1- A STUDY OF THE INTERACTION DYNAMICS OF THE RECOMBINANT IXOLARIS PROTEIN, INHIBITOR OF THE EXOGENOUS TISSUE FACTOR (TFPI) PATHWAY OF SALIVA GLAND IXODES SCAPULARIS, WITH COAGULATION FACTORS VII AND X.

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Ixolaris is an inhibitor of the exogenous tissue factor pathway (TFPI) characterized by the salivary gland of the tick Ixodes scapularis, vector of Lyme disease. This protein possesses two Kunitz domains and, in contrast to human TFPI, the second Kunitz domain binds to the exogenous heparin site (HBE) to factor Xa (FXa) (unlike TFPI, which binds to the active site) while the first domain binds to the active site of FVIIa in complex with tissue exporter. Another notable feature that differentiates Ixolaris from human TFPI is the ability to interact with FX, the zymogenic form of FXa, possibly through a precursor state of HBE. Ixolaris cDNA was previously expressed and purified on insect cells and was shown to inhibit FVIIa / TF induced FX activation with an inhibitory concentration of 50% (IC 50) in the picomolar range. However, taking into account several aspects of this method, such as difficulty in the incorporation of stable isotopes and the production of a therapeutic enzyme, some studies were carried out with the objective of improving the purification protocol and using the expression system in E. coli , in order to generate protein for NMR structural characterization suitably. Also in that same study, we used only the constructs composed of Kunitz-domain I and II sequences (namely K1 and K2) to further study the structural characteristics and mechanism of inhibiting blood clotting in each domain separately. In the present study, we reviewed the expression of the complete protein, as well as of the two domains, cloned in the pET32a expression plasmid, which fuses the protein of interest with the N-terminal TRX-His6 tag, facilitating the expression and purification of the recombinant protein. The TRX-His6 sequence is followed by an enterokinase protease cleavage site for removal of the N-terminal tag after purification of the protein. And we intend to verify the dynamics of the interaction of ixolaris and its two Kunitz domains with factors VII and X of coagulation, because both molecules play an essential role in key steps necessary for the maintenance of the blood coagulation cascade.

2- AGGREGATION-PRIMED MOLTEN GLOBULE CONFORMERS OF THE p53 CORE DOMAIN PROVIDE POTENTIAL TOOLS FOR STUDYING p53C AGGREGATION IN CANCER

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The functionality of the tumor suppressor p53 is altered in more than 50% of human cancers, and many individuals with cancer exhibit amyloid-like buildups of aggregated p53. An understanding of what triggers the pathogenic amyloid conversion of p53 is required for the further development of cancer therapies. Here, perturbation of the p53 core domain (p53C) with subdenaturing concentrations of guanidine hydrochloride and high hydrostatic pressure revealed native-like molten globule (MG) states, a subset of which were highly prone to amyloidogenic aggregation. We found that they bind the fluorescent dye 4,4 -dianilino-1,1′- binaphthyl-5,5′-disulfonic acid (bis-ANS) and have a native-like tertiary structure that occludes the single Trp residue in p53. Fluorescence experiments revealed conformational changes of the single Trp and Tyr residues before p53 unfolding and the presence of MG conformers, some of which were highly prone to aggregation. P53C exhibited marginal unfolding cooperativity, which could be modulated from unfolding to aggregation pathways with chemical or physical forces. We conclude that trapping amyloid precursor states in solution is a promising approach for understanding p53 aggregation in cancer. Our findings support the use of single-Trp fluorescence as a probe for evaluating p53 stability, effects of mutations, and the efficacy of therapeutics designed to stabilize p53.

3- ANALYSIS OF THE HEPATITIS C VIRUS CAPSID ASSEMBLY IN NUCLEOCAPSID-*LIKE* PARTICLES AND THEIR INTERACTION WITH PROTEIN p53

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Hepatitis C is a global public health problem. Currently, Hepatitis C virus (HCV) infects about 150 million people worldwide of this 71 million are chronically infected. In Brazil, there are 200,000 infected and the percentage of infected men and women is similar. The capsid protein, besides being involved in the assembly and protection of the viral RNA, is involved in different associated mechanisms of the host cell. Our group, and other studies, have shown that the N-terminal portion of the capsid protein (C124) is an intrinsically disordered protein being able to interact with several cellular and viral partners, among them p53 tumor suppressor protein, which may represent a mechanism of modulation of apoptosis as an important step for the replicative cycle of HCV and, consequently, for assembling the virus. To increase the knowledge on HCV assembly and pathogenesis mechanism we studied HCV core protein (C124), expressed heterologously in *E. coli* or in eukaryotic cell culture. Besides we analyzed the interaction of C124 with p53. With these purposes, we applied the followed techniques: sample turbidity assay techniques, fluorescence spectroscopy (FL), fluorescence polarization, circular dichroism (CD), confocal fluorescence microscopy, number and brightness (N&B) analysis, surface plasmons ressonance (SPR), binding assay and nuclear magnetic resonance (NMR). HCVcp can assemble into nucleocapsid-like particles (NLPs) *in vitro* in the presence of different and unspecific negative ligands, such as poly(GC) DNA, p53 consensus DNA, high molecular weight heparin, low molecular weight heparin and also when the pH is brought near to its isoelectric point. In addition, the formed NLPs are not stable due to the progressive reduction of turbidity as seen by turbidity analyzes for 5 hours. The peptides present in the sequence of C124 (pC22-39, pC50-67 and pC85-102) do not inhibit or favor C124 assembly process in NLPs, which, in the presence of pC50-87, remain bound after hours of assembly had been started. In order to identify intermediates of HCVcp assembly, different forms (C124, C179 and C191) and fused to the green fluorescent protein (GFP) on its N- (GFPC124, GFPC179 and GFPC191) or C-terminal (C124GFP, C179GFP and C191GFP), were expressed in Huh7 liver

cells. We found that C124GFP, C179GFP and C191GFP localizes into the nucleus and around the lipid droplets, whereas GFPC124, GFPC179 and GFPC191 appear diffuse throughout the cell. N&B analysis suggest a GFPC191 capsid protein present in dimeric form. Additionally, studies were performed between C124 and p53 in their entire form (p53full) and their central domain (p53C). Binding assays showed that C124 interacts more specifically with p53full. These data are confirmed by the nanomolar affinity observed during interaction by SPR assays and chemical shift variation, observed by NMR. Variations in the C124 spectrum were also visualized in the presence of p53C. In the presence of p53full a greater internalization of the tryptophan residues and greater gain of variation of the CD signal was verified. The data set obtained in these studies can help to elucidate the HCV assembly process, a virus of worldwide clinical importance and that causes many deaths annually. A better understanding of HCV assembly steps and the mechanisms of HCV proteins interaction can be used for the development of drugs and vaccines.

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4- BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF THREE-FINGER TOXINS FROM BRAZILIAN CORAL SNAKES

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The coral snakes belong to the Elapidic family (Elapidae) found around the world, are widely distributed over the American continent. These snakes have a potent cocktail of toxins that constitute their deadly venom. There are two different venom profiles in the species from America, one predominant in Three-finger toxins and the other in Phospholipases. In Brazil, the coral snake venoms are predominant in Three-finger toxins. The three-finger toxins are a family of small non-enzymatic proteins constituted by 58 to 74 amino acid residues. In all members of the family, the protein fold is based on three loops of beta stranded that assemble “fingers" directed to a globular and hydrophobic core, which is well structured by four conserved disulphide bonds. The high biotechnological potential of these proteins have been demonstrated in the literature. One exemple is the Mambalgin, a three-finger toxin from Black mamba venom, that interacts with the acid sensing ion channels (ASICs) and abolish pain. About the Brazilian three-finger toxins, very little is known, both on function and structure. Knowing this, we decided to study this family of small proteins searching mainly for a three-finger similar to the Mambalgin, as well as outstanding uncommon structure. Considering the great number of Brazilian three-fingers available in database and the diversity of sequences, we first conducted an in silico screening analyses. We utilized the Clustal and HHPred softwares to predict sequence similarity and structural homology, respectively, to form groups. With the sequences chosen, we used the Modeler software to model them and the PyMOL software to analyze the created structures. Later, we will express these sequences and pharmacologically characterize them. In our preliminary results, we found two proteins with a structural homology to Mambalgin. These proteins possess a great distribution of positive amino acid residues, which are important for the interaction with the ASIC. Furthermore, we chose two other proteins, one matching with a classical short neurotoxin, and the other classified as a non-conventional three-finger, which possess five disulphide bonds.

5- CHARACTERIZATION OF THE PRION PROTEIN FIBRILLIZATION PATHWAY INDUCED BY PHOSPHATIDIC ACID

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INTRODUCTION: The mechanism of prion protein (PrP) conversion to its misfolded isoform, prion scrapie (PrP^{Sc}), is not completely explained, but several studies have shown that this conversion can be induced by interaction with phospholipids. Phosphatidic Acid (PA) vesicles interact with recombinant PrP (rPrP), leading to the formation of amyloid fibrils, similar to PrP^{Sc} (data not published). **OBJECTIVES:** Our aim is to understand the detailed mechanisms of this aggregation pathway, investigating the conditions important for PA induced PrP fibrillization and fibril stability. **MATERIALS AND METHODS:** We subjected the PA-induced rPrP fibrils to different conditions (temperature and chaotropic agents), and used light scattering, circular dichroism, fluorescence and microscopic measurements to obtain structural and stability information. **RESULTS AND DISCUSSION:** We observed that lower temperatures favor PA-induced protein fibrillization. We observed fibrils with different morphologies. High temperatures decreased fibril signal, but did not affect secondary structure. Return to room temperature restored fibril signal. **CONCLUSIONS**: Our results suggest that changes over PrP dynamics and flexibility are important to follow a fibrillization pathway induced by PA. The quaternary structure of these fibrils seems to be sensible to high temperatures, although its secondary structure is stable, forming an intermediate capable of reorganizing into fibers.

Key words: Fibril formation, Phospholipid, Prion conversion.
Supported by: CNPq, FAPERJ, IFRJ, INBEB.

6- DOES THE RABBIT PRION PROTEIN INTERACTS DIFFERENTLY WITH BIOLOGICAL COFACTORS? IS THIS THE CAUSE OF RESISTANCE?

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Introduction: The cellular prion protein (PrP^{c}) is an α-helix rich protein that can suffer alterations in its native conformation, converting itself into a pathogenic isoform (PrP^{Sc}), turning to a β-sheet rich structure. This conversion may lead to the appearance of progressive and lethal diseases known as transmissible spongiform encephalopathy (TSEs), involving the formation of amyloid aggregates associated to the neurodegeneration process. There is still no effective treatment that prevents or fights TSEs efficiently. Glycosaminoglycans (GAGs) and Lipids are PrP ligands that have been tested as converters for prion conversion. One of the few mammals reported as relatively resistant to the causer agents of prion diseases are rabbits. Differences the way PrP interacs with cofactors may contribute to resistance. Objectives: We aim to evaluate the interaction of rabbit PrP (rabPrP) with different biological cofactors, such as heparin, dermatan sulfate, and the L-α-Phosphatidic acid (PA), elucidating protein structural changes, stability, aggregation and conversion. Material and Methods We obtained rabbit and mouse PrP by recombinant expression. We used light scattering, slow kinetics and fluorescence measurements in order to provide information on the chemical and physical properties of the interaction. Results and Discussion The results show that heparin and dermatan sulfate interacts with both PrP constructs, leading to transient PrP aggregation. Still, rabbit PrP aggregation is less robust. Interaction was pH dependent as observed for mouse PrP. After interacting with heparin and dermatan sulfate, the PrP can not bind again. The interaction of PA with PrP's is permanent. Conclusions: Heparin and dermatan Sulfate developed a protective effect on moPrP, while the rabPrP was protected only by dermatan sulfate. In both constructions the aggregation is transient when interacting whith glycosaminoglycans and permanent when interacting with L-α-phosphatidic acid.

7- EFFECT OF BOVINE LACTOFERRIN ON HUMAN PROSTATE TUMORIGENIC CELL LINES

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Introduction: Prostate cancer is the second most frequent type of cancer, and the largest fourth cause of death by cancer worldwide. According to estimates, more than 68.000 men will be diagnosed in 2018. The cellular and molecular processes underlying the pathogenesis, development and controlling of prostate cancer are poorly understood. Bovine lactoferrin (bLf) is an 80 kDa glycoprotein belonging to the transferrin family, found in body fluids, like milk, blood and saliva. It's an iron binding protein found in two different conformational state, the iron-saturated (holo-bLf) and iron-free lactoferrin (apo-bLf). It's a multifunctional protein that has been widely associated with immunologic effects and anticancer activity, besides other functions. **Objectives:** The aim of this study was to evaluate the influence of bLf on tumorigenic human prostate cell lines. **Materials and Methods:** PC-3 and DU-145 cells, from prostate cancer, were used to study anticancer activity of bLf. The morphological changes were analyzed in the optic microscope with the bLf in the cell lines Du-145 and PC-3 in 48 hours. We use MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays to analyze cytotoxicity of apo-bLf and holo-bLf at concentrations ranging from 1 to 40 mg/ml in 24, 48 hours of treatment. Proliferation assay, cell cycle and apoptosis were performed using flow cytometry after 48h treatment with bLf (1-40mg/mL). Confocal microscopy using FITC to observe apo- and holo-bLf internalization and cellular damage in DU-145. **Results and Discussion:** It was observed that bLf at 40 mg/ml induced approximately 70% of cell death in 24 hours of treatment in DU-145 cells. On PC-3 cells, we observed that 20 mg/ml bLf induced 50% of cell death in 48h compared to control. Apo-bLf caused morphological changes in PC-3 and DU-145. In PC-3, apo-bLf inhibited cell proliferation, altered cell cycle and increased cell death by necrosis and apoptosis. We were able to observe the internalization of bLF in DU-145 cells and the cell damage it caused with some differences between apo and holo. **Conclusions:** Until this point, our data support that apo-bLf induces cell death on PC-3 and DU-145 cell lines on a time and dose dependent manner. In this regard, bLf seems to be a potent inhibitor of cell growth, arrest cell cycle and increase apoptosis in prostate cancer cell lines, suggesting an effect in the modulation of human prostate cancer cell lines activity.

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8- EFFECTS OF NEW MICHAEL ACCEPTORS COMPOUNDS ON MUTANT P53 AGGREGATION

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Introduction and Objectives: The p53 protein is a tumor suppressor that inhibits tumor growth and induce cell death, usually through apoptosis pathway. In more than 50% of cancer cases this protein has at least one missense mutation. When mutated, p53 has a less stable conformation and a greater tendency to form intracellular amyloid aggregates, contributing to the accumulation of p53 in tumors. Therapy for Cancer, although vast, is still quite limited, make of extreme necessity the discovery and study new therapeutic targets, such as the aggregated p53 protein, as well as new molecules. PRIMA-1 is a Michael acceptor molecule drug candidate completing phase II study that has already been tested on different cancers. It is able to bind by covalent bond with cysteines of mutant p53 core domain and rescue the mutant p53 protein from its unfolded conformation to a wild-like conformation. Our main goal is investigate the mechanism of action of new drug candidates who are also Michael acceptors, as well as PRIMA-1, in the context of p53 aggregation. **Material and Methods:** New compounds, with Michael acceptor properties, were screened using ovarian and breast cancer cells lines. The selected compounds were used to treat cell lines expressing mutant p53 and the effects were analyzed by western blotting, immunoprecipitation and functional assays. **Results and Discussion:** Compounds were screened by the MTT reduction assay using the concentrations of 10 and 100 μM in the breast and ovarian cancer cell lines expressing mutant (MDA-MB-231 and OVCAR-3) or wild type p53 (MCF-7 and A2780). Three of them were selected for reducing the cell viability of mutant cells (RCP-03, RCP-17 and RCP-18), but the RCP-17 was effective only for the MDA-MB-231. Another was selected for reducing the cellular viability of the wild type cell lines (RCP-07). The MDA-MB-231 cell was treated with 10 μM of the selected compounds and immunoprecipitated by anti amyloid oligomers (A11), demonstrating that we have a reduction of the p53 levels in the amyloid fraction with RCP-07, -17 and -18 tratment. By western blotting, we found no difference in MDM2 and p53 levels in MDA-MB-231 after treatment, however, when treated with RCP-17, those levels are increased. Functional assays were performed with RCP-17 and -18. Both induced apoptosis by the Annexin / PI assay. Moreover, 1 μM of RCP-18 was able to induce spheroid formation in the MDA-MB-231 cell line, which is characterized as line that does not form spheroids by the protocol used, indicating a loss of function of mutant p53 in this line. Compounds RCP-17 and -18 demonstrated potential in the elimination of mutant p53 aggregates.

9- HYDROSTATIC PRESSURE EFFECTS ON PROTEIN STRUCTURE OF THE P53C MUTANT R249S INVOLVED IN HEPATOCELLULAR CARCINMA (HCC).

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Cancer is the second leading cause of death in the world. Mutations in the TP53 gene have been identified in half of all cases. These mutations result in inactivation of the p53 protein, which lose the activity as a tumor suppressor and frequently gain of oncogenic functions, besides contributing to the formation of protein aggregates with characteristic of amyloid-like. About 98% of p53 mutations occur in a highly conserved region, the DNA-binding domain (DBD). The mutation R249S is one of the six most common cancer-associated p53, mainly in hepatocellular carcinoma. The presence of the mutation destabilizes the thermodynamically stability of the p53 DBD, due to the loss of interactions that maintains the native conformation of the protein, which compromises the abilities to binds to the response element and the transcription of target genes. The function of the p53 protein depends on it three-dimensional structure, which makes it crucial the understanding of these molecular mechanisms that establish the protein folding. In this context, the hydrostatic pressure rise as a tool to investigate the structural transitions, the energetic and volumetric properties between the native (N) and unfolded (D) states. In our work, we investigated the effects of hydrostatic pressure on the structure of DBD mutant R249S (p53C R249S). The combination of hydrostatic pressure with low concentrations of guanidine (GdmCl) was used to stabilize intermediate states that have characteristics of the molten globule during equilibrium and kinetics between N / D state transitions. The Concentrations of GdmCl at 0.3, 0.5 and 0.8 M, lead to a decrease in the intrinsic fluorescence of p53C R249S, which shows that the tryptophan residue is partially exposed to the solvent, however the protein do not fully aggregates, suggesting a tertiary structure more flexible than the native structure. This conformation was able to bind the The fluorescent apolar probe bis-(8-anilinonaphthalene-1-sulfonate), Indicates that there is an increase in the exposure of hydrophobic pockets. Research by circular dichroism describe the similarities in secondary structure between this conformation and the native protein structure. Taken together, these data suggest that p53C acquires a partially unfolded conformation (MG) that precedes the formation of protein aggregation.

Keywords: Cancer, p53, Hydrostatic pressure, Fluorescence spectroscopy, Molten globule.

10- INVESTIGATING THE INTERACTION BETWEEN PRION AND LIPIDS

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Introduction: Cellular prion protein (PrP^C) can go through conformational change, assuming the structural characteristics of its pathological conformer, PrP^{Sc}.The process that leads to protein conversion involves the interaction between PrP^{Sc} and PrP^C, but endogenous molecules are believed to participate, facilitating this interaction and acting as cofactors. Lipids were shown to interact with prions and induce aggregation, but the details of these interactions remain to be clarified. **Objectives:** The present study investigates the structural aspects of the interaction between recombinant PrP^C and lipid vesicles *in vitro* characterizing aggregates, toxic mechanisms associated with the disease and its potential as cofactors. **Material and Methods:** Murine $rPrP^C$ was incubated with Phosphatidylethanolamine (PE) or Phosfatidic Acid (PA) vesicles. The samples were analyzed by Light Scattering (Rayleigh, Dynamic and SAXS) and fluorescence measurements to detect changes in protein conformation. Information about secondary structure was obtained by Circular Dichroism (CD) and FTIR. **Results and Discussion:** Increasing concentrations of PA vesicles raised rPrP light scattering, forming large aggregates. The tryptophan fluorescence emission intensity was increased and blue-shifted. In CD experiments, aggregates showed a beta-sheet negative peak, against the characteristic alpha-helix peaks from native structure. PE induced changes in fluorescence intensity, but had no impact on secondary structure, although SAXS measurements suggest aggregation. **Conclusions:** The results indicate that both PE and PA interact with PrP, but only PA induced changes in secondary structure, leading to fibril formation. It suggests that lipids, depending on its structure, interact with PrP through different aggregation pathways, which can be crucial for disease development.

Key words: Prion conversion, Aggregation, Phospholipid.
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11- INVESTIGATION OF THE INTERACTION BETWEEN PRION PROTEIN AND BOVINE LACTOFERRIN

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The cellular prion protein (PrP^C) is found in all tissues and abundantly in the central nervous system. PrP^C can suffer a structural modification on its endogenous rich α-helix form to a pathogenic isoform, PrP scrapie (PrP^{Sc}), turning into a β-sheet rich structure. This conversion triggers protein aggregation, which accumulates in the nervous tissue and progressively causes the loss of neuronal cells. Bovine lactoferrin (bLf) is an 80 kDa glycoprotein that belongs to transferrins family. It is an iron binding protein widely known by its multiple functions, such as antiviral, antimicrobial and antitumor activity. Lactoferrin is found in brain cells that were damaged by various neurodegenerative diseases, such as amyotrophic lateral sclerosis and Alzheimer's. In addition, the expression of lactoferrin is increased in neurons and glia of Alzheimer's patients. It was seen that lactoferrin binds to PrP^C and PrP^{Sc} and inhibits PrP^{Sc} formation. Since bLf can bind to PrP, it is important to investigate the possible antiprion activity of this protein. Our goal is to verify the interaction between recombinant PrP and bLf, characterizing the molecular details involved in this interaction. The structure of the complex PrP:bLf is monitored by spectroscopic techniques such as polarization. The dot-blot assay was used to assess whether apo and holo-bLf were able to decrease the presence of proteinase k resistant PrP in ScN2a cells. It was seen that increasing concentrations of bLf were able to decrease the presence of proteinase K resistant PrP. In addition, the RT-qUIC assay was performed to induce the formation of fibrillar aggregates in the presence and absence of apo and holo- bLf. In analyzing these data we noted that apo and holo-bLf were able to totally inhibit fiber formation even at the lowest concentrations. These studies are important to understand the possible application of bLf as an antiprion agent.

12- MAST CELLS COUPLE TISSUE PARASITISM TO INFLAMMATORY NEOVASCULARIZATION IN A HAMSTER MODEL OF *TRYPANOSOMA CRUZI* INFECTION

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1,2,5,6,7,8,9,10,11,12,13,16,18,19.FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 3.NATIONAL SCIENTIFIC AND TECHNICAL RESEARCH COUNCIL, BUENOS AIRES - ARGENTINA; 4.NATIONAL LABORATORY FOR SCIENTIFIC COMPUTING, PETRÓPOLIS - RJ - BRASIL; 14.FEDERAL FLUMINENSE UNIVERSITY, NITEROI - RJ - BRASIL; 15.FIOCRUZ, CAMPO GRANDE - MS - BRASIL; 17.UNIVERSITY OF TEXAS AT EL PASO, EL PASO - ESTADOS UNIDOS.

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Palavras-chave: Mast cells;Angiogenesis;Trypanosoma cruzi

Abstract

Microvascular leakage induced by proangiogenic factors fosters the formation of a provisional fibrin matrix that supports the migration of endothelial-tip cells at the onset of neovascularization. We reported that plasma leakage, a mast cell (MC)--driven inflammatory response propagated via cycles of bradykinin release and contact system activation, fuels heart parasitism, myocarditis and fibrosis. Here we investigated the impact of tissue parasitism in the microcirculation of the hamster cheek pouch (HCP) following challenge by wt Dm28c TCTs or Dm28c TCTs expressing GFP protein and luciferase. Intravital microscopy (IVM) showed angiogenesis in HCP at 7 d.p.i. Neovascularization was associated to PMN infiltration and increased density of MCs. Bioluminescence analysis revealed that Dm28c-luciferase reached other tissues, in a few cases, including the heart on 14, 21 and 30 dp.i. The link between parasitism and angiogenesis was supported by evidences that (i) injection of the same dose of Dm28c epimastigotes did not induce microvascular changes in the HCP (ii) Benznidazole (BZN) treatment initiated 24 h after TCT inoculation abrogated inflammatory neovascularization at 7 d.p.i. Since angiogenesis developed within a narrow time –window (3 days), we next examined the HCP at early time points (3 d.p.i.). There was no evidence of vessel sprouting in spite of the massive tissue parasitism observed at this time-point, although non-vascular fluorescence (dextran-FITC) was slightly increased, suggesting that endothelial barrier function is altered at 3 d.p.i. Next, we conducted proteomic analysis in infected HCP (3 d.p.i) versus infected/BZN-treated hamsters and found upregulated levels of chymase, a MC protease, previously appointed as a driver of angiotensin II-dependent angiogenesis in the hamster sponge model. T.cruzi- induced angiogenesis was blocked by an angiotensin II converting enzyme inhibitor (captopril), chymostatin and TY51469- a selective chymase inhibitor.

13- MECHANISMS OF TRANSNITROSYLATION BY HUMAN THIOREDOXIN (HTRX) USING NUCLEAR MAGNETIC RESONANCE

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Thioredoxins (Trxs) are redox and nitroso-active proteins whose redox active site is composed by the conserved CGPC residues. The active site cysteines are very important for the disulfide reductase activity, important for the regulation of numerous biological targets that interact with Trx in the cytoplasm[1]. Human and other mammalian thioredoxins (hTrx) have five cysteines. Cys32 and Cys35 provides the reactive thiols in the redox active site, while the other three cysteine residues Cys62, Cys69 and Cys73 are additional cysteines, not present in basal eukaryotes Trxs, responsible for transnitrosylation and S-thiolation post-translational modifications. Trx regulates various cellular and physiological processes through these mechanisms, such as cell cycle, vasodilation/vasoconstriction, and many others. One of the most important post-translational modifications studied is the nitrosylation (or nitrosation) of the additional cysteines present in hTrx[2,3]. hTrx system has capacity of nitrosylation and transnitrosylation of other cysteines from interacting targets in a cellular environment[4]. The mechanism of transnitrosylation and/or thiolation and the factors regulating is not fully undesrtood. Our goal is to describe mechanistically how hTrx receives and delivers the S-nitroso group. Our strategy consists of using mutants with containing only one cysteine to measure the kinetics of nitrosylation/thiolation of the different cysteines present in hTrx (Cys32, Cys35, Cys62, Cys69, Cys73) with S-nitroglutathione (GSNO) as S-nitrosylating agent. Our data sbows that Cys62 is responsible of stably store the –SNO group. Cys69 and Cys73 are more prone to thiolation. We also are calculating the structure and the structural changes of hTrx Cys62 and Cys62-SNO. Using these different approaches, in a near future, we seek to uncover the mechanisms involved in hTrx nitrosylation and thiolation.

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14- METABOLOMIC ANALYSIS OF UHT BOVINE MILK (ULTRA-HIGH-TEMPERATURE) USING: NUCLEAR MAGNETIC RESONANCE AND CHEMOMETRY ANALYSIS

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We used a metabolic approach to evaluate the hydrophilic low molecular weight metabolites present in UHT bovine milk in the Brazilian market. Seventy-two bovine milk samples were analyzed: Twenty-four samples of whole milk (3% of lipids), 24 of semi-skimmed milk (1% of lipids) and 24 of skim milk (0% of lipids). Samples were centrifuged (14,000 rpm for 10 minutes), filtered (0.22 μm) and analyzed by NMR using a 500 MHz Bruker spectrometer. The 1D 1H spectra and two-dimensional spectra ^{1}H / ^{1}H TOCSY and ^{1}H / ^{33}C HSQC were acquired at 25 ° C and were analyzed using the Topspin, AMIX and CCPN softwares. Multivariate chemometric analyzes were performed using MetaboAnalyst 3.0. Thirty-nine metabolites were assigned using literature databases, including choline, ethanolamine, phosphocholine and TMAO, which are increased in skim milk. We are now performing the analysis of the hydrophobic fraction of the milk.

15- METABOLOMIC PROFILE SECRETATED BY *IN VITRO* GROWTH OF *STREPTOCOCCUS MUTANS*

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The purpose of this study was to characterize the low molecular weight profile from the growth of *Streptococcus mutans* under different *in vitro* conditions, comparing to the degree of demineralization on bovine dental enamel. BHI Infusion culture medium with 2% sucrose and *S. mutans* strain ATCC 25175 (5 X 10 6 CFU / mL) was used in addition to 30 dental blocks, selected by surface microhardness (354.91 ± 8.6 KHN) , partially covered by acid-resistant varnish and distributed in 6 groups (n = 5) according to time of experiment: G1 - control (without microorganism); G2 - 2 days; G3 - 4 days; G4: 6 days; G5 and G6 - 4 and 6 days, respectively, both with change of culture medium every 48h. In addition, groups with *S. mutans* growth only in culture medium with no dental blocks (n = 5), were studied, being divided according to the growth time: G7 - 2 days; G8 - 4 days; G9 = 6 days; G10 - control (culture medium only). . The 1H RMN spectra from samples were acquired using a 500 MHz Bruker Spectrometer at 25ºC. After baseline correction, the spectra were submitted to the Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) (AMIX, Bruker, GER), and also to Kruskal-wallis and Mann-Whitney tests (SPSS 20.0, IL, EUA), with the confidence interval set at 95% ($p < 0.05$). The data demonstrated great distinction among the groups (ACC = 0.84), showing a time-dependent increasing in latic acid and butyric acid secretion ($p < 0.05$). The sucrose and aminoacids such as alanine, phenilalanine, and tyrosine presented a remarkable decreasing after 2 and 4 days compared to 6 days ($p < 0.05$). Reduced metabolic activity was observed in G6, with lower sucrose consumption and decreased amino acid release, compared to G5. Microhardness loss was statistically similar between G3, G4, G5 and G6 ($p> 0.9$). G7 presented higher amount of organic acid secretion ($p <0.05$) when compared to the groups with the blocks. It can be concluded that the organic acid profile secreated by *S. mutans* varied according to the substrate and this secretion is time-dependent. Additionally it was observed that the nutriente consumption decreases with the biofilm maturation. Different times and *in vitro* growth conditions of *S. mutans* caused changes in the amount of demineralization on bovine dental enamel and in the pattern of sucrose consumption and amino acid secretion, leading to different metabolomic profiles.

16- MOTHERS 'AND BABIES' BUCAL HEALTH IN THE BREASTFEEDING PERIOD

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The objective of this study was to describe the oral health condition as well as the saliva profile of women and their respective babies during breastfeeding, including breast milk. Mothers (n = 47) and their infants (n = 48) were interviewed to obtain demographic data. All have also received intraoral examination. The examination of the mothers began with oral mucosa, oral hygiene status (O'Leary), periodontal examination (da Silva-Boghossian et al. 2011) and DMF-T index (WHO, 2010). Babies had their buccal mucosa and examined teeth (dmf-t). Salivary samples of babies and mothers and breast milk were collected and analyzed by ^{1}H NMR spectroscopy with a 600 MHz spectrometer. Data were tabulated and analyzed in a statistical program (SPSS). The results showed that the mothers had a mean age of 27 years and the babies 132 days, 64.6% of whom were exclusively breastfeed. The oral conditions of the mothers revealed DMF-T 8,20; gingivitis 72.4% and biofilm 62%. The examination of the babies showed candidiasis and Bohn's nodule in 4.18% and 2.08% respectively, and dmf-t zero. The ^{1}H-NMR spectrum showed that exclusively breastfeed babies had increased sugar levels when compared to the others. Breast milk showed high lactose in the sugar region and saliva metabolites such as propionate, ethanol, lactate, acetate, butyrate, N-acetyl and sugar. It is concluded that babies have good health conditions and the mothers that participated in this study had poor oral health. NMR analyses of saliva samples showed differences between mothers and babies, and the intensity of metabolites, such as in the sugar region, showed that lactose is dominant in the saliva of babies when compared to mothers. Exclusive breastfeeding influences the salivary profile of infants.

17- NMR-SUPPORTED STRUCTURAL GENOMIC STUDIES OF TRYPANOSOMES

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Chagas disease, Sleeping sickness and Leishmaniasis are between the so called neglected diseases, endemic in poor countries of south-America and Africa. Sequencing of the genome of the kinetoplastids *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major* (Tritryp databank) open up new perspectives for drug research against these diseases. In each genome ca. 20,000 genes were identified ca. 50 % coding for proteins of unknown function. Proteomic studies confirmed the expression of several of these proteins in a life-cycle dependent manner. In the present work using bioinformatics tools available we mined the tritryp databank to end up with a list of 409 proteins up to 30 kDa, conserved in kinetoplastids, without orthologues in mammals, plant or fungi, without trans-membrane regions and without homologous sequences in the PDB that should be suitable for structural studies by solution NMR. From these proteins 20 were cloned in expression plasmids, and at least 6 were expressed as soluble and folded proteins in *E. coli*, as evidenced by their ^{1}H or ^{1}H-^{15}N HSQC nmr spectra. The expression of the remaining proteins is still being optimized. The NMR structures of six proteins were solved: Chagasin, a cysteine-protease inhibitor; FKBP12, a peptidyl-prolyl cis-trans isomerase; a protein containing a single domain of unknown function (DUF 1935), kinetoplastid specific related to the cysteine-protease Calpain; and the proteins Q4D059, Q4D6Q6 and Q4DY78. Interaction studies for some of these proteins with potential ligands are also described in this work.

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18- PRION PROTEIN COATED MAGNETIC BEADS FOR RAPID IDENTIFICATION OF POTENTIAL LIGANDS

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Prion diseases are characterized by protein aggregation and neurodegeneration. Conversion of native cellular príon protein (PrP^{C}) into the scrapied infectious isoform (PrP^{Sc}) is a critical step leading to the characteristic pathomorphologial hallmark of these diseases. Although the conversion mechanism is still not completely elucidated, the fact that ligands can act as cofactors or inhibitors in this aggregation process is widely accepted. In this context, magnetic beads were coated with the prion protein, characterized morphologically and used in bioaffinity assays, more specifically by the technique of ligand fishing, to isolate potential ligands from mixtures of organic synthetic compounds. Incubation and elution conditions were optimized, and the assay was applied to evaluate a library containing 22 quinone-quinolone derivatives. 5 compounds were fished out from this mixture and identified by ESI-MS. A control assay was performed using glycine-coated magnetic beads. The results showed good correspondence with those obtained by previously reported assays and demonstrated the reliability and rapidly of this assay in the isolation of potential inhibitors of the aggregation of the prion protein from complex mixtures.

19- PSD2 NMR ASSIGNMENT AND STRUCTURE

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PsD2 (Pisum sativum Defensing 2) is a samll cysteine-rich polypeptide (47 amino acids) from pea. This protein has been recognized to defend against fungus. Because it inhibit the fungus growth, the mechanism is unknow, but change the cell morphology. In this work, we assing fully resonances of HNCA, 15 N-HSQC, 13 C-HSQC, CBCACONH, HNCACB, HNCO, HBHACONH and HCCH-TOCSY using CCPNMR program. We also won calculate the structure at pH 3 and pH 5. For the structure calculation, we used the 2D-1H-1H-NOESY at ARIA2 program. The tertiary structure has shown different to Pisum sativum Defensin 1. Actually, two hydrogen bonds apprehend the α-helice in parallel to β-sheet, instead of diagonal on β-Sheet. Probable, that is why the selectivity is different of PsD1 at previous data.

20- ROLE OF CONFORMATIONAL EQUILIBRIUM IN MOLECULAR RECOGNITION AND CAPSID ASSEMBLY: THE CASE OF FLAVIVIRUS CAPSID PROTEINS

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Proteins are dynamic entities able to move in a wide range of timescales that goes from picoseconds to seconds. Dengue and Zika are major arthropod-borne human viral disease, for which no specific treatment is available. The flavivirus capsid protein is the trigger of virus assembly, they are located at the cytoplasm bound to lipid droplets (LD) or hydrophobic membrane interfaces. In Dengue, binding to LDs is essential for virus assembly. We showed that the positively charged N-terminal region of Dengue virus capsid protein prompts the interaction with negatively charged LDs, after which a conformational rearrangement enables the access of the central hydrophobic patch to the LD surface. We also showed the participation of the intrinsically disordered region in binding and possible regulation of capsid assembly. We probed the structure and dynamics of Dengue virus and Zika virus capsid proteins (DENVC and ZIKVC) by nuclear magnetic resonance. They bind lipid droplets (LD) in the cytoplasm, which mediates virus assembly in an unknown way. We showed that the dynamics of the capsid proteins are intrinsically involved in the mechanism of hydrophobic surface recognition, binding to LD, RNA and virus assembly. We also probed the assembly using small angle x-ray scattering and atomic force microscopy. The understanding of the participation of the intrinsically disordered N-terminal region and its dynamics helped us propose a mechanism for Dengue and Zika virus assembly and to develop a peptide with the potential to block virus assembly.

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21- SCREENING OF NEW COMPOUNDS FOR TUBERCULOSIS AND TRYPANOSOMIASIS: STRUCTURAL AND DYNAMICS STUDIES OF RIBOSE-5-PHOSPHATE ISOMERASE OF MYCOBACTERIUM TUBERCULOSIS AND TRYPANOSOMA CRUZI

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The neglected diseases are a serious public health problem generally found among the poorest populations in the world. These diseases affect 149 countries and are caused by infectious agents and parasites endemic in tropical and subtropical regions. Although they affect over one billion people, the pharmaceutical industry does not have interest on this research area. Due to limited resources, only 1.3% of the drugs produced between 1975 and 2004 targeted neglected diseases and tuberculosis. The ribose 5-phosphate isomerase (Rpi) catalyzes the interconversion of D-ribulose-5-phosphate and D-ribose-5-phosphate. This enzyme is important for cellular anabolism, which leads to the synthesis of molecules such as nucleotides and cofactors. There are two families of Rpi, called type A and type B. The RpiA is present in most eukaryotes, while RpiB is found, almost exclusively, in prokaryotes and some basal eukaryotes and fungi. Thus, the enzyme RpiB is a good target for drugs, since it is essential to cell growth and there is no human homologue. The purpose of this project is the identification of ligands for proteins RpiB of *Mycobacterium tuberculosis* (MtRpiB) and *Trypanosoma cruzi* (TcRpiB) and analyze the protein-ligand interaction by NMR techniques such as chemical shift mapping. Both enzymes were heterologous expressed in *Escherichia coli* and purified from cell lysate in a two-step process of nickel affinity and gel filtration chromatography. Isotopically labeled samples were produced and NMR data was acquired. Currently, MtRpiB backbone resonances were assigned and TcRpiB experiments are being acquired. In parallel, some screening experiments were performed and ligand candidates were selected. The structural, dynamic and interaction data obtained by NMR will be essential to generate information for design of high affinity and specificity inhibitors.

22- SPECTROSCOPIC AND IN SILICO ANALYZES REVEAL THAT ANTICANCER LUNASIN PEPTIDE EXHIBITS INTRINSIC DISORDER PROFILE IN PRE-MOLTEN GLOBULE-LIKE STATE AND STRUCTURAL PLASTICITY

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Lunasin is a 43 amino acid peptide from soybean exhibiting both preventive and therapeutic activities against cancer. In order to enable its therapeutic application, information regarding its structure and physical-chemical properties are important for a better understanding about its action and for the development of a pharmaceutical product. For this purpose, we performed analysis of secondary, tertiary and quaternary structure of lunasin, besides structural gain, stability and hydrodynamic properties. To this, we utilized spectroscopic and in silico analyzes, such as circular dichroism (CD), fluorescence spectroscopy, electrospray ionization-ion mobility-mass spectrometry (ESI-IMS-MS), size exclusion chromatography (SEC) and molecular dynamics (MD). Our CD analysis suggests lunasin as an intrinsically disordered peptide in pre-molten globule-like state with a small β-strand content. Additionally, Bis-ANS binding to Lunasin, verified by extrinsic fluorescence analysis, supports the presence of structural content. Structural plasticity of lunasin was suggested by α-helix gain in presence of trifluoroethanol. CD data also indicate lunasin stable until 100°C corroborating the intrinsic disorder. ESI-IMS-MS analysis indicates lunasin as monomeric in solution with propensity to oligomerization at highest pH. On the other hand, SEC analysis indicates lunasin mostly monomeric in solution since oligomeric populations are not observed. ESI-IMS-MS analysis with iodoacetamide indicates that lunasin presents propensity to form disulfide bond between residues Cys^{10}-Cys^{22}. Evaluation of hydrodynamics properties by SEC indicate compactness of lunasin structure, which has little influence of disulfide bond as suggested by MD analysis. MD data indicate possible influence of electrostatic interaction between N- and C-terminals of lunasin on its compactness. In conclusion, our data provide new information regarding lunasin structure, such as its structural plasticity, which can assist the understanding of its actions and, consequently, the development of a therapeutic product.

23- STUDY OF THE EFFECT OF ZIKA VIRUS INFECTION ON METABOLISM OF LIPID OF VERTEBRATE AND INVERTEBRATE CELLS

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The Zika virus (ZIKV) is an enveloped flavivirus belonging to the Flaviviridae family. The ZIKV genome consists of a single-stranded positive RNA. The replication cycle of this virus involves vertebrates and mosquitoes, maintaining an efficient cycle despite different lipid characteristics in these cells. The complex architecture of the cell membrane is maintained by means of the dynamic balance between membrane traffic, lipid synthesis and catabolism, being regulated at several levels in order to guarantee membrane homeostasis. However, it has been observed that in some cases viral infection is able to modulate lipid concentration and remodel cell membranes in organelles that are important during the viral replication process. The study of the effect of infection on the synthesis and uptake of lipids in both mammalian and mosquito allows to understand the efficient cycle that the arbovírus maintains in vertebrates and invertebrates. The objective of this work is to study the effect of ZIKV infection on the membrane organization of host cells, considering vertebrate (mammalian) and invertebrate (mosquito) hosts. Such as the effect of the infection on the lipid composition of these cells. In addition to verifying the thermostability of the viral particle and its structural impact. The effect of ZIKV infection on the cell lipid composition will be evaluated by Vero (green monkey kidney) or C6 / 36 (Aedes albopictus) cells by thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC). Changes in cellular energy metabolism during viral infection will be monitored through NADH fluorescence lifetime images in living cells using a multiphoton excitation microscope. The analysis of the life time allows to follow the changes in real time, drawing a trajectory of the changes of energy metabolism throughout the process of infection. The thermostability of the viral particle was verified by exposing the particle to different temperatures and verifying its infectivity by defining the titer. Also used was the Laurdan probe, a lipophilic probe that intercalates between the membrane phospholipids and changing its emission wavelength according to its contact with water, losing energy to it. It has an excitation wavelength at 360 nm. Another parameter analyzed was the scattering of light caused by the particle, and thus, determining the hydrodynamic volume of the ZIKV. Lipid analysis results showed that infected C6 / 36 cells exibithed clear enrichment of total phospholipids and neutral lipids, mainly triacylglycerol. While the infected cells exibithed a slight increase in the concentration of total phospholipids and cholesterol. Lifetime results show that ZIKV infection increases the concentration of NADH bound in infected cells from the third day of infection in

both cell lines, suggesting that the infection induces an oxidative characteristic in the metabolism of these cells. Laurdan's generalized polarization thermostability analyzes show that the ZIKV membrane begins to move to a less organized state as temperature increases, a result corroborated by the decrease in viral titer and the decrease in light scattering, indicating a possible decrease of the particle. These data suggest that ZIKV infection is capable of modulating metabolism and cellular lipid composition. In the meantime, further analysis will be made to more clearly understand the importance of these lipids in the replication cycle of this virus. It is also possible to state that there is a conformational change in the membrane as the temperature increases, which is concomitant with the fall in infectivity.

24-ESTUDO DA DINÂMICA ESTRUTURAL DO DOMÍNIO III DA GLICOPROTEÍNA E DO VÍRUS WEST NILE E SUA IMPORTÂNCIA NA INTERAÇÃO COM O HOSPEDEIRO.

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West Nile virus (WNV) belongs to the genus flavivírus that includes other viral individuals such as Dengue virus, Zika virus and yellow fever virus. West Nile virus can cause neurological conditions such as encephalitis, meningoencephalitis and others. The West Nile fever is common in Europe, Africa, Asia, australia and North America. In the United States thousands of cases are reported a year. WNV was isolated for the first time in Brazil in 2018 from tissue from a horse with encephalitis in Espirito Santo State.Viruses of the genus flavivírus have in their structure a capsid, which surrounds the genetic material, and the envelope, which contains two proteins, the glycoprotein E and the membrane structural protein. The glycoprotein E has three domains, DI, DII and DIII; the domain to be studied, DIII, its a important in the Dengue virus's pathways such as activation of the immune system and cellular invasion. The DIII domain is very conserved in flavivírus genus and this study with West Nile virus aim to obtain struture and dynsamic information of the DIII domain free and in complex with its host's receptors.The structural characterization of the DIII domain of West Nile virus glycoprotein E and its dynamics will be performed using the Nuclear Magnetic Resonance (NMR) technique. The domain was expressed in BL21 DE3 using pET3a and purified using ion exchange and size exclusion chromatography. The WNDIII gave good 1H/15N HSQC, with good dispersion and good stability. We are now acquiring and analysing the relaxation parameters of the free protein and then in complexes.

25- STRUCTURAL AND DYNAMICS STUDIES OF *MYCOBACTERIUM TUBERCULOSIS* FKBP12 PROTEIN: SCREENING AND DEVELOPMENT OF NOVEL ACTIVE COMPOUNDS

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Mycobacterium tuberculosis is the causative microorganism of the tuberculosis disease. In 2016, World Health Organization (WHO) reported 6.3 million new tuberculosis cases worldwide. Such disease still is a significant cause of morbidity and mortality, mainly in low-income and middle-income countries. Although many efforts had been done over the past years, WHO estimates that 490 000 new cases of multidrug-resistant *M. tuberculosis* has emerged in 2016 and this large number arise due to abandonment of the long duration treatment against active tuberculosis (at least 6 months for new cases), in which four different antibiotics are used. Treatment in such cases of multi-resistant strains has still low success rates. In this way, stronger efforts in the research of new and more effective tuberculosis drugs and targets are essential. The peptidyl-prolyl cis-trans isomerase enzyme (PPIase) FKBP12 was chosen as a target for these studies. Although FKBP12 human ortholog is already used as a target for compounds, this protein is also present in different causative microorganisms of diseases. The PPIases of microorganisms are known and validated as biological targets, and differ by about 40% in primary sequence compared to human ortholog. Putative FKBP12 from *Mycobacterium tuberculosis* (MtFKBP12) was overexpressed in *E. coli* BL21(DE3) strain at 37ºC and 0,5 mM IPTG. This protein was purified by nickel affinity chromatography and, subsequently, had its His-tag cleaved by TEV protease in 5:1 molar ratio per 16h at 4ºC. The second HisTrap step was performed in order to obtain MtFKPB12 with high homogeneity. To observe the oligomeric state of MtFKPB12 a size-exclusion chromatography using a Superdex-75 was performed. All steps were monitored by SDS-PAGE 15%. This protein was expressed isotopically labelled with 15N and 13C and had all double and triple resonance NMR experiments required for assignment done. Spatial correlational NMR experiments (NOESY) were performed too. The combinations of these with Residual Dipolar Coupling (RDC) techinic allowed us to determine its strucuture. To investigate the internal dynamics of the MtFKBP12 protein, 15N R1 and R2 relaxation experiments and heteronuclear 1H-15N NOE were performed. The relaxation data show little significant variation in the values of 15N R1, which are mostly in the range of 1.6-1.8 s^{-1} (mean R1 = 1.69 s^{-1}). For the values of R2, most of the residues present values between 7 and 9 s^{-1} (mean R2 = 8.05 s^{-1}). Most heteronuclear 1H-15N NOE values are in the range of 0.7 to 0.83 s-1 (mean NOE = 0.714 s^{-1}). The major significant variations in the N-terminus and loops between β1 and β2 and between π and β5 strands. For binding studies, the

thetrapeptide ALPF was used for titration. Perspectives include the identification of low affinity ligands that could subsequently become leading compounds for drug production.

26- STRUCTURAL CHARACTERIZATION OF GLYCOPROTEIN E DOMAIN III OF THE ZIKA VIRUS AND ITS INTERACTION WITH THE HOST

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Flavivirus is a genus that includes Dengue (DENV), Zika (ZKV), West Nile (WNV). The mature virion comprises three structural proteins: capsid, membrane protein and the envelope glycoprotein E that contains the cell receptor binding site and the fusion peptide. Protein E has 3 domains: DI, DII and DIII. The DIII domain is highly conserved between flavivirus and is related to virus binding to host cells and recognition of host antibody.We are currently working on the steps of expression, purification and renaturation of the DIII domain of the Zika virus in order to acquire ^{1}H, ^{15}N spectra and in future characterize the structure and observe its interaction with the GAG.We performed assays with the ZKV clone on pET28a and pET32a using different expression, purification and renaturation protocols. The recombinant protein goes into inclusion body and was solubilized with urea and guanidine chloride, in addition, we tested various protocols and found that recombinant protein did not rewrapped. We reanalyzed the domain sequence and added 8 residues at the C-terminus, removed the His-tag and added 2 residues at the N-terminus. We acquired the new construct in pET3a and expressed the DIII. In the current expression, DIII protein was shown to be stable in solution and the experiments show that it is folded. We are currently initiating the expression of the labeled protein to perform the experiments of measurement of the relaxation parameters to map its dynamics.

27- STRUCTURAL DETERMINATION OF NS2B PROTEIN ZIKA VIRUS AND INTERACTION STUDIES WITH NS3 VIRAL PROTEASE: THE SEARCH FOR NEW ANTIVIRAL COMPOUNDS

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The Zika virus (ZIKV) became an important global public health concern due to the Zika outbreak in 2015. ZIKV infection can cause severe neurological diseases such as, fetal microcephaly and Guillain-Barré syndrome in adults. In response, the structural virology field has explored the characteristics of ZIKV providing significant information about the pathogenesis and in the identification of novel targets for the design of new drugs. The ZIKV NS2B-NS3 protease has a crucial role in virus replication which make it an attractive target for antiviral drugs. Despite great efforts, several structural components of ZIKV, and other flaviviruses, remains unknown, especially, the transmembrane domains of the non-structural proteins, such as, the NS2B protein, the target of this study. All structural information related to these transmembrane domains will be of great relevance for understanding the pathogenesis of ZIKV and other flaviviruses. Thus, the aim of this project is the structural determination of transmembrane protein NS2B free and bound to NS3 protease domain of ZIKV using Nuclear Magnetic Resonance (NMR) in solution. The structural studies of membrane proteins by NMR are still a challenge, however, recently the use of smaller phospholipid nanodiscs allied to specific isotopic labeling strategies and NMR optimization became this strategy possible. We expressed NS2B ZIKV in *E. coli* BL21(DE3) and refolding and purification protocols has been performed to initiate the structural studies. The interaction and dynamics studies of NS2B/NS3 will allow the screening of leading compounds through fragment libraries and phage display searching to the development of new antiviral compounds.

28- STRUCTURAL DETERMINATION OF VEGFR-3 DOMAIN 2 AND ITS INTERACTION WITH VEGF INHIBITOR PEPTIDES BY NUCLEAR MAGNETIC RESSONANCE

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Vascular endothelial growth factor (VEGF) and its receptors (VEGFR) are key drivers not only in physiological but also in most pathological angiogenesis. VEGF receptor consist of an intracellular protein–tyrosine kinase domain, a single transmembrane segment and an extracellular component containing seven immunoglobulin-like domains, while domain 2 plays the major role for ligand binding. The binding of growth factors to receptors initiates the angiogenesis signaling pathways. Available anti-angiogenic therapies focus either on neutralization of VEGF binding or paths activated by these factors. However, small specific inhibitory molecules design has been a challenge in this field. Previous studies, using VEGF inhibitor peptides, suggested that VEGFR family members share a common binding site, which is extremely important for drug development. Therefore, structural studies of the interaction mechanism between peptides and VEGFRs are highly important to understand VEGFs signaling pathway and drugs design as well. Thus, the goal of this work is to determine VEGFR3-D2 structure and its interaction with VEGF inhibitory peptides using Nuclear Magnetic Resonance (NMR). VEGFR3-D2 was expressed in E. coli BL21 (DE3) strains for heterologous production. Different expression conditions were tested. Cell lysis was achieved through sonication, however, the protein remained in inclusion bodies. Therefore, refolding protocols associated with two steps of Nickel affinity chromatography was implemented in order to obtain the structured protein. All experiments were monitored by SDS-PAGE 15%. 1H-1D NMR spectra showed that VEGFR3 domain was structured and the NMR structural experiments will be performed immediately in order to calculate the structure and dynamics of this protein. The assignment of the inhibitor peptide PCAIWF has already been performed and the interaction studies of this peptide and VEGFR3 will be performed by NMR, which will allow the recognition of the peptide residues that interacts directly with VEGFR3-D2, as well, the region of the protein that interacts with this inhibitor. These studies could be of great importance to the design of new anti-angiogenic drugs.

29- STRUCTURAL DYNAMICS STUDY OF THERMOPHILIC PROTEINS BY NMR – TTHA0849 OF *THERMUS TERMOPHILUS*

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Proteins are dynamic macromolecules and undergo NMR-detectable conformational changes at various time scales. For example, movements of flexible loops that occur in the range of ns while the movement of domains occurs in the range of μs. These motions are important because they determine the function of the protein, allosteric events and modulate the interaction of proteins with different ligands. In this context, the thermophilic proteins represent a very interesting system to study these characteristics, since they perform functions similar to mesophilic proteins, but at high temperatures (40°C to 105°C). Analyzes of mesophilic and thermophilic homologous proteins show small differences: increase in the proportion of charged residues and salt bridges, increase in the number of hydrogen bonds and disulfide bonds, increasing their stability.TTHA0849, is a 147-residue Thermus thermophilus protein, which was identified by the RIKEN project. They determined the tertiary structure of this protein by X-ray crystallography, which presented an arrangement of α-helix and β-sheet with a hydrophobic cavity in the center of the protein. Its function is not yet known, but it has structural similarity with the proteins of the START (related lipid-transfer) and Betv1 superfamily. However the cavity of TTHA0849 is smaller than the like. It has more residues with hydrophobic and bulky side chains, such as tryptophan and phenylalanine and less space available for the accommodation of possible ligands. The much smaller space of the TTHA0849 cavity may indicate functional difference between this protein and Bet v 1, for example. Knowing this, TTHA0849 is an interesting study model by NMR. In this work, we try to understand how a protein of a thermophilic organism, maintain its structure and dynamics at high temperatures (~50°C) and compare the differences with a similar mesophilic organism protein.

30- STRUCTURE AND DYNAMICS OF THIOREDOXIN INTERACTING PROTEIN AND ITS CELLULAR TARGETS: THIOREDOXIN AND GLUT I.

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Thioredoxin-interacting protein (TXNIP), also known as vitamin D up-regulated protein, is strongly up-regulated by vitamin D. TXNIP belongs to the α-arrestin protein family and functions regulating oxidative stress response and glucose uptake. TXNIP binds directly to thioredoxin (hTrx) controlling all Trx-dependent regulatory processes, such as apoptosis and response to oxidative stress. It modulates inflammatory response, cardiac function, cell signaling and apoptosis. TXNIP plays a crucial role in several pathological conditions such as cancer, diabetes and cardiovascular diseases. Little structural information is available for this class of proteins. Our goals are to solve the structure of TXNIP domains and understand details of the interaction with hTrx.We cloned and expressed the N-terminal (1-149, D1) and C-terminal domain (163-301, D2) using the native sequence and mutating all cysteines by serine, with the exception of Cys63 for D1 and Cys247 for D2. We used standard methods to isotope label the cysteine-mutated domain (^{15}N and ^{15}N/^{13}C). D1 and D2 have been expressed in *E. coli* and purified. Nuclear magnetic resonance triple resonance experiments were collected. The software NMRPipe and CCPN-NMR were used for processing and assignment. We successfully expressed native and cys-mutated D1 and cys-mutated D2. D1 showed low-affinity interaction with hTrx. We almost fully assigned the resonances of D1 and map its interaction with hTrx. We constructed a NMR-derived model of the complex D1-Trx1. Contrary to D1, D2 showed strong interaction with hTrx.These data is crucial for understanding the mechanism of interaction with hTrx. We showed that D1 binds to hTrx through Cys37, possibly regulating the interaction, which occurs primarily thought D2, the hTrx-binding domain.

31- TRIGGERING OF APOPTOSIS BY BOVINE LACTOFERRIN IN VERO CELLS

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Introduction: Bovine lactoferrin (bLf) is a glycoprotein with 80 kDa belonging to the transferrin family. It can be found in many organic fluids such as tears, saliva and milk. This iron-binding protein is encountered in two forms: iron-free (apo-bLf) and iron-saturated (holo-bLf). Its vast range of functions has been intensively studied, like the antimicrobial, antiviral, anticarcinogenic and anti-inflammatory properties, by which lactoferrin acts enhancing the levels of cytokines and pro-apoptotic proteins leading the cell to programmed death. Despite all the research, the effects of bLf in the cell death mechanism and pathways are yet to be unraveled. **Objective:** This study aims to verify whether apo-bLf and holo-bLf induces apoptosis and the pathways affected by it. **Material and Methods:** The cytotoxicity effect of bLf was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, with concentrations ranging from 2 to 20 mg/mL with 3-day incubation. Flow citometry was used to analyze early apoptosis and necrosis events using annexin V and 7AAD markers. Besides, caspase-3/7 activity was assessed using Caspase-Glo 3/7 Assay (Promega®). **Results and Discussion:** Our results showed that apo-bLf has a negative effect on Vero cell viability, reducing it by 15% at 2 mg/mL and reaching a reduction of up 70% at higher concentrations. This can be correlated with the results observed by flow citometry, which pointed out signals for annexin V mostly after 24 and 48 hour-incubation and strong signals for 7AAD marker after 72 hour-incubation, suggesting early apoptosis and apoptosis followed by necrosis events respectively. We also observed that caspase-3/7 activity increased by around 60% when Vero cells were treated with apo-bLf. **Conclusion:** This study showed that apo-bLf leads Vero cells to death; our next steps are to extend these analyses to holo-bLf and determine which apoptotic pathway are affected by both forms of bLf.

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Keywords: Bovine Lactoferrin, apoptosis, Vero cells.

32- YELLOW FEVER VIRUS INDUCES CELL DEATH IN HUMAN MEGAKARYOCITES PROGENITORS

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Introduction: Yellow fever virus (YFV) is the causative agent of the haemorragic disease yellow fever. One of the classical symptoms of the disease is acute thrombocytopenia. During the platelet´s differentiation process, a single megakaryoblast can generate various megakaryocites and one megakaryocite can give origin to 10.000 platelets. Therefore, megakaryoblast alterations have a great impact on the platelet count. **Objectives:** In this study, we investigate the interaction between YFV and human megakaryoblasts, to better clarify the processes that the infection leads to thrombocytopenia. **Material and Methods:** For this study we used MEG-01 human megakaryoblast line and viral strain YFV 17DD. We evaluated infection through plaque assay and confocal fluorescence microscopy. Cellular death studies were realized through, trypan blue exclusion, flow cytometry and confocal fluorescence microscopy. **Results and Discussion:** Meg-01 lineage revealed itself to be susceptible and permissive to YFV. Susceptibility was demonstrated by the presence of viral proteins in the cell by fluorescence microscopy, starting 24h post infection. Permissibility was demonstrated by plaque assay and we observed infectious viral particle release, with maximum production at 4 days post infection. Cellular Death rates were evaluated by tripan blue exclusion and flow cytometry and we can observe more death on the infected group concerning to the control group starting on 5 days post infection. Moreover, we evaluated if there is activation of apoptosis by the virus, and for that, we used TUNEL assay and Annexin V assay, by which we observed more regular nuclear fragmentation and phosphatydilserine exposure starting on 5 days post infection. In addition, we also observed caspase 3/7 activation through luminescence with a peak on 3-4 days post infection. **Conclusions:** Our data shows that YFV infects and replicates in MEG-01 cells, with death induction starting on the fifth day post infection, with the participation of the apoptotic process.

Key Words: Yellow Fever; Virus; Megakarioblasts; Megakaryocites; Apoptosis.

33- YELLOW FEVER VIRUS MODULATES ENERGY METABOLISM OF HUMAN MEGAKARYOBLASTS

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INTRODUCTION: Yellow Fever Virus (YFV) is the causative agent of yellow fever. This hemorrhagic disease has great importance in Africa and South America. From july 2017 to july 2018, 1376 cases of yellow fever in Brazil were confirmed, with 483 deaths, calling once more the attention for this disease to the scientific community and the population. Viral infections can induce metabolic disfunctions, and this could be related to the pathogenesis. Yellow fever can cause symptoms like thrombocytopenia, that correlates to the frequent hemorrhagic events, that characterize the disease severity. Megakaryoblasts are responsible for platelet production, and a modulation of the metabolism of these cells during YFV infection can be related to the thrombocytopenia. **OBJECTIVES:** Investigate metabolic alterations in human megakaryoblasts infected by YFV. **MATERIALS AND METHODS:** Here, we used MEG-01 cells and YFV 17DD virus to investigate the infection, with a multiplicity of infection equal to 1. We detected infection through fluorescent confocal microscopy. Mitochondrial physiology was assessed by high-resolution respirometry and the Bound/Free NADH ratio by Fluorescence Lifetime Imaging Microscopy (FLIM), evaluating cellular metabolic profile. We measured lactate release during infection by spectrophotometry. **DISCUSSION AND RESULTS:** By using anti-YFV antibodies, we detected intracellular viral proteins since 24h post infection. Through respirometry, we observed reduction on routine and ATP coupled respiration on infected cells 144h post infection, compared to controls. By analyzing the Free/Bound NADH ratio, we observed a decrease in the free NADH fraction 72h post infection on infected cells, indicating a more oxidative profile compared to the control cells. We also observed an increased lactate release by infected cells until 96h post infection. **CONCLUSION:** Our data suggest that YFV can modulate mitochondrial physiology, by reducing cell routine and coupled respiration. Our results also suggest that infection leads to a more oxidative profile, through NADH metabolism and increased lactate release on MEG-01 cells.

Keywords: Megakaryoblasts, Yellow Fever Virus, Metabolism

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