Cyclins. Cyclin E normally accumulates at the G1/S boundary, where it promotes S phase entry and progression by activating CDK2. In normal cells, Cyclin E/CDK2 activity is associated with DNA replication-related functions. On the other hand, it has been shown that deregulation of Cyclin E leads to inefficient assembly of pre-replication complexes and insuficient levels of nucleotide, causing replication stress and eventually genomic instability. Cyclin E is frequently overexpressed in human cancers, correlating with decreased survival in breast cancer patients. However, the mechanisms by which Cyclin E deregulation causes genomic instability are not completely understood. Our group has demonstrated that high levels of Cyclin E1 delay S phase progression of mammary epithelial cells, allowing cells to enter into mitosis before completion of DNA replication. Incomplete DNA replication induces aberrant mitosis, including formation of anaphase bridges and micronuclei. High levels of Cyclin E1 leads to persistent chromosome aberrations, such as endoreduplication, chromosome pulverization, and premature separation of sister chromatids, eventually causing aneuploidy. Preliminary results indicate that Cyclin E1 deregulation interferes with cohesin complex formation. Our work aims at understanding how oncogene-induced replication stress and genomic instability contribute to human carcinogenesis.
Financial support: INCA, CNPq, CAPES, FAPERJ, PEW Foundation

SHORT TALK

Responsiveness of glioblastoma cells to γ-radiation and cisplatin treatments: a RHO -p53-mediated pathway?

Magalhães, Y.T.[1], Forti, F.L.[1]

[1] Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil.

Glioblastoma is the most frequent brain tumor in adults and despite aggressive therapies with combined surgery and radio-chemotherapy, the prognosis remains poor, with a two-year survival rate. The increased cell migration and invasiveness of these tumors are directly related to their aggressiveness, which has been linked to Rho GTPase pathways. Here we explored the effects of Rho pathway inhibition on glioblastoma cells with different p53 status after the γ-radiation and cisplatin treatments. U87-MG and T98G cells were subjected to inhibition of Rho GTPases by C3 toxin or the knockdown of downstream Rho pathway components. Cytotoxic and 3D clonogenic survival assays showed that Rho inhibition increases the sensitivity of gliomas to γ-radiation and cisplatin. From alkaline comet assays the Rho inhibition exhibited increased DNA damage and a delayed DNA repair capacity of both cells after γ-radiation. Immunofluorescence assays show that phosphorylation and foci formation of H2AX are compromised by Rho inhibition, as well as the 53BP1 foci formation. The impairment of Rho pathway decreases the phosphorylation of H2AX and increases the levels of phospho-Chk2 after genotoxic treatments, as demonstrated by immunoblotting analysis. This indicates a striking regulatory relationship between Rho and DNA damage response (DDR) pathways. Comparative analysis of the Rho activity on cells expressing wild-type or mutated p53 showed that p53 wild-type cells are more susceptible to the effects of Rho inhibition. This work shows that Rho pathway might be a fragile point in the resistance of gliomas against the usual therapies, being this effect dependent on p53

transcriptional activities. Acknowledgments: This work is supported by FAPESP (grants 2015/03983-0 and 2017/01451-7) and CNPq (grant 402230/2016-7).

SYMPOSIUM 2: Mechanisms of DNA damage bypass and replicative stress

Redefining replication stress response pathways in BRCA1-deficient cells.

Annabel Quinet[1], Stephanie Tirman[1], Jessica Jackson[1], Daniel Gonzalez[2], Juan Mendez[2], and **Alessandro Vindigni[1*]**

[1]Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis MO, 63104
[2] Spanish National Cancer Institute, Madrid, Spain

BRCA1-mutant cancer patients are often treated with platinum-based drugs, such as cisplatin, to create replication-blocking lesions and induce extended replication fork degradation mediated by the MRE11. However, this fork degradation phenotype was established with a single-dose treatment, neglecting the fact that patients are treated with multiple cisplatin doses in a clinical setting. We found that fork degradation is no longer detectable in *BRCA1*-deficient cells treated with cisplatin 24 hours after pre-exposure to this drug. Cisplatin pre-exposure upregulates and increases chromatin loading of PRIMPOL, a protein with primase and polymerase activity, but not of other canonical translesion synthesis polymerases, such as POL, REV1, or REV3L in *BRCA1*-mutant cells. By combining electron microscopy and single-molecule DNA fiber approaches, we found that PRIMPOL induction rescues fork degradation by virtue of its unique *de novo* priming activity and leads to accumulation of ssDNA gaps both at replication fork junctions and behind them. ssDNA gap accumulation triggers RPA recruitment, leading to exhaustion of the RPA pool and replication catastrophe. Our findings challenge the notion that DNA-damaging drug-sensitivity originates from the extended replication fork degradation observed after a single-dose treatment. Reversed forks are the entry point for MRE11 in *BRCA1*-deficient cells and suppressing fork reversal by RAD51 depletion prevents fork degradation. In addition to suppressing fork reversal, we found that RAD51 depletion leads to ssDNA gap accumulation on ongoing replication forks, mirroring PRIMPOL induction. We propose a model where PRIMPOL upregulation rescues fork degradation by reinitiating DNA synthesis past DNA lesions, while suppressing replication fork reversal.

Oncogenic and antioncogenic signals arising from the depletion of pol eta in the xeroderma pigmentosum variant disease.

María Belén Federico, Nicolás Calzetta,Natalia Paviolo, Carolina Campana, María Belén de la Vega and **Vanesa Gottifredi**.

Fundación Instituto Leloir, IIBBA/ CONICET, Buenos Aires, Argentina

Xeroderma Pigmentosa Variant is a cancer prone syndrome caused by the loss of translesion polymerase eta, which is specialized in the DNA synthesis across cyclobutane pyrimidine dimers (CDP). Pol eta replicates DNA across CPDs with outstanding accuracy. As a consequence, pol eta loss causes an increase in the rate of point mutations by less accurate specialized polymerases. However, DNA replication across UV damaged-DNA may not only be inaccurate but also, it could be inefficient as suggested by plasmid-based assays performed in cells depleted from pol eta. The consequences of the inefficiency of TLS in pol eta-depleted cells are currently unknown. Here we show that in the absence of pol eta, the DNA replication stress caused by UV irradiation is persistent when compared to control samples. Double strand breaks and cell death also increase in pol eta depleted cells after UV irradiation. Surprisingly, despite the recruitment of homologous recombination markers to replication factories, such DNA repair pathway plays no apparent role in the DDR response of UV-irradiated pol-eta depleted cells. Instead, pol eta knock down or depletion causes persistent cell cycle arrest in S phase, which is accompanied with massive

accumulation of single stranded DNA (ssDNA). Strikingly, the elimination of the MRE11 nuclease reduces ssDNA accumulation and cell death in UV-irradiated pol eta-depleted samples. Together, our results demonstrate that whilst pol eta elimination may certainly promote cancerogenesis triggered by error prone TLS, it can also trigger cell death mediated by ssDNA-accumulation in cell persistently arrested in S phase. This information can be of use when exploring the use of TLS inhibition for cancer treatment.

Maintenance of genomic stability in central nervous system development: Regulation of replicative stress by the ATR-ATRIP signaling pathway.

Gabriel E. Matos-Rodrigues[1], Pedro B. Tan[1], Letícia C. da Silva[1], Pierre-Olivier Frappart[2] and **Rodrigo A. P. Martins[1]***
1.Programa de Biologia Celular e do Desenvolvimento, ICB, UFRJ, Brazil
2. Department of Internal Medicine I, Ulm University, Germany.

While genetic changes and mutations are crucial for evolution, maintenance of genomic stability is crucial for proper development and disease prevention in most organisms. Unperturbed DNA replication as well as genotoxic agents may cause replicative stress leading to genomic instability. Prevention of replicative stress requires the ataxia telangiectasia and Rad3-related protein (ATR) kinase and its partner ATRIP (ATR-interacting protein). ATR knockout mice are embryonically lethal and mutations of these genes in humans lead to Seckel syndrome, a disorder characterized by growth impairment prior to birth, microcephaly and mental retardation. The high replicative index during neurogenesis and the unique metabolism of long-live neural cells have been proposed to contribute to the elevated sensitivity of the central nervous system (CNS) to genomic stability. To study the roles of Atr-Atrip signaling in replicative stress during CNS development in vivo, we first characterized how neural progenitor cells (NPC) respond to replicative stress induced by hydroxyurea (HU). HU treatment of retinal explants induced DNA damage, checkpoint activation and cell death. Pharmacological inactivation of Atr-Atrip signaling increased the sensitivity to replicative stress. To evaluate Atr-Atrip complex roles in vivo, we generated transgenic mice in which Atrip was inactivated specifically in the brain and/or in the retina. Loss of Atrip in NPCs resulted in developmental defects, microcephaly and postnatal lethality. Atrip inactivation in the retina led to DNA damage accumulation and increased cell death during embryogenesis. Severe dysplasia and neurodegeneration were observed in Atrip-deficient adult retinas. Besides the characterization of the molecular mechanisms by which NPC respond to replicative stress, these findings demonstrate that Atr-Atrip signaling is essential for neural progenitor cells survival and for CNS proper histogenesis in vivo. These results contribute to a better understanding of the roles of Atr and Atrip in development and in syndromes associated with replicative stress and genomic instability.

Unexpected role of the specialized polymerase zeta in DNA replication and genome stability.

Patricia Kannouche, Institut Gustave Roussy, Villejuif, France

DNA polymerase zeta (Pol), a key factor in the translesion DNA synthesis (TLS) process, is known to play an important role in the bypass of DNA damage induced by UV light as well as by cross-linking agents. Inactivation of the gene encoding its catalytic subunit (*Rev3L*) leads to embryonic lethality in the mouse, while deletion of the homologous *REV3* gene in *S.cerevisiae* is viable suggesting that Polζ has acquired an essential function during evolution. However, little is known about the role of Pol under physiological conditions. Here we show that S phase progression is impaired in *Rev3*$^{-/-}$ MEFs. Interestingly, genome-wide profiling of replication timing revealed that inactivation of *Rev3L* induces changes in the temporal replication program,

mainly in specific genomic regions whose the replication machinery is known to propagate at slower velocity. In parallel, loss of *Rev3L* leads to a global enrichment of repressive histone modifications as well as hypermethylation of major satellites DNA repeats, suggesting that fork movements can slow down or stall in specific locations, and a delay in restarting forks could promote heterochromatin formation. Consistent with that, we revealed that HP1□ directly interacts with Rev3L and Rev7 and recruits Rev3L to pericentromeric heterochromatin. As a direct or indirect consequence, we found by transcriptomic approach that several genes involved in growth and development are down-regulated in *Rev3*$^{-/-}$ MEFs, which might explain the embryonic lethality observed in *Rev3L* KO mice. We therefore propose that Pol□ has been co-opted by evolution to assist DNA polymerase □ and □ in duplicating specific condensed chromatin domains during mid and late S phase.

SHORT TALK

Redifining replication stress response pathways in BRCA1-deficient cells.

Quinet, A[1], Tirman, S.[1], Jackson, J.[1], Gonzalez, D.[2], Mendez, J.[2], and Vindigni, A[1]

[1] Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis MO, 63104; [2] Spanish National Cancer Institute, Madrid, Spain

BRCA1-mutant cancer patients are often treated with platinum-based drugs, such as cisplatin, to create replication-blocking lesions and induce extended replication fork degradation mediated by the MRE11. However, this fork degradation phenotype was established with a single-dose treatment, neglecting the fact that patients are treated with multiple cisplatin doses in a clinical setting. We found that fork degradation is no longer detectable in *BRCA1*-deficient cells treated with cisplatin 24 hours after pre-exposure to this drug. Cisplatin pre-exposure upregulates and increases chromatin loading of PRIMPOL, a protein with primase and polymerase activity, but not of other canonical translesion synthesis polymerases, such as POLη, REV1, or REV3L in *BRCA1*-mutant cells. By combining electron microscopy and single-molecule DNA fiber approaches, we found that PRIMPOL induction rescues fork degradation by virtue of its unique *de novo* priming activity and leads to accumulation of ssDNA gaps both at replication fork junctions and behind them. ssDNA gap accumulation triggers RPA recruitment, leading to exhaustion of the RPA pool and replication catastrophe. Our findings challenge the notion that DNA-damaging drug-sensitivity originates from the extended replication fork degradation observed after a single-dose treatment. Reversed forks are the entry point for MRE11 in *BRCA1*-deficient cells and suppressing fork reversal by RAD51 depletion prevents fork degradation. In addition to suppressing fork reversal, we found that RAD51 depletion leads to ssDNA gap accumulation on ongoing replication forks, mirroring PRIMPOL induction. We propose a model where PRIMPOL upregulation rescues fork degradation by reinitiating DNA synthesis past DNA lesions, while suppressing replication fork reversal. Acknowledgments: This work is supported by NIH grant R01GM108648 and DOD BRCP Breakthrough Award BC151728.

SYMPOSIUM 3: RNA and transcription on damaged template

Gene expression misregulation in DNA repair deficient cells.

Adrian Cambindo Botto, Juan C. Muñoz, Luciana Giono, Nicolás Nieto Moreno, Alberto R. Kornblihtt & **Manuel J. Muñoz**

DNA damage caused naturally by UV light exposure in skin cells triggers not only lesion repair mechanisms but also a global gene expression response that ultimately modulates cell functions. We recently demonstrated that repair of damaged DNA by the Nucleotide Excision Repair (NER) system generates single stranded DNA intermediates that in turn activate the ATR kinase. Active ATR triggers global hyperphosphorylation of RNAPII major subunit affecting gene expression at the quantitative and qualitative (alternative splicing isoforms) levels. Moreover, using CRISPR-Cas9 technology, we found that ablation of XPE, a lesion recognition factor, partially decreased the UV effect on AS, further demonstrating the crosstalk between repair and gene expression (Muñoz, 2017). To pursue the idea of the repair process acting as a gene expression controller in a genotoxic scenario, we knocked-down different repair factors to evaluate repair and gene expression upon UV irradiation of skin cells. The NER factors can be divided in two groups, those in charge of lesion recognition (XPE/XPC) and those in charge of the actual repair (XPA/XPB/XPD/XPF/XPG). We found that impairment in lesion recognition or in the actual repair have different consequences at the gene expression level. While it is clear that all XP patients have an increased risk of developing skin cancer, some other puzzling clinical features are characteristic of the specific factor being mutated. Therefore we propose that some of the clinical features of XP patients are due to defects in gene expression modulation triggered by the DNA repair pathway.

Chemical Inhibition of APE1 and its transcriptional consequences.

Lucymara Fassarela Agnez-Lima- Universidade Federal do Rio Grande do Norte

The APE1/REF-1 plays an important role in cellular response due to its DNA repair and transcriptional regulatory activities. It is an essential enzyme to life, since its inactivation leads to embryonic lethality. Due to its functions, this enzyme has been studied as a therapeutic target, however little is still known about the regulation and coordination of its distinct functions. In this work, we evaluated the effects of chemical inhibition on APE1, using specific inhibitors of its redox and DNA repair functions. Transcriptomes of treated cells were obtained, which were analyzed by bioinformatics tools, thus revealing transcriptional consequences associated to the absence of the main APE1 activities, which will be presented during this lecture.

Modulation of RNA decay pathways improves functionality of cells with impaired telomere maintenance.

Wilson Fok[1], Alexandre Vessoni[1], Siddharth Shukla[2], Roy Parker[2,3] and **Luis Batista[1]**

[1]Department of Medicine, Washington University, St. Louis; [2]Department of Biochemistry, University of Colorado at Boulder; [3]Howard Hughes Medical Institute (HHMI)

Telomere attrition causes bone marrow failure in dyskeratosis congenita (DC). Some of the most

severe mutations in these patients are found in dyskerin (*DKC1*), a component of the telomerase complex responsible for ribonucleoprotein assembly and stability of *TERC* (telomerase RNA component). Due to a lack of adequate models of study, therapeutic alternatives for patients with DC remain obscure. To circumvent that, we used genetically engineered human embryonic stem cells (hESCs) carrying disease-associated mutations in *DKC1* (DKC1_A353V) to study *TERC* stability and decay during hematopoiesis. We show that in DKC1_A353V hematopoietic progenitors *TERC* is rapidly degraded by the exosome complex, dependent on its adenylation by PAPD5. This leads to reduced telomerase activity and accumulation of DNA damage leading to reduced hematopoietic output, modeling therefore the bone marrow failure phenotype observed in patients. In these same cells, modulation of exosome activity, by silencing of the exosome component 3 (EXOSC3) or PAPD5, significantly increases *TERC* levels. Targeted deep sequencing analysis shows that silencing PAPD5 reduces poly-adenylation at both the mature and extended 3' *TERC* reads in DKC1_A353V mutants. Additionally, modulation of PAPD5 increased telomerase activity and promoted lengthening of telomeres in DKC1_A353V hematopoietic cells, concomitantly reducing the amount of DNA damage. Importantly, functional blood differentiation assays show that modulation of RNA decay improves derivation of definitive hematopoietic progenitors in DKC1_A353V, to levels similar to wild-type. Moreover, we observe a significant reduction in primitive hematopoietic output, a characteristic of cells with dysfunctional telomeres (stress erythropoiesis). Our data establishes that modulation of RNA degradation pathways in hematopoietic cells is a viable strategy to improve blood formation in DC and could be potentially used as a clinical alternative to this devastating disease.

SHORT TALK

DNA damage levels, oxidative stress and mitochondrial alterations in patients with type 2 diabetes mellitus.

Lima, J.E.B.F.[1], Conceição, R.F.[3], Xavier, D.J.[1]; Moreira, N.C.S.[1]; Foss-Freitas, M.C.[3]; Sakamoto-Hojo, E.T.[1,2].

[1] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo – USP, Ribeirão Preto, SP, Brazil; [2] Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo – USP, Ribeirão Preto, SP, Brazil; [3] Department of Internal Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo – USP, Ribeirão Preto, SP, Brazil.

Type 2 diabetes mellitus (T2DM) is mainly characterized by hyperglycemia. The high glucose levels can lead to several alterations, especially due to increased production of reactive oxygen species (ROS), which may increase oxidative stress and DNA damage. We aimed to assess alterations in mitochondrial parameters (mass, membrane potential, ROS levels) using fluorescent dyes and flow cytometry in blood samples of T2DM patients. Besides, we investigated the effects of a dietary restriction (DR) along four weeks of hospitalization evaluated before and after the intervention; these effects were studied in terms of DNA damage levels evaluated by the comet assay (n=6), and 8-oxo-dG (8-oxo-7,8-dihydro-20-deoxyguanosine), which is a biomarker of oxidative stress, present in the nucleotide pool, analyzed by an ELISA assay (n=7). DR consisted of a protein restriction (10g per kg/day), while calories were maintained at a basal level. DR during four weeks led to improvements on metabolic status of patients (reduced glucose, cholesterol and triglycerides levels). The protein restriction led to decreased levels of DNA damage ($p<0.01$) and there was a slightly reduction ($p>0.05$) of 8-oxo-dG levels after the intervention period. Regarding the mitochondrial parameters, only alterations in membrane potential was observed ($p<0.05$) in T2DM patients. Thus, DR was effective in reducing DNA damage levels in T2DM patients, compatible with improvements in glycemic and lipid control, although a significant effect on status of oxidative stress could not be observed in the nucleotide pool. Alterations on mitochondrial dynamics were not observed in T2DM patients, requiring

additional analyses with more patients. Acknowledgments: This work is supported by CAPES, CNPq and FAPESP.

LECTURE 2

Role of chromatin and DNA damage response functions in R loop-mediated genome instability.

Andrés Aguilera. Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER, Universidad de Sevilla, Seville, 41092, Spain

Coordination of DNA replication with DNA-damage sensing, repair and cell cycle progression ensures with high probability genome integrity during cell divisions. One important type of genome instability is that associated with transcription. R loops, structures formed by a DNA-RNA hybrid and the displaced single-stranded DNA (ssDNA) molecule, are transcriptional by-products that can be formed naturally as key intermediates in specific cellular processes. Nevertheless, they are also a major source of transcription-associated genome instability and compelling evidence supports that this is mainly caused by replication fork impairment. To explore further the role that replication functions and the DNA damage response (DDR) have on R loop-mediated genome instability we have determined the impact that R loops have on replication fork progression in normal cells and in cells accumulating high levels of R loops. Our results show that this transcription- and RNA-mediated genome instability is not necessarily caused or linked to a lower fork velocity, but to a higher level of obstacles that impair RF progression at sites scattered throughout the genome. Then, after a screen searching for new genes involved in R loop-mediated genome instability among a collection of genes involved in DNA metabolism, we selected 21 genes whose depletion in HeLa cells increased R loops apart of the previously reported role of the Fanconi Anemia/BRCA factors. Our in-depth analysis of R loop-mediated instability in human cells depleted of these factors as well as the THO complex involved in RNA biogenesis reveals a new role for chromatin modifications in R loop accumulation and DDR. The implications for the role of chromatin and DDR functions in both R loop formation and transcription-associated genome instability will be discussed.

SYMPOSIUM 4: Nuclear stress and DNA repair

The Long and Short of It: Mammalian Base Repair Pathways for Oxidative DNA Lesions.

Bruce Demple, Stony Brook University, School of Medicine, Stony Brook, NY, USA

Oxidative DNA damage is generated by endogenous processes, by many environmental and cancer therapy agents, and it is implicated in age-related human diseases. The complex array of oxidative DNA lesions includes ~20% of various abasic sites and sugar fragments. Some lesions cause extra problems for DNA repair. C1-oxidized 2-deoxyribonolactone (**dL**) forms at levels similar to 8-oxoguanine and, like other abasic lesions, interferes with DNA polymerases and lacks genetic information, potentiating mutagenesis. Unlike other abasic sites, dL can derail attempted repair: during "short-patch" base excision DNA repair, DNA polymerase β (**Pol**β) replaces the missing nucleotide and uses a separate lyase activity to excise the abasic residue. Attempted excision of dL leads to a dead-end product: Polβ covalently trapped in a DNA-protein crosslink (**DPC**). Our recent work shows that Polβ-DPC are formed abundantly in mammalian cells treated with dL-forming oxidants, dependent on the Polβ lyase active site. Ubiquitylation and the proteasome act rapidly to start clearing the DPC from the genome, and we have identified the Polβ residues required for this targeting and processing. We have also shown that several other repair proteins with abasic lyase activity also form DPC in cells treated to generate dL lesions in the genome. We have also been developing methods to probe the deployment of single-nucleotide and "long-patch" base excision repair, and data resulting from this effort will be presented.

Loss of function of the Fanconi anemia A protein leads to nucleolar stress and altered ribosomal biogenesis.

Filippo Rosselli, Institut Gustave Roussy, Villejuif, France

Fanconi anaemia (FA), the most frequent and genetically heterogeneous inherited bone marrow failure syndrome (iBMF), is associated with developmental defects, cancer predisposition and chromosomal fragility. In contrast to other major iBMFs (dyskeratosis congenita, Shwachman-Diamond syndrome, Diamond-Blackfan anaemia), in which BMF is due to hereditary defects in ribosome biogenesis (RiBi)3, FA is generally attributed to genetic instability and loss of activity of the 21 FA-associated (FANC) proteins, followed by activation of growth inhibitory and anti-proliferative pathways. The canonical function of the FANC proteins is to cope with damaged DNA and stalled replication forks. However, the presence and consequences of nucleolar and/or RiBi alterations have not been explored in FA. Here, we demonstrated that FANCA loss-of-function, which is responsible for more than 65% of FA cases, leads to a DNA damage signalling-independent nucleolar stress. These changes were associated with nucleolar protein mislocalisation, reduced rDNA transcription and rRNA processing, increased rDNA rearrangements, and alterations in RiBi and translational rate as well as p53- and DDR-independent p21 activation. Importantly, nucleolar and RiBi abnormalities were FANCA depletion-specific traits and were absent or scantly observed in cells depleted of FANCC, FANCD2, or FANCD1/BRCA2. Thus, we identified novel nucleolar and translational consequences associated with FANCA loss-of-function, challenging the current hypothesis on the physiopathology of FA and supporting a FANC pathway-independent role of FANCA.

The recombinase Rad51 plays a key role in events of genetic exchange in *Trypanosoma cruzi.*

Carlos Renato Machado - Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Braz

Detection of genetic exchange has been a limiting factor to deepen the knowledge on the mechanisms by which Trypanosoma cruzi is able to generate progeny and genetic diversity. We show that incorporation of halogenated thymidine analogues, followed by immunostaining, is a reliable method not only to detect T. cruzi fused-cell hybrids, but also to quantify their percentage in populations of this parasite. Through this approach, we were able to detect and quantify fused-cell hybrids of T. cruzi clones CL Brener and Y. Given the increased detection of fused-cell hybrids in naturally-occurring hybrid CL Brener strain, which displays increased levels of RAD51 and BRCA2 transcripts, we further investigated the role of Rad51 – a recombinase involved in homologous recombination – in the process of genetic exchange. We also verified that the detection of fused-cell hybrids in T. cruzi overexpressing RAD51 is increased when compared to wild-type cells, suggesting a key role for Rad51 either in the formation or in the stabilization of fused-cell hybrids in this organism.

The histone chaperone HJURP facilitates DSB repair and promotes radioresistance to astrocytoma cells.

Rodolfo B. Serafim, Cibele Cardoso, Luis F. M. Di Cristofaro, Enilza M. Espreafico, Maria L. Paçó-Larson, Brendan D. Price and **Valeria Valente.**

The Holliday Junction-Recognition Protein (HJURP) was reported as overexpressed in several cancers, including: lung, breast, brain, liver and ovary tumors. HJURP expression was also strongly correlated with poor prognosis of patients, especially in glioblastoma (GBM), the most common and deadly type of primary brain tumor. HJURP is responsible for loading the histone H3 variant - Centromeric Protein A (CENPA) - at the centromeres, being required for proper chromosome segregation. This activity is well established and quite characterized, regarding mechanisms, protein domains involved and the regulation during cell cycle. Additionally, this protein was preliminarily associated with DNA repair, but further evidences supporting this function are not yet available. In this scenario, we further investigated the involvement of HJURP with DNA repair in GBM cells. Our results revealed the recruitment of HJURP/CENPA to sites of double-strand breaks (DSBs) immediately after damage induction by irradiation. We also demonstrated that recruitment is dependent on PARP and is required for proper ATM activation and H2AX phosphorylation, an important response in DNA damage signaling. Furthermore, HJURP knockdown impaired the anchoring of CtIP and RAD51 at DNA lesions and lessened repair activity by the homologous recombination pathway. These defects promoted an overall reduction in DSB repair competence, once larger amounts of broken DNA persisted for extended periods in silenced cells. Finally, we observed that HJURP overexpression conferred proliferative competence and resistance to ionizing radiation for GBM cells. Altogether, these results show that HJURP/CENPA favors DNA repair, proliferative capacity and radioresistance for tumor cells, being directly correlated with GBM aggressiveness.

SHORT TALK

Replication Protein A from trypanosomatids: A new perspective for a well-known complex.

Pavani, R.S.[1], Zoltner, M.[2], Fragoso, S.P.[3], Field, M.C.[2], Elias M.C.[1].

[1] Cell Cycle Special Lab, Butantan Institute, São Paulo, SP, Brazil; [2] Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom; [3] Carlos Chagas Institute, Fiocruz-PR, Brazil

Trypanosoma cruzi and *Trypanosoma brucei* are parasitic protozoa responsible for causing Chagas disease and sleeping sickness that result in a high number of deaths annually. These parasites possess a complex life cycle that alternates between replicative and non-replicative lifeforms. Despite the characterization of a great number of molecular pathways, there are still many gaps in the understanding of the processes that coordinate the DNA metabolism of these organisms. Replication protein A (RPA), the major eukaryotic single-stranded binding protein, is a heterotrimeric complex formed by three subunits RPA-1, RPA2 and RPA-3 that participates in various vital functions during replication, repair and checkpoint signaling. RPA from trypanosomatids present significant structural peculiarities compared to other eukaryotes such as the lacking of DBDF-domain that interacts with proteins majorly involved in DNA damage response (DDR) pathways and aminoacids substitutions in conserved regions, raising questions regarding the conservation of canonical functions described in mammals and yeast. In this work, we show that RPA from trypanosomatids can interact with single-stranded DNA and is indeed involved in replication and DNA damage response pathways. Moreover, we could find new features concerning trypanosomatids RPA such as the discovery of (i) non-described post-translation modifications (ii) a new RPA-like protein that seems to be exclusive of trypanosomatids interacting with RPA complex and (iii) a nucleus-cytoplasm shuttle that is lifecycle dependant. Acknowledgments: This work is supported by FAPESP grants 2013/07467-1, 2014/02978-0 and 2016/24255-6.

SYMPOSIUM 5: Making and Mending Breaks on DNA

RAD51 generates genetic instability through non-homologous recombination.

[1]Josée Guirouilh-Barbat, [1]Ayeong So, 1Camille Gelot, [1]Elodie Dardillac [2]Wei Yu, [2]Chloé Lescale, [2]Ludovic Deriano and **[1]Bernard S. Lopez**

[1]CNRS UMR 8200, Institut de Cancérologie Gustave Roussy, Université Paris Sud, Equipe labélisée “LIGUE 2014”, Villejuif, France
[2] Institut Pasteur, Paris, France

DNA double-strand breaks (DSB) are very harmful lesions that can generate genome rearrangements. We used intrachromosomal reporters to compare both the efficiency and accuracy of end-joining occurring with close (34 bp apart) *vs.* distant DSBs (3200 bp apart) in human fibroblasts. We showed that a few kb between two intrachromosomal I-SceI-induced DSBs are sufficient strongly affect repair efficiency and to foster deletions and capture/insertions at the junction scar. Captured sequences are mostly coupled to deletions and can be partial duplications of the reporter (i.e., sequences adjacent to the DSB) or insertions of ectopic chromosomal sequences (ECS). Interestingly, silencing 53BP1 stimulates capture/insertions with distant but not with close double-strand ends (DSEs), although deletions were stimulated in both case. This shows that 53BP1 protects both close and distant DSEs from degradation and that the association of unprotection with distance between DSEs favors ECS capture. Reciprocally, silencing CtIP lessens ECS capture both in control and 53BP1-depleted cells. We propose that close ends are immediately/rapidly tethered and ligated, whereas distant ends first require synapsis of the distant DSEs prior to ligation. This "spatio-temporal" gap gives time and space for CtIP to initiate DNA resection, suggesting an involvement of single-stranded DNA tails for ECS capture. We therefore speculate that the resulting single-stranded DNA copies ECS through microhomology-mediated template switching. Because arrest of replication forks generate single-ended DSB, end-joining of such DSBs, which involve distant DSEs, should lead to genetic instability. Therefore we addressed the question as whether any mechanisms can protects against the joining of distant DSBs, specifically in the S phase. We show that depletion of cohesion complex proteins specifically stimulates the end-joining (both C-NHEJ and A-EJ) of distant, but not close I-SceI-induced DSEs, in S/G2 phase. At the genome level, whole-exome sequencing showed that ablation of RAD21 or Sororin produces large chromosomal rearrangements (Translocation, duplication, deletion). Moreover, cytogenetic analysis showed that RAD21 silencing leads to the formation of chromosome fusions synergistically with replication stress, which generates distant single-ended DSEs. These data reveal a role for the cohesion complex in protecting against genome rearrangements arising from the ligation of distant DSEs in S/G2 phase (both long-range DSEs and those that are only a few kb apart), while keeping end-joining fully active for close DSEs. Therefore, this role likely involves limitation of DSE motility specifically in S phase, rather than inhibition of the end-joining machinery itself. Finally, we will discuss an unexpected and provocative role for RAD51 and BRCA2 in generating ECS and genome rearrangements through non-homologous recombination

Quantitative Analysis of DNA Damage Signaling Responses.

Francisco M. Bastos de Oliveira, UFRJ, Brazil

Phosphorylation-mediated signaling is essential for maintenance of the eukaryotic genome. The evolutionarily conserved kinases ATR and ATM sense specific DNA structures generated upon DNA damage or replication stress and mediate an extensive signaling network that impinges upon most nuclear processes. ATR/ATM signaling is highly regulated and can function in a context dependent manner. Thus, the ability to quantitatively monitor most, if not all, signaling events in

this network is essential to investigate the mechanisms by which kinases maintain genome integrity. We developed a method for the Quantitative Mass-Spectrometry Analysis of Phospho-Substrates (QMAPS) to monitor *in vivo* DNA damage signaling in a systematic, unbiased, and quantitative manner. Using the model organism *Saccharomyces cerevisiae,* we provide an example for how QMAPS can be applied to define the effect of genotoxins, illustrating the importance of quantitatively monitoring multiple kinase substrates to comprehensively understanding kinase action.

DNA single-strand breaks, genome stability, and human disease.

Keith W Caldecott, Genome Damage and Stability Centre, University of Sussex, UK

Chromosomal DNA single-strand breaks (SSBs) are amongst the most frequent DNA lesions arising in cells and if not repaired correctly can threaten both genetic stability and cell survival. Moreover, SSB repair defects are associated with hereditary neurodegeneration in humans. This is illustrated by cerebellar ataxias that reminiscent of ataxia telangiectasia and in which enzymatic components of DNA end processing are mutated such as *ataxia oculomotor apraxia-1* (AOA1; mutated in *APTX*), *spinocerebellar ataxia with axonal neuropathy-1* (SCAN1; mutated in *TDP1*), and *ataxia oculomotor apraxia-4* (AOA4; mutated in *PNKP*). More recently, we have identified a human patient with bi-allelic mutations in XRCC1; the scaffold protein that interacts with the enzymes encoded by these genes and which promotes their stability and/or function. Collectively, these observations establish the importance of XRCC1 protein complexes for normal neurological function and identify a possible therapeutic approach for treating DNA strand break repair-defective neurodegenerative diseases. Here, I will describe recent progress in our work aimed at understanding how SSBs are sensed, signalled, and repaired in human cells, and how these processes help maintain genome stability and human health.

XRCC1 Mutation is Associated with PARP1 Hyperactivation and Cerebellar Ataxia

Nicolas C. Hoch[1,2], Hana Hanzlikova[1], Stuart L. Rulten[1], Emilia Komulainen[1], Limei Ju[1], Grace Yoon[3] & Keith W. Caldecott[1]

1 Genome Damage and Stability Centre, University of Sussex, Brighton, UK
2 Department of Biochemistry, University of São Paulo, São Paulo, Brazil
3 The Hospital for Sick Children, University of Toronto, Toronto, Canada

Mutations in several DNA repair genes are associated with rare hereditary diseases characterized by neurodevelopmental and/or neurodegenerative phenotypes. However, the underlying molecular mechanisms are largely unknown, precluding the identification of potential therapeutic strategies. Here we describe a novel disorder caused by biallelic mutations in the human XRCC1 gene and implicate elevated levels of protein ADP-ribosylation in the associated neuropathology. DNA single-strand breaks (SSBs) are arguably the most abundant form of DNA damage in cells and their repair is initiated by poly-ADP-ribose polymerases (PARPs), which modify proteins surrounding the break site with poly-ADP-ribose (PAR) chains. This leads to the recruitment of XRCC1, which binds, stabilizes and stimulates the DNA end-processing enzymes that process and subsequently ligate damaged DNA termini. Single-strand break repair plays critical roles in the brain, particularly the cerebellum, as mutations in many of these factors result in rare hereditary diseases characterized by cerebellar atrophy, ataxia and oculomotor apraxia. We describe the first XRCC1-deficient patient with these clinical features and show that patient-derived cells exhibit not only reduced rates of single-strand break repair but also increased levels of DNA damage-induced protein ADP-ribosylation. Interestingly, aberrant ADP-ribose levels

were also observed in cells from a patient with a related syndrome caused by mutations in the XRCC1 partner protein PNKP and in the cerebellum of untreated Xrcc1-deficient mice. Remarkably, genetic deletion of PARP1 rescued normal ADP-ribose levels both in human cells and mouse cerebellum, suggesting that delayed repair of SSBs leads to overt PARP1 signalling of these lesions. Remarkably, PARP1 loss also reduced the loss of cerebellar neurons and ataxia in these animals, identifying a molecular mechanism by which endogenous single-strand breaks trigger neuropathology. Collectively, these data establish the importance of XRCC1 protein complexes for normal neurological function and identify PARP1 as a therapeutic target in DNA strand break repair-defective disease.

SHORT TALK

Role of the DNA polymerase kappa in the regulation of the kinase CHK1.

Dall'Osto, M. [1 2], Pierini, L.[2], Hoffmann J.S.[1], Pillaire M.J.[1],
[1]Cancer Research Center of Toulouse, INSERM Unit 1037; University Toulouse 3-CNRS ERL 5294; 2 avenue Hubert Curien, CS 3717, 31007 Toulouse
[2]*Equally contributing authors*

The replication of the genome is constantly challenged due to endogenous or exogenous fork barriers leading to fork stalling which is a cause of replicative stress (RS) [1]. When stalled forks fail to restart, it can give rise to DNA breaks or chromosomal rearrangements. To prevent this genetic instability, the fork blockage is signalled by a set of proteins during the S phase trough the ATR/Chk1 pathway. We now provide data showing that in addition to its role at the stalled forks [2], Pol Kappa is also involved to maintain the pool of Chk1. We observe that the depletion of Pol Kappa induces a Chk1 protein level decrease in mice and mammalian cells. Since the pool of Chk1 in the nucleus supports the genetic stability, we focused on the impact of Pol Kappa on the Chk1 protein level in the nuclear compartment. We proved that this regulation is specific to Pol Kappa among the Y-family of specialized DNA polymerases and does not depend of exogenous RS since this regulation also happens in unperturbed cells. By different approaches, we show that Pol Kappa belong to the same complex. Here, we provide data carried out by DNA spreading and showing defects in the forks restart after RS when Pol Kappa or Chk1 are depleted by RNA interference. The expression of ectopic Chk1 can rescued the fork restart in both cases arguing that Pol kappa and Chk1 work in the same pathway. Taken together, these findings lead us to propose a model in which Pol Kappa maintains a reservoir of Chk1 in the nucleus making cells ready to answer to stress induced by the replication forks barriers.

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SYMPOSIUM 6: Mechanisms for maintaining genome integrity

Damage sensor role of UV-DDB during base excision repair: stimulation of APE1 and OGG1.

Sunbok Jang[1], Muwen Kong[1,2], Samuel H. Wilson[3], **Bennett Van Houten[1,2]**

[1]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Hillman Cancer Center, Pittsburgh, PA 15213, USA; [2] Molecular Biophysics and Structural Biology Program, University of Pittsburgh, Pittsburgh, PA 15260 USA; [3]Genomic Integrity & Structural Biology Laboratory, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, USA.

A common oxidative base lesion, 8-oxoguanine (8-oxoG), is repaired through base excision repair (BER). During this process, the damaged site is recognized by 8-oxoguanine DNA glycosylase (OGG1), which removes the oxidized base, followed by a slow lyase activity that generates a 3' nick at the remaining sugar moiety. This resulting repair intermediate is then recognized by AP-endonuclease 1 (APE1), which cleaves on the 5' side of the sugar moiety, creating a 3'OH and a one base gap that is filled by DNA polymerase β and sealed by DNA ligase I. Both OGG1 and APE1 have also been shown to have difficulty processing lesions within nucleosomal DNA in chromatin. We sought to understand if additional repair factors may help overcome this barrier to repair. To this end, we have found that UV-DDB, commonly known to participate in the removal of UV-induced photoproducts during nucleotide excision repair, exhibits tight binding affinity to abasic sites (Kd = 4 nM), the substrate of APE1. Surprisingly, we discovered that UV-DDB stimulates APE1 strand cleavage of a 37-bp duplex containing one abasic site by 8-fold. Furthermore, we demonstrated that UV-DDB also stimulates OGG1 removal of 8-oxoG and lyase activity by 3-fold. Single molecule analysis using our DNA tightrope imaging platform indicate that UV-DDB can form complexes on DNA with APE1 or OGG1. UV-DDB in the presence of magnesium slides on DNA with ~40% of the molecules showing motility. Both APE1 and OGG1 decrease the motile fraction of UV-DDB. UV-DDB helps to dissociate OGG1 from DNA. These data suggest that UV-DDB may be a general sensor of DNA damage and, through association with an E3-ligase complex consisting of Culin4A and Rbx, could work to relax chromatin around certain types of base damage to facilitate efficient BER in chromatin. This work is supported by NIH 5R01ES019566-07 to BVH.

Mfn1 is essential to oocyte fertility in mice whereas Mfn2 is required in oocytes to filter out mutant mitochondrial DNA.

Marcos R. Chiaratti, Federal University of São Carlos, São Carlos, SP, Brazil

Mitochondria play a fundamental role during oocyte development, distinguishing from other cells by being fragmented, small and round. These characteristics rely on mitochondrial dynamics, which determine organelle activity, transport and degradation. We have been investigating the role of mitochondrial dynamics in heteroplasmic mouse oocytes through *Zp3-Cre* conditional knockout of *Mfn1* and/or *Mfn2*. Females lacking *Mfn1* in oocytes are infertile due to arrest of oocyte growth and folliculogenesis, which associate with downregulation of the Pi3k-Akt signaling pathway. As a result, mitochondrial DNA (mtDNA) content does not increase in *Mfn1*-null oocytes, remaining as low as in non-grown oocytes. Yet, the mutant NZB mtDNA is selectively filtered out in these oocytes similarly to fully-grown wild-type (WT) oocytes, suggesting this filter does not rely on mtDNA replication. Regarding *Mfn2*, its ablation impacts slightly on oocyte growth and mtDNA accumulation, enabling fertilization and development to term. However, these oocytes fail to filter out NZB mtDNA, suggesting *Mfn2* is needed to eliminate mutant mtDNA during oogenesis. As a consequence, mice born to *Mfn2*-null oocytes

inherit more NZB mtDNA than those born to WT oocytes. Finally, double knockout of *Mfn1* and *Mfn2* enables oocyte growth, but impairs fertility as ovulated oocytes are not capable of resuming the meiotic division due to dysfunction of mitochondria and the endoplasmic reticulum. Also, NZB mtDNA accumulate in these oocytes, confirming the finding that *Mfn2* is necessary to filter out mutant mtDNA during oogenesis. In conclusion, single ablation of *Mfn1* prevents oogenesis whereas ablation of *Mfn2* interferes with inheritance of mutant mtDNA.

Twinkle and the maintenance of mitochondrial DNA.

Marcos T. Oliveira, Universidade Estadual Paulista, Jaboticabal, SP, Brazil

Defects in the mechanisms of mitochondrial DNA (mtDNA) maintenance are correlated with mitochondrial dysfunctions that are recognized as a common cellular basis for countless human diseases, including classical mitochondrial encephalomyopathies, diabetes, deafness, neurodegenerative diseases, and cancer. The mtDNA helicase Twinkle has a well-recognized role in mtDNA replication, but recent data indicate it can also participate in recombinational repair inside mitochondria. Overexpressing Twinkle in the animal model *Drosophila melanogaster* increases mtDNA copy number up to 40%, without affecting any phenotypic parameter analyzed to date. This is accompanied by an increase in the abundance of mtDNA replication and recombination intermediate molecules, and a decrease in the typical replication pausing at the binding sites for the transcription termination factor DmTTF, as judged by two-dimensional neutral agarose gel electrophoresis. In addition, Twinkle overexpression leads to deletions of repeated elements in the major non-coding region of the mitochondrial genome, where replication initiation and/or termination take place. The number of these repeated elements has been associated with fluctuating levels of mtDNA copy number in wild-type strains of *D. melanogaster*. How Twinkle acts in the interplay between mtDNA replication and recombination, and how its overexpression accounts for the findings described above are still under investigation. Our goal is to describe how the modulation of Twinkle levels in mitochondria may impact positively the maintenance of the mitochondrial genome, both in healthy and disease states.

Cross-talk between phospholipid metabolism and DNA Damage Response in *Saccharomyces cerevisiae.*

José R Cussiol[1,2] , Caio Chede[2], Nadja C Souza-Pinto[2]

[1] Departmento de Bioquímica, Universidade Federal de São Paulo, Brazil
[2] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil

During the DNA Damage Response (DDR), the budding yeast checkpoint kinase Mec1 protects genome integrity through the initiation of the DNA damage checkpoint signaling. Mec1 transduces the signal to the kinase effector Rad53, which elicits a canonical checkpoint response. Failure to establish a proper response to genotoxic insult can lead to genomic instability, which is a hallmark of cancer. Whereas the regulatory role of Mec1 in cellular processes, such as DNA repair, replication and cell cycle, is well accepted, much less is known about its regulation of metabolic processes. We have previously found that Mec1 phosphorylates Opi1, a transcriptional repressor of genes involved in phosphatidylinositol biosynthesis, and that Rad53 hyperactivation leads to a significant decrease in Opi1 phosphorylation by Mec1. Therefore, we sought to investigate the contribution of Opi1 for the DNA damage response. Here, we show that yeast cells lacking Opi1 are sensitive to genotoxic stress induced by methyl methanesulfonate (MMS). Moreover, MMS sensitivity of *opi1Δ* cells can be rescue by growing cells in the absence of inositol, a precursor of several phospholipids, suggesting that inositol overproduction affects the DDR. Moreover, Rad53 hyperactivation impaired cell growth in the absence of inositol, which

was rescued by *OPI1* deletion. Therefore, we propose that Opi1 is a key regulator of phospholipid metabolism during the DDR. However, further investigation is still required to identify whether Opi1 phosphorylation by Mec1 affects its role in phospholipid biosynthesis regulation.

SHORT TALK

Decreased PGC-1 expression correlates with ΔTG mutation in *XPC* gene but not with decreased expression of PGC-1A target genes.

Mori, M. P.[1], de Souza-Pinto, N. C.[1]

[1] Department of Biochemistry, Instituto de Química, Universidade de São Paulo, São Paulo.

Xeroderma pigmentosum (XP) is an inherited autosomal recessive syndrome with a 1,000-fold elevated skin cancer rate in areas exposed to UV light. XP is caused by mutations in one of seven genes (*XPA-XPG*) of the nucleotide excision repair pathway or in one translesion DNA polymerase (*POLH*). We have recently demonstrated that XP-C cells show increased mitochondrial H_2O_2 production with an adaptive shift between respiratory complexes I and II, leading to increased sensibility towards mitochondrial stress. A very significant decrease in expression of the transcriptional coactivator PGC-1α, which has a major role in controlling mitochondrial biogenesis, was found in four different XP-C cell lines carrying the c.1643_1644delTG mutation (ΔTG) in *XPC*. The same reduction of PGC-1α expression was not observed in an XP-C cell line carrying a different mutation, regardless of nearly absent XPC protein levels, in agreement with results showing that siRNA-mediated XPC knockdown in normal fibroblasts did not alter PGC-1α expression. Furthermore, cell lines from the heterozygous parents of one patient carrying ΔTG mutation also show decreased PGC-1α expression. This is in line with the observation that some mutations can generate new non-coding RNAs, with a gain-of-function new molecular phenotype. However, PGC-1α expression was also low in cells carrying a third *XPC* mutation and in another unrelated normal cell line. Interestingly, in all cell lines investigated here expression levels of the PGC-1α target genes TFAM and SDHA did not correlate with PGC-1α levels. As expression of PGC-1 α is almost undetectable in fibroblasts, it is possible that PPRC1, the third member of PGC family, levels are more relevant to mitochondrial biogenesis in this cell type. Acknowledgments: This work is supported by FAPESP grants 2016/15407-7 and 2017/04372-0.

LECTURE 3

Genome maintenance protects from aging and cancer: the impact of nutrition.

Jan H.J. Hoeijmakers, Dept. of Molecular Genetics, Erasmus MC, Rotterdam, The Netherlands

Ageing in various organisms appears remarkably plastic: e.g. suppressing insulin signaling extends lifespan. However, virtually all premature aging syndromes in man link with genome instability. We have generated mouse models which strikingly mimic human DNA repair deficiency syndromes and display cancer predisposition and/or wide-spread accelerated aging. E.g. *Ercc1*$^{\Delta/-}$ mice defective in four repair pathways show numerous accelerated aging features limiting lifespan to 4-6 month. Simultaneously they exhibit an anti-aging, anti-cancer 'survival response', which suppresses growth/IGF1 and enhances maintenance, resembling the longevity response induced by dietary restriction (DR). Interestingly, subjecting these progeroid mutants to 30% DR tripled lifespan, and drastically retarded accelerated aging, e.g. DR animals retained 50% more neurons and maintained full motoric function. The same findings in repair-deficient *Xpg*$^{-/-}$ mice extended this observation beyond *Ercc1*$^{\Delta/-}$. Interestingly, *ad libitum Ercc1*$^{\Delta/-}$ liver expression profiles showed gradual preferential extinction of expression of long genes, consistent with genome-wide accumulation of stochastic, transcription-blocking lesions, which affect long genes more than short ones. DR largely prevented this transcriptional decline, indicating that DR prolongs genome function. We present conditional DNA repair mutants accelerating aging in selected organs, and striking parallels with Alzheimer's disease. Our findings strengthen the link between DNA damage and aging, establish *Ercc1*$^{\Delta/-}$ mice as powerful model for identifying interventions to promote healthy aging, reveal untapped potential for reducing endogenous damage and hence also cancer, provide new venues for understanding the molecular mechanism of DR, and suggest a counterintuitive DR-like therapy for human progeroid genome instability syndromes and DR-like interventions for preventing neurodegenerative diseases.

SYMPOSIUM 7: Carcinogenesis and stem cells

The Repertoire of Mutational Signatures in Human Cancer

Ludmil B. Alexandrov, University of California, San Diego, CA, USA

Cancer is the most common human genetic disease. All cancers are caused by somatic mutations. These mutations may be the consequence of the intrinsic slight infidelity of the DNA replication machinery, exogenous or endogenous mutagen exposures, enzymatic modification of DNA, or defective DNA repair. In some cancer types, a substantial proportion of somatic mutations are known to be generated by exogenous carcinogens, for example, tobacco smoking in lung cancers and ultraviolet light in skin cancers, or by abnormalities of DNA maintenance, for example, defective DNA mismatch repair in some colorectal cancers. Each biological process causing mutations leaves a characteristic imprint on the genome of a cancer cell, termed, mutational signature. In this talk, I will present the largest analysis of mutational signatures in human cancer. Using 84,514,689 somatic mutations from 4,684 whole cancer genome and 18,864 exome sequences encompassing most cancer types, we characterized 49 single base substitution, 11 doublet base substitution, four clustered base substitution, and 17 small insertion and deletion mutational signatures. Some signatures are present in many cancer types, notably mutational signatures attributed to clock-like mutational processes, whereas others are confined to a single cancer class. Certain signatures are associated with age of the patient at cancer diagnosis, known mutagenic exposures or defects in DNA maintenance, but many are of cryptic origin. The results reveal the diversity of mutational processes underlying the development of cancer, with potential implications for understanding of cancer etiology, prevention, and therapy.

Blocking LINE-1 reverse transcriptase activity in TREX1-deficient cells rescues neurotoxicity in Aicardi-Goutières syndrome.

Charles A. Thomas[1], Leon Tejwani[1], Cleber A. Trujillo[1], Priscilla D. Negraes[1], Roberto H. Herai[2], Pinar Mesci[1], Angela Macia[1], Yanick J. Crow[3], and **Alysson R. Muotri[1]***

1- Department of Pediatrics/Rady Children's Hospital San Diego and Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA, USA.
2- School of Medicine, Graduate Program in Health Sciences, Pontificia Universidade Catolica do Parana, Curitiba, Parana, Brazil.
3- INSERM UMR 1163, Laboratory of Neurogenetics and Neuroinflammation, Paris Descartes-Sorbonne Paris Cite University, Institut Imagine, Hopital Necker, Paris, France.
*Corresponding author

LINE-1 (or L1) retrotransposons are repetitive sequences that are spread through the human genome with the ability to generate copies of themselves. Recent studies have demonstrated that L1 elements are able to mobilize and generate high number of copies in neurological and neurodevelopmental diseases, giving insights that L1 activity can be detrimental when de-regulated. In order to define the impact of L1 activity, we generated a human model of Aicardi-Goutières syndrome (AGS) in a dish. AGS is a developmental disease characterized by neuroinflammation and accumulation of DNA species derived form endogenous retroelements, with onset in early infancy. AGS arise when TREX1 (Three-prime repair exonuclease 1), an anti-viral enzyme that cleaves nucleic acids in the cytosol, is mutated. Thus, we developed a human stem cell cortical organoid model using iPSCs derived from patients with AGS. Interestingly, cortical organoids exhibited a reduced size compared to controls, neurons become importantly affected and suffer apoptosis, and astrocytes produce an increase secretion of type I interferon (IFN). Importantly, we observed abundant extrachromosomal DNA in TREX1-deficient cells, of which active L1 elements were a major source. In order to control L1 activity in these cells, we

chronically treated AGS cells with reverse-transcriptase inhibitors (RTis) that prevent the generation of new L1 copies in the genome. We observed a rescue en the neurotoxicity of AGS neurons and cortical organoids as well as reduced expression of type I IFN to near-control levels. These results suggest that L1 reverse-transcriptase activity induces TREX1-deficient astrocytes to produce a more toxic environment for neurons via the secretion of factors, highlighting their potential utility in therapeutic regimens for AGS and related disorders.

Targeted Apoptosis of Senescent Cells against Aging and Cancer.

Peter L.J. de Keizer- Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, the Netherlands

Aging is the main risk factor for the development of a majority of diseases. As we age, we accumulate cellular damage, which can eventually result in cells becoming “senescent”. Senescent cells cease to divide, but chronically secrete a wide range of factors that permanently alter their environment. As such, they are thought to impair tissue function and promote cancer progression, migration and therapy resistance by permanently enforcing a state of stemness[1]. I will discuss how we identified the damage-associated protein FOXO4 as a pivot in senescent cell viability and how we designed a D-Retro-Inversed FOXO4 peptide, FOXO4-DRI, to selectively eliminate senescent cells[2]. In vivo, this approach was applicable to restore fitness, fur density and renal function of fast and naturally aged mice, arguing that it is, at least to an extent, possible to restore healthspan once it has already declined. It will be crucial to dissect senescence heterogeneity and identify molecular mechanisms that dictate sensitivity or resistance. Current research is focused on these mechanisms and further optimization of FOXO4-DRI for eventual clinical translation.

1. de Keizer, P.L., *The Fountain of Youth by Targeting Senescent Cells?* Trends Mol Med, 2017. 23(1): p. 6-17.
2. Baar, M.P., et al., *Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging.* Cell, 2017. 169(1): p. 132-147 e16.

DUOX1 Silencing in Mammary Cells Alters the Responses to Genotoxic Stress.

Rodrigo Soares Fortunato, Universidade Federal do Rio de Janeiro, RJ, Brazil

DUOX1 is an H_2O_2-generating enzyme related to a wide range of biological features, such as hormone synthesis, host defense, cellular proliferation, and fertilization, among others. DUOX1 is frequently down regulated in lung and liver cancers, suggesting a tumor suppressor role for this enzyme. Here we show that DUOX1 expression is decreased in breast cancer cell lines and also in breast cancers when compared to the non-tumor counterpart. In order to address the role of DUOX1 in breast cells, we stably knocked down the expression of DUOX1 in non-tumor mammary cells (MCF12A) with shRNA. This led to higher cell proliferation rate, decreased migration and adhesion properties, which are typical features for transformed cells. After genotoxic stress induced by doxorubicin, DUOX1-silenced cells showed reduced IL-6 and IL-8 secretion, and increased apoptosis levels. Furthermore, cell proliferation rate was higher in DUOX1-silenced cells after doxorubicin in comparison to control cells. In conclusion, we demonstrate here that DUOX1 is silenced in breast cancer, which seems to be involved in breast carcinogenesis.

SHORT TALK

DNA damage response in Human Embryonic stem cells with short telomeres.

Vessoni, A.T.[1], Batista, L.F.Z.[1]
[1] School of Medicine, Washington University in St. Louis, St. Louis, MO, USA.

Telomeres are repetitive DNA sequences (TTAGGG) located at the end of our chromosomes. Their main functions are to buffer against loss of important genetic information (end-replication problem) and to allow assemble of *shelterin*, a multi-protein complex that protects the chromosomes ends from been recognized as DNA breaks. Telomeres gradually become shorter with aging, and upon reaching a dysfunctional length they induce DNA damage response (DDR). In human fibroblasts, telomere dysfunction induces a permanent G1/S arrest (senescence) via the P53 and the Rb1 pathways. Telomere shortening can be prevented by telomerase, a ribonucleoprotein complex that uses an RNA template to elongate telomeres. Its expression is restricted to a few cell types, such as stem and progenitor cells, and is critical to preserve adult stem cells function and tissue homeostasis. Still, telomerase expression is not enough to prevent aging-induced telomere shortening in several adult stem cells, and how stem cells respond to telomere shortening is not yet fully understood. A better comprehension of this phenomenon may provide new insights into how aging is associated to loss of regenerative potential and tissue dysfunction. In this work, we developed a new model to study the impact of telomere shortening on human stem cells. Using state-of-the-art genome engineering technology, we developed isogenic human embryonic stem cells (hESC) lines *knocked out* for *TP53*, *RB1*, or both, and in which telomerase can be turned ON/OFF at will. We assess the effect of progressive telomere shortening on DDR, cell fate, gene expression and metabolism of these cells. Financial Support: Philip W. Majerus M.D. Fellowship, American Federation for Aging Research (AFAR), The Longer Life Foundation (LLF).

POSTER SESSION ABSTRACTS

P01 - CHARACTERIZATION OF DMC1 GENE IN *TRYPANOSOMA CRUZI*

Silva V.S.[1], Oliveira C.F.B.[2], Felicori L.F.[2], Tahara E.B.[2], Macedo A.M.[2], Franco G.R.[2], Pena S.D.J.[2], Machado C.R.[2]

[1,2] Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, UFMG, Brazil

e-mail: veronicasantana_444@hotmail.com

It is known that the Trypanosoma cruzi, the etiologic agent of Chagas disease (CD), reproduce predominantly by clonal way (asexual) however researches show that *T.cruzi* can present rare events of genetic exchange by cellular fusion and formation of hybrids. Others trypanosomatids like *Trypanosoma brucei* can be able to realize genetics exchange by meiosis and formation of haploids gametes. These parasites present specific genes of meiosis in their genome and T. cruzi also presents one of these genes (DMC1). We are investigating if *T. cruzi* also can be able to perform meiosis. Thus, we intend to study DMC1, a specific gene of meiosis, and characterize its role in *T. cruzi.* A TcDMC1 polyclonal antibody was produced. Then we analyzed the expression of this protein in different strains of this parasite (CL Brener - a hybrid cell, and Dm28c – a non-hybrid cell). The preliminaries results showed that CL Brener parasites expresses DMC1 protein already in epimastigote form. On the other hand, we cannot see clearly a DMC1 expression in Dm28c cells. Curiously, in the imunofluorescence assays we can observe that some cells present a colocalization of DMC1 protein in the kinetoplast. These preliminary results suggest that the protein DMC1 is expressed in proliferative epimastigotes form of *T. cruzi*, especially in hybrid cells. Currently, we are investigating the role of DMC1 in the generation of hybrid cells.

Keywords: Trypanosoma cruzi, DMC1, meiosis

Acknowledgments: FAPEMIG, CNPq, CAPES

<u>P02</u> -EVALUATION OF GALECTIN-3 / PARP1 INTERACTION IN THE CELLULAR RESPONSE TO DNA DAMAGE

Silva, T.S.L. [1,3], Pereira, T.M.[1], Carvalho, R.S.[1,2]

[1] Laboratory of Biotechnology Research, Faculty of Pharmacy, UFRJ, Rio de Janeiro - RJ, Brazil; [2] Faculty of Pharmacy, Health Sciences Center, UFRJ, Rio de Janeiro - RJ, Brazil; [3] Pharmaceutical Science Pos Graduate Program, Health Sciences Center, UFRJ, Rio de Janeiro - RJ, Brazil.

e-mail: ttaianalopes@gmail.com

Galectin is a protein family that bind β-galactosides. Among them, galectin-3 stands out as the only chimeric member. In tumor cells, galectin-3 levels are increased, promoting the proliferation and survival of these cells. Our group identified poly ADP-ribose polymerase 1 (PARP1) as a novel protein-interacting partner of galectin-3. PARP1 promotes the addition of ADP-ribose polymers to proteins involved in DNA damage repair processes. PARP1 is compromised in repairing DNA single and double-strand break. The work purpose is to analyze the performance of galectin-3 in the repair steps to DNA damage dependent on PARP1. The interaction between galectin-3 and PARP1 was analyzed by immunoprecipitation using total extracts of HeLa cells. HeLa galectin-3 silenced cells (shGAL3) and HeLa control silenced cells (shSCRB) cells were treated with cisplatin and PARP1 inhibitor (PJ34) at different concentrations for 24 h and then subjected to a colorimetric cell viability test by tetrazolium salt reduction (MTT). Using the same experimental approach, the combination of irradiation and PJ34 was evaluated. The activity of PARP1 in irradiated cells for different periods of time was evaluated by detecting poly ADP-ribose polymers by immunofluorescence. The interaction between Gal3 and PARP1 was observed. Treatment with cisplatin was not able to decrease the viability of HeLa shGAL3 cells. However, HeLa shGAL3 cells treated with cisplatin and PJ34 exhibited higher sensitivity to the treatment. Moreover, HeLa shGAL3 cells shows less poly ADP ribose focus than control cells. The data show the response of HeLa shGAL3 cells to the cisplatin is influenced by the inhibition of PARP1.

Keywords: DNA repair; DNA damage; cancer.

Acknowledgments: CAPES and CNPq

P03 - CHARACTERIZATION OF ENDONUCLEASE XPF FROM *TRYPANOSOMA CRUZI* AND ITS INVOLVEMENT ON REPLICATION STRESS

Resende B.C.[1], Oliveira K.A.[1], Machado C.R.[1]
[1] Departmento de Bioquímica e Imunologia - ICB, Universidade Federal de Minas Gerais - UFMG, Belo Horizonte, MG
e-mail: bruno_carvalhoo_resende@hotmail.com

DNA repair mechanisms are crucial for maintenance of the genome and for the success of parasitic infections since the parasite is exposed to DNA damaging agents derived from its host, besides the endogenous sources from metabolic processes, leading to replication stress. Genome analyses of trypanosomatids have identified differences in the DNA maintenance mechanisms in comparison to other eukaryotes. The XPF gene encodes an endonuclease that acts in nucleotide excision repair. It is also involved in repair of interstrand crosslinks and could be acting as a relief for replicative stress caused by DNA damage in *T. cruzi*. The aim of this work is analysis the role of XPF gene in genomic stability upon induced replication stress by DNA damaging agents. Initially, for production of XPF single knockout, a cassette containing neomycin resistance gene flanked by XPF 5' and 3'UTR was constructed into pCR-2.1TOPO vector. After transfection of *T. cruzi* CL Brenner cells, modified strain was selected by adding neomycin in the culture. The impact of this modification in parasite proliferation was assessed and results showed a reduced growth rate in modified cells compared to wild type strain. An antibody against an internal motif of XPF was generated. Imunofluorescence assay showed a perinuclear localization of XPF after treatment with cisplatin. We are studying other phenotypes of XPF modified cell.

Keywords: DNA Repair, *Trypanosoma cruzi,* replicative stress

<u>P04</u> - EFFECT OF DNA DAMAGE INDUCED BY CISPLATIN OR UVC LIGHT IN THE CELL CYCLE OF HUMAN XP VARIANT CELLS

Corradi, C.[1], Castro, L.P.[1], Munford, V.[1], Menck, C.F.M.[1]

[1] Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.

e-mail: camilacorradi@gmail.com

Xeroderma Pigmentosum (XP) is an autosomal recessive disorder characterized by high sensitivity to the ultraviolet (UV) light which causes DNA damage such as cyclobutane pyrimidine dimers and 6, 4 photoproducts, distorting the double helix and blocking replication and transcription that lead to deleterious consequences, such as cell death, mutagenesis and cancer. XP patients may have a defect in Nucleotide Excision Repair (NER) or may belong to the variant group of XP (XP-V). XP-V patients are unable to replicate their DNA after UV irradiation due to a deficiency in the DNA polymerase η (POLH) protein required for the Translesion Synthesis (TLS) apparatus. We analyzed cell cycle behavior of XP-V SV40 transformed fibroblasts and the same cells complemented with POLH after UVC irradiation or cisplatin treatment, in the presence of VE-821, an inhibitor of ATR kinase (iATR) and important DNA damage sensor, which acts during replication stress. XTT and colony formation assays were performed to analyze cell sensitivity. Cell cycle behavior was investigated with flow cytometry in the presence of γH2AX antibody, propidium iodide and nocodazole, an inhibitor of cell progression through mitosis. Our results revealed a strong increase in S-phase arrest in presence of iATR and higher levels of H2AX phosphorylation mainly for XP-V cells irradiated with UVC. Although cisplatin induced DNA damage is bypassed efficiently by POLH, neither increase of S-phase arrest in the presence of iATR nor higher levels of γH2AX were detected in theses TLS deficient cells, suggesting different earlier signaling induced by UVC or cisplatin DNA damage.

Keywords: cisplatin, UVC, DNA damage, Xeroderma Pigmentosum, cell cycle.

Acknowledgments: This work is supported by FAPESP grant 2017/24418-5, CAPES, CNPq.

P05 - MOLECULAR DOCKING OF NEK1 INHIBITORS EMPLOYING DIHYDROPYRIMIDINONES

Batista, D.P.[1], Kist, R.[3], Caceres, R.A.[3], Bigioli, M.N.G.[2], Moura, D.J.[1]
[1] Laboratory of Toxicological Genetics; [2] Laboratory of Chemistry; [3] Laboratory Bioinformatics, Graduate Program in Biosciences, Federal University of Health Sciences, Porto Alegre, RS, Brazil.
daniellepbatista@gmail.com

Gliomas are the most frequently occurring primary malignancies in the central nervous system. World Health Organization classified the gliomas into four stages. Glioblastoma multiforme (GBM) classified as grade IV is the most prevalent and aggressive. The glioblastomas showed a low survival rate, which is 9-12 months, and the treatments include surgery, radiation therapy and temozolomide (TMZ). Several studies have shown that NEK1, member of the serine/threonine kinases NIMA family, is overexpressed in glioblastomas. The function of this protein has been related to DNA damage and cell cycle progression control. In this work, we are looking for new pyrimidine compounds which can act by inhibiting Nek1 applying *in silico* strategies. These compounds were based on dihydropyrimidinones structure and other compounds, which already shown excellent biological activity as Nek1 inhibitors. The Nek1 complex was obtained through molecular modeling of missing loops of Protein Data Bank. Thirty dihydropyrimidinones derivates were designed through PyMOL and were parameterized employing Automated Topology Builder server. The DataWarrior webserver based on DrugBank were employed in order to generate molecular conformers, totalizing 2026 distinct conformers. Molecular docking experiments were carried out using AutoDock Vina. The ADMETox properties were evaluated in order to stablish the best scored conformers, lower docking energy and absence of mutagenic, tumorigenic, irritant and reproductive effects. After that, eleven dihydropyrimidinones showed excellent potential to Nek1 inhibition and these molecules will be synthesized and tested *in vitro*. The development of a Nek1 inhibitor presents a possibility of establishing a new perspective in glioblastoma treatment.

Keywords: Molecular docking; Glioblastoma; Nek1 inhibitors; Dihydropyrimidinones.

Acknowledgments: This work is supported by FAPERGS/MS/CNPq grants PPSUS n° 17/2551-0001388-3

P06 - MODULATION OF DOXORUBICIN-INDUCED TOXICITY BY PARP-1 INHIBITOR AND ANGIOTENSIN-(1-7) IN RAT CARDIOMYOBLASTS

Guecheva, T.N.[1], Meirelles, N.L.[1], Baptista, A.[1], Morais G[1], Irigoyen, M.C.[1,2]
[1] Institute of Cardiology/ University Foundation of Cardiology, Porto Alegre, Brazil;
[2] Heart Institute (InCor), Faculty of Medicine, University of São Paulo, São Paulo Brazil.
e-mail: tgesheva@gmail.com

Doxorubicin (DOX) is one of the most commonly used antitumor drugs, which acts inducing DNA strand breaks and oxidative stress, triggering cell death. The use of DOX in cancer therapy has been limited by its serious side effects, especially cardiotoxicity. The Renin-Angiotensin System (RAS) with its receptors (AT1R, AT2R and Ang-(1-7) Mas receptor), has been implicated in these processes. The poly(ADP-ribose) polymerase-1 (PARP-1) is induced in response to DNA strand breaks and was identified as a negative transcriptional regulator of the AT2R. In this study, we aimed to clarify the effect of Losartan (AT1R antagonist), Ang-(1–7) (Mas receptor agonist) and PARP-1 inhibitor (DPQ) on DNA damage and apoptosis induction by DOX in rat cardiomyoblasts H9c2. The genotoxic effect of DOX was evaluated in the Comet assay and cell death induction using AnnexinV-PE and 7-Amino-Actinomycin detection by Flow Cytometry. The 24-hour DOX treatment induced dose-dependent increase in the formation of DNA strand breaks at concentrations of 0.1-2µM DOX. The co-treatment with DPQ (10µM) or Ang- (1-7) (100nM) showed decrease in DNA damage formation and apoptosis induction for concentration of 0.1µM DOX. A combined co-treatment with Ang- (1-7) and Losartan (100µM) also decrease apoptosis at this DOX concentration. The same protection was not achieved at 1 µM DOX treatment. Co-treatment with DOX 1µM and DPQ led to increase of necrotic cell death. Our results suggest that activation of the Ang-(1-7)/Mas axis of RAS and PARP-1 inhibition have protective effect against cell death induction at therapeutic DOX concentrations in rat cardiomyoblasts.

Keywords: Doxoruicin, PARP-1, Renin-Angiotensin System

Acknowledgments: Work supported by FAPIC/IC-FUC, CAPES and CNPq.

P07 - RESPONSIVENESS OF GLIOBLASTOMA CELLS TO γ-RADIATION AND CISPLATIN TREATMENTS: A RHO-p53-MEDIATED PATHWAY?

Magalhães, Y.T.[1], Forti, F.L.[1]

[1] Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil.

e-mail: yuli.magalhaes@usp.br

Glioblastoma is the most frequent brain tumor in adults and despite aggressive therapies with combined surgery and radio-chemotherapy, the prognosis remains poor, with a two-year survival rate. The increased cell migration and invasiveness of these tumors are directly related to their aggressiveness, which has been linked to Rho GTPase pathways. Here we explored the effects of Rho pathway inhibition on glioblastoma cells with different p53 status after the γ-radiation and cisplatin treatments. U87-MG and T98G cells were subjected to inhibition of Rho GTPases by C3 toxin or the knockdown of downstream Rho pathway components. Cytotoxic and 3D clonogenic survival assays showed that Rho inhibition increases the sensitivity of gliomas to γ-radiation and cisplatin. From alkaline comet assays the Rho inhibition exhibited increased DNA damage and a delayed DNA repair capacity of both cells after γ-radiation. Immunofluorescence assays show that phosphorylation and foci formation of H2AX are compromised by Rho inhibition, as well as the 53BP1 foci formation. The impairment of Rho pathway decreases the phosphorylation of H2AX and increases the levels of phospho-Chk2 after genotoxic treatments, as demonstrated by immunoblotting analysis. This indicates a striking regulatory relationship between Rho and DNA damage response (DDR) pathways. Comparative analysis of the Rho activity on cells expressing wild-type or mutated p53 showed that p53 wild-type cells are more susceptible to the effects of Rho inhibition. This work shows that Rho pathway might be a fragile point in the resistance of gliomas against the usual therapies, being this effect dependent on p53 transcriptional activities.

Keywords: Glioblastoma, Rho GTPase, DNA repair, p53, DDR.

Acknowledgments: This work is supported by FAPESP (grants 2015/03983-0 and 2017/01451-7) and CNPq (grant 402230/2016-7).

P08 - SEARCH FOR NOVEL PLAYERS OF CDC42 GTPASE PATHWAY IN GENOMIC INSTABILITY

Da Silva, L.E.[1*], De Souza, R.C.[1], Kitano, E.S.[2], Monteiro, L.F.[1], Iwai[2], L.K., Forti, F.L[1*]

[1] Laboratory of Signalling in Biomolecular Systems (LSSB), Department of Biochemistry, Institute of Chemistry, University of Sao Paulo, Sao Paulo-SP, Brazil;
[2] Laboratory of Applied Toxinology (LETA), Centre of Toxins, Immune-Response and Cell Signalling (CeTICS), Butantan Institute, Sao Paulo-SP, Brazil.
*e-mail: luiz_silva@usp.br; flforti@iq.usp.br

Cdc42, a member of the Rho family of GTPases, is an intracellular signalling protein known for its roles in regulating the cytoskeleton and, more recently, in apoptosis and senescence triggered by genotoxic stress. In some tumour cells, the overactivation of Cdc42 through the expression of constitutively active mutants (G12V or Q61L), GEFs activation or GAPs downregulation functions as an anti-proliferative or pro-aging mechanism. This work examined the interactions between Cdc42 and proteins involved in human cell lines subjected to UV radiation triggered DNA damage. For this purpose, two cell lines with different status of the p53 tumour suppressor gene, MRC-5 (immortalized human fibroblasts) and HeLa (human cervical adenocarcinoma) were exposed to UV radiation and the obtained lysates were subjected to pull-down experiments, using the recombinant proteins GST, GST-Cdc42-WT or GST-Cdc42-G12V as baits, followed by mass spectrometry. Combined proteomic and interactome analysis unveiled putative interactions between Cdc42 and SMC2, MCM7, PHB-2, P53, H1.5, 14-3-3 η, APE1, PAK-4 and PARP1 proteins, which are involved in cell cycle regulation, chromatin remodeling and DNA repair. Experimental validation so far has been conducted using pull-down assays followed by immunoblotting, co-immunoprecipitation and colocalization analysis. In addition, the expression profile of these possible interaction partners is under investigation in clonal sublines of HeLa cells with different backgrounds of Cdc42 GTPase. Altogether these results will certainly contribute to a better understanding of the Cdc42 role in the context of DNA damage response triggered by genotoxic stress.

Keywords: Cdc42, DNA Damage Response, UVC radiation, Protein-protein interaction.

Acknowledgments: This work is supported by CAPES, FAPESP and CNPq.

<u>P09</u> - GENOTOXIC EFFECT ON HUMAN CELLS INFECTED BY *TRYPANOSOMA CRUZI*

Florentino, P.T.V.[1]; Martins D.J.[1]; Leandro G.S.[1]; Vitorino F.N.L.[2]; Cunha J.P.C.[2]; Mortara R.A.[3]; Menck, C.F.M.[1]

[1] Universidade de São Paulo; [2] Instituto Butantan; [3] Universidade Federal de São Paulo

Trypanosoma cruzi is the etiologic agent of Chagas' disease. In their life cycle parasites infect host cell, multiply in the cytoplasm which leads to host cell death. When infected with *T. cruzi*, host cells activate several responses that promote unbalance of reactive oxygen species (ROS; $O_2^{\cdot}$, H_2O_2, $ONOO^-$). It was previously reported that ROS induced by *T. cruzi* infection causes 8-oxoguanine lesions and DNA strand breaks that could activate poli-adenosine-ribose polimerase 1 (PARP-1). We have infected HeLa cells with trypomastigotes forms in order to evaluate host cell DNA damage at early and late stages of *in vitro* parasite cycle. We have shown that during *T. cruzi* infection, expression of nuclear factor erythroid 2–related factor 2 (NRF2), regulator of cellular resistance to oxidants, is downregulated what could promote an oxidative stress leading to genotoxic effects that may help to support effective infection. In fact, host cells treated with glutathione precursor, N-acetyl cysteine (NAC) or NRF2 activator (Sulforaphane) were able to significantly reduce parasite burst. Moreover, oxidative stress in infected cells increased carbonilated chromatin associated-proteins. Also, *T. cruzi* induces γH2Ax staining and 53BP1 foci, indicating the formation of double strand break in infected HeLa cells. Thus, we propose that parasites could benefit from oxidative stress, interfering with genomic stability in HeLa cells and promoting cell death.

Financial Support: FAPESP, CNPq and CAPES

P10 - RAPID DNA REPLICATION FORK BREAKAGE BY ARTEMIS AND XPF LIMITS GENETIC INSTABILITY UPON ACUTE REPLICATIVE STRESS.

Pelegrini, A.L.[1,2], Bétous, R.[2], Rugy, T.G.[2], Hoffmann, J.S.[2,3], Menck, C.F.M.[1]
[1]DNA Repair Laboratory, Institute of Biomedical Science, University of São Paulo, Brazil; [2]Laboratoire d'Excellence Toulouse Cancer LABEX TOUCAN - Cancer Research Center of Toulouse, Inserm U1037, CNRS ERL5294; [3] University Paul Sabatier, Toulouse, France.
e-mail: aleepelegrini@gmail.com

DNA replication stress (DRS) leads to the accumulation of stalled DNA replication forks leaving a fraction of genomic loci incompletely replicated, a source of chromosomal rearrangements during their partition in mitosis. MUS81 is known to limit the occurrence of chromosomal instability by processing these unresolved loci during mitosis. Here, we unveil that the endonucleases ARTEMIS and XPF-ERCC1 can also induce stalled DNA replication forks cleavage through non-epistatic pathways all along S and G2 phases of the cell cycle. We also showed that both nucleases are recruited to chromatin to promote replication fork restart. Finally, we found that rapid chromosomal breakage controlled by ARTEMIS and XPF is important to prevent mitotic segregation defects. Collectively, these results reveal that Rapid Replication Fork Breakage (RRFB) mediated by ARTEMIS and XPF in response to DRS contributes to DNA replication efficiency and limit chromosomal instability.

Keywords: DNA replication; DNA damage; DNA endonucleases; DNA replication fork; fork breakage; Chromosomes segregation defects

Acknowledgments: This work was supported by FAPESP, Institut National du Cancer, Agence Nationale de la Recherche (ANR) and La Ligue contre le Cancer.

P11 - REVEALING TEMOZOLOMIDE RESISTANCE MECHANISMS VIA GENOME- WIDE CRISPR LIBRARIES

Rocha, C.R.R.[1], Rocha, A.R.[2], Gomes, L.[1], Molina, M.S.[1], Menck, C.F.M.[1]
[1] Institute of Biomedical Science, University of São Paulo (USP), Brazil; [2] Institute of Theoretical Physics, State University of São Paulo (UNESP), Brazil.
e-mail for contact: calsmah@usp.br

Introduction and objectives: Glioblastoma (GBM) is a severe type of brain tumor with a poor prognosis and few therapy options. Temozolomide (TMZ) has been widely used to treat glioma, however with limited success. TMZ therapeutic failure is mainly due to tumor resistance. The aim of this work was to identify genes that modulate TMZ resistance in GBM. Material and methods: Genome-wide CRISPR-Cas9 lentiviral screen libraries for gene knockout and activation were transduced in human GBM cell line U138MG. Next-generation sequencing was used to identify gRNAs that were enriched in the knockout or activation screen libraries upon TMZ treatment compared to untreated cells. Results: Pathway analysis of gene candidates on knockout screening revealed that mismatch repair and Sonic hedgehog pathway were significantly enriched. Gene silencing of genes ranked on top 10 list (MSH2, PTCH2 and CLCA2) greatly protect the cells from TMZ-induced death. Also, activation genome-wide screen library revealed that NRF2 and WNT pathways are involved on TMZ resistance. Overexpression of FZD6, CTNNB1 or NRF2 was able to significantly increase cell survival upon TMZ treatment. Using TGCA RNA-seq dataset of glioblastoma patients, we confirmed that expression levels of NRF2 and related genes significantly correlate with patient survival rates. Furthermore, several gene candidates from knockout or activation screening are targetable by inhibitors or small molecules, and some of them are already been used in clinics. Conclusions: Overall, our results have identified a number of genes that when the expression is absent or constitutively active contribute to TMZ resistance in human glioma cell.

Keywords: Temozolomide; cancer resistance; glioblastoma; CRISPR library; NRF2

Acknowledgments: FAPESP (Sao Paulo, Brazil) and CNPq (Brasília, Brazil).

P12 - PALB2 GERMLINE MISSENSE MUTATIONS: FUNCTIONAL EVALUATION THROUGH BRCA1 AND BRCA2 PROTEIN INTERACTION

Gomes, T.T.[1,2], De Gregoriis, G.[1], Suarez-Kurtz, G.[1], Monteiro, A.N.[1], Carvalho, M.A.[1,2]

[1] Nacional Institute of Cancer, Clinical Research Program, Rio de Janeiro, RJ, Brazil;
[2] Federal Institute of Rio de Janeiro, Molecular Genetics Laboratory, Rio de Janeiro, RJ, Brazil.

e-mail: thiago.torres.456@gmail.com

Germline mutations in the *PALB2* tumor suppressor gene are associated with an increased risk of hereditary breast and pancreatic cancers. The PALB2 protein plays a pivotal role in the maintenance of the genomic integrity through homologous recombination (HR)-mediated DNA damage repair. PALB2 works as a scaffold protein that mediates the formation of a complex with BRCA1 and BRCA2 (via its N and C-terminal region, respectively) that promotes RAD51 recombinase activity. While nonsense and frameshift mutations are usually pathogenic due to the great impact in the protein structure and function, missense variants of uncertain significance (VUS) represents a challenge. Curation of literature led to the identification and selection of a set of 44 VUS PALB2 for functional evaluation. The variants were generated by site-directed mutagenesis strategies and the functional evaluation were performed by a mammalian two-hybrid approach using PALB2 N- or C-terminal region as prey and BRCA1 C-terminal or BRCA2 N-terminal as bait, respectively. Our data suggest a pathogenic behavior for 4 PALB2 VUS located in the N-terminal and 6 in the C-terminal region, as they present a consistent impairment in the ability to interact with BRCA1 and BRCA2, respectively. Mutations that compromise the formation of BRCA1/PALB2/BRCA2 complex may confer an increase in cancer risk and its identification is an important approach to help surveillance of high-risk patients. Here we report the first functional analysis of a large set of PALB2 VUS, identifying mutations that lead to functional impairment. However, more studies are needed to define the precise impact of these VUS in cancer predisposition.

Keywords: Hereditary cancer, PALB2, Germline mutations.

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P13 - CDK9 55k, A NEW PLAYER IN DNA DAMAGE REPAIR

Fernandes, V.C.[1], Nepomuceno, T.C[1], Suarez-Kurtz, G.[1], Monteiro, A.N.[2], Carvalho, M.A.[1,3]

[1]Instituto Nacional de Câncer, Rio de Janeiro, Brazil; [2]H. Lee Moffitt Cancer Center and Research Institute, Tampa, USA; [3]Instituto Federal de Educação Ciência e Tecnologia do Rio de Janeiro, Rio de Janeiro, Brazil.

Email: camaravf@gmail.com

Genomic instability allows mutations which lead to carcinogenesis. DNA damage response (DDR) pathway plays a pivotal role restraining these events. To better understand this network, our group performed a study to identified proteins involved in DDR. We identified the Cyclin Dependent Kinase 9 (CDK9) as a putative interactor of BRCA1, BARD1 and PTIP. CDK9 has two isoforms, the 42k form plays a role in transcription elongation and homologous recombination (through interaction with BRCA1 and BARD1). However, CDK9 55k isoform role remains unclear. In this work we intend to understand functional differences between CDK9 55k and 42k, exploring 55k interaction with PTIP and its possible role in non-homologous end joining (NHEJ) pathway. To evaluate CDK9/PTIP and CDK9/BRCA1 interactions, we performed GST-pulldown assays using PTIP tBRCTs or BRCA1 tBRCT and CDK9 55k or 42k ectopic expression in human cells. Both CDK9 isoforms interact with all three PTIP tBRCT domains, however only CDK9 42k interacts with BRCA1 tBRCT domain. NHEJ pathway occurs rather in G1 than S/G2 phases, therefore, we hypothesize that CDK9 expression may be regulated throughout cell cycle. Synchronized human cells showed an extensive fluctuation of CDK9 55k levels throughout the cell cycle, displaying high levels of CDK9 55k in G1/G2/M in contrast to low levels observed in S phase. Next, we intend to check CDK9 55k RNA levels during cell cycle. We are also generating a human cell line silenced for the 55k isoform using CRISPR/Cas9 technology.

Keywords: CDK9, DNA damage, repair pathway

Acknowledgments: Ministério da Saúde, Fundação do Câncer, CNPq, FAPERJ

P14 - DNA DAMAGE RESPONSE IN HUMAN EMBRYONIC STEM CELLS WITH SHORT TELOMERES

Vessoni, A.T.[1], Batista, L.F.Z.[1]
[1] School of Medicine, Washington University in St. Louis, St. Louis, MO, USA.
e-mail: alexandre.vessoni@wustl.edu

Telomeres are repetitive DNA sequences (TTAGGG) located at the end of our chromosomes. Their main functions are to buffer against loss of important genetic information (end-replication problem) and to allow assemble of *shelterin*, a multi-protein complex that protects the chromosomes ends from been recognized as DNA breaks. Telomeres gradually become shorter with aging, and upon reaching a dysfunctional length they induce DNA damage response (DDR). In human fibroblasts, telomere dysfunction induces a permanent G1/S arrest (senescence) via the P53 and the Rb1 pathways. Telomere shortening can be prevented by telomerase, a ribonucleoprotein complex that uses an RNA template to elongate telomeres. Its expression is restricted to a few cell types, such as stem and progenitor cells, and is critical to preserve adult stem cells function and tissue homeostasis. Still, telomerase expression is not enough to prevent aging-induced telomere shortening in several adult stem cells, and how stem cells respond to telomere shortening is not yet fully understood. A better comprehension of this phenomenon may provide new insights into how aging is associated to loss of regenerative potential and tissue dysfunction. In this work, we developed a new model to study the impact of telomere shortening on human stem cells. Using state-of-the-art genome engineering technology, we developed isogenic human embryonic stem cells (hESC) lines *knocked out* for *TP53*, *RB1*, or both, and in which telomerase can be turned ON/OFF at will. We assess the effect of progressive telomere shortening on DDR, cell fate, gene expression and metabolism of these cells.

Keywords: Telomeres, DNA damage, human embryonic stem cells (hESCs).

Financial Support: Philip W. Majerus M.D. Fellowship, American Federation for Aging Research (AFAR), The Longer Life Foundation (LLF).

P15 - CYTOTOXIC EVALUATION OF THE COMPOUNDS LQFM20 AND LQFM23 IN NORMAL PROSTATE CELL LINE PnT2 AND HEPATOCELLULAR CARCINOMA CELL LINE HuH7

Stinglin, M.R.R.[1], Piva, M[1], Nunes, H.L.[1], Cardoso, C. R. P.2, Pazini, F.[2], Cólus, I.M.S[1].

[1]Department of General Biology, State University of Londrina, Londrina, PR, Brazil; [2]Department of Pharmacology, Federal University of Mato Grosso, Sinop, MT, Brazil.

e-mail: nanastinglin@yahoo.com.br

In this study, we investigated the possible cytotoxic profile of LQFM20 and LQFM23, two compounds synthetized based on the hybridization of drugs Milrinone and Cilostazol, used to control cardiovascular diseases. Cytotoxicity assays with different targets: MTT, Neutral Red (NR), LDH and Oxidative Stress-ROS, were made using cell lines PnT2 (normal prostate) and HuH7 (hepatocellular carcinoma). LQFM20 induced cytotoxicity in the two cell lines used at different concentrations. In the NR assay, LQFM20 was cytotoxic from 50µM to the normal and 150µM to the tumor line. In MTT assay, the cytotoxic concentrations to PnT2 were □100µM and to HuH7 cell line, equal to 300µM and 500µM. In PnT2 cells the concentration of 150 µM was cytotoxic in the LDH assay and 300µM induced oxidative stress after 1 hour of treatment. LQFM23 induced cytotoxicity in PnT2 cells in the follow assays: NR (from 200µM); MTT (from 250µM) and LDH (only 400µM and 500µM). HuH7 cells showed cytotoxicity in the NR (from 50µM), MTT (from 300µM) and LDH (500µM) assays. In both cell lines, no significant results were obtained by ROS assay. Comparison of the responses of the two cell types to the treatments with the two synthetic drugs evaluated showed that, in general, the normal prostate cell line responds positively from lower drug concentrations than the tumor cell line. However, with the exception of the neutron red assay, in all others assays the cytotoxic concentrations were above LD_{50} calculated. Thus, these drugs appear to offer no danger if used in low concentrations.

Keywords: MTT; Neutral Red; LDH; ROS

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P16 - TOPOISOMERASE II INHIBITORS-INDUCED LESIONS – AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS

Busatto, F.F.[1,2], Viero, V.P.[1], Rocha, J.C.[1], Masson, J.Y.[3,4], Saffi, J.[1,2]

[1] Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, Brazil; [2] Post–Graduation Program in Cellular and Molecular Biology - Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; [3] Genome Stability Laboratory, Centre Hospitalier Universitaire de Québec Research Center, Québec City, Canada; [4] Department of Molecular Biology, Medical Biochemistry and Pathology, Laval University Cancer Research Center, Québec City, Canada

e-mail: franbusatto@gmail.com

Topoisomerase II inhibitors form stabilized drug-DNA-topoisomerase complexes. Processing of these complexes creates DNA double-strand breaks (DSBs). We have already shown that the Nucleotide Excision Repair (NER) pathway is involved in this process. Therefore, the aim of this study was to understand the role of CSB, a NER protein, in response to topoisomerase II inhibitors, Doxorubicin (DOX) and Mitoxantrone (MXT), and the relation with DSB repair pathways. U2OS cells were transfected with siRNAs for different NER genes, treated with MXT and DOX and followed by 53BP1 and $_{\gamma}$H2AX foci IF analysis; the influence of NER knockdowns in the Homologous Recombination (HR) rates was done using a CRISPR based reporter assay. To evaluate the interaction of CSB and Topo II a co-IP was performed. NER-deficient fibroblasts were treated with DOX and MXT and evaluated for R-Loops formation through IF, and nuclear intensity was measured. IF analysis showed an increase in 53BP1 and $_{\gamma}$H2AX foci after MXT treatment, but there was no difference between NER knockdowns (siERCC6, siXPC and siXPA) and the siCTRL. Interestingly, we observed an increase in HR in siXPC cells. We detected a co-IP of Topo II and CSB in response to MXT treatment. A preliminary result of an IF showed an increase in R-Loops formation after the treatments, mainly DOX; and it seems to be more prominent in CSB-deficient cells.
Our results indicate an interaction between CSB and Topo II, which could explain the implication of CSB in DSBs repair pathway in response to Topo II inhibitors induced lesions.

Keywords: Topoisomerase II inhibitors, NER, double-strand breaks

Funding Agencies: CAPES, CNPq, FAPERGS

P17 - DNA REPAIR MODULATION IN HUMAN BREAST CANCER CELLS SUBMITTED TO A CLINICAL CHEMOTHERAPY PROTOCOL

Busatto, F.F.[1, 2]; Viero, V.P.[1]; Saffi, J.[1,2]

[1] Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, Brazil; [2] Post–Graduation Program in Cellular and Molecular Biology - Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.

e-mail: franbusatto@gmail.com

Among the therapies used to treat breast cancer, chemotherapy with genotoxic agents is still widely applied. Nevertheless, these tumors can often be low or non-responsive to this approach, that can be associated to a range of molecular aspects, such as the DNA repair processes. Currently, in vitro models used to study chemotherapy failure are limited to the evaluation of cells responses after a step-mediated resistance induction to a drug, which in fact does not simulate what happens in clinical protocols. Therefore, our main objective was to evaluate the DNA repair modulation during a clinical chemotherapy protocol. Thus we treated breast cancer cells (MCF-7) following a clinical protocol that uses Doxorubicin and Paclitaxel and evaluated cells viability as well as the cumulative population doubling (CPD) rate during and after the treatment. Moreover, RNAs were extracted at the end of each cycle for further DNA repair expression analysis through qPCR.

We found a reduction in CPD during the protocol, however when cells were maintained in culture after that, the CPD was reestablished. qPCR analysis showed an increase in XPA, XPC, CSB and XPF genes expressions compared to non-treated cells. Moreover, when we compare gene expression after 4 cycles of treatments there is a loss of the pattern found after one cycle, indicating that during the protocol these genes are constantly changing their expressions. Although we did not find a resistance profile during the treatment, the proposed model allows us to study different cell mechanisms that can be modulated during all chemotherapy phases.

Keywords: cancer resistance, breast cancer, DNA repair

Funding Agencies: CAPES, CNPq, FAPERGS

P18 - INFLUENCE OF FERROCENE COMPOUNDS ON THE VIABILITY OF GLIOBLASTOMA CELLS TREATED WITH TEMOZOLOMIDE

Henn, J.G.[1]; Poletto, F.[2]; da Rosa, R.G.[3]; Moura, D.J.[1]
[1] Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre; Porto Alegre, RS, Brazil; [2] Department of Organic Chemistry, Institute of Chemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil;
[3] Department of Inorganic Chemistry, Institute of Chemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.
e-mail: jefersonghenn@gmail.com

Glioblastoma (GBM) is the most common and aggressive neoplastic brain tumor, and its treatment has proved to be a challenge. For decades its therapy has been based on the use of temozolomide (TMZ), but the survival rate remains low. However, the discovery of the cytotoxic properties of ferrocenes has suggested new approaches in the use of these ferric compounds coupled to chemotherapeutics in the treatment of several cancers. We investigated whether functionalization of ferrocenes with TMZ inside cubic-shaped liquid crystal particles (cubosomes) induce a decrease in viability of GBM cells T98G. The effects of ferrocene complexes on cell viability were investigated by neutral red uptake assay (NRU). As results, monopeguilated and dipeguilated ferrocenes did not induce cytotoxicity at any of the concentrations tested. On the other hand, when incorporated in cubosomes, the cell viability was slightly reduced in a dose-dependent manner. Finally, when we added TMZ to the ferrocenes cubosomes, the cytotoxicity increased considerably, even more than the formulation containing only TMZ inside cubosomes. Together, our data suggest that formulations based on ferrocene compounds within cubosomes may contribute to increase the cytotoxicity of TMZ in GBM cells, making it a possible alternative in the treatment of this disease.

Keywords: glioblastoma; temozolomide; ferrocenes; cubosomes.

P19 - DNA DAMAGE AND TRANSCRIPT EXPRESSION PROFILE OF GENES ASSOCIATED WITH OXIDATIVE STRESS AND DNA REPAIR IN ELDERLY INDIVIDUALS WITH DIFFERENT DEGREES OF COGNITIVE DECLINE

Vieira, J. A.[1], Amorim, R. S[2]., Tanimoto, M. H.[1], Moriguti, J. C.[2], Sakamoto-Hojo, E. T.[1,3]

[1] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil; [2] Department of Medical Clinic, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil;

[3] Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

e-mail: vieirajohnathan@usp.br

Aging is marked by functional and cognitive decline with increased susceptibility to age-related diseases, being a main risk factor for neurodegenerative diseases. The age-related decay of normal cognition is characterized as Mild Cognitive Impairment (MCI). However, there are few reports in the literature about oxidative stress levels in patients with MCI, who are more likely to develop dementias than elderly with normal cognition (N-MCI). The aim of this study is to analyze DNA damage levels in Peripheral Blood Mononuclear Cells (PBMCs) of MCI patients compared to elderly age-matched controls, by using the comet assay with and without hOGG1 treatment applied to N-MCI: n=10, and MCI: n=6 groups; for few individuals of both groups, we also carried out a preliminary evaluation of transcript expression for genes associated with oxidative stress, cell cycle progression, and DNA damage: *ATM*, *ATR*, *CDKN1A* and *MTH1* genes, by means of the RT-qPCR method. The comet assay results showed a statistically significant ($p < 0,01$) high level of oxidative DNA damage in MCI compared to N-MCI, but a lack of significant difference ($p > 0.05$) was observed between the studied groups for the conventional comet assay. Regarding transcript expression, *CDKN1A* gene was up-regulated in MCI, but in contrast, the expression profiles of *ATM*, *ATR* and *MTH1* were similar in both groups, MCI and N-MCI. Currently, this study is under way to increase the number of individuals in both groups of patients. The preliminary results showed high levels of oxidative DNA damage and up-regulation of *CDKN1A* gene.

Keywords: Mild Cognitive Impairment; DNA repair; DNA damage; oxidative stress.

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P20 - FUNCTIONAL EVALUATION OF BRCA1 VARIANTS LOCATED IN COILED-COIL DOMAIN

Elias, A. B. R.[1], De Gregoriis, G.[2], Suarez-Kurtz, G.[1], Monteiro, A.N.[3], Carvalho, M.A.[1,4]

[1] Clinical Research Program, National Institute of Cancer, Rio de Janeiro – Brazil;
[2] Department of Oncology, The CR-UKMRC Oxford Institute for Radiation Oncology, University of Oxford – UK; [3] H. Lee Moffitt Cancer Center, Tampa, FL. – USA;
[4] Federal Institute of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro – Brazil.

The majority of hereditary cases of breast cancer can be attributed to alterations in *BRCA1*, a crucial gene for the maintenance of genome integrity. Gene sequencing helps in the early identification of individuals in high-risk of developing cancer, benefits affected individuals to seek guidance and to adopt preventive procedures. However, many of mutations found in genetic studies cannot be classified because they do not have a clear designation regarding cancer association. In these cases, mutations remain as variants of unknown significance. Functional assays may work to circumvent this problem. In this work *BRCA1* missense variants coded in the coiled-coil region were functionally evaluated using two approaches: the BRCA1 transcriptional activation ability and its competence to interact with PALB2. An initial set of 7 *BRCA1* variants was identified in the Breast Cancer Information Core database all *missense* variants of natural occurrence in population and not functionally evaluated yet. A second set of 14 *missense* variants was selected base on their position in the coiled-coil structure and their *in silico* prediction for cancer association. Variants were generated by site-directed mutagenesis and assays were performed in HEK293FT cells. Considering the whole set of 21 variants, 10 presented a suggestive pathogenic profile in transcriptional activation assay (all from the second set) and seven in the PALB2 interaction assay (two from the first set and five from second). Both approaches contribute to built variants functional behavioral profile, however, further analyzes are required for a final classification.

Keywords: Variants, PALB2, Functional Assay, Breast cancer.

Funding agencies: FAPERJ, CNPq, Ministério da Saúde, Fundação do Câncer.

P21 - DNA DAMAGE LEVELS, OXIDATIVE STRESS AND MITOCHONDRIAL ALTERATIONS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Lima, J.E.B.F.[1], Conceição, R.F.[3], Xavier, D.J.[1]; Moreira, N.C.S.[1]; Foss-Freitas, M.C.[3]; Sakamoto-Hojo, E.T.[1,2].

[1] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo – USP, Ribeirão Preto, SP, Brazil; [2] Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo – USP, Ribeirão Preto, SP, Brazil; [3] Department of Internal Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo – USP, Ribeirão Preto, SP, Brazil.

e-mail: jessicaellen.lima@usp.br

Type 2 diabetes mellitus (T2DM) is mainly characterized by hyperglycemia. The high glucose levels can lead to several alterations, especially due to increased production of reactive oxygen species (ROS), which may increase oxidative stress and DNA damage. We aimed to assess alterations in mitochondrial parameters (mass, membrane potential, ROS levels) using fluorescent dyes and flow cytometry in blood samples of T2DM patients. Besides, we investigated the effects of a dietary restriction (DR) along four weeks of hospitalization evaluated before and after the intervention; these effects were studied in terms of DNA damage levels evaluated by the comet assay (n=6), and 8-oxo-dG (8-oxo-7,8-dihydro-20-deoxyguanosine), which is a biomarker of oxidative stress, present in the nucleotide pool, analyzed by an ELISA assay (n=7). DR consisted of a protein restriction (10g per kg/day), while calories were maintained at a basal level. DR during four weeks led to improvements on metabolic status of patients (reduced glucose, cholesterol and triglycerides levels). The protein restriction led to decreased levels of DNA damage ($p<0.01$) and there was a slightly reduction ($p>0.05$) of 8-oxo-dG levels after the intervention period. Regarding the mitochondrial parameters, only alterations in membrane potential was observed ($p<0.05$) in T2DM patients. Thus, DR was effective in reducing DNA damage levels in T2DM patients, compatible with improvements in glycemic and lipid control, although a significant effect on status of oxidative stress could not be observed in the nucleotide pool. Alterations on mitochondrial dynamics were not observed in T2DM patients, requiring additional analyses with more patients.

Keywords: Type 2 Diabetes Mellitus; protein restriction; oxidative stress; DNA damage; mitochondrial parameters.

Acknowledgments: This work is supported by CAPES, CNPq and FAPESP.

P22 - DECREASED PGC-1 A EXPRESSION CORRELATES WITH ΔTG MUTATION IN *XPC* GENE BUT NOT WITH DECREASED EXPRESSION OF PGC-1A TARGET-GENES

Mori, M. P.[1], de Souza-Pinto, N. C.[1]

[1] Department of Biochemistry, Instituto de Química, Universidade de São Paulo, São Paulo.

Xeroderma pigmentosum (XP) is an inherited autosomal recessive syndrome with a 1,000-fold elevated skin cancer rate in areas exposed to UV light. XP is caused by mutations in one of seven genes (*XPA-XPG*) of the nucleotide excision repair pathway or in one translesion DNA polymerase (*POLH*). We have recently demonstrated that XP-C cells show increased mitochondrial H_2O_2 production with an adaptive shift between respiratory complexes I and II, leading to increased sensibility towards mitochondrial stress. A very significant decrease in expression of the transcriptional coactivator PGC-1α, which has a major role in controlling mitochondrial biogenesis, was found in four different XP-C cell lines carrying the c.1643_1644delTG mutation (ΔTG) in *XPC*. The same reduction of PGC-1α expression was not observed in an XP-C cell line carrying a different mutation, regardless of nearly absent XPC protein levels, in agreement with results showing that siRNA-mediated XPC knockdown in normal fibroblasts did not alter PGC-1α expression. Furthermore, cell lines from the heterozygous parents of one patient carrying ΔTG mutation also show decreased PGC-1α expression. This is in line with the observation that some mutations can generate new non-coding RNAs, with a gain-of-function new molecular phenotype. However, PGC-1α expression was also low in cells carrying a third *XPC* mutation and in another unrelated normal cell line. Interestingly, in all cell lines investigated here expression levels of the PGC-1α target genes TFAM and SDHA did not correlate with PGC-1α levels. As expression of PGC-1α is almost undetectable in fibroblasts, it is possible that PPRC1, the third member of PGC family, levels are more relevant to mitochondrial biogenesis in this cell type.

Keywords: Mutation, regulation of gene expression, xeroderma pigmentosum, non-coding RNA.

Acknowledgments: This work is supported by FAPESP grants 2016/15407-7 and 2017/04372-0.

P23 - IDENTIFICATION OF A NETWORK OF MITOTIC KINASES AND COEXPRESSED GENES ASSOCIATED WITH PATIENT OUTCOME IN CANCER

Immich, B.F.[1,2], De Bastiani, M.A.[3], Bracco, P.A.[4], Moura, D.J.[2], Lenz, G.[1], Saffi, J.[1,2]
[1] Center of Biotechnology, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil; [2] Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil; [3] Laboratory of Cellular Biochemistry, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil; [4] Postgraduate Program in Epidemiology, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.
e-mail: brunaimmich@gmail.com

Mitotic kinases have been shown to impact tumor aggressiveness and are good candidates for targeted therapies in cancer. We aimed to assess the impact of mitotic kinases and their network of co-expressed genes in patient survival. We analyzed RNA-seq samples from 14 tumors in the TCGA (The Cancer Genome Atlas) cohorts. Through Cox multiple regression analysis, we found that *AURKA*, *AURKB*, *CDK1*, *NEK2* and *PLK1* expression are associated with increased patient risk in renal clear cell, liver, lung, and pancreatic carcinomas. Among these kinases, *NEK2* and *PLK1* expression were the best prognostic markers in lung adenocarcinoma, whereas *PLK1* and *CDK1* were independently associated with patient risk in liver and pancreatic tumors, respectively. Additionally, *NEK5* was revealed as a potential prognostic marker in thyroid carcinoma. A survival network for co-expressed genes was built, and revealed that several genes involved in cell cycle regulation and DNA repair, including downstream targets of mitotic kinases, are also risk predictors. The expression of transcription factor *FOXM1*, which stimulates transcription of essential mitotic genes and participates in the DNA damage checkpoint, increased patient risk in 6 tumors, and its effect was independent from mitotic kinases expression in predicting patient risk in cutaneous melanoma. Taken together, our results reveal new potential prognostic markers and therapy targets.

Keywords: Mitotic kinases; cancer.

Funding: CNPq

P24 - ANALYSIS OF THE ROLE OF RAD52 IN THE OXIDATIVE DAMAGE REPAIR AND MITOCHONDRIAL BIOENERGETICS

Machado, F.T.[1]; Santos, V.T.; Nascimento, D.; Souza-Pinto, N.C.[1]
[1] Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo – SP, Brasil.
e-mail: felippetruglio.m@gmail.com

The RAD52 protein is involved in the repair of DNA double-strand breaks (DSB) through homologous recombination (HR), single stranded annealing, or the newly characterized RNA-DNA repair. In mammals, the role of RAD52 is not well characterized. RAD52-knockout mice showed nearly normal phenotype, without significant alterations in HR activity. RAD52, however, may play a back-up role in cells deficient in HR proteins like BRAC1, BRAC2 and RAD51. We have previously demonstrated that RAD52 increases the activity of the oxoguanine DNA glycosylase OGG1, involved in the Base Excision Repair (BER) pathway. In mitochondria, the BER pathway is well characterized and thought to play an important role due to the higher load of oxidized bases in the mitochondrial DNA (mtDNA), as a consequence of exposure to oxidants generated by the oxidative phosphorylation (OXPHOS). Our study intends to analyze the role of RAD52 in mtDNA maintenance, as well as the effects of its absence in cellular bioenergetics. Using two different human cell lines we demonstrated that RAD52 localizes in mitochondria under normal growth conditions, and exposure to hydrogen peroxide induces mitochondrial translocation of at least one of the RAD52 isoforms. However, RAD52 binding to mtDNA was not detected under these conditions, suggesting that it may not be directly involved in DSB repair in mtDNA. To analyze RAD52 functional role in mitochondria we generated HEK293T cells with RAD52 expression constitutively knockdown (KD) via shRNA. Interestingly, RAD52-KD cells showed increased maximal respiration supported by NAD-linked substrates when compared to wild-type or scrambled shRNA control cells, indicative of alterations in the OXPHOS Complex I in these cells. These results may suggest that although the absence of RAD52 does not have significant effects on the development and phenotype of mice, cellular bioenergetics may be altered, and compensatory mechanisms are triggered to maintain cell viability. We are now investigating whether other respiratory complexes are altered in these cells.

Keywords: Mitochondrial DNA Repair, oxidative damage, bioenergetics, RAD52

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P25 - DUSP3 SILENCING AFFECTS CELL CYCLE REGULATORS AND PROLIFERATION OF XERODERMA PIGMENTOSUM CELL LINEAGES

Jessica O. Farias; Lilian C. Russo; Fábio L. Forti
Laboratory of Signaling in Biomolecular Systems - Department of Biochemistry, Institute of Chemistry – University of Sao Paulo
e-mail: jessicafarias@usp.br

The dual-specificity protein phosphatase 3 (DUSP3) has been described as a potential mediator of DNA repair processes, (by mechanisms yet not fully understood), and variations in its expression have been described in different types of cancers. The aim of this work was to evaluate fine-tune regulation of cell proliferation after DUSP3 silencing in two NER-deficient cell lines (XPA and XPC) and in two NER-proficient cell lines (MRC-5 and XPV, which is deficient in the translesion DNA synthesis). Proliferation of the cell lines, exposed or not to UV radiation, was evaluated by growth curves and the expression of cyclin-dependent kinases, cyclins, p53 and p21 and cyclin-dependent kinase inhibitors was evaluated by Western blot. It was observed that the silencing of DUSP3 did not significantly affect the proliferation of the XPC and XPV deficient cells, unlike the XPA and MRC-5 cells in which silencing caused a decrease in proliferation. After UV radiation treatments all the cell lines had significant decline in their growth, being more expressive in XPC and XPA, but independently of DUSP3 silencing. Proliferation was accompanied by changes in the expression of cyclins (A, B, D, E), CDKs (1, 2, 4, 6), and CKIs (p21, p53) according to the differences between lineages, but in overall the DUSP3 deficiency increased sensitivity of NER-deficient cells to UV. In conclusion, DUSP3 maintains cell proliferation, in a lineage dependent manner, even in the presence of UV damage, and plays role in DNA repair very likely leading cells to accumulate DNA lesions along generations.

Keywords: DUSP3, NER-deficient cells, cell proliferation, cell cycle, DNA damage repair

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P26 - MECHANISMS OF TUMOR RESISTANCE TO CISPLATIN: IMPACT OF CELLULAR DETOXIFICATION AND DNA LESIONS PROCESSING

Matheus Molina Silva[1], Clarissa Ribeiro Reily Rocha[1], Alessandra Luiza Pelegrini[1], Inês Chaves[2], Gijsbertus T. J. van der Horst[2], Carlos Frederico Martins Menck[1]

[1] Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [2] Molecular Genetics Department, Erasmus University Medical Center, Rotterdam, The Netherlands.

e-mail: matheusmolina@usp.br

The most common lung cancer treatment is chemotherapy with cisplatin, however, changes in the protection systems of the cell may arise, causing resistance. Among these changes, the increased expression of XPF, ERCC1 and NRF2 proteins in tumor cells stand out. Thus, this project aims to characterize two lung cancer cell lines (A549 and NCI H23) and one control lung cell line (IMR-90) for their sensitivity to cisplatin, understanding the relation between sensitivity, levels of XPF, ERCC1 and NRF2 expression, and the processing of DNA injuries. Initially, the cells were treated with cisplatin and sensitivity was evaluated by XTT colorimetric assay. The level of the proteins of interest was investigated by western blot. We also measured the glutathione levels and the mRNA levels of genes involved in its synthesis by qPCR. The viability experiments showed that the most sensitive cell line is NCI H23, while the western blot indicate that this cell line has higher levels of the DNA repair proteins. On the other hand, this cell line also has lower levels of glutathione and proteins involved in its synthesis, along with NRF2. By synchronizing the circadian cycle of these cells we saw that the sensitivity to cisplatin oscillates during the day, together with the NRF2 levels. Therefore, cisplatin resistance in these cell lines are not determined by the ERCC1 and XPF protein levels. However, glutathione, which is regulated by the activity of the transcription factor NRF2 on genes like *GCLM*, seems to be crucial for the tumor resistance to cisplatin.

Keywords: Cisplatin, Lung Cancer, DNA Repair, NRF2, Glutathione, Circadian Cycle

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P27 - ATR INHIBITION SENSITIZES HPV +/- CERVICAL CANCER CELLS TO CISPLATIN

Padilha, E.[1], Munford, V.[1], Bocardo, E. [1]; Menck, C.F.M.[1]
[1] Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.
e-mail: eduardo.padilha@usp.br

Cervical cancer is one of the main types of cancer in women, being responsible for about 4% of the cancer-related deaths worldwide. Most of these cases (99.7%) are associated with infection by the Human Papillomavirus (HPV), also associated to mouth, throat, anus and penile cancers. The HPV oncogenic potential is due to the E6 and E7 genes, which mediate degradation of the tumor suppressor proteins p53 and pRb, respectively. The most common treatment involves cisplatin, a chemotherapeutic agent that forms bulky lesions in the DNA double-strand. The ATR kinase is the main responsible for initiating the DNA damage response in the presence of cisplatin-induced lesions, in order to maintain the replicative viability of the cells. Thus, our goal was to investigate if ATR inhibition in cervical cancer cell lines (C33A, HeLa and SiHa) would potentiate the effect of cisplatin treatment in these cells. The cell viability, clonogenic survival, real time cell proliferation and Sub-G1 assays showed that ATR inhibition increased cell death after cisplatin treatment. Also H2AX phosphorylation was higher when ATR was inhibited, indicating increase in the amount of DNA damage and breaks. These results indicate that ATR inhibition affected cells ability to cope with DNA damage, inducing cell death even under very low doses of the drug. Considering that most cervical tumors (as well as other head, neck, penile and rectum tumors) are HPV positive or present defects in p53, this may be a viable way towards potentiating the therapeutic protocol of these tumors and surpassing tumor resistance.

Keywords: Cervical cancer; Cisplatin; ATR inhibitor; DDR - DNA Damage Response.

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P28 - REPLICATION PROTEIN A FROM TRYPANOSOMATIDS: A NEW PERSPECTIVE FOR A WELL-KNOWN COMPLEX

Pavani, R.S.[1], Zoltner, M.[2], Fragoso, S.P.[3], Field, M.C.[2], Elias M.C.[1].
[1] Cell Cycle Special Lab, Butantan Institute, São Paulo, SP, Brazil; [2] Wellcome Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee, United Kingdom; [3] Carlos Chagas Institute, Fiocruz-PR, Brazil
e-mail: rapavani@gmail.com

Trypanosoma cruzi and *Trypanosoma brucei* are parasitic protozoa responsible for causing Chagas disease and sleeping sickness that result in a high number of deaths annually. These parasites possess a complex life cycle that alternates between replicative and non-replicative lifeforms. Despite the characterization of a great number of molecular pathways, there are still many gaps in the understanding of the processes that coordinate the DNA metabolism of these organisms. Replication protein A (RPA), the major eukaryotic single-stranded binding protein, is a heterotrimeric complex formed by three subunits RPA-1, RPA2 and RPA-3 that participates in various vital functions during replication, repair and checkpoint signaling. RPA from trypanosomatids present significant structural peculiarities compared to other eukaryotes such as the lacking of DBDF-domain that interacts with proteins majorly involved in DNA damage response (DDR) pathways and aminoacids substitutions in conserved regions, raising questions regarding the conservation of canonical functions described in mammals and yeast. In this work, we show that RPA from trypanosomatids can interact with single-stranded DNA and is indeed involved in replication and DNA damage response pathways. Moreover, we could find new features concerning trypanosomatids RPA such as the discovery of (i) non-described post-translation modifications (ii) a new RPA-like protein that seems to be exclusive of trypanosomatids interacting with RPA complex and (iii) a nucleus-cytoplasm shuttle that is lifecycle dependant.

Keywords: Replication Protein A; RPA; Trypanosoma.

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P29 - MOLECULAR CHARACTERIZATION OF DNA REPAIR IN MOUSE OLFACTORY SENSORY NEURONS

Rowies, F. T.[1]; Malnic, B.[1]; Souza-Pinto, N. C.[1]

[1] Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo – SP, Brasil.

e-mail: fer.rowies@usp.br

Olfactory Sensory Neurons (OSNs) undergo neurogenesis and migration through the olfactory epithelium (OE) during maturation process; therefore their lifespan is shorter than most neurons within the brain. Olfactory dysfunction has been reported as an early clinical symptom in many neurodegenerative diseases, which are associated with impaired DNA repair. Whether DNA repair mechanisms play a role in OSNs genomic maintenance, however, is still unknown. Our study intends to describe DNA repair pathways in mature and precursors of OSNs. For that, we analyzed data from two different transcriptomes to characterize the expression pattern for DNA repair enzymes with age and stage of neuronal differentiation. In order to confirm the results obtained from the *in silico* analysis, we performed RT-PCR for selected DNA repair targets from newborn (4-7 days) and 3 weeks old mice OE samples. Our results from *in silico* analysis suggest that representative DNA repair enzymes of most repair pathways are reduced in young mice, when compared to newborns. On the other hand, non-homologous end joining (NHEJ) pathway is apparently increased, which may indicate a preference for non-homologous repair pathways for repairing double strand breaks due to lack of homology in differentiated cells. However, our preliminary results from the RT-PCR analysis suggest that, at least for base excision repair (BER) components, the expression pattern indicates an increase with age. This analysis also confirmed the increased expression of NHEJ components with age. Despite being preliminary, our results already show that there are differences in the expression pattern of DNA repair proteins in OSNs throughout development, which has never been reported before.

Keywords: DNA repair, olfactory sensory neurons, neurodegeneration.

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P30 - MATHEMATICAL MODELING ON CELLULAR RESPONSES TO MOLECULAR EFFECTS OF LOW INTENSITY IONIZING RADIATION

Yamada Sittoni M.[1], Ramos A.F.[1,2]
[1] Department of Radiology and Oncology, Faculty of Medicine; [2] School of Arts, Sciences and Humanities (EACH), University of São Paulo, São Paulo, SP, Brazil.
e-mail: misakicristine@gmail.com

Biological effects of ionizing radiation have been studied intensely since the beginning of the 20th century; however, with enhancement in technologies and improvement in experimental techniques in the field of molecular biology, some biological phenomena peculiar to low intensity ionizing radiation have started to be documented recently. Furthermore, with the advent of the advance in computational capacity in the area of informatics, the presence of mathematical modeling and computational simulation of natural phenomena have been increasingly witnessed in various branches of science, including biology. Among the topics of radiobiology, effects related to low intensity radiation and their mechanisms are the most challenging to be investigated. These types of effects appear after a period of latency which can last decades, and the probabilistic nature of their occurrence makes the collection of sufficient quantity of epidemiological data to reach statistically meaningful results difficult. In these conditions, computational simulations of mathematical models can help clarify the mechanisms and also predict future effects. With these in mind, we conducted computational simulations of a stochastic (probabilistic) model in order to investigate biological effects caused by low intensity ionizing radiation, notably cell survival associated with DNA lesions, and possible influences of random fluctuation (noise) on it. The stochastic model was constructed by us from a deterministic model based on *in vitro* experiments, selected from recent literature, which describes the dynamics of different cellular populations exposed by low intensity ionizing radiation.

Keywords: ionizing radiation, DNA, biological effects, mathematical modeling, simulation.

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P31 - PROGNOSTIC IMPACT CHANGES IN NUCLEOTIDE EXCISION REPAIR AND TRANSLESION POLYMERASES EXPRESSION IN SPORADIC COLORECTAL CANCER

Gloria, H.C.[1], Leguisamo, N.M. [1,3], Laporte, G.A. [1,2], Azambuja, D.B. [1,2], Martins, T.V. [2] Kalil, A.N.[1,2]., Saffi, J.[1]

[1] Laboratory of Genetic Toxicology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, Brazil; [2] Oncology and Colorectal Surgery, Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Porto Alegre, Rio Grande do Sul, Brazil; [3] Institute of Cardiology/University Foundation of Cardiology, Porto Alegre, Rio Grande do Sul, Brazil.

e-mail: helenadecastroegloria@gmail.com

Inappropriate DNA repair may result from imbalances in several pathways, including nucleotide excision repair (NER) and translesion synthesis (TLS). Clinically, it may be associated with insufficient response to chemotherapy, clinical features of aggressiveness and poor survival. Pre-treatment tumor samples from 70 patients with sporadic colorectal adenocarcinoma were assessed for NER and TLS key-components genes and proteins expression. MLH1 protein expression was used to determine the MMR status (proficient or deficient) of each patient. The molecular data were analyzed in relation to clinical features and TNM staging as prognosis predictor. Regarding the NER components: the reduction of CSB and XPG levels were associated with poor clinicopathological outcomes (poorly differentiated tumors, advanced TNM stages and more invasive tumors). Higher levels of ERCC1 and XPF were associated with unfavorable pathological outcomes, such as tumor poorly differentiated and mucinous, and the presence of lymphatic invasion. Gene and protein expression of ERCC1, XPD and XPG were strongly correlated. No associations were found between XPA mRNA levels and pathological features. Concerning the TLS components, we identified increases the expression of DNA polymerases kappa and eta, but not theta. Overexpression of DNA polymerases kappa and theta were associated with poor pathological outcomes features, such as low grade tumours and metastatic lymph nodes. No associations were found between TLS polymerases or NER genes expression and MMR status. We concluded the heterogeneous expression pattern of DNA repair pathways may provide a molecular signature to refine CRC staging and prognosis.

Keywords: Colorectal cancer; nucleotide excision repair; translesion synthesis; mismatch repair; prognosis.

P32 - READTHROUGH COMPOUND AS SUPPRESSION AGENT OF NONSENSE MUTATIONS IN *BRCA1*

Abreu, R. B. V.[1], Gomes, T. T.[1], Fuchshuber-Moraes, M.[1], Monteiro, A. N.A.[3], Suarez-Kurtz, G.[1], Carvalho, M. A.[1,2]

[1] Programa de Pesquisa Clínica, Instituto Nacional de Câncer, Rio de Janeiro, Brazil; [2] Departamento de Biotecnologia, Instituto Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [3] H. Lee Moffitt Cancer Center and Research Institute, Tampa, USA.

Mutations in *BRCA1* are responsible for most cases of hereditary breast and ovarian cancer syndrome (HBOC). Nonsense variants account for ~ 13% of mutations in *BRCA1* gene and they are characterized by a premature stop codon (PTC) that encodes a truncated protein. Different studies have shown that some compounds, like aminoglycosides, can induce readthrough of PTCs, restoring the protein function. The use of these compounds may represent an important strategy for cancer prevention in HBOC patients. Our study intends to evaluate the use of aminoglycosides on the restoration of tumor suppressor activity of nonsense variants of the *BRCA1* gene. Nonsense variants coding PTC in the BRCA1 C-terminus were generated and cloned into a retroviral vector in fusion with EGFP or GAL4 DBD cassettes. HeLa cells constitutively expressing the nonsense variants were tested in the presence and absence of G418 to evaluated full-length protein synthesis restoration using flow citometry and confocal microscopy. However, restoration of full-length protein levels does not reflect their biological functional status. BRCA1 ability to interact with CtIP was used to circunvent this issue. Functional restoration was observed for a limited group of variants. *BRCA1* missense variants representing the most probable acquired mutations consequence of the readthrough event of the original nonsense mutants were identified. Their impact in BRCA1 biological function was evaluated using a transcription activation assay approach. The results corroborate BRCA1-CtIP interaction data. This is the first study that evaluates the readthrough of nonsense variants with clinical relevance in *BRCA1* gene.

Keywords: *BRCA1*, nonsense, readthrough.

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P33 - MUTAGENICITY PROFILE CAUSED BY UVA AND UVB LIGHT IN CELLS FROM PATIENTS WITH XERODERMA PIGMENTOSUM GROUP C

Quintero-Ruiz, N.[1], Moreno, N.C. [1], Corradi, C. [1], Souza, T.A. [1], Menck, C.F.M. [1]
[1]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.
e-mail: cfmmenck@usp.br, nathaliaquinteroruiz@usp.br

Solar ultraviolet radiation (UV) is an ubiquitous environmental carcinogen that causes structural damage in DNA, such as pyrimidine dimers (i.e. CPD, 6,4-PP) and oxidized bases. UV is typically divided into three spectral ranges according to the wavelength, UVA/UVB/UVC, but only UVA and UVB reach the Earth's surface. Despite this, most reports focus on the UVC impact in mutagenesis. The nucleotide excision repair (NER) pathway removes bulky DNA lesions, as those induced by UV. Defects in NER proteins result in a clinical phenotype that includes an extremely high incidence of skin cancer in sunlight exposed areas, denominated Xeroderma Pigmentosum (XP). Our aim is to establish and compare the mutagenicity profiles induced by UVA and UVB light in XP-C deficient cells, by sequencing the exome of cellular clones, using next generation sequencing strategy. The results indicate that UVA and UVB: increased cell death of XP-C cells compared to the control lineage, and induced the CPD formation in both cell lines. The exome data from UVA irradiated cells points out a significant mutagenesis increase in XP-C cells. In fact, the C>T transition, considered the mutational signature of UVC/UVB radiation, is the main type of point mutation observed. Suggesting, at least for XP-C cells, that UVA mutagenesis is probably more related to CPD induction than with the generation of oxidized bases. The identified mutations and sequence context is being analyzed in order to better understand what patterns of mutations are generated, and check for eventual differences after UVB-irradiation.

Keywords: ultraviolet radiation; Xeroderma Pigmentosum; DNA damage; mutagenesis.

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P34 - IDENTIFYING POINT MUTATION PROFILES IN UVA-LIGHT MUTAGENESIS BY WHOLE-EXOME SEQUENCING OF HUMAN CELLS

Souza T.A.[1], Moreno N.C.[1], Garcia C.C.M.[2], Munford V.[1], Rocha C.R.R.[1], Castro L.P.[1], Corradi, C.[1], Quintero, N.R.[1], Ienne, S.[1] and Menck C.F.M.[1]

[1] Institute of Biomedical Sciences, University of Sao Paulo (USP), Sao Paulo, SP, Brazil; [2] NUPEB & Biological Sciences Department, Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

e-mail: tiagoantonio@gmail.com

Exposure to sunlight induces direct and indirect lesions leading to cell death and mutagenesis. Although UVA composes most of the sunlight that reaches Earth surface, little is known about its impact in mutagenesis. Patients with Xeroderma Pigmentosum Variant (XP-V) have impairment in the function of polymerase eta (pol eta) showing an increased incidence of skin cancer, which is associated with sunlight exposure. Thus, we decided to use a whole-exome sequencing approach to investigate UVA mutagenesis in normal and pol eta-deficient cells. Cells were cloned and expanded for genomic DNA extraction from each clone population and subjected to whole-exome enrichment followed by Illumina HiSeq sequencing. Reads were aligned with BWA and SNVs were called using GATK, then annotated with ANNOVAR and filtered to pick up only exclusive SNVs for each sample, compared to all other samples. Mutational signatures were extracted using SomaticSignatures, and SNV profiles were evaluated using woland (github.com/tiagoantonio/woland) - a Perl and R multiplatform tool implemented to explore point mutation profiles from resequencing genomics data. The results indicate that both UVA-light induced direct damage, and oxidative-related DNA damage have mutagenic consequences, especially in XP-V cells. Basically, there was no evidence for a pol eta role on inserting A in TT-dimers ("A rule") in UVA-irradiated cells. Altogether, the results demonstrate the use of a successful exome-wide method for detection and evaluation of mutations driven by an exogenous mutagen and the relations with DNA damage in human cells.

Keywords: exome, mutational signatures, mutagenesis, UVA light, XP-V patients.

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P35 - THE ROLE OF MITOCHONDRIAL TRANSCRIPTION FACTOR A (TFAM) IN PROTECTING DNA FROM OXIDATIVE DAMAGE

Alencar, R.R., Souza-Pinto, N.C.
Department of Biochemistry, Institute of Chemistry
e-mail: rralen@icloud.com

The mitochondrial transcription factor A (TFAM) is a nuclear encoded high mobility group box protein that is essential for mitochondrial DNA (mtDNA) transcription, replication, and for regulation of mtDNA copy number. TFAM is also directly involved in mtDNA structure and organization through its packaging of mtDNA into nucleoids. TFAM non-specifically binds to and coats the entire mtDNA and packages it into a nucleoproteic complex, similar to the manner in which histones package nuclear DNA. Since the mtDNA is contained in the inner side of the inner mitochondrial membrane, it is prone to oxidative damage by reactive oxygen species (ROS) that accumulate as byproducts of the electron transport chain. It has been suggested that TFAM could play a role in protecting mtDNA from oxidative damage by blocking access to damaging species. On the other hand, we have shown that TFAM binding to DNA delays damage removal by restricting access to DNA repair proteins. In this study we will dissect the role of TFAM in protecting mtDNA from oxidative damage. Furthermore, we propose to investigate how TFAM binding modulates the balance between mtDNA damage and repair, and what protein interacting partners are involved. For this we propose to investigate: *i.)* whether TFAM binding to DNA will protect it form induced oxidative damage by photoactive dyes in vitro *ii.)* if TFAM levels affect cell survival after induced oxidative damage by photoactive dyes, and *iii.)* protein interactions that modulate TFAM binding to mtDNA. These results will help to determine if TFAM binding to mtDNA protects it from oxidative damage, and how this binding is modulated by possible protein interacting partners.

Keywords: mtDNA, nucleoids, DNA damage

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P36 - MUSASHI-1 KNOCKDOWN IMPACTS ON BLADDER CARCINOGENESIS AND ON CHEMO AND RADIOTHERAPY RESISTANCES

André Luiz Ventura Sávio[1], Kátia Ramos Moreira Leite[2], Suzanne Burns[3], Glenda Nicioli da Silva[4], Pedro Alexandre Favaretto Galante[5], Luiz Otavio Ferraz Penalva[3], Daisy Maria Fávero Salvadori[1]

[1] UNESP - São Paulo State University, Botucatu, SP, Brazil; [2] USP - University of São Paulo, São Paulo, Brazil; [3] Children's Cancer Research Institute, UTHSCSA, San Antonio, TX, USA; [4] UFOP - Federal University of Ouro Preto, Ouro Preto, MG, Brazil; [5] Sírio-Libanês Hospital, São Paulo, SP, Brazil.

Bladder cancer (BC) is the most common urothelial malignancy, with high rates of recurrence, aggressiveness and progression to invasive muscular disease. Recently, the neuronal sub-group (NSG) was included in the new molecular classification of bladder muscle-invasive tumors, being the most aggressive and treatment-resistant. Among the altered genes in NSG, Musashi1 (*MSI1*) encodes a RNA binding protein that can modulate multiple molecular pathways, including tumorigenesis. This study aimed to investigate the relationship between *MSI1* overexpression and BC development. *MSI1* expression was evaluated in 405 bladder cancer samples (384 high- and 21 low-grade tumors) from the TCGA data bank and 31 from Brazilian cohorts (20 high- and 11 low-grade tumors). Increased expression of *MSI* strongly associated with poor prognosis was identified in high-grade tumors. In addition, *MSI1* knockdown in three high grade bladder cancer cell lines (J82, T24 and UMUC3, with different MSI1 expression levels) showed decreased cell proliferation, migration, invasion and viability, and increased rates of apoptosis. A strong association between *MSI1* expression and resistance to chemo and radiotherapy was also detected in the UMUC3 cell line. Currently, molecular *MSI1* mRNA targets are under investigation by RNAseq and CLIPseq analyses. In conclusion, MSI1 is related to poor prognosis and chemo and radiotherapy resistance in bladder cancer. Therefore, *MSI1* can be a promisor target for therapeutic strategy

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P37 - REVEALING MICROENVIRONMENT-MEDIATED CISPLATIN RESISTANCE IN BREAST CANCER CELLS

Gomes, L.R.[1], Rocha, C.R.R.[1], Martins, D.J.[1], Menck, C.F.M.[1]
[1] Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil
e-mail: luciana.gomes@usp.br

Tissue architecture and cell-extracellular matrix (cell-ECM) interaction determine organ specificity, however the influence of these factors on anticancer drugs preclinical studies is neglected. For considering such aspects, three-dimensional (3D) cell culture models are relevant tools for accurate analysis of cellular response to chemotherapy. Here, we compared the MCF-7 breast cancer cells response to cisplatin in traditional two-dimensional (2D) and in 3D laminin-rich ECM cell culture models. The results showed a substantial increase of cisplatin resistance mediated by 3D microenvironment. This phenotype was independent of p53, autophagy and true to other cellular models, such as lung cancer cells. Such strong decrease on cellular sensitivity was not due to differences on drug-induced DNA damage, since similar levels of γ-H2AX and cisplatin-DNA adducts were detected under both conditions. However, the processing of these cisplatin-induced DNA lesions was very different in 2D and 3D culture. Unlike cells in monolayer, cisplatin-induced DNA damage is persistent in 3D-cultured cells, correlating with high senescence induction. Moreover, only 3D-cultured cells were able to progress through S cell cycle phase, upregulating translesion (TLS) DNA polymerases expression and triggering the ATR-Chk1 pathway, with unaffected DNA replication fork progression. Co-treatment with VE-821, pharmacological inhibitor of ATR, blocked the 3D-mediated changes on cisplatin response, including low sensitivity and high TLS capacity. Altogether, the results demonstrate that microenvironment-associated resistance to cisplatin is due to an efficient induction of TLS, mediated by ATR. Thus, co-treatment with ATR inhibitors might be a promising strategy for enhancement of cisplatin treatment efficiency in breast cancer patients.

Keywords: Breast cancer, 3D cell culture, cisplatin, Translesion DNA Synthesis (TLS), A*taxia* Telangiectasia and Rad3 related (ATR), senescence

Acknowledgments: This work is supported by FAPESP grant 2014/15982-6, CNPq and CAPES.

P38 - INTRA-S PHASE CHECKPOINT IS REGULATED BY ATR IN *Trypanosoma brucei* AFTER IONIZING RADIATION TREATMENT

Marin, P.A.[1], Araujo, C.B.[1], da Silva, M.S.[1], Machado, C.R.[2], Cestari, I[3]. , Lima, A.A[1], Elias, M.C[1]

[1] Cell Cycle Laboratory (LECC) - Center of Toxins, Immune Response and Cell Signaling (CeTICS), Butantan Institute, São Paulo, São Paulo; [2] Biochemical and Immunology Department, Institute of Biomedical Science, ICB, Federal University of Minas Gerais (UFMG), Minas Gerais, Belo Horizonte; [3] Institute of Parasitology, McGill University, Montreal, Canada.

e-mail: pamm0414@usp.br

Trypanosoma brucei, like other trypanosomatids, has the striking ability to gain, lose or rearrange DNA. The molecular mechanisms controlling genome maintenance pathways after DNA breaks is unknown in *T. brucei*, however recombination of natural DNA breaks is proposed to be important for parasite survival. Hence, we investigated the role of ATR kinase in response to DNA breaks generated by ionizing radiation (IR). To establish the ability of IR-exposed parasites to progress through the cell cycle, insect stage procyclic form parasites were treated with ATR and ATM inhibitors before IR and then double labelled with thymidine analogs IdU and CldU to monitor cell cycle using microscopy. The Co-detection of IdU/CldU from different time points after IR indicated that ATR kinase plays a role in regulating the intra-S checkpoint while ATM kinase function in G1/S checkpoint. ATR regulation of intra-S checkpoint was confirmed using RNA interference. Furthermore, both G1-damaged and S-damaged cells returned to cell cycle 4 and 3 hours after damage, respectively. Using DNA combing assays, we observed that the blockage of DNA replication is due to fork stalling. These forks were recovered after damage and new origin firing were detected at the same molecules. Our data suggest a role for ATR kinase in regulating the intra-S checkpoint in *T. brucei* after double strand DNA break, perhaps by orchestrating DNA synthesis recovery and dormant origin firing after repair of double strand DNA break. In contrast, ATM exhibited an inferior role in S-phase cells but essential for G1/S transition after DNA damage.

Keywords: DNA damage response; Intra-S phase checkpoint; Ionizing Radiation; ATR.

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P39 - FUNCTIONAL CHARACTERIZATION OF TOS4 AND UBP12 INTERACTION DURING REPLICATION STRESS

Soares, B.L. [1]; Bastos de Oliveira, F.M. [1]
[1]Instituto de Biofísica Carlos Chagas Filho, Program of Molecular and Structural Biology, Universidade Federal do Rio de Janeiro
e-mail: barbara-luisa@hotmail.com

TOS4 (*Target of SBF 4*) is a *Saccharomyces cerevisiae* cell cycle-dependent gene. During cell cycle progression, *TOS4* transcription is activated at the end of G1 and inhibited while cells progress into S-phase. Although the function of *TOS4* is still unclear, it was demonstrated that its transcription is sustained during S-phase in response to replication stress. Tos4 is characterized by the presence of a protein interacting Forkhead Associated (FHA) domain. Interestingly, we found that Tos4 mutant cells lacking functional FHA domain showed increased sensitivity to hydroxyurea. To better understand the role of Tos4 during replication stress, we performed an immunoprecipitation assay followed by mass spectrometry analyses and found that Tos4 interacts with Ubp12 (Ubiquitin-specific Protease 12). Ubp12 interaction was confirmed by co-immunoprecipitation and demonstrated to be dependent on Tos4 FHA domain. Also, by performing a cycloheximide chase assay in S-phase progressing cells, we showed that FHA mutated Tos4 has a short half-life compared to wild type. Based on previous evidences that ubiquitination is important to regulate the turnover of Tos4 during S-phase, our results suggests that Ubp12 interaction may be important to counteract Tos4 degradation, contributing to sustain its activity during replication stress.

Keywords: cell cycle gene; replication stress; Tos4 and Ubp12 interaction

Acknowledgments: This work is supported by FAPERJ Nº E-26/111.232/214 and CNPQ Nº446143/2014

<u>P40</u> - CHARACTERIZATION OF MISSENSE MUTATIONS FOUND ON DNA REPAIR GENES IN HEREDITARY BREAST AND OVARIAN CANCER SYNDROME PATIENTS

Carvalho, S.C.S.[1], Rosa, R.C.A. [1], Brotto, D.B.[1], Gomes, T.T.[2], Carvalho, M.A.[2], Ferraz, V.E.F. [1], Silva Jr, W.A. [1].

[1] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil; [2] Clinical Research Program, Nacional Institute of Cancer, Rio de Janeiro, RJ, Brazil.

e-mail: simonedacostaesilva@usp.br

Many studies showed that besides *BRCA1/BRCA2*, other DNA repair genes confer risk to Hereditary Breast and Ovarian Cancer Syndrome (HBOC). We performed a mutation screening in 21 DNA repair genes, and besides *BRCA1*/*BRCA2*, thirteen genes presented missense variants of unknown clinical significance. Seven of them carried mutations significantly associated to HBOC. Until now, we evaluated the effect of five variants found on our study to the genome instability and BRCA1 binding function. We performed microsatellite instability (MSI) analyses in three patients: one of them carrying a *MSH2* variant and two patients carrying one *PMS2* variant each. We also evaluated the effect of two *PALB2* variants on BRCA1 binding. We used a two-hybrid assay system, and the PALB2-BRCA1 interaction was measured through the reporter luciferase transcriptional activity. The *MSH2* mutation is classified as benign by ClinVar, but the *in silico* analyses predicts it as damaging. The *PMS2* mutations present conflicting data on pathogenicity in both ClinVar and *in silico* predictors. The MSI analysis showed the same allele amplification pattern in both tumor and blood DNA samples from each patient, suggesting that these variants do not increase genomic instability. *PALB2* missense mutations also presented conflicting data on pathogenicity (ClinVar and *in silico* predictions), however their luciferase activity were similar to the wild type *PALB2* allele, suggesting that they do not interfere in the *BRCA1* binding. In summary, our data suggest that these variants are not associated with an increased risk to HBOC by genomic instability promotion or BRCA1 binding impairing.

Keywords: HBOC; variant characterization; genomic instability; BRCA1 binding.

Acknowledgments: This work is supported by CNPq grant 141974/2015-0.

P41 - INVOLVEMENT OF RAD51 IN MITOCHONDRIAL HOMOLOGOUS RECOMBINATION REPAIR IN *TRYPANOSOMA CRUZI*

Ferreira, W.R.R.[1], Repolês, B.M.[1], Machado, C.R.[1]

[1] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

e-mail: wesleyrrferreira@gmail.com

Trypanosoma cruzi is the etiological agent of Chagas disease, one of the 17 neglected tropical diseases. An important feature of trypanosomatids is the presence of a single mitochondria, known as kinetoplast. Our objective in this work is to study homologous recombination repair in *T. cruzi* kinetoplast. Although we have evidence of the occurrence of recombination in the mitochondria of *T. cruzi*, it is not yet known which recombinase acts in this process on the kinetoplast. In this context, we intend to study the effect of Rad51 depletion on *T. cruzi* kDNA using a strain deficient for the Rad51 recombinase gene. Epimastigote forms of *T. cruzi* Cl Brener strain as well TcRad51 single knockout were used on the experiments. After exposing these strains to agents that are able to cause lesions on the nuclear DNA and on the kDNA, we observed that the response for TcRad51 single knockout is distinct to WT control strain. When exposed to methyl methane sulfonate (MMS) TcRad51 single knocknout present an arrest on growth, while WT strain can recover faster. Since MMS treatment targets both, nuclear and kDNA of *T. cruzi,* we are using some drugs that are specific to mitochondria. After exposure to chlorambucil-target-mitochondria (mt-Cbl) it was evidenced that TcRad51 single knocknout are more sensitive to the treatment. This result could suggest a role for TcRad51 on the maintenance of *T. cruzi* kDNA. We are now verifying the extent of the damage that persists on *T. cruzi* DNA in the absence of normal TcRad51 levels.

Keywords: DNA repair; *Trypanosoma cruzi*; Recombination repair; kDNA.

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P42 - IN VITRO INITIAL STUDIES ON THE CITOTOXICITY OF THE SYNTHETIZED COMPOUNDS LQFM11, LQFM21 AND LQFM22

Piva, M.1, Stinglin, M.R.[1], Nunes, H.L. [1], Serpeloni, J.M. [1], Guembarovski, R.L.[1], Cardoso, C. R. P. [2], Pazini, F.[2], Cólus, I.M.S.[1]

[1] Department of General Biology, State University of Londrina, Londrina, PR, Brazil;
[2] Department of Pharmacology, Federal University of Mato Grosso, Sinop, MT, Brazil.
e-mail: maiarapiva@gmail.com

The compounds LQFM11, LQFM21 and LQFM22 were synthetized through molecule hybridization of two inhibitors of myocardial phosphodiesterase III, Cilostazol and Milrinone, drugs that increase the heart's contractility. To measure the possible cytotoxic activity of these compounds, MTT, neutral red (NR), LDH and ROS assays were conducted in concentrations 10-400µM for 24 hours, on two cell lines: PNT2 (normal prostate) and HuH7 (hepatocellular carcinoma). The tumor line, when treated for 24 hours with LQFM11, showed decreased cellular viability by the neutral red assay (250 to 350µM) and increased LDH levels (100 to 350µM). On the other hand, both cell lines were very sensitive to LQFM21 treatment, with marked decrease in viability by NR assay (10-400µM). LDH level were slightly elevated at higher doses (350 and 400µM) in the normal cell line PNT2. ROS and MTT assays had no significant changes in any of the cell lines evaluated. Although ROS levels demonstrated no change, LQFM22 treatment reduced 40% of cell viability of both cell lines on MTT assay but it was decreased only in PNT2 when performed NR assay. LDH levels were elevated in HuH7 (100, 300 and 350µM) and in PNT2 (400µM) cell lines. In conclusion, neither compounds increased reactive oxygen species, although LDH levels were found moderately elevated. The results obtained allow us to state that the three drugs had low cytotoxicity for both cell lines evaluated. However, at higher concentrations the LQFM11 drug presented a selective cytotoxicity to the tumoral line.

Keywords: cytotoxicity; reactive oxygen species; LDH, neutral red

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P43 - DUSP12 AND DNA REPAIR PROTEINS: A PROTEOMICS APPROACH

Monteiro, L.F.[1], Forti, F.L.[1]

[1] Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil.

e-mail: lucasfalcaomonteiro@hotmail.com

Dual Specificity Phosphatase 12 is the largest enzyme in the atypical DUSP family, possessing a zinc-finger domain besides the catalytic domain. It has been implicated in diverse processes, including ribosome maturation, cell cycle, redox response, development and survival. Despite reports of protein targets of DUSP12, such as the MAP kinase p38, and an extensively described interaction with the maturing ribosome, most of the biological functions are not fully understood.

The DUSP family of Protein Tyrosine Phosphatases is able to dephosphorylate tyrosine and serine/threonine residues, or even more elusive targets such as lipids. Since other DUSPs – such as DUSP3 – have been linked to DNA Repair, we challenged A549 (lung adenocarcinoma) and MCF-7 (breast adenocarcinoma) cells to genotoxic stimuli (namely gamma-radiation, UVB radiation and the double-strand break-inducing drug bleomycin). We subsequently purified potential targets from nuclear lysates with the pull-down assays, using DUSP12 variants as baits.

According to MS/MS analyses, we have identified several potential targets related to DNA Repair, and this gene set was overrepresented among the hits with statistically significant FDR values. Other biological functions are overrepresented, and they are related to previously described DUSP12 functions, such as rRNA processing and cell cycle, and other previously unreported functions, such as mRNA splicing and DNA replication. We chose select targets for validation, including HP1BP3, NAT10 and CENPJ, which are reportedly involved in genomic stability in the context of the cell cycle. This report may lay the groundwork for the validation of novel DUSP12 targets and mechanistic insight into biological functions.

Keywords: DNA repair; DNA damage; Dual Specificity Phosphatase; Protein Tyrosine Phosphatase; Proteomics.

Acknowledgments: This work is supported by FAPESP (grants 2015/03983-0 and 2017/01451-7) and CNPq (grant 402230/2016-7).

P44 - ANALYSIS OF THE GERMLINE MUTATION RATE IN PROBAND DESIGNED AFTER PARENTAL EXPOSURE TO CESIUM-137 IONIZING RADIATION IN GOIÂNIA-BRAZIL (1987): A CASE REPORT

Rodrigues, LHNS [1,7]; Costa, EOA [1,3]; Pinto, IP [1,5]; Caetano, AR[4]; Leite-Filho, HP[5]; Nunes, HF[7], da Silva, JF[1]; da Cruz, AS[1], Silva, DM[7]; da Silva, CC [1,2,5,6], Pereira, RW[3]; da Cruz, AD [1,2, 5,7]

[1] Pontifical Catholic University of Goiás, Department of Agricultural and Biological Sciences, Genetics Master's Program, Replicon Research Group; [2] Human Cytogenetics and Molecular Genetics Laboratory, Public Health Central Laboratory Dr. Giovanni Cysneiros, Health Secretary of Goiás State, Goiânia-GO, Brazil; [3] Catholic University of Brasilia, Genomic Sciences and Biotechnology Graduate Program, Brasilia-DF, Brazil; [4] Embrapa Genetic Resources and Biotechnology, Biological Station Park, Brasilia, DF, Brazil; [5] University of Brasília, Biotechnology and Biodiversity PhD Program, Brasilia-DF, Brazil; [6] State University of Goiás, UEG, Goiânia, Goiânia-GO, Brazil; [7] Federal University of Goiás, Graduate Program in Genetics and Molecular Biology, Institute of Biological Sciences, Goiânia-GO, Brazil.

e-mail: lhnsr@hotmail.com

Exposure to ionizing radiation (IR) may lead to an accumulation of mutations in the offspring of irradiated parents. It has been well documented that even low absorbed doses of radiation may increase the frequency of aneuploidies and genomic mutations. Copy number variations (CNVs) can occur naturally as well as be induced by some environmental factors. Chromosomal Microarray Analysis (CMA) is an effective tool for high resolution genomic analysis to detect of gains or losses resulting in CNVs using SNP-based arrays. The current study reports a 20-year-old woman, born to both parents accidentally exposed to IR during the radiological accident involving 137CsCl in Goiânia – Brazil (1987), with absorbed doses ≤0.2Gy. The conception occurred one month after parental exposure, the mother was 26 and the father 24 years old at the time of conception. CMA was performed using CytoScan HD (Affimetrix® / ThermoFisher®). The child presented low rates of germline mutation for both CNVs and SNPs when compared to her brother conceived previously parental exposure and to the control group comprised of 8 children born in Goiânia from parents with no history of radiation exposure. If there is a linear or exponential dose response or a discrete threshold in which the biological consequences are uncommon, they remain to be understood.

Keywords: Cesium-137; CMA; CNV; SNPs; Mutation rate

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P45 - NEUROVASCULAR DYSFUNCTION AND NEUROINFLAMMATION IN A COCKAYNE SYNDROME MODEL

Kajitani, G.S.[1,2], Trevino-Villareal, J.H.[1], Trocha, K.[1], Mitchell, S.[1], Menck, C.F.M[2], Mitchell, J.R[1].

[1] Harvard T.H. Chan School of Public Health, Department of Genetics and Complex Diseases, Boston, MA, USA; [2] Dept. of Microbiology, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil.

e-mail: gustkajitani@gmail.com

Cockayne Syndrome (CS) is a rare, autosomal genetic disorder characterized by premature aging-like features, such as cachectic dwarfism, retinal atrophy and progressive neurodegeneration. The underlying genetic defect in CS lies in genes associated with the transcription-coupled arm of the nucleotide excision DNA repair (NER) pathway, although how defective DNA repair leads to the particular symptoms of CS is not yet clear. In this work, we used a mouse model of severe CS with total loss of NER, termed the CX model, which recapitulates several CS-related phenotypes, resulting in premature death of these mice at approximately 20 weeks of age. We used a cell culture and the CX in vivo model in order to assess the role of endothelial and glial cells in the etiology of this disease. Although the CX mice exhibit a severe progeroid phenotype, we found no evidence of a cell autonomous vascular dysfunction in this model. However, in spite of this observation, we detected a blood barrier dysfunction and a significant increase in inflammatory markers and glial activation in the brains of these animals, which indicates that neuroinflammation could play a role in the neurodegenerative phenotype observed in this model and in the human disease. These findings have implications for the etiology of this disease and could contribute to the study of novel therapeutic targets for the treatment of Cockayne Syndrome patients.

Keywords: DNA Repair, Cockayne Syndrome, Neuroinflammation, Glia activation.

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P46 - AUTOPHAGY AS A SURVIVAL MECHANISM TO DNA DAMAGE IN *TRYPANOSOMA CRUZI*

Trivelato Junior, R.R.[1], Repoles, B.M.[1], Resende, B. C.[1], Silva, H.M.C.[1], Machado, C.R.[1]

[1] Department of Biochemistry and Immunology, Institute of Biological Sciences, UFMG, Belo Horizonte, MG, Brazil.

e-mail: robertotrivelatojr@yahoo.com.br

Trypanosoma cruzi is the etiological agent of Chagas disease. Autophagy is a catabolic process where damaged or unused cellular components are segregated in vesicles and later degraded by the lysosome. It is responsible for maintenance of genomic integrity, cellular homeostasis and remodeling. Although its molecular mechanisms are well known in mammals and fungi, its role in *T. cruzi* has been the objective of few studies. The DNA damage response system, DDR, is responsible for the detection of lesions and subsequent signaling, leading to activation of cell cycle arrest and DNA repair pathways. Earlier studies from our research group, have shown that after DNA damage induction by UV irradiation, *T. cruzi* presents rapid cell death, that can be inhibited by caffeine, although the mechanism behind this process is still unknown. In this study our goal was to describe how inhibition of autophagy affects the response to DNA damage in *T. cruzi*, and how it is involved in UV damage cell death. By examining dose-response curves in the presence of pharmacological agents and analyzing survival rates and growth curves with inhibition of both autophagy and the DDR, we concluded that autophagy is an important process for cell survival after DNA damage induction, and that its inhibition lead to lower survival rate, followed by longer cell cycle arrest and slower cell growth. Inhibition of DDR lead to higher survival rates, however, when combined with inhibition of autophagy it was not able to prevent cell death.

Keywords: DNA damage; Autophagy; DDR; *Trypanosoma cruzi*.

Acknowledgments: This work is supported by CAPES and CNPq.

P47 - PARP-1 INHIBITION SENSITIZES TEMOZOLOMIDE-TREATED GLIOBLASTOMA CELL LINES AND OVERCOME DRUG RESISTANCE INDEPENDENTLY ON MGMT ACTIVITY

Ana Paula Montaldi[1], Larissa O. Piassi[1], Sarah Caroline G Lima[1], Elza Tiemi Sakamoto-Hojo[1,2].

[1] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo – USP, Ribeirão Preto, SP, Brazil; [2] Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo – USP, Ribeirão Preto, SP, Brazil.

e-mail: larissa.piassi@usp.br

Sensitizing effects of poly-ADP-ribose polymerase (PARP) inhibitors against tumor cells have been studied in experimental cancer research, although a clear definition of predictive biomarkers of tumor responses to temozolomide (TMZ) remains under investigation, such as MGMT (O6-methylguanine-DNA methyltransferase) activity, which has been associated with tumor resistance in glioblastoma (GBM). In this work, our purpose was to study the effects of NU1025 (PARP-1 inhibitor) on cell responses to TMZ treatment in several GBM cell lines. Experiments/assays were carried out to analyze the effects of combined treatments (TMZ plus NU1025) on DNA damage responses and cell death, such as antiproliferative activity (cell viability), G2/M arrest, DSBs (γH2AX detection) and apoptosis induction (annexin-V assay). We found that NU1025 effectively sensitizes TMZ-resistant, T98G and LN18 cell lines (proficient for MGMT activity) and TMZ-sensitive cells (U251MG, MGMT deficient), while the same effect was not observed for NU1025 tested as a single-treatment. In contrast, a similar response was not observed in U87MG TMZ-sensitive cells (MGMT deficient), suggesting that other genetic alterations possibly influence drug responses. Surprisingly, following 20 days of recovery after three consecutive days of TMZ treatment, we found that in TMZ resistant cells, TMZ combined to NU1025 was notoriously efficient in causing cell death in T98G and LN18 cells, thus revealing the potential of drug combination in inducing cell lethality. Therefore, independently on the MGMT activity, the combination of TMZ with the PARP-1 inhibitor seems to be a promising therapy strategy to be further investigated in GBM.

Keywords: PARP-1 inhibition, glioblastoma, temozolomide, MGMT.

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P48 - REVEALING BIOCHEMICAL MECHANISMS OF DEATH IN *TRYPANOSOMA CRUZI*

Assis, A. V.[1], [1], Machado, C.R.[1]

[1] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

e-mail: antoniiofs@gmail.com

Trypanosoma cruzi is the etiologic agent of Chagas' disease, a weaken disease that currently is incurable and with low treatment alternative. Previous studies from our research group have demonstrated that high dose of ultraviolet radiation and gamma radiation leads to partially or total death of *Trypanosoma cruzi* CL Brener strain in their epimastigote form. Ultraviolet (UV) radiation generates DNA lesions that deplete the replication and transcription process, having predominant products CPD and 6,4-cyclobutane, and gamma radiation causes double strand break. We have seen that 1500J/cm² of UV decreases 33% of cells population in the first 4 hours after treatment, and 3000gy of gamma radiation maintains cells in a state of dormancy for approximately 5 days and then total cell death occurs after 8 days. To investigate biochemical and biological mechanisms in the death of the parasites in these tests, we are standardizing the tests of annexin V with propidium iodide and TUNEL (Terminal deoxinucleotidyl transferase dUTP Nick-End Labeling) with propidium iodide in flow cytometry for investigation which kind of death (apoptosis or necrosis) occurs. In addition, we are investigating using γ-H2AX antibody, how long the DNA lesions persists after genotoxic treatment.

Keywords: DNA repair; *Trypanosoma cruzi*; Apoptose; DNA damage.

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P49 - NRF2 IS ACTIVATED IN THE ABSENCE OF MGMT PROVIDING RESISTANCE TO TMZ IN GLIOBLASTOMA CELL LINES

Bastos, A.U.[1], Rocha, C.R.R.[1], Menck, C.F.M.[1]
[1] Department of Microbiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil
e-mail: uchibastos@yahoo.com.br

Temozolomide (TMZ) is an alkylating agent that causes DNA methylation and DNA damage, leading to cell death. Although it is the main drug used to treat glioblastoma, several mechanisms of TMZ resistance have been described. One of the main mechanisms is the ability of MGMT (Methyl-Guanine Methyl-Transferase) to directly reverse the lesions caused by TMZ. Additional mechanisms were associated with resistance to TMZ, and our group showed that glutathione (GSH) - a peptide that plays a crucial role in the maintenance of redox cell homeostasis - prevents cell death caused by TMZ, by a mechanism mediated by the transcription factor NRF2. However, it is unclear how NRF2 modulates TMZ cytotoxicity. Thus, by over-expressing MGMT in glioma cells, our aim was to investigate the role of MGMT and NRF2 resistance mechanisms in the TMZ induced cell death. In this work, U138-MG and U251-MG glioma cell lines were transduced with a MGMT expressing vector. These cells express high amounts of MGMT protein and their viability after TMZ treatment was assessed by XTT assay. MGMT and NRF2 expression were determined by Western blot. Surprisingly, no significant difference in cell viability was observed comparing cells expressing or not expressing MGMT. TMZ treatment leads to NRF2 induction both in U138-MG and U251-MG cell lines. However, the expression of NRF2 was not modulated in the cells expressing MGMT. Thus, we propose that, although MGMT can provide cell resistance to TMZ treatment, in cells lacking MGMT, NRF2 plays a crucial role on cell survival upon TMZ treatment.

Keywords: Temozolomide, glioblastoma, MGMT, NRF2, DNA damage

Acknowledgments: This work is supported by FAPESP (grant 14/15982-6), CNPq and CAPES.

<u>P50</u> - COMPARISON OF *DE NOVO* CNVS IN THE F1 GENERATION OF OCCUPATIONALLY EXPOSED INDIVIDUALS TO CESIUM 137

Campos, C.B.M[1,2]; Silva, J.F.[1]; Pinto, I.P.[1]; Costa, O.A.C.[1]; Gonçalves, M.W[1]; Rodrigues, L.H.N.S[1,2]; Da Silva, C.C.[1]; Da Cruz, A.D.[1,2]
[1] Replicon Research Center, Pontifical Catholic University of Goiás, Goiânia, Brazil;
[2] Postgraduate Program in Genetics and Molecular Biology, Federal University of Goiás, Goiânia, Brazil
e-mail: calebe_campos26@hotmail.com

Ionizing radiation (IR) is one of the environmental components that causes most cellular stress in complex organisms. Exposure to ionizing radiation induces breaks in nucleic acids, especially, DNA double and single strand breaks. The study aim was to compare the effect of exposure to IR on the formation of microduplications and microdeletions in each chromosome between case and control group of an exposed human population occupationally to ionizing radiation from Cesium-137 during the accident in Goiania. The exposed group consisted of 07 families, of which at least one parent was occupationally exposed to ionizing radiation from Cesium-137, including a total of 25 individuals, do not know the absorbed dose of the military who were occupationally exposed to ionizing radiation. A group of 11 families not exposed to IR was used as control group, totaling 33 individuals with no history of exposure to IR. The genotyping microarray was conducted in CytoScan HD system (Affymetrix®/Thermo Fisher®) then analyzes were performed in ChAS® software. The statistical test used was MannWhitney U. All analyzes were performed using the statistical package SPSS 21.0, with a significance level of 5% ($p <0.05$). Differences in the variance of CNV's mutation frequencies per locus per generation per chromosome observed in the exposed and control groups were statistically significant for chromosomes 5 and 18 for microdeletions. For microduplications, there were no statistical differences. MR_{CNVl} (Mutation rate of CNV's losses) had a greater contribution to elucidate the aesthetic effect of parental exposure to IR.

Keywords: microdeletion, microduplication, ionizing radiation, microarray, 137 CsCl.

Acknowledgments: FAPEG; CNPQ; CNEN.

P51 - GENOTYPIC CHARACTERIZATION OF BRAZILIAN XERODERMA PIGMENTOSUM PATIENTS AND SEARCH FOR FOUNDER EFFECTS

Castro, L.P.[1], Santiago, K.[2], Munford, V.[1], Moura, L.[3], Souza, T.A.[1], Ezquina, S.[4], Valle, F.F.[5], Bertola, D.R.[4], Lajus, T.B.P.[6], Souto, R.[7], Chaibub, S.C.[8], Achatz, M.I.W.[2,9], Menck, C.F.M.[1]

[1] Institute of Biomedical Sciences, University of São Paulo, SP, Brazil; [2] A.C. Camargo Cancer Center, São Paulo, Brazil; [3] Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil; [4] Center for Human Genome and Stem-Cell research (HUG-CELL), University of São Paulo, SP, Brazil; [5] Federal University of Amazonas, AM, Brazil; [6] Advanced Center for Oncology (Cecan), Natal, RN, Brazil; [7] Secretariat of Health, Goiânia, GO, Brazil; [8] General Hospital of Goiania, Goiânia, GO, Brazil; [9] National Cancer Institute, Bethesda, Maryland, USA.

Human syndromes deficient on nucleotide excision repair (NER) or DNA damage tolerance are known to display photosensitivity and/or neurological problems, such as xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). Case reports and genotypic descriptions of patients have been published worldwide, mainly in North America, Europe, Africa and Japan. The follow up of these patients for decades and the study with these cells led to an understanding of what is currently known about the molecular pathways and genetic defects involved in the clinical phenotypes of these syndromes. In Brazil, a few case-reports describe some patients with these phenotypes, and genetic and molecular characterizations are scarce. With the possibility to identify mutations directly with New Generation Sequencing (NGS) techniques, we initiated a project to diagnose the mutations involved in these NER syndromes. Thus, up to now, we identified mutations for 77 Brazilian patients, including 17 XP-V patients from Faina, GO, isolated community (Munford and Castro et al, 2016). Many of these mutations are novel (22/37) and include 33 XPV, 31 XPC, five XPE, three XPA, three XPG and two mutated at XPD gene, one patient with XP and the other with TTD phenotypes. It is important to highlight that 54% of the XP-C patients carry a mutation previously found at Comorian Islands, close to Mozambique in the Indian Ocean (Cartault et al, 2011). The molecular diagnosis of these patients offers not only data for the distribution of mutations of NER patients in Brazil, and genetic counseling, but also gives them and their families the possibility to search for more health and social support.

Key words: NER disorders, Genetic clusters, Brazilian patients.

Financial Support: FAPESP (2018/05216-5, São Paulo, Brazil) and CNPq (Brasília, Brazil).

P52 - PARTICIPATION OF POL IOTA IN THE MECHANISMS OF TRANSLESION SYNTHESIS IN UV-IRRADIATED HUMAN CELLS

Martins, D.J.[1]; Quinet, A.[2]; Menck, C.F.M.[1]
[1] Institute of Biomedical Science, University of São Paulo (ICB-USP), São Paulo, Brazil; [2] Saint Louis University. Missouri, USA
e-mail: davi.martins@usp.br

Ultraviolet (UV) light is a highly carcinogenic agent to which man is exposed, damaging the cellular components including the DNA molecule, resulting in cell death or genome instability. The lesions formed by UV light are usually removed by the nucleotide excision repair pathway (NER). When unrepaired lesions remain until S-phase, translesion synthesis (TLS) is of the mechanisms of tolerance to damage, performed by specific enzymes, the TLS polymerases (pols). This process may occur by the action of one or more TLS pols. Among them, Pol eta (η) is the most studied and its absence leads to a milder phenotype of xeroderma pigmentosum (XP) syndrome, the variant form (XP-V). However, it is not yet known how TLS occurs in the absence of Polη, leaving open an issue about the high frequency of mutagenesis (and skin tumors) in XP-V patients. It is known that Pol iota (ι), another TLS pol, is found together with Polη in replication, but its function in TLS is not yet clear. In this context, the main objective of the present project is to investigate the role of Polι in the bypass of UV-induced photoproducts in NER- deficient human cells. For this, we used cell lines silenced for Polι and/or Polη, previously established in our laboratory. The results obtained so far indicate that Polι may be involved in the TLS of UV lesions in the absence and presence of Polη, and that the absence of both polymerases being extremely harmful.

Keywords: Translesion synthesis (TLS); Pol iota; Pol eta; XP-C cells; UV lesion; NER

Financial Support: FAPESP; CAPES; CNPq (Brazil)

P53 - DNA REPAIR GENES *XRCC1*, *XRCC3* AND *XPD* POLYMORPHISMS AND LARYNGEAL CANCER: THEIR CONTRIBUTION IN THE TUMOR DEVELOPMENT RISK, METASTASIS AND SECOND PRIMARY TUMOR.

Firigato, I.[1], Gonçalves, F.T.[1], Curioni, O.A.[2], De Antonio, J.[1], Silva, M.R.[1], Gattás, G.J.F.[1]

[1] Departamento de Medicina Legal, Etica Medica, Medicina Social e do Trabalho, Instituto Oscar Freire, LIM-40, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, SP, Brazil; [2] Department of Head and Neck Surgery and Otorhinolaryngology, Hospital Heliopolis, Sao Paulo, Brazil.

e-mail: isabela.firigato@usp.br

Laryngeal cancer is the second most common head and neck cancer worldwide becoming a serious global health problem and presents high morbidity and mortality rates. Genetic alterations identified in the genes *XRCC1 (*Base Excision Repair- BER), *XPD* (Nucleotide Excision Repair- NER) and *XRCC3* (*Homologous recombination repair* - HRR) are critical linked to the individual repair DNA damage ability, leading cancer development and its progression. Moreover, there are inconclusive results about the association between those polymorphic genes and the tumor risk, metastasis and second primary tumor in laryngeal cancer. Thus, the aim of this study was the evaluation of a possible association of *XRCC1* (Arg194Trp - rs1799782; Arg399Gln - rs25487), *XPD* (Lys751Gln - rs13181) and *XRCC3* (Thr241Met - rs861539) and a laryngeal cancer risk, metastasis and/or second primary tumor of the disease. A hospital based case-control study was carried out comprising 149 laryngeal cancer patients and in 448 controls from Heliópolis Hospital, São Paulo, Brazil. The polymorphisms were determined by TaqMan SNP Genotyping Assays. We found a decreased risk association of laryngeal cancer in individuals who presented heterozygous genotype (OR 0.63, 95% CI 0.41-0.96) as well as the mutated homozygous genotype (OR 0.29, 95% CI 0.13-0.66) of the SNP *XRCC1*-rs25487 (Arg399Gln). None of the gene polymorphisms was associated with the development of metastasis and/or second primary tumor events. In conclusion, only the variant genotypes (399Gln) of *XRCC1* in homozygous and heterozygous were associated with the decreased laryngeal cancer risk.

Keywords: *XRCC1* Arg399Gln, laryngeal cancer risk, metastasis, second primary tumor.

Acknowledgments: This work is supported by FAPESP grant 2014/16092-4 and LIM40-FMUSP

P54 - CYTOTOXIC AND MUTAGENIC EFFECTS OF BRACHYDIN A, A FLAVONOID, IN NORMAL AND TUMOR PROSTATE CELL LINES

Nunes, H.L.[1], Serpeloni, J.M.[1], Da Rocha, C.Q.[2], Cólus, I.M.S[1]
[1] Department of General Biology, Center of Biological Sciences; State University of Londrina, Londrina, PR, Brazil; [2] Department of Chemistry, Center of Science and Technology, Federal University of Maranhão, São Luis, MA, Brazil.
e-mail: higorlopesn@gmail.com

The gradual increase in cancer cases has reinforced the importance of studying possible agents to prevent or help in the treatment of this disease. Some phytochemicals, chemicals extracted from plants, e.g. Paclitaxel, has been used to treat the cancer. The phytochemical Brachydin A was extracted from the roots of the species *Fridericia platyphylla* (Cham.) L.G.Lohmann. The traditional use of teas prepared from roots of this plant for treatment of kidney stone and arthritis is already known. Brachydin A was evaluated for its possible cytotoxic (MTT, Neutral red and LDH assays) and mutagenic effects (Micronucleus assay) in normal (PNT2) and tumoral (PC-3) prostate cell lines. Nine concentrations of brachydin A (0.24 to 30.72µM) were chosen based on previous studies of our laboratory and were evaluated in the cytotoxic assays. The four highest concentrations were cytotoxic in the neutral red assay for both cell lines. By the MTT assay, some selectivity could be noticed, where only the highest concentration (30.72µM) was cytotoxic in PNT2 against three concentrations (15.36, 24.00 e 30.72 µM) in PC-3. In LDH assay, two concentrations were cytotoxic in PNT2 against three in PC-3. Three non-cytotoxic concentrations were chosen to analyze the mutagenic effect (0.24, 0.96 and 3.84 µM). Brachydin A revealed a mutagenic effect, inducing nucleoplasmic bridges at 0.96µM and micronuclei plus nucleoplasmic bridges at 3.84µM at both cell lines. The results of this research are important to verify the security of traditional teas and maybe, in future, produce medicines based on this compound.

Keywords: Mutagenesis; Phytochemical; LDH assay; Micronucleus

Acknowledgments: This study was supported by FAPESP grant 125796/2016-1, CNPq grant 401516/2016-4 and CAPES/PROAP.

P55 - CANCER RISK LINKED TO BAD LUCK HYPOTHESIS AND EPIGENOMIC MUTATIONAL SIGNATURES

Belizário J.E.[1]

[1] Institute of Biomedical Sciences, Department of Pharmacology, University of São Paulo, São Paulo, SP, Brazil.

e-mail: jebeliza@usp.br

Exposure to pathogen infection, occupational and environmental agents contribute to induction of most types of cancers through different mechanisms. Cancer is defined and characterized by accumulation of mutations and epimutations that lead to changes in the cellular genome and epigenome. Accordingly to a recent "Bad Luck Hypothesis", random error mutations during DNA replication in a small sub-population of stem cells may be implicated in two-thirds of variation of cancer risk in 25 organs and tissues. What determine stem cell vulnerability and risk of malignancy across the spectrum of organs, such brain, bone marrow, skeletal muscles, skin, and liver? Did stem cell pool in particular tissue or organ evolved some critical ability to deal with DNA damage in presence of extrinsic environmental factors? Epigenetic imprinting rather than catastrophic mutational processes may be important factor in dictating the evolutionary trajectory of single cancer cells to multiple cancer clones. Remarkably, Klustein and colleagues (Proc. Natl. Acad. Sci. USA 2017, 114(9), 2230-2234) found the strongest correlation between DNA methylation and lifetime cancer risk ($R = 0.8$, $P < 0.0001$) in normal tissues and organs of people as function of age to 70 year old. In this presentation, it will be discussed on epigenomic mutational cancer signatures that will help is to approximate the probability for relative cancer risk variation between organs and tissues due to heredity or mutagenic factors and evidence of random copy errors in stem cells as origin of cancers.

Keywords: cancer, cancer stem cells, DNA repair, epigenome, cancer risk.

Acknowledgments: This work is supported by CNPq grant 486048/2011 and 312206/2016-0.

P56 - ANGIOTENSIN (1-7) RECEPTOR MAS MODULATION IMPROVES MCF-7 CELLS RESPONSE TO DOXORUBICIN BUT NOT TO MITOXANTRONE

Nogueira LJ[1,2], Grudzinski, P. [1], Saffi J[2], Leguisamo, NM[1,2]

[1] Laboratório de Cardiologia Celular e Molecular, Instituto de Cardiologia/Fundação Universitária de Cardiologia, Porto Alegre, RS, Brazil; [2] Laboratório de Genética Toxicológica, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil

e-mail: laurajnogueira@gmail.com

Renin-angiotensin system (RAS) peptides - mainly mediated by Angiotensin II and its receptors AT1 and AT2- have pleiotropic effects, including neoangiogenesis, proliferation and metastasis of cancer cells. Ang(1-7), another RAS peptide, acts through Mas receptor and is known for counteracting Angiotensin II actions in several physiopathological settings, but its potential as a coadjuvant in cancer therapy has not been explored. Thus, we aimed to evaluate the effect of Mas receptor modulation in breast cancer cells treated with DOX or MTX and the potential mechanisms involved. Breast cancer cells lineage, MCF7, were treated with DOX (0.1 nM and 1nM) or MTX (0.1 µM and 1µM) in combination with an agonist, Ang(1-7) (100Nm) or an antagonist, A-779 (10µM) of Mas receptor for 24 hours followed by 24 hours of washout. MCF-7 cells viability was assessed by MTT assay, cell death induction by Annexin V and 7-AAD detection by Flow Cytometry and migration capacity by Transwell Assay. DNA damage were assessed by comet assay. Stimulation of Mas receptor by its agonist Ang(1-7) increased the cytotoxic effect of DOX and MTX in both doses. Co-treatment of MTX with Ang(1-7) changed death pattern from predominant apoptosis to necrosis in comparison to MTX alone. Co-treatment of Ang(1-7) and MTX reduced MCF-7 migration capacity. These data suggest that the addition of Ang(1-7) in first-line breast cancer treatment may increase the cytotoxic effect of MTX and DOX and prevent its metastatic spread.

Key terms: Breast Cancer, anthracyclines, Mas receptor, Angiotensin (1-7)

Acknowledgments: Work supported by FAPIC/IC-FUC, CAPES and CNPq

P57 - EVALUATION OF THE ANTIPROLIFERATIVE POTENTIAL OF *STRYPHNODENDRON ADSTRINGENS* (MART.) COVILLE IN THE MODEL *ALLIUM CEPA*

da Silva, K.R.X.[1], Almeida, S.S., Santos[1], Y.Q., Caldeira[1], A.J., Bailão[1], E. F. L. C., Ayres[2], F.M.

[1] Universidade Estadual de Goiás – CCET, Anápolis, GO, Brasil; [2] Universidade Estadual de Goiás – Campus Goiânia, Goiânia, GO, Brasil.

e-mail: flavioayres@yahoo.com

Stryphnodendron adstringens (Striph-A) was identified as a cerrado species used by patients on oncological treatment at Hospital Araújo Jorge, Goiânia-GO. In situ monitoring of substances by the Allium cepa test is validated as a bioindicator of cytotoxicity, mutagenicity and anti-mutagenicity, increase and decrease of cell proliferation. By this method, the root has direct contact with the substance tested, which commonly agrees with other genotoxicity tests. Considering that Striph-A may have action on the mitotic index (MI), we evaluated the cellular proliferation index of Striph-A in different concentrations by the Allium cepa test. The analyzes were carried out in Striph-A decoction at concentrations of 20%, 10%, 5% and 2,5% and the negative control with mineral water. In total, 6,000 cells were counted per condition. The observed MI corresponded to 12% (± 3.9), 17% (± 4.7), 15% (± 2.0) and 15% (± 4.6) at concentrations of 20%, 10%, 5% and 2.5%, respectively. Because these MIs are below that observed in the negative control group, we concluded that Striph-A decocts have potential antiproliferative action.

Keywords: Cerrado, medicinal plant, cytotoxicity.

Acknowledgments: This work is supported by Fundação de Amparo à Pesquisa do Estado de Goiás - FAPEG/CAPES

P58 - PALB2 GERMLINE MISSENSE MUTATIONS: FUNCTIONAL EVALUATION THROUGH BRCA1 AND BRCA2 PROTEIN INTERACTION

Gomes, T.T.[1,2], De Gregoriis, G.[1], Suarez-Kurtz, G.[1], Monteiro, A.N.[3], Carvalho, M.A.[1,2]

[1] Nacional Institute of Cancer, Clinical Research Program, Rio de Janeiro, RJ, Brazil; [2] Federal Institute of Rio de Janeiro, Molecular Genetics Laboratory, Rio de Janeiro, RJ, Brazil; [3]H. Lee Moffitt Cancer Center, Tampa, FL, USA.

e-mail: thiago.torres.456@gmail.com

Germline mutations in the *PALB2* tumor suppressor gene are associated with an increased risk of hereditary breast and pancreatic cancers. The PALB2 protein plays a pivotal role in the maintenance of the genomic integrity through homologous recombination (HR)-mediated DNA damage repair. PALB2 works as a scaffold protein that mediates the formation of a complex with BRCA1 and BRCA2 (via its N and C-terminal region, respectively) that promotes RAD51 recombinase activity. While nonsense and frameshift mutations are usually pathogenic due to the great impact in the protein structure and function, missense variants of uncertain significance (VUS) represents a challenge. Curation of literature led to the identification and selection of a set of 44 VUS PALB2 for functional evaluation. The variants were generated by site-directed mutagenesis strategies and the functional evaluation were performed by a mammalian two-hybrid approach using PALB2 N- or C-terminal region as prey and BRCA1 C-terminal or BRCA2 N-terminal as bait, respectively. Our data suggest a pathogenic behavior for 4 PALB2 VUS located in the N-terminal and 6 in the C-terminal region, as they present a consistent impairment in the ability to interact with BRCA1 and BRCA2, respectively. Mutations that compromise the formation of BRCA1/PALB2/BRCA2 complex may confer an increase in cancer risk and its identification is an important approach to help surveillance of high-risk patients. Here we report the first functional analysis of a large set of PALB2 VUS, identifying mutations that lead to functional impairment. However, more studies are needed to define the precise impact of these VUS in cancer predisposition.

Keywords: Hereditary cancer, PALB2, Germline mutations.

Acknowledgments: This work is supported by FAPERJ, CNPq, Ministério da Saúde and Fundação do Câncer.

P59 - GENOTOXIC EFFECTS AND LEVELS OF CATALASE IN ZEBRAFISH (*DANIO RERIO*) EXPOSED TO WATER OF CAVEIRAS RIVER (LAGES, SC)

Neves, G.B.[1], Uczay, M.[1], Rodrigues, R.B.[2], Bandieri, L.O.[3], Vogel, C.I.G.[1]

[1] Department of Animal Production and Food, Program Biochemistry and Molecular Biology, State University of Santa Catarina, Lages, SC, Brazil; [2] Department Animal Science, Program Animal Science, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil; [3] Department of animal production and food, Faculty of Veterinary Medicine, State University of Santa Catarina, Lages, SC, Brazil.

e- mail: gabi_zootec@yahoo.com.br

Caveiras River, located in Lages (SC), is part of the Guarani Aquifer System and source of aquifer recharge. In view of this, it is important monitoring the water quality of this river using biomarkers of genotoxicity and oxidative stress. The objective of this study was to analyze the catalase activity and DNA damage levels in fish (*Danio rerio*) exposed to water of Caveiras River. To evaluate the water quality, 90 fish were used, 45 in control water and 45 in exposure to the river's water. First, both groups were kept for 15 days in control water for acclimatization, after this period (day 0) the experimental group was exposed to the river's water for 21 days to be finally replaced in the control water for more seven days (day 28). It was collected blood and muscles in five period (0, 7, 14, 21 and 28 days). Catalase levels were measured in the lateral muscle of the fish. To evaluate levels of DNA damage in erythrocytes it was used the alkaline comet assay modified with the enzyme foraminopyrimidine-glicosylase (FPG). A significant difference ($p<0.05$) in catalase levels was observed on the seventh day between control and exposed group. For the DNA damage levels it was observed an increase in the frequency of DNA percentage in the comet tails of the exposed group on days 14, 21 and 28. These results indicated that the water analyzed may contain substances with potential to cause genetic damage and water quality must to be monitored more frequently.

Keywords: Comet assay; Oxidative stress; DNA damage.

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P60 - INVESTIGATION OF CYTOTOXICITY AND GENOTOXICITY OF ANTIDEPRESSANT VORTIOXETINE *IN VITRO*

Almeida, I.V.[1], Prazeres, J.A.[1], Vicentini, V.E.P.[1]
[1] Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Maringá, PR, Brazil.
e-mail: igoralmeida.bio@gmail.com

Depression, a common societal problem, is a multifactorial disease characterized by a severe mental disorder that affects about 350 million people worldwide, affected by low productivity, the need for special medical care and the adjustment of their lifestyles. Characterized as one of the main causes of morbidity and mortality in Western countries, due to the coexistence in a society very advanced in technological and social aspects, resulting in a higher collection in terms of productivity, competitiveness and economic response, leaving the emotional relegated. Vortioxetine is a second generation of antidepressant, approved by Anvisa in 2016, with multimodal activity, capable of acting on the pathways of the dopamine, histamine, acetylcholine, glutamate and serotonin neurotransmitters, acting in depression and also in the improvement of problems, with fewer side effects. Cytotoxicity tests are indicated to assess whether the compounds affect cell viability, such as the MTT assay, and those of genotoxicity that identify substances that produce primary DNA damage and mutagenesis, such as the Comet assay. In the present work, these two assays were performed in HepG2/C3A cells (human hepatocarcinoma), evaluating the action of different concentrations of Vortioxetine, calculated from the plasma concentrations of daily doses of 5, 10 and 20 mg. Statistical analyzes showed that the medicine did not reduce cell viability and did not induce DNA fragmentation in the treated groups when compared to the controls. New parameters should be investigated using other assays and cell lines.

Keywords: Brintellix; HepG2/C3A; major depressive disorder.

P61 - CYTOTOXIC EFFECTS OF FLAVONOIDS BRACHYDINS 'B' AND 'C' IN NORMAL AND TUMOR PROSTATE CELLS

Oliveira, L.C.B.[1], Nunes, H.L.[1], Rocha, C.Q.[2], Cólus, I.M.S.[1], Serpeloni, J.M.[1]

[1] Department of General Biology, Center of Biological Sciences; State University of Londrina, Londrina, PR, Brazil; [2] Department of Chemistry, Center of Science and Technology, Federal University of Maranhão, São Luis, MA, Brazil.

e-mail: julianaserpeloni@yahoo.com.br

The increase in the incidence of prostate cancer, high treatment cost, and adverse effects of traditional therapies reinforce the importance of searching new therapeutic approaches, such as the development of chemotherapeutic agents from natural sources. *Fridericia platyphylla* (Cham.) L. G. Lohmann is popularly known as 'cervejinha do campo' and the consumption of teas from their roots is popularly known for the treatment of kidney stone and arthritis. Flavonoids brachydins 'B' and 'C', which differ in only one radical, were evaluated for their effects on cell viability (MTT, neutral red and LDH assays) in normal (PNT2) and tumor (PC3) prostate cell lines at concentrations previous stablished at our lab (0.24 to 30.72 μM). The five highest concentrations (3.74 to 30.72 μM) decreased cell viability in the MTT and neutral red assays in both cell lines. In addition, in the LDH assay brachydin 'B', induced cytotoxicity at the concentration of 0.75 μM in normal cells but not in tumor cells. While MTT and neutral red assays evaluate cell viability, cells with damaged membranes release LDH enzymes into the extracellular medium, a marker for cell death. Our results indicate caution in the traditional consumption of teas obtained from this plant, and suggest further studies investigating the possibility of using the cytotoxic effects of these flavonoids in the possible development of chemotherapeutic agents.

Keywords: cytotoxicity; prostate cancer; brachydin.

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P62 - Mre11, Rad50 AND Nbs1 PEPTIDE SEQUENCES INDICATE MITOCHONDRIAL IMPORT

Muta, L.Y.M.[1], Souza-Pinto, N.C.[1]
[1]Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil.
e-mail: lais.muta@usp.br

In the nucleus DNA double strand breaks are predominantly repaired by the Non-Homologous End Joining and the Homologous Recombination pathways. In mammals, the MRN complex, formed by the Mre11, Rad50 and Nbs1 subunits, binds to the break and stabilizes it for further processing. Rad50 and Nbs1 subunits localization in mammal mitochondria has not been demonstrated. The main route to import mitochondrial proteins involves the translocase of outer membrane (TOM), wherein the Tom20 subunit recognizes specific targeting motifs for imported proteins. Thus, we analyzed the MRN subunits for the presence of canonical mitochondrial targeting sequences and Tom20-recognizing motifs. The mitochondrial transcription factor A (TFAM) and the DNA glycosylase UDG were used as positive controls. The software iPSORT only identified mitochondrial targeting presequences in all TFAM transcripts and in one of the UDG transcripts. The software MitoFate recognized Tom20-binding elements in all proteins, in at least one of the annotated transcripts. Experimental evidences in yeast with chimeric presequences showed that the N-terminal Tom20-binding element efficiently promoted binding of the presequence to Tom20. Therefore, the presence of Tom20-recognizing motifs suggests that the MRN subunits are imported into the mitochondria. According to the literature, a second Tom20 motif enhances the mitochondrial import efficiency. We identified two Tom20-binding elements in at least one transcript of TFAM, UDG and Mre11, which has already been localized in mitochondria, thus suggesting that this approach can predict mitochondrial localization. The possible mitochondrial localization of Rad50 and Nbs1 will be confirmed biochemically.

Keywords: DNA repair; homologous recombination, MRN complex.

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P63 - EVALUATION OF REACTIVE OXYGEN SPECIES INDUCED LESIONS IN NUCLEOTIDE EXCISION REPAIR DEFICIENT HUMAN CELLS FROM PATIENTS WITH DIFFERENT CLINICAL PHENOTYPES

Leandro G.S.[1], Mendes D.[1], Nascimento-Luz, L.S.[1], Menck C.F.M.[1]

[1] Institute of Biomedical Sciences, University of Sao Paulo (USP), São Paulo-SP, Brazil.

e-mail: giovanasl@usp.br

In humans, mutations in genes related to nucleotide excision repair pathway culminate in some syndromes as xeroderma pigmentosum (XP), where patients present as main feature the sensitivity to sunlight, resulting in high frequency of skin cancer. These mutations are also found in diseases where patients present clinical manifestations related to neurodegeneration and premature aging symptoms as Cockayne syndrome (CS) and trichothiodystrophy (TTD). Considering the hypothesis that accumulation of oxidized bases may interfere in the transcription process, we aim to evaluate how human cells holding different mutations in NER genes (that result in diverse phenotypes in relation to premature aging and neurodegeneration (as XP-D XP/CS and CS) will deal with oxidative stress induction. Our results showed that TTD, XP/CS and CS cells are more sensitive to damage caused by agents that induce oxidative stress (such as potassium bromide, hydrogen peroxide and methylene blue) than XP-D and wild type cells measured by cell viability, host cell reactivation and cellular growth. Moreover, we found evidences that such oxidative stress induced damage can be related to accumulation of DNA-RNA hybrids in those cells, especially in those from TTD patients. Therefore, we expect these results will contribute to the comprehension of oxidative stress and DNA repair effects. Our perspective is to establish a better connection among the different clinical phenotypes caused by NER genes mutations, especially those related to neurodegeneration and premature aging. Understanding these processes may help us to understand the process of normal aging.

Keywords: Nucleotide excision repair, oxidative stress, neurodegeneration.

Financial Support: FAPESP, CNPq and CAPES.

P64 - ATR AND ATM DUAL INHIBITION IMPROVES THE ACTIVITY OF TRABECTEDIN AND LURBINECTEDIN BY DISTURBING THE DDR AND HRR

Lima, M.S.[1,2], Bouzid, H.[1], Soares, D.G.[1,3], Selle, F.[3], Morel, C.[1], Galmarini, C.M.[4], Henriques, J.A.P.[2,5], Larsen, A.K.[1], Escargueil, A.E.[1]
[1] Sorbonne Universités, UPMC Univ Paris 06, INSERM UMR_S938, Centre de Recherche Saint-Antoine, Paris, France; [2] Departamento de Biofísica/Centro de Biotecnologia, UFRGS, Porto Alegre, Brazil; [3] Medical Oncology Department, Hospital Tenon, Public Assistance Hospitals of Paris (AP-HP), Alliance Pour la Recherche En Cancérologie (APREC), Paris, France; [4] Cell Biology Department, PharmaMar, Poligono Industrial La Mina, Madrid, Spain; [5] Instituto de Biotecnologia, UCS, Caxias do Sul, RS, Brazil
e-mail: michelle.cacoal@gmail.com

Trabectedin (Yondelis®, ecteinascidin-743, ET-743) is a marine-derived natural product approved for treatment of advanced soft tissue sarcoma and relapsed platinum-sensitive ovarian cancer. Lurbinectedin is a novel DNA minor groove binder structurally related to trabectedin. The structural variation of lurbinectedin is accompanied by important modifications of the pharmacokinetic and pharmacodynamic properties in cancer patients although the preclinical activities of this drug remain close to those observed for trabectedin. Both ecteinascidins generate DNA double-strand breaks that are processed through homologous recombination repair (HRR), thereby rendering HRR-deficient cells particularly sensitive. Until now, no strategy has been evaluated to inhibit or to perturb this repair pathway although this approach is likely to improve the activity of ecteinascidins by mimicking HRR deficiency. In this study, we characterize the DNA damage response to trabectedin and lurbinectedin in human carcinoma cell lines. Our results show that trabectedin and lurbinectedin activate the ATM/Chk2 (ataxia-telangiectasia mutated/checkpoint kinase 2) and ATR/Chk1 (ATM and RAD3-related/checkpoint kinase 1) pathways. Pharmacological inhibition of Chk1/2, ATR or ATM is not accompanied by any significant improvement of the cytotoxic activity of the ecteinascidins. Interestingly, simultaneous inhibition of both ATM and ATR strongly potentiates the activity of both ETs against human cervical and ovarian carcinoma cells by efficiently blocking the foci formation of HRR proteins following exposure to ecteinascidins, resulting in extensive chromosome damage. Together, our data identify ATR and ATM as central coordinators of the DDR to ecteinascidins and provide a mechanistic rationale for combining these compounds with ATR and ATM inhibitors.

Keywords: DNA double strand breaks; DNA alkylators; DNA replication; Homologous recombination; Checkpoint abrogators.

Acknowledgments: This work was supported by CAPES/COFECUB (French-Brazilian collaborative research grant No. 583/07) and PharmaMar.

P65 - DIFFERENT SENSITIVITY TO DNA DAMAGE OF IPS AND NEURON PROGENITOR CELLS DERIVED FROM COCKAYNE SYNDROME PATIENTS

Luz-Nascimento LS[1], Vessoni AT[2], Muotri A[3] and Menck CFM[1]

[1] Institute of Biomedical Sciences, University of São Paulo, USP - São Paulo, São Paulo, Brazil; [2] Washington University in Saint Louis - St. Louis, Missouri, USA; [3] University of California San Diego, School of Medicine - San Diego, California, USA

E-mail: livialuz307@gmail.com

Cockayne Syndrome (CS) is characterized by neurological abnormality, neurodegeneration, growth failure and photosensitivity. Patients are deficient in CSA or CSB proteins, which are part of the transcriptional coupled nucleotide excision repair pathway and their roles in neural impairment are not fully understood. Skin fibroblasts (primary or transformed) are the main models to study this syndrome, but they do not fully represent the complexity of the – most affected - neural tissue. Here, using reprogrammed fibroblasts into induced pluripotent stem cells (iPSCs) and iPSC-derived neural precursor cells (NPC), we characterized their susceptibility to DNA damage agents: ultraviolet light C (UVC) and potassium bromate (KBrO3) – a well described oxidative stressor. We analyzed cellular viability, cell death, caspase-3 activation (apoptosis signaling), PARP-1 activity and protein carbonylation. As expected, all CS cells – fibroblast, iPSC and NPC - were more sensitive to UV irradiation than proficient TC-NER cells. Surprisingly, only CS iPSC were sensitive to KBrO3, while no difference in sensitivity was detected for their isogenic fibroblasts. Moreover, the use of N-acetylcysteine (NAC), a glutathione precursor and important antioxidant, improves cellular viability to oxidative stress on iPSC. However, NPC seems to have little, if any, increase in sensitivity to oxidative stress caused by KBrO3. We believe these results are an initial step to better understand the CS phenotype and propose the use of these models to study DNA damage effects in this syndrome's neuronal precursors cells and possibly differentiated neural tissue.

Keywords: Cockayne syndrome, neurodegeneration, oxidative stress, DNA lesion, DNA repair, n-acetylcysteine, NAC

Financial support: FAPESP, CAPES and CNPq.

P66 - TRANSCRIPTIONAL PROFILE OF COCKAYNE SYNDROME CELLS AFTER OXIDATIVE DNA DAMAGE CHALLENGE

Maira Rodrigues de Camargo Neves[1], Livia Luz Souza Nascimento[1], Alexandre Teixeira Vessoni[2], Carlos Frederico Martins Menck[1]

[1] Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo/SP, Brazil; [2] Department of Medicine, Washington University in Saint Louis, Saint Louis/MO, USA

e-mail: maira.rn@gmail.com

DNA repair pathways are critical to the preservation of the cell's genome. One of the most studied pathways present in eukaryotic cells is nucleotide excision repair (NER). This pathway branches to two sub pathways: global genome repair (GGR), which repairs bulky lesions on the DNA throughout the genome, and transcription-coupled repair (TCR), which although faster than GGR, only affects actively transcribed regions of the genome. TCR is recruited after RNA polymerase II is stalled by a lesion on the DNA, and the proteins CSA and CSB play a major role in this recruitment. Mutations on those genes can cause a human syndrome called Cockayne Syndrome (CS), with severe neurological involvement and progeroid clinical features. RNA synthesis arrest after UV irradiation is a defining cellular phenotype in CS. Skin lesions are common in CS patients after sun exposure, however nervous tissues are not exposed to UV irradiation to justify the neurological symptoms. It is possible that DNA damage agents other than UV are affecting the nervous system in CS. In the present work, we investigate the transcriptional profile of pluripotent stem (iPS) CS cells after treatment with Potassium Bromate, which induces oxidative stress in the cell, causing oxidative damage in the DNA such as 8-oxoguanine. RNAseq experiments were performed on CS and wild type iPS cells, targeting poly-A positive mRNA. No differentially expressed transcripts were found on wild type cells after treatment (q-value < 5%), however more than 100 genes were differentially expressed in CS, including evidence of alternative splicing.

Keywords: Transcriptomics, Cockayne syndrome, transcription-coupled repair, iPS cells.

Funding Agency: CAPES and FAPESP (Brazil).

P67 - ALKYLADENINE DNA GLYCOSYLASE (AAG/MPG) IS NECESSARY FOR UPR ACTIVATION IN RESPONSE TO ALKYLATING AGENTS

Milano L[1,2], Andreguetti R[1], Thome MP [2], Samson LD [3], Lenz G [2], Henriques JAP[2], Nohturfft A [4], Meira LB [1]

[1] Department of Clinical and Experimental Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK; [2] Department of Biophysics, UFRGS, Porto Alegre, Brazil; [3] Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, USA; [4] Molecular and Clinical Sciences Research Institute, St. George's University of London, London, UK

e-mail: milano.lari@gmail.com

Alkylating agents are widely used in cancer chemotherapy, so a better understanding of the cellular responses to alkylation-induced damage could lead to improvements in cancer treatment. To investigate cellular outcomes to alkylation, gene expression changes in response to the alkylating agent methyl methane sulfonate (MMS) were quantified and compared in mice deficient or proficient for alkyladenine DNA glycosylase (Aag/Mpg), the enzyme initiating base excision repair (BER) of alkylated DNA bases. MMS exposure resulted in a robust transcriptional response in mouse liver of Aag proficient animals, but Aag deficient mice showed a diminished response that was also unique, with minimum overlap between genotypes. Analysis of transcriptional networks associated with MMS exposure identified an Aag-dependent expression of unfolded protein response (UPR). The UPR is an adaptive signal transduction pathway orchestrated by the ER and important for the maintenance of a functional proteome. We next used human cell lines expressing different AAG levels to investigate the mechanism underpinning alkylation-induced UPR activation. AAG is required for UPR induction as evidenced by the fact that alkylation-induced expression of the chaperone BiP and activation of the transcription factor XBP1 was suppressed in AAG knock-down cells. In addition, AAG expression influences cell survival upon alkylation and ER stress induction. Taken together, these findings uncover a new role for AAG coordinating cellular responses to alkylation-induced damage. Importantly, our results suggest a rationale for a novel cancer therapeutic strategy combining alkylating agents to pharmacological activators of the UPR.

Keywords: alkylating agents, AAG/MPG, unfolded protein response.

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P68 - DIFFERENTIAL EXPRESSION OF NEK1 IN GLIOBLASTOMA CELLS

Morás, A.M.[1], Steffens, L.[1], Moura, D. J.[1]
[1] Toxicology Genetics Laboratory, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil.
e-mail: anamoiramoras@gmail.com

Gliomas are primary cancer which represent 80% of all malignant CNS tumors. The class IV gliomas, known as Glioblastoma Multiforme (GBM), are highly prevalent among all gliomas type (54.7%), being the most aggressive form, with worse prognosis. The low survival and absence of alternatives to standard chemotherapy cycles based on Temozolomide (TMZ) administration justify the need to better understand the molecular signature and the nature of the resistance, as strategy in search of new targets for a more effective GBM treatment. Nek1 protein has been associated with tumor resistance in glioma samples, showed an overexpression directly related to the degree of tumor severity, proliferation rate and resistance to TMZ. Moreover, the patient samples analyses indicate a correlation with survival prognosis. This protein has been associated with DNA damage response, however, there isn't description of how this protein could be associated with tumor severity an/or resistance. Thus, the aim of this work was to evaluate the role of Nek1 in response to DNA damage induced by co-treatment of TMZ and a radiomimetic drug (RD) in glioblastomas cells. Our results of sensitivity and expression profile performed on T98G and U87 demonstrated that Nek1 was differentially expressed after exposure to TMZ and RD. Additionally, immunofluorescence results indicated a shift of Nek1 to the nucleus, which was correlated with the phosphorylation of H2AX. These results indicate that Nek1 protein is involved in the response to TMZ and RD, but its role is still under investigation.

Keywords: DNA damage, Nek1, glioblastoma.

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P69 - MUTATIONAL PROFILE OF SKIN TUMORS FROM XERODERMA PIGMENTOSUM (XP-V) PATIENTS.

Vilar, J.B.[1]; Corradi[1], C.; Souza, T.A.[1]; Castro, L.P.[1]; Carvalho, F.; Chaibub, S.C.W.; Menck, C.F.M.[1]

[1]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.

e-mail: jbvilar@usp.br

Skin cancer (SC) is the most common form of cancer in the Caucasian population. Therapy options include both surgical and non-surgical modalities, even though surgical approaches are most commonly used to treat these tumors. Xeroderma Pigmentosum (XP) is an autosomal recessive disease associated with high UV-sensitivity and a 1000-fold increased risk of developing cutaneous neoplasms compared to the general population. The management of XP patients is a difficult therapeutic challenge, since they generally become maimed due to the high number of surgeries they undergo. Nowadays, there is no specific non-surgical treatment or prophylaxis (except a total sun protection) for such kind of patients. To understand the genetic profile that leads XP patients to such severe phenotype and the differential genetic panel that characterizes their tumors, we performed the whole exome sequencing of 11 SC samples from different XP-V patients from Araras, GO, where two founder mutations are responsible for a high frequency of this disease. The samples were analyzed in relation to its mutation signature and context. The most prevalent mutation in all tumors was the transition C>T, which is the canonical UV induced signature. These results highlight the main role of UV-induced pyrimidine dimers in skin carcinogenesis in XP-V patients, regardless of the cancer type (melanoma or non-melanoma). Another major contributor were the C>A transversions, possibly in consequence to UVA-induced oxidated damage. The third most common pattern found were the T>C transition, which can be a result of the exposure to certain inflammatory environments, such as NOS3. We can conclude the exposure to UV light is the major factor for the mutagenesis found in the tumoral samples, being in complete agreement with the environmental exposure expected for the skin.

Keywords: Xeroderma Pigmentosum, Skin Cancer, Next Generation Sequencing; UV signature; DNA repair; DNA damage.

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P70 - **UVA LIGHT ACTIVATE DNA DAMAGE RESPONSE AND PROMOTE DELETERIOUS AND MUTAGENIC EFFECTS IN XP VARIANT HUMAN CELLS**

Moreno NC[1], Souza TA[1], Garcia CCM[2], Munford V[1], Rocha CRR[1], Castro LP[1], Corradi, C[1], Quintero, NR[1], Ienne, S[3] and Menck CFM[1].

[1] Institute of Biomedical Sciences, University of Sao Paulo (USP), Sao Paulo, SP; [2] NUPEB & Biological Sciences Department, Federal University of Ouro Preto, Ouro Preto, MG; [3] Core Research Support Facility (CEFAP-USP), University of Sao Paulo (USP), Sao Paulo, SP, Brazil.

E-mail: ncmoreno@usp.br

UVA light induces DNA damage and participate in skin tumor formation. The role of DNA polymerase eta (pol eta) in protecting against sunlight-induced tumors is evidenced in Xeroderma Pigmentosum Variant (XP-V) patients, who present increased frequency of skin cancer. However, the role of UVA-light in carcinogenesis of these patients is not completely understood. Working with XP-V and pol eta complemented cells, UVA (120 kJ/m2) induced DNA damage but lesion removal was particularly affected in XP-V cells, possibly due to the oxidation of proteins. UVA irradiation promoted replication fork stalling and cell cycle arrest in the S-phase for XP-V cells. The participation of ATR kinase in protecting XP-V cells after receiving UVA (20 kJ/m2) was demonstrated by Chk1 protein phosphorylation and the use of a specific inhibitor promoted a remarkable increase in sensitivity and genotoxic stress. Interestingly, when cells were treated with the antioxidant N- acetylcysteine (NAC), all these deleterious effects were consistently reverted, revealing the role of redox process in these processes. Moreover, whole exome sequencing of cloned UVA-irradiated cells showed CPD is the main type of lesion responsible by UVA mutagenesis in XP-V cells as demonstrated by increase of mutations (C>T) in pyr-pyr sequences. Also, G>T mutations (8-oxoG signature) was increased at basal level in XP-V cells, indicating the participation of oxidized bases in spontaneous mutations in these cells. Finally, we confirmed the participation of pol eta in controlling UVA-induced mutagenesis and in addition, we revealed a previously unreported pol eta function regarding the redox process control.

Keywords: UVA light, XP-V patients, redox process, DNA damage, ATR pathway, N-acetyl cysteine, mutagenesis.

Financial support: FAPESP, CNPq and CAPES.

P71 - CHK1 IS A POTENTIAL THERAPEUTIC TARGET FOR BRAF INHIBITOR-RESISTANT MELANOMA

Gonçalves de Carvalho D.[1*], Kenski J.C.N.[2*], Peeper D.S.[2], Possik P.A.[1]
[1] Brazilian National Cancer Institute, Rio de Janeiro, RJ, Brazil; [2] The Netherlands Cancer Institute, Amsterdam, NH, The Netherlands.
[*] Authors equally contributed to this work
e-mail: ppossik@inca.gov.br

Melanoma is a highly aggressive type of skin cancer. BRAF and MEK inhibitors have revolutionized melanoma treatment but resistance is a major clinical challenge. Checkpoint kinase 1 (Chk1) has an important role in DNA damage response and cell cycle checkpoint activation and is currently being explored as a therapeutic target in cancer. This study aimed to investigate the role of Chk1 and its potential as a therapeutic target for BRAF inhibitor-resistant (BRAFiR) melanomas. We demonstrate that BRAFiR melanoma cells are more sensitive to Chk1 inhibitor (Chk1i) than treatment-naïve cells. Upon Chk1 inhibition, melanoma cells accumulate in S-phase and show impaired BrdU incorporation. This is more pronounced in BRAFiR cells and is accompanied by increased levels of phosphoRPA32$^{S4/6}$ and γH2AX. Fluorescence ubiquitination cell cycle indicator (FUCCI) labelling and time-lapse microscopy show that, while treatment-naïve cells continue cycling upon Chk1 inhibition, BRAFiR cells appear to lose viability during S/G2/M phases. The remaining cells fail to complete cell division and become polyploid. DNA Combing assay shows stalling of DNA replication and increased origin firing after Chk1 inhibition in both BRAFiR and treatment-naïve cells. However, even in the absence of Chk1i, BRAFiR cells have a significant increase in origin firing. In conclusion, we show that BRAFiR melanomas are hypersensitive to Chk1 inhibition. This vulnerability may be related to a more pronounced Chk1 inhibitor-induced replication stress and failure of BRAFiR cells to recover from the resulting DNA damage. Our study may contribute to the identification of resistance biomarkers and more efficient therapeutic approaches for melanoma.

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P72 - REDEFINING REPLICATION STRESS RESPONSE PATHWAYS IN BRCA1-DEFICIENT CELLS

Quinet, A[1], Tirman, S.[1], Jackson, J.[1], Gonzalez, D.[2], Mendez, J.[2], and Vindigni, A[1]
[1] Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis MO, 63104; [2] Spanish National Cancer Institute, Madrid, Spain
e-mail: annabel.quinetdeandrade@health.slu.edu

BRCA1-mutant cancer patients are often treated with platinum-based drugs, such as cisplatin, to create replication-blocking lesions and induce extended replication fork degradation mediated by the MRE11. However, this fork degradation phenotype was established with a single-dose treatment, neglecting the fact that patients are treated with multiple cisplatin doses in a clinical setting. We found that fork degradation is no longer detectable in *BRCA1*-deficient cells treated with cisplatin 24 hours after pre-exposure to this drug. Cisplatin pre-exposure upregulates and increases chromatin loading of PRIMPOL, a protein with primase and polymerase activity, but not of other canonical translesion synthesis polymerases, such as POLη, REV1, or REV3L in *BRCA1*-mutant cells. By combining electron microscopy and single-molecule DNA fiber approaches, we found that PRIMPOL induction rescues fork degradation by virtue of its unique *de novo* priming activity and leads to accumulation of ssDNA gaps both at replication fork junctions and behind them. ssDNA gap accumulation triggers RPA recruitment, leading to exhaustion of the RPA pool and replication catastrophe. Our findings challenge the notion that DNA-damaging drug-sensitivity originates from the extended replication fork degradation observed after a single-dose treatment. Reversed forks are the entry point for MRE11 in *BRCA1*-deficient cells and suppressing fork reversal by RAD51 depletion prevents fork degradation. In addition to suppressing fork reversal, we found that RAD51 depletion leads to ssDNA gap accumulation on ongoing replication forks, mirroring PRIMPOL induction. We propose a model where PRIMPOL upregulation rescues fork degradation by reinitiating DNA synthesis past DNA lesions, while suppressing replication fork reversal.

Keywords: damage tolerance, DNA degradation, repriming, fork reversal

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P73 - MOLECULAR CHARACTERIZATION OF ENDOMETRIAL TUMORS FOR THE PROFICIENCY OF THE DNA-MISMATCH REPAIR SYSTEM: A UNIVERSAL SCREENING FOR LYNCH SYNDROME

Rosa, R. C. A[1], Chahud, F.[2], Silva, A. R.[2], Silva-Júnior, W. A.[1], Ferraz, V. E. F.[1]
[1] Department of Genetics; [2] Department of Pathology and Legal Medicine Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.
e-mail: reginaldoallves@usp.br

Endometrial cancer (EC) is the most common gynecologic malignancy in the world. About ninety percent of the cases of EC are sporadic and the remaining cases are associated with genetic factors. Lynch syndrome (LS), a genetic disorder of hereditary predisposition to cancer, is responsible for a large portion of cases of hereditary CE. Tumors developed in LS carriers are generally deficient of the DNA mismatch repair system (MMR). Thus, we aimed to characterize endometrial tumors for the proficiency of the MMR system in a Brazilian series of unselected EC. Cases of EC were characterized for the proficiency of the MMR repair system and the molecular data were correlated with clinicopathologic characteristics. A total of 127 tumors were characterized for the MMR proficiency and were classified as: Methylated MMR-deficient (M-dMMR), Non-Methylated MMR-deficient (NM-dMMR) and MMR-proficient (pMMR). 17/127 (13.39%) tumors were classified as M-dMMR; 52/127 (40.94%) as NM-dMMR and 58/127 (45.67%) endometrial tumors were classified as pMMR. The presence of tumor invasion in the angiolymphatic region was significantly ($p = 0.006$ - Chi-square test) associated with the MMR repair system deficiency. M-dMMR tumors exhibited angiolymphatic invasion more frequently than the NM-dMMR tumors ($p = 0.002$ - Chi-square test) and more frequently than pMMR tumors ($p=0.0129$ - Chi-square test). No statistical difference was observed between the NM-dMMR and the pMMR endometrial tumors. The analysis of germline mutations in genes associated with Lynch syndrome is strongly recommended for women whose endometrial tumors were characterized as being deficient in the MMR repair system.

Keywords: Endometrial cancer. DNA Mismatch repair. Lynch Syndrome.

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P74 - THE PROTECTING ROLE OF XPG ENDONUCLEASE IN REDOX STRESS INDUCED BY HYDROGEN PEROXIDE

Davi Mendes[1], Giovana da Silva Leandro[2], Pilar Tavares Veras Florentino[2], Clarissa Ribeiro Reily Rocha[2], Carlos Frederico Martins Menck[2]
Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo.
e-mail: davi2.mendes@usp.br

Mutations in genes related to nucleotide excision repair (NER) in the majority cases. Culminates in two human syndromes such as Xeroderma Pigmentosum (XP) and Cockayne syndrome. Patients portraying a mutation in that truncates the NER-endonuclease XPG (ERCC5) develop a syndrome that is composed by the UV hypersensitivity of Xeroderma Pigmentosum added by the neurodegeneration and premature aging characteristics from Cockayne syndromes, called XP/CS. Otherwise, mutations that inactivates the endonuclease activity cause only the Xeroderma Pigmentosum syndrome. It was demonstrated by several groups that, XP/CS cells have increased death after redox stress, while the XP cells does not demonstrate the same behaviour. Thus, our goal is to verify the role of XPG endonuclease in redox protection.. For that, we over expressed the wild type XPG allele in immortalized cells from a patient with XP/CS and whether the complementation could improve the response to reactive oxygen species (ROS). After re-expressing XPG, we observed an increased in cell viability and a decrease in sub-G1 population after ROS induction by hydrogen peroxide as well was observed in response to UVC induced damage. Moreover, we observed an increase on repair capacity in lesions caused by ROS from photoactivated methylene blue. Together these data confirms that XPG protein is important to deal with DNA lesions provoked by ROS, preventing cell death.

Keywords: XPG, redox stress, XP/CS

Funding Agency: FAPESP and CAPES

P75 - ROLE OF THE DNA POLYMERASE KAPPA IN THE REGULATION OF THE KINASE CHK1

Dall'Osto, M. [1 2], Pierini, L.[2], Hoffmann J.S.[1], Pillaire M.J.[1],
[1]Cancer Research Center of Toulouse, INSERM Unit 1037; University Toulouse 3-CNRS ERL 5294; 2 avenue Hubert Curien, CS 3717, 31007 Toulouse
[2]*Equally contributing authors*
e-mail: marina.dall-osto@inserm.fr

The replication of the genome is constantly challenged due to endogenous or exogenous fork barriers leading to fork stalling which is a cause of replicative stress (RS) [1]. When stalled forks fail to restart, it can give rise to DNA breaks or chromosomal rearrangements. To prevent this genetic instability, the fork blockage is signalled by a set of proteins during the S phase trough the ATR/Chk1 pathway. We now provide data showing that in addition to its role at the stalled forks [2], Pol Kappa is also involved to maintain the pool of Chk1. We observe that the depletion of Pol Kappa induces a Chk1 protein level decrease in mice and mammalian cells. Since the pool of Chk1 in the nucleus supports the genetic stability, we focused on the impact of Pol Kappa on the Chk1 protein level in the nuclear compartment. We proved that this regulation is specific to Pol Kappa among the Y-family of specialized DNA polymerases and does not depend of exogenous RS since this regulation also happens in unperturbed cells. By different approaches, we show that Pol Kappa belong to the same complex. Here, we provide data carried out by DNA spreading and showing defects in the forks restart after RS when Pol Kappa or Chk1 are depleted by RNA interference. The expression of ectopic Chk1 can rescued the fork restart in both cases arguing that Pol kappa and Chk1 work in the same pathway. Taken together, these findings lead us to propose a model in which Pol Kappa maintains a reservoir of Chk1 in the nucleus making cells ready to answer to stress induced by the replication forks barriers.

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Participants of the VI Fundamental Aspects in DNA Repair and Mutagenesis- VI FARM-DNA

Name	e-mail
Alain Sarasin	alain.sarasin@gustaveroussy.fr
Alessandra Luíza Pelegrini	aleepelegrini@gmail.com
Alessandro Vindigni	alessandro.vindigni@health.slu.edu
Alexandre Ludmil	l2alexandrov@ucsd.edu
Alexandre Teixeira Vessoni	alexandre.vessoni@wustl.edu
Aline Amorim	aline.amorim@usp.br
Allyson Muotri	muotri@ucsd.edu
Amanda Soares Hirata	amandashirata@gmail.com
Ana Carolina Silva Santos	acss.1303@gmail.com
Ana Maria Barbuena	anybarbuena@gmail.com
Ana Moira Morás	anamoiramoras@gmail.com
André Luiz Ventura Sávio	savio.alv@gmail.com
André Uchimura Bastos	uchibastos@yahoo.com.br
Andres Aguillera	aguilo@us.es
Anna Beatriz Ribeiro Elias	annaribeiro.elias@gmail.com
Annabel Quinet	annabel.quinetdeandrade@health.slu.edu
Antônio Vinícius de Assis	antoniiofs@gmail.com
Augusto Akira Mori	augusto.akira.mori@usp.br
Barbara Colatto Fernandes	barbara.colatto@gmail.com
Bárbara Luísa Soares	barbara-luisa@hotmail.com
Ben Van Houten	vanhoutenb@upmc.edu
Bernard Lopes	bernard.lopez@gustaveroussy.fr
Bruce Demple	bruce.demple@stonybrook.edu
Bruna Frielink Immich	brunaimmich@gmail.com
Bruno Repolês	marcalrepoles@yahoo.com.br
Bruno Resende	bruno_carvalho_resende@hotmail.com
Calebe Bertolino Marins de Campos	calebe_campos26@hotmail.com
Camila Carrião Machado Garcia	carriao@ufop.edu.br
Camila Corradi	camilacorradi@gmail.com
Camila dos Santos Xavier	camiladsxavier@gmail.com
Carlos Menck	cfmmenck@usp.br
Carlos Renato Machado	crmachad1967@gmail.com
Catarina S. Takahashi	cstakaha@usp.br
Catarina Satie Takahashi	cstakaha@usp.br

Clarissa Rocha	calsmah@usp.br
Daisy Salvadori	dfavero@fmb.unesp.br
Danielle Batista	daniellepbatista@gmail.com
Danilo Batista Veira de Melo	danilobio@usp.br
Davi Jardim Martins	davi.martins@usp.br
Davi Mendes	davimendes4@gmail.com
Debby Roubicek	droubicek@sp.gov.br
Deborah Roubicek	debby.roubicek@gmail.com
Dinara Jaqueline Moura	dinjamoura@gmail.com
Eduardo Padilha Antonio	eduardo.padilha@usp.br
Elza Tiemi Sakamoto	etshojo@usp.br
Elza Tiemi Sakamoto-Hojo	etshojo@usp.br
Fabio de Mello	fabionunesmello@gmail.com
Fabio Forti	flforti@iq.usp.br
Fabrícia Heloísa Cavicchioli Sugiyama	fabricia.sugiyama@gmail.com
Felippe Truglio Machado	felippetruglio.m@gmail.com
Fernanda Rowies	fer.rowies@gmail.com
Filipo Rosselli	filippo.rosselli@gustaveroussy.fr
Flávio Ayres	flavioayres@yahoo.com
Franciele Faccio Busatto	franbusatto@gmail.com
Francisco Meirelles Bastos de Oliveira	fmboliveira@gmail.com
Gabriela Maciel Lopes	gabrielamaciel.gml@gmail.com
Gabriela Reis	gabrielaboltesreis@gmail.com
Gabriella Bassi Neves	gabi_zootec@yahoo.com.br
Giovana da Silva Leandro	giovanabiousp@gmail.com
Giovana Fioravante Romualdo	gi_romualdo@hotmail.com
Giovanna Napolitano	giovanna.napolitano77@gmail.com
Glaucia Regina Martinez	grmartinez@ufpr.br
Gustavo Kajitani	gustkajitani@gmail.com
Gustavo Siconello dos Santos	gustavo_siconello@hotmail.com
Helena Gloria	helenadecastroegloria@gmail.com
Higor Lopes Nunes	higorlopesn@gmail.com
Igor Vilela de Sousa	igorcefet14@gmail.com
Igor Vivian Almeida	igoralmeida.bio@gmail.com
Isabela Firigato	isabela.firigato@usp.br
Jan Hoeijmakers	j.hoeijmakers@erasmusmc.nl
Jean Yves Masson	jean-yves.masson@fmed.ulaval.ca

Jeferson Gustavo Henn	jefersonghenn@gmail.com
Jenifer Saffi	jenifer.saffi@gmail.com
Jessica Degaspare	jdegaspare@gmail.com
Jéssica Ellen Barbosa de Freitas Lima	jessicaellen.lima@usp.br
Jessica Farias	jessicafarias@usp.br
Joana Alves Teixeira	joanaifsp@gmail.com
Johnathan Andrade Vieira	vieirajohnathan@usp.br
Jonatas Alécio Prazeres	jonatasap2008@hotmail.com
Jose Belizario	jebeliza@usp.br
José Djaci Augusto	djaciaugusto8@gmail.com
José Renato Cussiol	jrcussiol@gmail.com
Juliana Bradstetter Vilar	jbvilar@usp.br
Juliana da Silva	juliana.silva@ulbra.br
Juliana Mara Serpeloni	julianaserpeloni@yahoo.com.br
Juliano de Oliveira Silveira	juliano2309@gmail.com
Keith W. Caldecott	k.w.caldecott@sussex.ac.uk
Laís Bianchoni Manoel	laisbima@hotmail.com
Laís Yoshie Morikawa Muta	lais.muta@usp.br
Larissa Milano de Souza	milano.lari@gmail.com
Larissa Piassi	larissa.piassi@gmail.com
Laura Nogueira	laurajnogueira@gmail.com
Leandra Ernst Kerche Silva	leakerche@gmail.com
Leonardo Karran Teixeira	lkteixeira@inca.gov.br
Leonardo Menezes Souza	menezeslcs@gmail.com
Leticia Pinto	ll.eticiapinto@gmail.com
Ligia Castro	licastro@usp.br
Lívia Luz Souza Nascimento	livialuz307@gmail.com
Lorena Ferreira	l.orena@hotmail.com
Lucas Falcão Monteiro	lucasfalcaomonteiro@hotmail.com
Lucas Henrique Nascimento Silva Rodrigues	lhnsr@hotmail.com
Lucas Peres	lucas.peres@fm.usp.br
Luciana Rodrigues Gomes	luciana.gomes@usp.br
Lucymara F Agnez Lima	lfagnez@gmail.com
Lucymara F. Agnez Lima	lfagnez@ufrnet.br
Luis Batista	lbatista@wustl.edu
Luis Mariano Polo	l.m.polo@sussex.ac.uk
Luiz Eduardo da Silva	luiz_silva@usp.br

Maiara Piva	maiarapiva@gmail.com
Maira Neves	maira.rn@gmail.com
Manuel Munoz	manolo2003@gmail.com
Marcela Teatin Latancia	marcela.latancia@gmail.com
Marcelo Alex de Carvalho	marcelo.carvalho@ifrj.edu.br
Marco Batista	marco.batista@usp.br
Marco Foiani	marco.foiani@ifom.eu
Marcos Chiaratti	chiarattimr@gmail.com
Marcos T Oliveira	mtoliveira@fcav.unesp.br
Maria Carolina Elias	carolina.eliassabbaga@butantan.gov.br
Mariana Simões Ferreira	marianasimoesf@gmail.com
Mariana Stinglin Rosa Ribas	nanastinglin@yahoo.com.br
Marina Andrade Tomaz	mndrd05@gmail.com
Marina Dall'Osto	m.dallosto1@gmail.com
Marina Storti Corbani	maricorbani@gmail.com
Marina Tenório Botelho	marinatenoriobotelho@gmail.com
Mateus Prates Mori	mori_mateus@yahoo.com.br
Mateus Priolo Grejo	mateus.grejo@usp.br
Matheus Becker	matheusbeckerfreitas@gmail.com
Matheus Hikaru Tanimoto	tanimoto@usp.br
Matheus Silva	matheusmolina95@gmail.com
Michelle de Souza Lima	michelle.cacoal@gmail.com
Misaki Sittoni	misakicristine@gmail.com
Nadja Cristhina de Souza Pinto Lardner	nadja.lardner@gmail.com
Natália Cestari Moreno	nacestari@hotmail.com
Natalia Leguisamo Meirelles	nmleguisamo@gmail.com
Nathalia Quintero Ruiz	natalita26@gmail.com
Nicolas Carlos Hoch	nicofar@gmail.com
Patrícia Abrao Possik	ppossik@inca.gov.br
Patricia Kannouche	patricia.kannouche@gustaveroussy.fr
Paula Andrea Marin Muñoz	pamm0414@gmail.com
Paula Pellenz Tomasini	paula-tomasini@hotmail.com
Peter de Keizer	peterdekeizer@gmail.com
Phillipe Franklin Magalhães	phillipefcm@gmail.com
Pilar Veras	pilar.veras@gmail.com
Raphael Pavani	rapavani@gmail.com
Raysssa Feitosa	ray_melofeitos@hotmail.com

Rebeca Alencar	rralen@icloud.com
Rebeca Buenos Alves	rebecabuenoalves@gmail.com
Reginaldo Rosa	reginaldoallves@usp.br
Renan Pongilio	renanpaschoalinop1998@gmail.com
Renata Barbosa Vahia de Abreu	rbvabreu@gmail.com
Renato Sampaio Carvalho	rscarvalho@pharma.ufrj.br
Roberto Trivelato Júnior	robertotrivelatojr@yahoo.com.br
Rodrigo de Almeida	drigo_jau@hotmail.com
Rodrigo Galhardo	rgalhard@usp.br
Rodrigo Martins	rodrigo.martins@icb.ufrj.br
Rodrigo Soares Fortunato	rsfortunato@yahoo.com.br
Scarlet Ferreira de Souza	scarletfsouza@gmail.com
Shaila Almeida	shaila.almeida@hotmail.com
Sheila Winnischofer	sheilambw@ufpr.br
Simone da Costa e Silva Carvalho	simonedacostaesilva@gmail.com
Solange Mara Carvalho Peixoto	solmaracarvalho@gmail.com
Stefany Cristine Rodrigues da Silva	scristine73@gmail.com
Taiana Sousa	ttaianalopes@gmail.com
Tamara Gomes	tamarinea@gmail.com
Temenouga Guecheva	tgesheva@gmail.com
Thiago Torres Gomes	thiago.torres456@gmail.com
Thiago Yan Machado Prates	thiagoyanmp@gmail.com
Tiago de Souza	tiagoantonio@gmail.com
Tirzah Braz Petta Lajus	tirzah_bp@yahoo.com
Valeria Valente	valenteval@gmail.com
Vanessa Câmara Fernandes	camaravf@gmail.com
Vanessa Gottifredi	vgottifredi@gmail.com
Veridiana Munford	vmunford@usp.br
Verônica Elisa Pimenta Vicentini	veronicaepvicentini@gmail.com
Verônica Santana Silva	veronicasantana_444@hotmail.com
Wesley Roger Rodrigues Ferreira	wesleyrrferreira@gmail.com
William Kleber Martins Vieira	williamklebermartins@gmail.com
Yuli Thamires Magalhães	yuli_thamires@hotmail.com